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*Printed copy is uncontrolled. Refer to electronic copy for current version.*
## REVISION HISTORY

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01 OVERVIEW

DNA-01-01 OVERVIEW

1 DNA Standard Operating Procedures

The DNA Standard Operating Procedures Manual (SOP) specifies analytical procedures and processes for routine examination and analyses of biological evidence and for nuclear DNA testing. In addition, the SOP incorporates the quality assurance elements necessary to ensure accuracy, reliability, quality, and the uniformity of analyses and reported conclusions.

A. The DNA discipline is composed of three primary examination processes: biological evidence screening, male screening and DNA testing.

1. Basic biological evidence screening includes the identification of biological fluids, collection/preservation of relevant stains and collection/preservation of trace evidence.

2. Male screening includes determination of presence or absence of male DNA on items, collection/preservation of relevant stains and collection/preservation of trace evidence.

3. Forensic DNA analysis is conducted on probative biological evidence to identify human genetic markers and to determine possible sources.

B. The Texas Department of Public Safety (DPS) DNA laboratories participate in the National DNA Index System (NDIS) which contains DNA profiles of convicted offenders and forensic samples.

C. Abbreviations used with the examination documentation must be from the approved Standard Abbreviations List in the DNA SOP, the Glossary of Terms in the DNA SOP, the Terms and Definitions chapter of the Crime Laboratory Service Manual, a common American English dictionary, or commonly recognized abbreviations. Additional abbreviations not captured in the above mentioned sources must be defined in the case record or in a local document.

2 Quality Manual

The Crime Laboratory Service Manual (CLS) is considered the Crime Laboratory Service Quality Manual, which contains both laboratory policy and defines quality assurance processes.

3 DNA Training Manual

The DNA training manual contains supplementary materials, reference materials, and resources adequate to ensure competence of forensic scientists in the fields of biological evidence screening, male screening and DNA analysis.

4 Safety Manual

Crime Laboratory Service requirements for safety, exposure control, and chemical hygiene are addressed in the Safety Manual.

5 Oversight of DNA Testing Laboratories

A. DPS is required by state and federal law to maintain compatibility with NDIS which includes following the NDIS procedures prescribed by the FBI and compliance with the FBI Quality Assurance Standards.
B. The Texas DPS DNA Advisory Board (DPS DAB) is the Advisory Board for DNA and has goals and tasks as defined in the Crime Laboratory Service Manual. The Texas DPS DAB offers recommendations to the Crime Laboratory Service on technical issues related to biological evidence and DNA procedures.

6 Case Prioritization

As part of a commitment to public safety, reduction of case backlogs, providing a quality and timely completion of case analysis, and in response to State and Federal statutes, the DPS Crime Laboratory Service DNA discipline prioritizes crimes against person cases including homicide and sexual assault cases over property crime cases such as burglary. Additionally, the Service strives for completion of the analysis of sexual assault kits within 90 days.
# DNA-01-02 STANDARD ABBREVIATIONS LIST

## 1 Scope

This is the list of approved abbreviations for use in DNA examination documentation. If abbreviations are used that are not contained within this list, the Glossary of Terms in the DNA SOP, the Terms and Definitions chapter of the Crime Laboratory Service manual, a common English Dictionary, or commonly recognized abbreviations, then they must be defined in the case record or in a local document.

## 2 Abbreviations

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<td>✓</td>
<td>used/accepted/completed</td>
</tr>
<tr>
<td>+ or pos</td>
<td>positive reaction</td>
</tr>
<tr>
<td>Ø, (-), neg, NR</td>
<td>negative, no reaction, no result</td>
</tr>
<tr>
<td>1, 2, 3, 4</td>
<td>strength of reaction weak to strong, e.g., 4+ for strong positive reaction</td>
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<td>2P</td>
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<td>24plex, I24</td>
<td>Investigator 24plex QS</td>
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<td>4P</td>
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<td>none</td>
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<tr>
<td>A</td>
<td>Allele any</td>
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<tr>
<td>AD</td>
<td>additional data</td>
</tr>
<tr>
<td>AE</td>
<td>additional evidence</td>
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<td>Af Amer</td>
<td>African American</td>
</tr>
<tr>
<td>AH</td>
<td>Arrestee Hit</td>
</tr>
<tr>
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<td>allelic ladder</td>
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<td>alternate light source, includes Polilight, crime scope, Luma-Lite, etc.</td>
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<td>positive amplification control</td>
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<td>amount</td>
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<td>acid phosphatase</td>
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<td>apparent</td>
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<td>analysis/analytical threshold</td>
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<td>BBOX</td>
<td>brown box</td>
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<td>BC</td>
<td>blood card</td>
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<td>diH2O, dH2O</td>
<td>Deionized water</td>
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<td>Bad injection</td>
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<td>Blood-like substance</td>
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<td>basepair</td>
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<td>Cannot be excluded</td>
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<td>Cofiler</td>
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<td>Coll</td>
<td>collected</td>
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<td>Cond</td>
<td>Condition or conditioned</td>
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<td>CPI</td>
<td>Combined probability of inclusion</td>
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<td>CPO</td>
<td>Crotch press out</td>
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<tr>
<td>FH</td>
<td>Forensic hit</td>
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<td>Fluor</td>
<td>Fluorescence</td>
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<td>FOV</td>
<td>Field of view</td>
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<tr>
<td>FSO</td>
<td>Female sexual organ</td>
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<tr>
<td>FTC</td>
<td>Found to contain</td>
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<td>H</td>
<td>Human</td>
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<tr>
<td>Hd</td>
<td>Defense’s hypothesis</td>
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<td>HDHE</td>
<td>High does hook effect</td>
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<td>HEM</td>
<td>HemaTrace</td>
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<td>H/F</td>
<td>Heads per field</td>
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<td>HIS</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Hp</td>
<td>Prosecutor’s hypothesis</td>
</tr>
<tr>
<td>HPF</td>
<td>High-power field (400x)</td>
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<tr>
<td>IA</td>
<td>Investigation aided</td>
</tr>
<tr>
<td>I/B</td>
<td>Inside back</td>
</tr>
<tr>
<td>Id, ID</td>
<td>Identifer</td>
</tr>
<tr>
<td>Id +, ID +</td>
<td>Identifer Plus</td>
</tr>
<tr>
<td>I/F</td>
<td>Inside front</td>
</tr>
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<td>Imix</td>
<td>Indistinguishable mixtures</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>--------------------------------------------------</td>
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<tr>
<td>NE</td>
<td>not examined</td>
</tr>
<tr>
<td>NFR</td>
<td>nuclear fast red</td>
</tr>
<tr>
<td>NIK</td>
<td>not in kit</td>
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<tr>
<td>NM</td>
<td>no match</td>
</tr>
<tr>
<td>NN</td>
<td>not needed</td>
</tr>
<tr>
<td>NOC</td>
<td>number of contributors</td>
</tr>
<tr>
<td>NOE</td>
<td>not opened or examined</td>
</tr>
<tr>
<td>NP</td>
<td>no profile</td>
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<td>NPM</td>
<td>Non-peak morphology</td>
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<tr>
<td>NTC</td>
<td>Non template control</td>
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<td>NS</td>
<td>No stains seen</td>
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<tr>
<td>O/B</td>
<td>Outside back</td>
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<tr>
<td>Obs</td>
<td>observed</td>
</tr>
<tr>
<td>O/F</td>
<td>outside front</td>
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<td>offender hit</td>
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<td>off-ladder</td>
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<tr>
<td>OMR</td>
<td>outside marker range</td>
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<td>organic</td>
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<tr>
<td>Orig</td>
<td>Original</td>
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<tr>
<td>OS</td>
<td>Off-scale</td>
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<td>P</td>
<td>partial</td>
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<td>PAC</td>
<td>Positive amplification control</td>
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<td>Positive control</td>
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<td>Peak height</td>
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<td>phenolphthalein</td>
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<td>PHR</td>
<td>Peak height ratio</td>
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<td>picroindigocarmine</td>
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<td>Pkg</td>
<td>package</td>
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<tr>
<td>Pn, Pty</td>
<td>panties</td>
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<tr>
<td>Pro (+)</td>
<td>Profiler Plus</td>
</tr>
<tr>
<td>Pro/Co</td>
<td>Profiler Plus/Cofiler</td>
</tr>
<tr>
<td>PS, P/S</td>
<td>properly sealed</td>
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<tr>
<td>P&lt;AT</td>
<td>peaks below analytical threshold</td>
</tr>
<tr>
<td>P&lt;ST</td>
<td>Peaks below stochastic threshold</td>
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<tr>
<td>Q</td>
<td>Questioned sample</td>
</tr>
<tr>
<td>QF</td>
<td>Quantifiler</td>
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<tr>
<td>Std</td>
<td>standard</td>
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<tr>
<td>Stn(s)</td>
<td>stains(s)</td>
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<tr>
<td>QNS</td>
<td>Quantity not sufficient (for further analysis)</td>
</tr>
<tr>
<td>QP</td>
<td>Quick prep</td>
</tr>
<tr>
<td>Qplate</td>
<td>Questioned plate</td>
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<tr>
<td>R, Rt</td>
<td>Right</td>
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<tr>
<td>RA, ReA</td>
<td>reamplify</td>
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<tr>
<td>RB</td>
<td>Reagent blank</td>
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<td>reagent blank known</td>
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<td>RBQ</td>
<td>Reagent blank questioned</td>
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<td>RBS</td>
<td>Reagent blank sperm</td>
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<td>Rev</td>
<td>Review, revision</td>
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<td>RFU</td>
<td>Relative fluorescence units</td>
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<td>RI</td>
<td>reinject</td>
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<td>RMP</td>
<td>random match probability</td>
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<td>RS</td>
<td>retained sample</td>
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<td>Rxn</td>
<td>reaction</td>
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<td>S, Sus, Susp</td>
<td>suspect</td>
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<td>(S)</td>
<td>stained</td>
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<td>SAK, SA kit</td>
<td>sexual assault kit</td>
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<td>sexual assault nurse examiner</td>
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<td>SBBX</td>
<td>swab box</td>
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<td>sealed brown paper bag</td>
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<td>Sec</td>
<td>Second</td>
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<td>sealed manila envelope</td>
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<td>Sp, SC</td>
<td>sperm cell</td>
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<td>specimen</td>
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<td>spike</td>
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<tr>
<td>SR</td>
<td>stochastic region</td>
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<tr>
<td>SS</td>
<td>Single source</td>
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**Printed copy is uncontrolled. Refer to electronic copy for current version.**
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<td>ST</td>
<td>Stochastic threshold</td>
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<td>Sub</td>
<td>substandard</td>
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<td>Swb(s)</td>
<td>swabs</td>
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<td>SYE</td>
<td>Sealed yellow envelope</td>
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<td>TC</td>
<td>Thermal cycler</td>
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<tr>
<td>TMB</td>
<td>tetramethylbenzidine</td>
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<tr>
<td>TL</td>
<td>Technical leader</td>
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<tr>
<td>TNTC</td>
<td>Too numerous to count</td>
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<tr>
<td>U</td>
<td>unknown</td>
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<td>unstained</td>
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<td>uninterpretable</td>
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<td>Q24</td>
<td>Investigator 24plex QS</td>
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<td>V, Vic, Vict</td>
<td>victim</td>
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<td>vaginal</td>
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<td>VS, Vs</td>
<td>Vaginal swab</td>
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<td>with</td>
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<td>W, wk</td>
<td>Weak reaction</td>
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<td>WBOX</td>
<td>White box</td>
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<td>WE</td>
<td>Water extract</td>
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<td>White envelope</td>
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<td>Wht</td>
<td>white</td>
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<td>WPB</td>
<td>White paper bag</td>
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<tr>
<td>w/o, w/out</td>
<td>With out</td>
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<tr>
<td>X</td>
<td>Injection not used</td>
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<td>XL</td>
<td>extra large</td>
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<td>Y23</td>
<td>PowerPlex Y23 kit</td>
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<td>yellow</td>
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<td>Yfiler</td>
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DNA-01-03 GLOSSARY OF TERMS

1 Scope
This document defines terms used in the DNA SOP.

2 Definitions
Additive Effects: A characteristic of data that occurs due to allele sharing between contributors or sharing of stutter and allele peaks in a DNA profile.

Allele: Any peak that meets or exceeds the established analytical threshold, has Gaussian morphology, and is not an artifact.

Allele Stacking: A possible occurrence in a DNA mixture when multiple contributors in a DNA profile share an allele and cause an increase in peak height.

Allelic Drop-out: An occurrence with low-template and/or degraded samples when at least one allele at a particular locus fails to amplify to a level above analytical threshold.

Analytical Threshold: The minimum height (RFU) at and above which detected peaks can be reliably distinguished as artifacts or alleles from background noise.

Artifact: A non-allelic product of the amplification process (e.g., stutter, non-template nucleotide addition, or other non-specific product), an anomaly of the detection process (e.g., pull-up or spike), or a by-product of primer synthesis (e.g., dye blob).

Characterization: The designation of a profile as full, partial, single source, or mixture.

Comparison: Determination whether the alleles/genotypes of a reference profile are included within or excluded from an evidentiary profile.

Combined Profile: A DNA profile generated by combining typing results obtained from amplifications of the same DNA extract using different STR amplification kits. (e.g., combining typing results from a Profiler Plus amplification and a Cofiler amplification to create a more complete profile).

Composite Profile: A DNA profile generated by combining typing results from different loci obtained from multiple injections of the same amplified sample and/or multiple amplifications of the same DNA extract from an evidentiary sample while using the same STR kit. When separate extracts from a given item are combined prior to amplification, the resulting DNA profile is not considered a composite profile.

Complex Mixture: An unresolvable mixture of three or more contributors with potential allelic drop-out.

Conclusion: The results of a comparison; these may be inclusion, exclusion, or uninformative.

Contamination (Investigator 24plex): The unintentional introduction of exogenous DNA into a DNA sample or control. This can be recognized by observation of one or more called alleles at or above analytical threshold in a blank or negative control; three or more reproducible peaks attributable to exogenous DNA above 30 RFU in a reagent blank, negative control, positive control, or sample; the presence of an analyst’s DNA type in a sample, and/or evidence of carry-over between samples. Carry over from epithelial cell and sperm cell fractions of the same sample is not considered contamination. (This definition applies to current amplification kit data and not legacy amplification kit data.)
Contamination (legacy amplification kit data): The unintentional introduction of exogenous DNA into a DNA sample or control. This can be recognized as observation of one or more called alleles at or above analytical threshold in a blank or negative control; the observation of three or more reproducible, recognizable peaks below analytical threshold in a blank or negative control; the presence of any analyst’s DNA type in a sample, and/or evidence of carry-over between samples. Carry-over from epithelial cell and sperm cell fractions of the same sample is not considered contamination.

Deconvolution: Examining a profile for the different possible genotype combinations that could pair together to create the detected results as well as eliminate genotype combinations as unreasonable based on quantitative peak information and any interpretation assumptions.

Distinguishable Mixture (major/minor): A DNA mixture in which relative peak height ratios allow for deconvolution of the profiles of major/minor contributor(s).

Dye Blobs: Reproducible artifacts that may be the result of “free” fluorescent dye or dissociated dye tags from primers. They typically exhibit broadened peak morphology, low RFU values, and may be present in any dye color.

Effective Sample Size (ESS): This value is the actual number of independent samples proposed by the STRmix software, since neighboring iterations of an MCMC are not independent. This is used in the highest posterior density, an interval which most likely contains the true value, for MCMC weight variability.

Elevated Baseline: Higher than typical fluorescent background noise.

Environmental Sample: A sample that cannot be assumed to have been in direct contact with a particular individual.

Foreign: A contribution of DNA material not originating from a particular source.

Full/Complete Profile (single source): A DNA profile that exhibits a complete genotype at each locus tested.

Full/Complete Profile (mixture): A DNA profile that exhibits called information at every location tested.

Genotype: The representation of an allele pair at a specific locus.

Hd: The tested hypothesis of a likelihood ratio in the denominator; referred to as the Defense Hypothesis. Also known as H₂ or scenario 2.

Hp: The tested hypothesis of a likelihood ratio in the numerator; referred to as the Prosecution Hypothesis. Also known as H₁ or scenario 1.

Highest Posterior Density (HPD): This distribution takes into account sampling uncertainty in population databases. By utilizing the 99% 1 sided lower bound HPD, we are 99% confident that the true LR value is larger than the LR value we are reporting.

Indigenous Sample: A sample that is known to have been in direct contact with an individual for a prolonged period of time such that the presence of the individual’s DNA profile can be expected (e.g. clothing, bedding).

Indistinguishable Mixture: A mixture in which the relative mixture ratios do not allow for the determination of a major contributor.

Interpretation: Any characterization, comparison or conclusion concerning any evidentiary profile. Statistical calculations applied to any comparison are also considered interpretation.
**Intimate Sample**: Samples which originate directly from an individual’s body (e.g. fingernails, oral swab, vaginal swab, swabbing from any skin surface). Underwear (male or female) and feminine hygiene products are also considered intimate samples.

**Legacy Amplification Kit Data**: DNA typing data that was produced with an amplification kit that is no longer used by the laboratory for the generation of typing data (e.g. Profiler Plus, Cofiler, Identifiler, Identifiler Plus data).

**Longest Uninterrupted Sequence (LUS)**: The longest stretch of basic repeat motifs within an allele. It is synonymous with a theory of stutter formation that is allele specific and based on DNA sequence rather than allele size.

**Markov-Chain-Monte-Carlo (MCMC)**: A class of algorithms for sampling from probability distributions based on constructing a Markov chain that has the desired distribution as its equilibrium distribution.

**Method**: A combination of procedural steps used to perform a specific technical process. The method includes the validated steps, reagents, and critical instruments needed to perform the process or portion of a process. The same method may be conducted using different equipment (automated versus manual) when appropriately validated.

**Methodology**: Categories of methods used to perform a stage of a DNA typing technology or technologies. For example, methodologies for STR technology can include extraction, quantification, amplification, and detection.

**Minus A**: An artifact of PCR amplification observed as a minor peak one base pair shorter than the main allele peak resulting from incomplete adenylation. Minus A is often detected when an excessive quantity of template DNA is amplified.

**Mixture**: A DNA typing result originating from more than one individual.

**Mixture Proportions**: A method of assessment for the relative contributions of donors within a mixture. This is calculated by dividing the sum of RFU values for a single contributor by the sum of total RFU values at that locus.

**Mixture Ratio**: A method of assessment for the relative contributions of donors within a mixture. This is calculated by dividing the sum of RFU values for one contributor by the sum of the RFU values for the other contributor.

**No Result**: The statement given when no alleles for a given locus are detected at or above analytical threshold.

**Null Allele**: An allele that is not detected usually due to a mutation in the template DNA that prevents a PCR primer from binding properly; can result in a true heterozygote being called a homozygote.

**Obligate Allele (required allele)**: Designation of an allele that must come from a particular donor. The designation is used in mixture deconvolution and CODIS.

**Off Ladder Allele**: The designation for an allele falling outside of an established bin in the analysis software.

**Off Scale Data**: Data that exceeds the saturation point of the CCD camera of the instrument in the raw data.
Partial DNA Profile (single source): A DNA profile for which locus and/or allelic drop-out is suspected based on the evaluation of the entire profile, and a complete genotype is not obtained at all tested loci due to DNA degradation, inhibition of amplification, a low-quality template, and/or a low-quantity template.

Partial DNA Profile (mixture): A DNA profile with contributions from more than one donor that exhibits complete locus drop-out at one or more loci.

Peak: A relatively symmetric arch, tapering to a point, which is visibly distinguished from background noise and which may or may not represent an amplified allele.

Peak Height Ratio: The RFU height of the smaller allele divided by the RFU height of the larger allele at a particular locus, expressed as a percentage.

Primer Binding Site Mutation: Sequence polymorphism known to occur within or around an STR repeat region potentially resulting in a null allele causing allelic drop-out or peak height imbalance at a particular locus.

Pull-up: The result of dye bleed-through from one dye lane into another, usually because of off-scale peaks due to amplification of excessive template DNA. It may also be an indication that a new spectral calibration is needed.

Reference Sample (known): A DNA sample taken directly from a known individual (e.g. liquid blood, buccal swabs, pulled hair, bone) to serve as a standard for comparison to evidentiary profiles.

Relative Fluorescence Units (RFU): A scale used to measure intensity of fluorescence and can be an indicator of how much amplified product is present.

Single Source Profile: A DNA profile that exhibits genetic information determined to originate from one individual.

Spike: An artifact that manifests as sharp peaks that may occur in a single dye or be exhibited across multiple dyes. Most spikes in electropherograms are not reproducible because they are not an inherent part of the sample being separated and detected.

Stochastic Effects: Random disproportionate amplification of alleles in low quantity template samples (e.g. intra and inter locus peak imbalance or allelic drop out) that may result in false homozygous results if one of the heterozygous alleles fails to be detected or falls below threshold.

Stochastic Region: The area on an electropherogram where the peak height of any given allele is greater than the analytical threshold but equal to or less than the stochastic threshold.

Stochastic Threshold: The peak height value above which it is reasonable to assume that, at a given locus, allelic drop-out of a sister allele has not occurred.

Stutter: A reproducible artifact of PCR amplification that is typically one repeat unit less or one repeat unit more than the corresponding main allele peak resulting from strand slippage during amplification.

Technology: The type of forensic DNA analysis performed in the laboratory such as RFLP, STR, YSTR, XSTR, SNP, microhaylotypes or mitochondrial DNA.

Tri-Allelic Pattern: Three allelic peaks observed at a single locus in a profile that would otherwise appear to be single source in origin. The three peaks seen at the locus may or may not be of equal peak height.

Unambiguous Allele: A peak defined by the analyst as a true allele and as such, is not reasonably defined as an artifact.
Uninformative Comparison: A determination during the comparison of a casework reference sample to a forensic sample that statistical analyses provides equal support for exclusion or inclusion.

Uninterpretable: A determination that DNA data cannot be interpreted (e.g. due to poor or limited data quality, data that is too complex to confidently assign a number of contributors, data that fail to meet laboratory quality requirements). Uninterpretable profiles might not be able to be fully characterized and are not suitable for comparison.

Uninterpretable Locus: A locus which is determined to be unsuitable for comparison or statistics.
02 QUALITY ASSURANCE

DNA-02-01 PERSONNEL

1 Scope

This document provides information related to DNA personnel. It includes Technical Leader responsibilities, contingency plans, job descriptions, procedure for scientific literature review, and general information concerning training, analyst approval, and proficiency testing.

2 Related Chapters/Documents

Forensic Biology Proficiency Instructions

CLS Manual – Part III: Personnel

3 Technical Leader Responsibilities

A. Each DNA laboratory must have an assigned or appointed Technical Leader who is responsible for oversight of training, quality assurance, safety, proficiency testing, technical problem solving, and evaluation of DNA methods used.

B. The Technical Leader is accountable for the DNA quality assurance program in the laboratory to the extent that they have the authority to initiate, suspend and resume DNA analytical operations for the laboratory or an individual.

C. Laboratory organizational charts reflect the position of the Technical Leader in the management structure.

D. It is the responsibility of the Technical Leader to support and promote the management system by communicating the management system and related policies to all employees within their discipline. Specifically, the Technical Leader will:

1. Oversee the technical operations of the laboratory.
   a) Technical Leaders appointed on or after July 1, 2020 must be currently or previously qualified in all technologies used in the laboratory within a year of appointment as the Technical Leader.

2. Act as advisor with respect to technical issues. Resolve technical problems or issues between casework analyst and reviewer that may be identified in a technical review.

3. Communicate and investigate conditions or situations in the laboratory that may lead to noncompliance with policy or procedure, and ensure appropriate preventive action and self-improvement.

4. Evaluate and document approval of all validations and methods used by the laboratory.

5. Direct and document an annual review of the quality system as applicable to DNA. This review shall include the Crime Laboratory Service Manual, system-wide safety documents, General Laboratory Training Manual, and local documents. This review will be documented on the Annual Controlled Document Review form (LAB-508). The DNA SOP and training manual review will be performed by the DNA Advisory Board and documented on the Annual Controlled Document Review form (LAB-508).
6. Review and approve the training, quality assurance and proficiency testing programs in the laboratory. Review all proficiency test results reported as uninterpretable, uninformative, and/or any inconclusive comparisons for compliance with laboratory guidelines.

7. Review results of the proficiency test and document such review on the Examiner Assessment Report. Inform the casework CODIS administrator of all non-administrative discrepancies that affect the typing results and/or conclusions at the time of discovery.

8. Document approval of corrective actions prior to implementation of corrective actions. Implement and document quality action plans.

9. Ensure that personnel are adequately trained and qualified for assigned duties, to include continuing education opportunities.

10. Review the academic transcripts and training records for newly qualified analysts and technical reviewers and approve their qualifications prior to independent work analysis and document such review.

11. Review the training records and verify completion of required elements of the training program for newly qualified technicians and approve their qualifications prior to independent work analysis and document such review.

12. Assess a DNA analyst’s or technician’s previous training and ensure it is adequate and documented. If a modified training program is determined to be appropriate, document such modification.

13. Make recommendations for analyst/technician approval to perform independent case work.

14. Review internal and external DNA Audit documents and, if applicable, approve corrective action(s) to ensure that findings, if any, were appropriately addressed; document such review.

15. Approve program for the annual review of scientific literature that documents the analysts’ ongoing reading of scientific literature.

16. Document approval of the technical specifications of the outsourcing agreement with a vendor laboratory before it is awarded. Such documentation shall be maintained by the NDIS participating laboratory. Document prior approval of the technical specifications of the outsourcing agreement and/or document approval of acceptance of ownership of the DNA data prior to uploading or acceptance of DNA data for upload to or search in CODIS from any vendor laboratory or agency.

17. Complete the FBI sponsored auditor training within one year of appointment, if such training has not been previously obtained.

18. Review requests by any contract employees for employment by multiple NDIS participating and/or vendor labs. If no potential conflict of interest exists, document the approval of such requests.

19. Document approval of analysts’ review of validation studies and archived procedures for legacy amplification kits.
E. Newly appointed Technical Leaders shall also be responsible for the documented review of the following:

1. Validation studies and methodologies currently used by the laboratory
2. Validation studies and archived procedures for legacy amplification kits that were used by the laboratory
3. Training records of currently qualified technicians
4. Educational qualifications and training records of currently qualified analysts and technical reviewers

F. Contingency plan for the loss of the Technical Leader

1. In the event that a Technical Leader position is vacated or if the Technical Leader is unable to regularly respond to requests for a period of greater than 30 consecutive days, a former Technical Leader who is qualified under the current DNA Audit document or another current Technical Leader within the system will be immediately appointed by the Laboratory Director as acting Technical Leader until a permanent replacement is appointed.

2. If another Technical Leader is not available, an analyst qualified to be a Technical Leader will be appointed as acting Technical Leader until a permanent replacement is appointed.

3. The laboratory must notify the State CODIS Administrator and the NDIS Custodian regarding the loss of the Technical Leader and identify the acting Technical Leader. Appendix B from the most recent QAS Audit document is used for notification.

G. Contingency plan for labs with less than two full-time employees who are qualified DNA analysts

1. If the Technical Leader position is vacant and the laboratory has less than two full-time employees who are qualified DNA analysts, in addition to the plan for loss of a Technical Leader outlined in section F, another analyst currently qualified in the methods used by the laboratory and authorized for independent work will be appointed by the Laboratory Director to perform/review DNA casework onsite at the laboratory for at least 1 day a week until the laboratory has at least two full-time employees who are qualified DNA analysts that are authorized for independent work.

2. If the Technical Leader position is not vacant and the laboratory has less than two full-time employees who are qualified DNA analysts, an offsite technical reviewer who is currently qualified in the methods used by the laboratory and authorized for independent work will be appointed by the Laboratory Director to perform technical review for all casework completed during this time (including mentored/supervised work).

3. The laboratory will not issue DNA work and will forward DNA work to another DPS system lab until the number of qualified DNA analysts authorized for independent work reaches two full-time employees.

4. Mentored/supervised casework may be performed by the laboratory during this time as long as there is a full-time employee that is a qualified DNA analyst onsite that is approved to mentor/supervise trainees.
5. Mentored/supervised casework will not be issued by the laboratory unless an analyst currently qualified in the methods used by the laboratory and authorized for independent work who is not the mentor completes a second review of the mentored/supervised cases.

6. The Technical Leader or designated Technical Leader for the laboratory must review the academic transcripts and training records for any analysts or technical reviewers temporarily assigned to the laboratory and approve their qualifications prior to independent work analysis and document such review.

7. If there is a Technical Leader onsite at the laboratory, profiles may be entered into CODIS for NDIS upload after the profiles for upload have been reviewed by two qualified analysts authorized for independent work.

8. If there is no Technical Leader onsite at the laboratory, profiles may be entered into CODIS for SDIS upload only until a Technical Leader is onsite and the profiles for upload have been reviewed by two qualified analysts authorized for independent work.

9. The laboratory must consult with both the State CODIS Administrator and the NDIS Custodian to receive approval for the plan. The plan should not continue for longer than 90 days. Appendix B from the most recent QAS Audit document is used for notification.

4 Job Descriptions

The personnel job descriptions for the Crime Laboratory Service are updated periodically and distributed by Human Resources of the Department of Public Safety. All education, coursework, and experience requirements are listed in the respective job descriptions. The date of hire as a DNA analyst will be used to determine the applicable version of the Quality Assurance Standards for assessing educational requirements.

5 Training

A. Training will be conducted according to the system-wide training manuals. The DNA training manual references the competency requirements and addresses the qualifying examinations for personnel approval. Training beyond what is required in the system-wide training manuals will be performed at the discretion of the Technical Leader and/or supervisor.

B. Each approved DNA analyst/technician will attend at least eight cumulative hours of continuing education annually in a subject area related to DNA analysis and such attendance will be documented. Programs based on multimedia or internet delivery shall be subject to the approval of the Technical Leader. Participation in such programs shall be formally recorded and its completion shall be submitted to the Technical Leader for review and approval. The documentation shall include the time required to complete the program.

6 Review of Scientific Literature

A. Personnel assigned to the DNA section that perform analysis on casework samples are responsible for the ongoing reading of scientific literature and documentation of such.

B. The DNA Advisory Board shall make a list of at least four articles relevant to the DNA discipline that must be read for continuing education during the calendar year. The names of these articles will be forwarded to system QA through the Biology Program Coordinator or designee.
C. Documentation of completion of the readings will be maintained through Qualtrax.

D. The DNA Technical Leader indicates continued approval of the program for review of scientific literature through participation in the annual review of the quality system as applicable to DNA.

E. The four articles selected by the DNA Advisory Board must be read by all members of the DNA discipline that perform analysis on evidence during the calendar year to satisfy the requirement for annual review of scientific literature.

F. If any other members of the discipline become aware of an article they think would be appropriate for distribution, they should forward the article to the Technical Leader for consideration.

G. Members of the DNA section may meet to discuss the reading of scientific literature during the year as directed by the Technical Leader and/or Section Supervisor.

7 Analyst/Technician Approval

A. Analyst/Technician approval is required for the following sub disciplines/examinations:
   1. Biological Evidence Screening
   2. Male Screening (Sample Preparation and Process)
   3. Analytical DNA
   4. DNA Interpretation

B. New technology or methodology requires continued analyst/technician approval and analysts/technicians currently or previously qualified by DPS through completion of a specific unit of training (e.g. Biological Evidence Screening, Male Screening, Analytical DNA, or DNA Interpretation) will undergo training in order to add new methods in the unit of training for which they are already authorized for independent work.
   1. The minimum training requirements for the new method must cover technical skills and knowledge required to perform the new method and will include completion of readings (as selected from the relevant section of the training manual) and a competency set.
      a) Training must be included that covers quality control and case record documentation expectations for the new method.
      b) For analysts that will also perform technical review of the new method, this aspect must be addressed during training.
      c) A separate competency test is not required for technical review of the new method unless that is the only aspect the analyst will be qualified to perform on the new method. Analysts that qualify on performing and/or interpreting the new method become familiar with the expected documentation and results and as such are considered competent in technical review.

   2. Practice samples, a written exam and mentored/supervised work is not typically required for continued authorization and may be undertaken at the discretion of the Technical Leader.

   3. The training plan will be recorded on the Additional Methods Training Checklist (LAB-DNA-50).
4. The Quality Manager and Technical Leader will review and authorize continued work by the analyst/technician in order for them to use the technology or methodology. The approval documentation should be forwarded to the Quality Assurance Unit for inclusion with the analyst/technician’s credential file.

5. For examiners/technicians part of a validation team, the experience gained while conducting a validation with a technology or methodology will be accepted as the competency test. Documentation must be available to indicate that the involvement in the validation was representative of the extent the personnel will be involved in the casework applications.

C. Non-routine examinations which include use of DPS approved procedures not encountered by the analyst/technician during training can only be conducted on casework after competency has been demonstrated on at least five (5) known or previously analyzed samples. The Quality Manager and Technical Leader will review and authorize continued work by the analyst/technician in order for them to use the technology or methodology. The approval documentation should be forwarded to the Quality Assurance Unit for inclusion with the analyst/technician’s credential file.

D. Analysts/technicians previously qualified by DPS through completion of a specific unit of training (e.g. Biological Evidence Screening, Male Screening, Analytical DNA, or DNA Interpretation) may undergo retraining as determined by Technical Leader evaluation. Retraining may be necessitated due to extended absence or leave of absence from DPS, voluntary lapse of proficiency, or for cause. The Technical Leader will review the circumstances and previous work of the examiner/technician to determine the extent of retraining necessary, and a documented retraining plan will be developed. Once the Technical Leader has approved the retraining plan, the most current version of the DNA Training manual will be used to complete the training. If readings/exercises are necessary beyond what is required in the training manual, these will be listed (along with documentation of completion) on the retraining plan. Competency must be demonstrated prior to the analyst/technician resuming mentored/supervised or independent work. It is recommended that the Section Supervisor (if applicable) is involved in this process.

8 Proficiency Testing

A. Each DNA analyst, technical reviewer, and technician is required to successfully complete a proficiency test in DNA analysis performed to the full extent in which they participate in casework twice each year from an approved provider.

1. Based on qualification, a test must be completed using each technology (STR or YSTR) at least once per calendar year.

2. Based on qualification, a test must be completed using each amplification kit (Yfiler, Y23, Minifiler, Investigator 24plex) at least once per calendar year, with the exception of legacy amplification kits.

3. Based on qualification, a test must be completed using at least one method in each methodology (extraction, quantification, amplification, detection) at least once per calendar year.

B. One proficiency test must be performed in the first six months of the calendar year and the second in the last six months of the calendar year. The interval between consecutive tests must be at least four months and not to exceed eight months. The proficiency-testing interval is based on the date the results are submitted to the provider.
C. At least one proficiency test each calendar year must include body fluid identification for each qualified analyst.

D. Individuals whose only qualification is male screening preparation must perform male screening preparation on at least one proficiency test per calendar year.

E. Two proficiency tests per calendar year must include male screening process for each qualified analyst/technician unless that analyst/technician is also qualified in DNA analysis. If the analyst/technician is also qualified in DNA analysis, then only one test each calendar year must include male screening process.

F. Individuals whose sole responsibility is performing technical review of DNA cases shall be tested in technical review of data from each technology (STR, Y-STR) and each typing kit which they are qualified to review at least once per year.

G. If the laboratory uses DNA technicians or a team approach to casework examination, they may also do so on proficiency tests. However, each analyst performing interpretation and reporting must be assigned their own test to complete the interpretation and reporting portions.

H. Employees are considered qualified analysts/technicians in the relevant category of testing once the Laboratory Director has authorized them to conduct work in that category.

I. The competency test for newly qualified biological evidence screening (non-DNA) analysts and those qualified in male screening sample preparation only may serve as the proficiency test for the first year. These analysts/technicians shall then complete a proficiency test at least once per year to the full extent in which they participate in casework.

J. Newly qualified DNA analysts/technicians and those newly qualified in male screening process shall be added to the examiner assessment schedule within eight months of the date of qualification by the Laboratory Director.

1. An analyst/technician is considered compliant with the semiannual proficiency requirement if the initial proficiency test is taken within eight months of qualification. After the initial test, they are required to complete semiannual proficiency tests for any subsequent years.

K. Typing of all CODIS core loci shall be attempted for each technology performed.

L. If a proficiency test cannot be performed or completed due to analyst leave, the analyst shall complete the missed proficiency test upon their return to work. This test shall serve as proof of continued competency after a leave of absence.

1. For these situations, the analyst must return to the proficiency testing cycle within eight months of re-authorization to conduct work.

M. Proficiency tests are evaluated by System Quality Assurance. The grading criteria used for DNA proficiency tests include an evaluation of the correctness (including uninterpretable results or inconclusive/uninformative comparisons) of all reported inclusions, all reported exclusions, and all reported genotypes and/or phenotypes. A satisfactory grade is attained for a proficiency test when there are no errors for the DNA profile-typing data or the reported conclusions.

N. If any of the results are inconsistent with manufacturer's results, then the Technical Leader/supervisor and Quality Manager will be notified of the potential inconsistency.
O. If there is sufficient time before the provider’s due date to evaluate and correct inconsistent results, they may be corrected by the examiner before submission to the provider. A Quality Incident must be initiated for technical issues and any corrections made are documented in the record.

P. The results are evaluated by the Biology Program Coordinator for consensus within the system.

Q. There is no proficiency test requirement for analysts that participate in reinterpretation and review of legacy amplification kit data.

9 Analysts Participating in Reinterpretation and Review of Legacy Amplification Kit Data

A. Analysts that participate in the reinterpretation and/or review of legacy amplification kit data (including CODIS Administrators/Alternate CODIS Administrators assessing moderate stringency CODIS matches) must have been a previously qualified analyst in the legacy amplification kits (including most current interpretation procedures) or complete an initial training program related to interpretation and review of data generated using legacy DNA amplification kits.

B. Analysts that participate in the reinterpretation and/or review of legacy amplification kit data (including CODIS Administrators/Alternate CODIS Administrators assessing moderate stringency CODIS matches) must review the validation studies and standard operating procedures related to the legacy amplification kit they are reinterpreting and/or reviewing once every two calendar years. This review must be approved by the Technical Leader.

C. If an analyst that participates in the reinterpretation and/or review of legacy amplification kit data (including CODIS Administrators/Alternate CODIS Administrators assessing moderate stringency CODIS matches) has participated in a proficiency test using the legacy amplification kit within two years of performing legacy amplification kit data reinterpretation and review, they are not required to complete the review of the validation and standard operating procedures for the legacy amplification kit that was used to complete the proficiency test.

10 Records

DNA Technical Leader Checklist (LAB-DNA-48)
DNA-02-02 FACILITIES

1 Scope
The laboratory will be designed to provide adequate security and minimize contamination. Security, limited controlled access, and system-wide facilities requirements are described in the Crime Laboratory Service Manual.

2 DNA Work Areas

2.1 Overview
A. The DNA laboratory will have space for evidence examination, DNA extraction, PCR setup, and amplified DNA product handling, which are designated on a floor plan of the laboratory.

B. The evidence examination area, DNA extraction area, and PCR setup area will be separate from each other.
   1. This can be accomplished by maintaining separate physical spaces for each task or by conducting these tasks at separate times.
   2. If conducted in the same space at separate times, the space will be decontaminated between tasks.

C. The amplified DNA product area will be physically separate from all other areas. The amplified DNA product area will have a door maintained in a closed position.

2.2 Evidence examination
The examination of evidence will be performed in an evidence examination area. The tasks performed may include screening, trace evidence collection, body fluid identification, and selection and cutting of stains. Microscopy may also be performed in this area.

2.3 DNA Extraction area
Male screening lysis, DNA extraction, DNA purification, and DNA concentration are performed in a DNA extraction area at separate times or in separate spaces from quantification and amplification.

2.4 PCR Set-up area
All quantification and amplification set-up steps are performed at separate times or in separate spaces from extraction and each other. A PCR set up area may be a different room or an enclosed space within the lab such as a laminar flow hood or PCR set-up enclosure designated for quantification and amplification set-up.

2.5 Amplified DNA product area
The generation and analysis of amplified DNA product will be performed in the amplified DNA product area. This includes performing quantification using real-time PCR analysis. Once amplified, no samples will leave the amplified DNA product area unless securely packaged. Equipment, reagents, and supplies in the amplified product area are dedicated to that area and will not be removed unless properly decontaminated. Each removable item will be readily identified as dedicated for use in the amplified DNA product area only.
3 Good Laboratory Practices

3.1 Prevention and Decontamination

A. The following may be used to remove residual DNA and/or decontaminate surfaces: 10% bleach, a commercial DNA decontaminant, UV irradiation, or ethanol.

When cleaning with bleach, use a freshly prepared (weekly) dilution as its effectiveness declines over time.

B. Wear disposable gloves during all testing. Change gloves frequently and whenever gloves may have become contaminated. Discard gloves when leaving a work area.

C. A face mask should be worn when appropriate to guard against sample contamination.

D. A lab coat with cuffs that is dedicated to the process being performed (e.g. screening or DNA) should be worn during all testing.

E. Centrifuge all liquid to the bottom of closed tubes before opening. A de-capper should be used.

F. Use sterile, disposable lab supplies during testing (e.g. pipette tips, spin baskets, transfer pipettes, tubes).

G. Use aerosol-resistant pipette tips while working with any sample that may be DNA typed.

H. If the sample is already present in the tube, pipette tips must be changed between samples. It is recommended that once sample has been added, only one sample tube should be opened at a time.

I. Limit talking during sample handling.

J. In the evidence examination area:

   1. Clean work surfaces thoroughly at least at the end of each evidence examination session.

   2. Use disposable bench paper whenever possible and change at least at the end of each evidence examination session.

   3. Use a clean cutting surface such as a weighing paper or small tablet of paper for each piece of evidence. Protect supplies of this paper from dust and other particulates or aerosols.

   4. Clean instruments (scissors, forceps, etc.) between evidence samples. Alternatively, use a fresh scalpel blade with each sample.

   5. To prevent contamination of other standards or evidence, handle liquid samples such as a blood standard one at a time and with no other evidence open in the immediate work area.

K. In the DNA extraction area, clean work surfaces thoroughly at least at the end of each DNA extraction session.

L. In the PCR set-up area, add DNA template last to the PCR set-up tubes to minimize inadvertent transfer between set-up tubes. Clean work surfaces thoroughly after use.

M. In the amplified DNA product area, wear a dedicated, disposable lab coat when handling amplified samples. Do not wear the lab coat outside the amplified DNA product area. These lab coats will be disposed of when necessary. Clean work surfaces thoroughly after use.
3.2 Cleaning glassware

A. Wear gloves when cleaning.

B. In general, clean glassware after each use with an appropriate detergent and water. Rinse with deionized or distilled water and allow to air-dry inverted.

C. Glassware used in the amplified DNA product area will be rinsed thoroughly with water after each use, rinsed in a final rinse of distilled or deionized water, and inverted to air-dry.

D. Glassware used for DNA analysis may be sterilized by autoclaving or UV irradiation.
DNA-02-03  VALIDATION

1 Scope
Validation is the process of establishing documentation and objective evidence to demonstrate that the Laboratory is operating competently and is able to generate valid results. Validation provides a degree of assurance that a specific process, procedure, or method will consistently produce a result which meets its predetermined specifications and quality attributes. This document covers the validation process related to DNA.

2 Related Documents
CLS Manual: Validations and Performance Verifications
CLS Manual: Laboratory Equipment

3 Practices

3.1 Validation of Methods and Equipment
A. A Validation Plan (LAB-407) is required before any validation work may begin for Initial (System) Validation studies or Modification/Revision validation studies of methods and equipment.
B. A developmental validation is required prior to implementation of any new methods used for DNA analysis.
   1. The laboratory will rely on developmental validations performed in other non-DPS laboratories.
   2. The citation and/or publications referencing the developmental validation are maintained and available within the DPS laboratory where the method is used.
C. Validation studies shall be documented and summarized.
   If any studies are determined to be not applicable, the summary must acknowledge and address the reason why this determination was made.
D. The Technical Leader is responsible for approving methods used by the laboratory. Technical Leaders may engage in or direct such activities within their laboratories.
E. Initial (System) Validation studies:
   1. Shall include as applicable: known and non-probative evidence samples or mock evidence samples, accuracy and precision (including reproducibility and repeatability), sensitivity and stochastic studies, mixture studies, and contamination assessment.
   2. May be shared between the laboratories in the System;
      a) Each regional laboratory shall complete site-specific precision, sensitivity and contamination assessment studies as part of an implementation validation prior to using the method.
      b) The summary of any shared validation data must be available at the regional laboratory.
   3. Shall include evaluation using an appropriate and available certified reference material such as a NIST or NIST-traceable reference material if the method affects amplification through characterization procedures.
4. Shall define quality assurance parameters and interpretation guidelines, including mixture interpretation and application of appropriate statistics as applicable.

5. Shall be required if substantial changes such as changes to typing test kit or instrument model platform are made.

F. Implementation Validation studies:
   1. Shall include site-specific precision, sensitivity and contamination assessment studies.
   2. Shall include evaluation using an appropriate and available certified reference material such as a NIST or NIST-traceable reference material if the method affects amplification through characterization procedures.

G. Modification/Revision Validation studies:
   1. Are conducted if a procedure is modified such that a protocol change is required (i.e. a material method modification),
   2. Shall evaluate the modified procedure by comparing the original procedure to the modified procedure.
   3. May be shared between the laboratories in the System if the laboratories use the same procedure on the same reagents and significant equipment.
      a) Each regional laboratory may need to complete site-specific precision, sensitivity, and contamination assessment studies if the modification impacts the efficacy or reliability of the test.
      b) The summary of any shared validation data must be available at the regional laboratory.

3.2 Required Studies for Methods and Equipment

A. Initial system validation of CE instruments must include the following studies and associated summaries:
   1. Precision study comparing at least 10 allelic ladder injections from the same run to justify the +/- 0.5 bp window
   2. Sensitivity (dilution) and stochastic studies (includes signal-to-noise, limit of detection, limit of quantitation, and peak height ratio evaluation)
   3. Optimal DNA template range determination
   4. Verification using an appropriate and available certified reference material
   5. Known/non probative samples
   6. Accuracy (repeatability and reproducibility) studies
   7. Mixture study
   8. Contamination assessment (including the evaluation of controls and known samples)
   9. Other appropriate studies as needed
B. Initial system validation or implementation validation of thermal cyclers must include the following studies and associated summaries:
   1. Temperature verification, temperature uniformity, and diagnostic tests
   2. Amplification and analysis of an appropriate and available certified reference material for concordance.

C. Initial system validation of Real-Time PCR systems must include the following studies and associated summaries:
   1. Verification using an appropriate and available certified reference material
   2. Sensitivity study (includes limit of detection and limit of quantitation)
   3. Precision study
   4. Reproducibility study
   5. Contamination assessment (including the evaluation of controls and known samples)
   6. Mixtures, as applicable (i.e. male screening)

D. Initial system validation of amplification kit must include the following studies and associated summaries:
   1. Verification using an appropriate and available certified reference material
   2. Sensitivity study (includes optimal template, peak height ratio, signal-to-noise)
   3. Precision study
   4. Reproducibility study
   5. Stochastic study
   6. Contamination assessment (including the evaluation of controls and known samples)
   7. STRmix assessment
   8. Known and non-probative samples

E. Validation of a robotic workstation must be conducted. The studies performed will depend upon the procedure being automated. Initial system validation must include verification using an appropriate and available certified reference material and may also include as appropriate:
   1. Sensitivity study
   2. Precision study
   3. Reproducibility study
   4. Contamination study (including the evaluation of controls and known samples)
   5. Changes to the robot programming after validation which may affect DNA results will require additional validations.
3.3 **Software Validation**

A. A Validation Plan Form (LAB-407) is required before any validation work may begin for Initial (System) Validations or Modification/Revision validations of software.

B. Developmental validation is required prior to implementation of any new software used for DNA analysis.
   1. The laboratory will rely on developmental validations performed in other non-DPS laboratories.
   2. The citation and/or publications referencing the developmental validation are maintained and available within the DPS laboratory where the software is used.

C. New software or new modules of existing software used as a component of equipment, for the analysis and/or interpretation of DNA data or for statistical calculations shall be evaluated to assess the suitability of the software for its intended use and to determine the necessity of validation studies or software testing. This evaluation shall:
   1. Be performed at the direction of the Technical Leader,
   2. Include determination of which studies will and will not be conducted,
   3. Be documented,
   4. Specify the type of software being evaluated (equipment, analysis/interpretation, or statistical),
   5. Specify if the analytical process is impacted or not, and
   6. Include software tools developed by the laboratory.

D. When performed, validation studies shall be documented and summarized.
   1. If any studies are determined to be not applicable, the summary must acknowledge and address the reason why this determination was made.

E. Whenever major revision (i.e. one that impacts the analytical process, interpretation, or statistical calculations) is made to software, the software shall require modification/revision validation prior to implementation and shall include functional (performance) testing, reliability testing, and regression testing.
   1. Validation of major revision to software used for analysis and/or interpretation of DNA data shall also include as applicable: precision and accuracy studies, sensitivity, and specificity studies.
   2. Validation of major revision to software used for statistical calculations shall also include as applicable: precision and accuracy studies.

F. Whenever a minor revision is made to the software (i.e. one that does not impact the analytical process, interpretation, or statistical calculations), a performance verification (functional test) is required prior to implementation.

G. Each laboratory in a multi-laboratory system may share software validation and software testing studies as long as the summary of the shared validation data is available at each site and each site completes applicable reliability testing.

H. The Technical Leader is responsible for reviewing and approving software validation and testing prior to implementation. Technical Leaders may engage in or direct such activities within their laboratories.
3.4 Required Studies for Software

A. Validation of new software or new modules of existing software that are a component of equipment must include the following studies and associated summaries:
   1. Functional testing (performance verification)
   2. Reliability testing

B. Validation of new software or new modules of existing software used for the analysis and/or interpretation of DNA data must include the following studies and associated summaries:
   1. Functional (performance verification) testing
   2. Reliability testing
   3. Precision and Accuracy studies (as applicable)
   4. Sensitivity studies (as applicable)
   5. Specificity studies (as applicable)

C. Validation of new software or new modules of existing software used for statistical calculations must include the following studies and associated summaries:
   1. Functional (performance verification) testing
   2. Reliability testing
   3. Precision and Accuracy studies (as applicable)

3.5 Technical Review of Validation Studies

A. Technical review of validation studies should address the following components at a minimum:
   1. All the necessary studies relevant to the method/equipment as listed above have been addressed for initial (system) validations.
   2. As applicable, concordance with the previous method has been assessed and found to be similar or better than the previous method. Concordance is required for method modification/revision validations.
   3. For implementation validation,
      a) An initial (system) validation has already been performed and is accessible to the regional laboratory.
      b) Site-specific precision, contamination, and sensitivity studies have been completed.
   4. A copy of the developmental validation paper is accessible to the regional laboratory for the method/instrument.
   5. The materials and methods used to complete the validation are clearly listed in a summary and are adequate for each study. The detail is sufficient that if another analyst wanted to repeat the study, they would be able to do so by looking at the validation paperwork.
6. Results for each study are summarized and supported by data.
   a) The data record is complete and includes, as applicable, required forms for extraction, quantification, and amplification and data analysis project lists for any capillary electrophoresis runs.
   b) Any raw data or electropherograms are accessible and could be produced for inspection upon request.
   c) The data record clearly indicates any changes made to parameters listed in the current DNA SOP (e.g. changes to thermal cycling parameters, CE run voltage and time, etcetera).

7. DNA documents (both system and local) have been reviewed to determine if they are applicable to the validated method/instrument.
   a) Any proposed deviation to current DNA documents has been tested during the validation.

8. The need for further studies to address any issues with the method/instrument that were revealed during the validation has been assessed.

B. Technical review of validation studies should not be performed by personnel that were directly involved in the completion of the validation.
   1. The Technical Leader must document approval of validation studies.
   2. While this documentation may be in the form of a technical review, if the Technical Leader was directly involved in the completion of the validation study, they may document their approval of the validation through means other than documented technical review.

C. Technical review of validation studies should be completed by personnel that are qualified in the general discipline under which the validation falls. For example, a validation of a real-time PCR quantification kit might be technically reviewed by an analyst who is qualified in DNA analysis or male screening involving the use of quantification by real-time PCR.

D. A validation study that was performed at one DPS laboratory may be technically reviewed by qualified personnel at another DPS laboratory.

E. The elements listed above may also be used by the Technical Leader to review and approve validation studies conducted by outsource laboratories.

4 Records
Validation and Verification Plan Form (LAB-407)
Validation and Verification Form (LAB-408)

5 Literature References and Supporting Documentation


DNA-02-04 ANALYTICAL CONTROLS

1 Scope
Controls are required to assess the effectiveness, accuracy and/or precision of the analytical procedures. Appropriate controls must be analyzed with each sample or set of samples. Appropriate controls may include, but are not limited to, reagent blanks, negative controls, and positive controls.

2 Related Chapters/Documents
Response to Quality Issues
Contamination Log Workflow
Legacy Amplification Kit Data Reinterpretation
CLS Manual: Laboratory Equipment
CLS Manual: Standards, Reference Materials/Collections, Databases, and Controls

3 Biological Evidence Screening
The reagents and methods used in screening for body fluids must be tested with controls and documented on the day of use.

4 DNA Analysis
4.1 Extractions
A. Process Separation
1. The manual extraction of known samples must be performed at a separate time or location from the extraction of evidentiary samples to eliminate the potential for known to unknown sample contamination.
2. Time and date are documented on the DNA Extraction Worksheet or equivalent.
B. Reagent Blank
1. A reagent blank will be prepared for each set of DNA extractions and will contain all reagents used in that extraction process with the exception that it will not contain sample. It will be extracted concurrently and in the most sensitive volume of the extraction set.
2. Multiple reagent blanks may be used per extraction set. All reagent blank requirements will apply to each associated blank except that only one reagent blank must be amplified per primer set and instrument used. If any reagent blank yields a detectable DNA quantification result, amplification and typing of at least the reagent blank yielding the greatest signal is required.
3. A reagent blank per extraction set will be processed through the entire analysis and will be handled in such a way that it will detect contamination during the extraction procedure. If a sample extract is processed further, the corresponding reagent blank must also be processed in a similar manner.
4. Multiple extraction procedures will be monitored with a reagent blank for each type or group of extraction reagents used.
5. Although reagent blanks are considered work product, any remaining reagent blank must be retained.
6. The reagent blank shall be amplified in the same concentration conditions as required by the forensic sample(s) containing the least amount of DNA. For example, if 15 µL is the greatest amount of template amplified for any evidence sample in the batch, 15 µL of reagent blank must be used as template during the amplification.

7. A reagent blank shall be amplified utilizing the same primers and thermal cycler model of the extraction set.

8. If any sample requires re-amplification using the same primer set, the associated reagent blank need not be amplified a second time if it has been shown to not exhibit contamination during the first amplification.

9. The reagent blank shall be typed utilizing the same CE instrument model and most sensitive injection conditions of the extraction set.

10. For samples extracted prior to July 1, 2009, if the reagent blank(s) from the extraction set has been depleted or has insufficient volume remaining, it is not required to complete steps 6-9 above.

11. The reagent blank must not exhibit contamination. If contamination is exhibited refer to the Response to Quality Issues and Contamination Log Workflow chapters in the DNA SOP for resolution.

   a) For Investigator 24plex samples, contamination can be recognized as observation of one or more called alleles at or above the analytical threshold and/or the observation of three or more reproducible peaks attributable to exogenous DNA above 30rfu in a reagent blank.

   b) For information about controls for legacy amplification kits, refer to the Legacy Amplification Kit Data Reinterpretation chapter.

   c) For information about controls for Minifiler and Y-STR kits, refer to local laboratory policy. If no local laboratory policy exists, use the definition located in the Legacy Amplification Kit Data Reinterpretation chapter.

C. Quantification Controls

1. Standards must be used as part of the quantification process.

2. A quantification blank will be used and will contain all reagents used in the quantification process with the exception that it will contain TE/dilution buffer in place of sample.

3. If a value is obtained in the quantification blank, this may indicate the need to run a background plate, check the assay setup, software setup, reagents or Real Time PCR system. The information from this plate may be used if a true negative (such as a reagent blank) is present on the plate.

4.2 Amplification Controls

A. Negative Control

1. The negative control will contain all PCR set-up reagents except DNA template.

2. The negative control should be the last sample processed in the set and will be handled in such a way that it will detect contamination occurring during manual PCR set-up. Refer to the DNA Amplification – Investigator 24plex QS Using Tecan
chapter and the *DNA Amplification – Yfiler Using Tecan* chapter in the DNA SOP for automated processing.

3. The negative control must not exhibit contamination. If contamination is exhibited refer to the *Response to Quality Issues* chapter in the DNA SOP for resolution.

4. Contamination can be recognized as observation of one or more called alleles at or above the analytical threshold and/or the observation of three or more reproducible peaks attributable to exogenous DNA above 30rfu in a negative control.

5. For Yfiler, the female negative control used during Y-STR analysis will be amplified only once during the QC of the Y-STR amplification kit and must not exhibit a DNA profile. If a DNA profile is apparent in the female negative control, notify the technical leader for assistance in resolution. There is no female negative control for the Y23 kit.

B. Positive Control

1. The positive control tests for proper amplification of samples, as well as ensuring that the DNA analysis software is working properly.

2. A positive control is included in the amplification kits.

3. The positive control must exhibit the following appropriate DNA profile:

### Investigator 24plex QS

<table>
<thead>
<tr>
<th>Amel</th>
<th>TH01</th>
<th>D3S1358</th>
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<th>TPOX</th>
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<td>8,9</td>
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<td>D12S391</td>
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<td>D10S1248</td>
<td>D22S1045</td>
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<td>18,24</td>
<td>23,2,26.2</td>
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<td>FGA</td>
<td>D16S539</td>
<td>CSF1PO</td>
<td>D13S317</td>
<td>D5S818</td>
<td>D7S820</td>
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### MiniFiler

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### Y23

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Yfiler

<table>
<thead>
<tr>
<th>Allele Peaks</th>
<th>QS1</th>
<th>QS2</th>
<th>Interpretation</th>
<th>Possible Solution</th>
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<tbody>
<tr>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Successful profile</td>
<td>n/a</td>
</tr>
<tr>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>No Result</td>
<td>Reamplification (consider cleanup of extract prior to)</td>
</tr>
<tr>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>PCR Failure</td>
<td>Cleanup or dilution of extract prior to reamplification or amplify using less target DNA</td>
</tr>
<tr>
<td>Ski-slope profile</td>
<td>Present</td>
<td>Absent</td>
<td>Possible inhibition</td>
<td>Consider reamplification using more target DNA</td>
</tr>
<tr>
<td>Ski-slope profile</td>
<td>Present</td>
<td>Present</td>
<td>Possible degradation</td>
<td></td>
</tr>
</tbody>
</table>

4. If the profile from the positive control contains peaks that correspond to artifacts such as spikes, pull up, minus A, or stutter, the analyst may designate the peaks as artifacts by labeling them with the identity of the artifact and use the data from the run. Alternatively, the analyst may choose to use the data from the run and reference a reinjection of the same positive control from a previous or subsequent run.

5. If the appropriate DNA profile is not exhibited (such as a partial profile is obtained, a mixture profile is obtained, or incorrect genotype is obtained), notify the Technical Leader for assistance in resolution.

C. Quality Sensors for Investigator 24plex QS

1. The Investigator 24plex QS kit contains two internal PCR controls: QS1, which appears at 74 bp and QS2, which appears at 435 bp.

2. Peaks heights for QS1 and QS2 may vary up to 30% under normal conditions. If the QS2 peak is more than 30% lower than the QS1 peak, this may indicate inhibition is occurring in the sample.

3. The table below summarizes possible interpretation for the QS sensors and may be used to determine if additional work is needed for a sample.
4.3 Software Evaluation of Controls

A. Approved DNA analysis software may be used to check for concordance of all ladders, positive controls, negative controls, and reagent blanks.

B. All negative controls and reagent blanks must be viewed manually to check for below threshold peaks and verify the presence of primer peaks.
DNA-02-05 RESPONSE TO QUALITY ISSUES

1 Scope
The quality issues specifically addressed by this chapter are contamination and sample switches. Samples can become contaminated with DNA from the environment (i.e. analyst, consumables, etc.), from other samples during sample preparation, or from amplified DNA product from a previous amplification. Reagent blanks and negative amplification controls are used to detect contamination. For preventative measures refer to the Facilities chapter in the DNA SOP. Like contamination, there is inherent risk for sample switches during the analysis process. Numerous preventative measures are taught to ensure the risk is minimal. However this chapter serves as a contingency plan, should one occur.

2 Related Documents
Contamination Log Workflow
CLS Manual: Quality Incident (QI) and Quality Action Plan Process (QAP)

3 Practices

3.1 Staff Profiles
A. All laboratory personnel DNA profiles should be developed and retained by the Technical Leader for the purpose of identifying possible sources of contamination.
B. The personnel DNA profiles from all laboratories are also available on SharePoint.
C. The personnel DNA profiles posted on SharePoint are reviewed and/or updated as needed, but at a minimum of once a year.

3.2 Contamination and Sample Switch Assessment
A. If contamination or a sample switch is detected, the analyst must notify the Technical Leader immediately. The Technical Leader will define and direct the actions taken.
B. All suspected quality issues must be investigated.
C. Contamination may be introduced after the start of a controlled forensic analysis. The start of a controlled forensic analysis is defined as the point that the evidence packaging is opened.
D. If appropriate, the analyst will work backwards through the sample processing steps to determine at which step contamination or a sample switch may have occurred and if the incident can be resolved.
E. Determining the appropriate actions to be taken will depend upon which step the suspected issue had occurred or in which sample/control it was observed.
   1. If contamination or the sample switch is still evident, no new DNA cases will be started by the analyst/technician until the cause is identified or until the incident is resolved.
   2. In batching situations, further assessment may be necessary to determine the extent of the effect of the contamination or the sample switch. For these situations, the Technical Leader will determine if processing should be ceased and to what level.
3. If contamination is suspected, associated buffers and prepared reagents may be discarded; reagent bottles thoroughly cleaned, decontaminated and autoclaved; and fresh reagents and buffers prepared.
   
   a) To test the fresh reagents, a known sample set may be re-extracted, re-amplified, or re-set up for CE (depending on the nature of the contamination).

F. All documentation pertaining to the incident must be placed in the case record. The Technical Leader should also maintain a copy of the supporting documentation related to the resolution of the incident.

G. If determined to be necessary, the quality incident/action plan process should be initiated as soon as practicable after such determination has been made.

   1. If corrective action was determined to be necessary, the quality action plan must have the documented approval of the Technical Leader prior to implementation.
   
   2. Preventative measures taken to minimize reoccurrence of the issue must also be addressed.
   
   3. Corrective action is necessary if the incident was unable to be resolved, was resolved after release of information to the customer, or if the incident was determined to be severe (e.g. pervasive contamination in the laboratory, analysts involved in repeated events of a similar type, the same equipment determined as a cause of repeated events, etcetera.)

3.3 Sample Switch Incidents
Sample switch incidents that are identified during technical and/or administrative review or after results have been released to the customer will be documented on a Quality Incident Report (LAB-510 or electronic equivalent).

3.4 Resolved Contamination

   A. Resolved contamination events include those where contamination in a reagent blank or samples is no longer evident after new CE set up, reamplification or re-extraction.
   
   B. Any resolved contamination investigations that are resolved through reamplification or re-extraction or those that consume additional reagent blank will be documented using the contamination log workflow (refer to the Contamination Log Workflow chapter).
   
   C. A Quality Incident Report (LAB-510 or electronic equivalent) is not required for resolved contamination investigations that consume additional sample, extract or reagent blank unless the resolution occurs after the results have been released to the customer.
   
   D. If contamination is resolved by re-extraction of the sample, both extractions must be reported to the customer.

      1. No results will be reported and a statement that a quality event occurred will be included in the report for the contaminated extract.
      
      2. Comparisons will only be made and reported for the non-contaminated extract.
      
      3. Refer to the Report Writing Guidelines chapter.
3.5 Unresolved Contamination

A. Unresolved contamination events include those where contamination in a reagent blank or samples remains evident despite re-extraction attempts or where it is not possible to re-extract the sample due to depletion and contamination is still evident.

B. Any unresolved contamination event will be documented using the contamination log workflow (refer to the Contamination Log Workflow chapter). Additionally, a Quality Incident Report (LAB-510 or electronic equivalent) is required for these incidents.

C. If contamination is unresolved, no results will be reported and a statement that a quality event occurred will be included in the report.

D. Any unresolved contamination event that was determined to have occurred as a result of something that happened to the evidence prior to submission to the laboratory will be documented on a Quality Incident Report (LAB-510 or electronic equivalent). Results may be reported for these situations.

E. In batching situations, if the analyst is unable to work backwards for a particular sample due to depletion then the profile from that sample is considered to have unresolved contamination and a Quality Incident Report is required for the case related to that sample.
   1. For contamination involving reagent blanks, the remaining samples in the batch may be used provided the analyst was able to work backwards and obtain a resolution to the contamination for the associated reagent blanks. The cases associated with the reagent blanks with resolved contamination will be listed on the contamination log workflow.
   2. For point contamination in the batch involving either single or multiple samples, the remaining samples in the batch that were unaffected by the contamination may be used. The cases related to the unaffected samples do not have to be listed on the contamination log workflow.

4 Records

Quality Incident Report (or electronic equivalent) (LAB-510)

Action Plan (or electronic equivalent) (LAB-511)

Action Plan/Supplement (LAB-512)
DNA-02-06 CONTAMINATION LOG WORKFLOW

1 Scope
This document provides instructions for completion of the contamination log workflow found in Qualtrax. The workflow is used to record contamination events that occur as a result of laboratory actions.

2 Related Chapters
Response to Quality Issues

3 Record Entry

A. In Qualtrax, select “Workflows” and then “DNA – Contamination Log” to open the record entry step.

B. The Record Entry step is generally completed by the Requestor.

C. Complete all fields in the record entry step: DNA Discipline, Laboratory Group, Technical Leader, Date Discovered, Requestor, Associated Individual(s), and Related Work Number(s).

D. The Requestor field indicates the person making the report of contamination. The Requestor may be the same or a different individual than the Associated Individual(s).

E. The Associated Individual(s) field is used to list all current or former employees of the laboratory who had a role in the contamination and could have reasonably prevented the contamination event. If there are no associated individuals (e.g. manufacturer contamination or robotics error), then indicate by selecting “Not Applicable”.

F. The Related Work Number(s) field is used to record the case numbers that were affected by the contamination. If the DNA is extracted in a batch, it is not sufficient to include the batch number in this field. The field must include case numbers separated by semicolons with a space in between each entry. Do not record individual sample names in this field.

G. Select the “Record Entry” button to move to the next step in the process.

4 Submission

A. The Submission step is generally completed by the Requestor.

B. Complete all fields in the submission step if not already completed during the record entry step: Requestor, Associated Individual(s), Related Work Number(s), Incident Description (Detail), Nature of Contamination, QI-Correction(s) Performed to Resolve Nonconformance, and Resolved.

C. The Incident Description (Detail) field is used to record specific details of the incident including what happened, when it happened, who was involved, where, was other work impacted, frequency of occurrence, and other details that may be beneficial for evaluation such as risk, liability, agency core values, and accreditation/certification. Individual sample names may be recorded in this field.

D. The Nature of Contamination field contains a drop down selection to describe the type of contamination that occurred. It is recognized that more than one selection may apply to any given contamination event. Selections are made based on what was contaminated and where in the process the contamination seems to have occurred.
1. Select Reagent Blank if the contamination was determined to be in the reagent blank(s) for the batch.
   
a) *If both samples and reagent blanks were determined to be contaminated at the same time within the same batch, select reagent blank.*

2. Select Amplification controls if the contamination was determined to be in the amplification step and either the positive or negative amplification control was contaminated.

3. Select Sample(s) if the contamination was determined to be in the DNA extract or the amplification step, none of the controls are contaminated, and one or more samples show contamination.

E. The QI – Correction(s) Performed to Resolve Nonconformance field is used to record what steps were taken to resolve the contamination. The QI designation is in the workflow to allow auto-population of fields should a QI result from the contamination event; however, filling out this field does not mean that a QI is necessary.

F. The Resolved field indicates if the contamination was resolved or not. Yes should be selected if the contamination event was resolved prior to the release of information to the customer. No should be selected if the contamination event was unable to be resolved or if it was resolved after release of information to the customer.

G. Select the “Submit for Review” button to move to the next step in the process.

5 Technical Review

A. The Technical Review step is completed by the DNA Technical Leader.

B. Complete all fields in the technical review step if not already completed in the submission step: Technical Leader, Requestor, Associated Individual(s), Related Work Numbers, Incident Description (Detail), Nature of Contamination, QI-Correction(s) Performed to Resolve Nonconformance, Resolved, QIQAP.

C. The DNA Technical Leader will review information to ensure accuracy and completeness. If corrections are needed, the DNA Technical Leader may return the process to the Requestor for revisions by clicking the “Return for Revision” button. A comment concerning the reason for the return may be included in the comment field.

D. The QIQAP selection is made by the DNA Technical Leader. If “yes” is selected, the system will automatically begin a QI/QAP workflow related to the contamination event.

1. Select “no” if the contamination was resolved prior to release of information to the customer.

2. Select “yes” if the contamination was unable to be resolved, was resolved after release of information to the customer, or if the incident was determined to be severe (e.g. pervasive contamination in the laboratory, analysts involved in repeated contamination events of a similar type, the same equipment determined as cause of repeated contamination events of a similar type, etcetera).

E. Select the “Review Completed” button to move to the next step in the process.
6 Program Coordinator Review

A. The Program Coordinator step is completed by the Biology Program Coordinator.

B. The Biology Program Coordinator will review information to ensure completeness. If corrections are needed, the Biology Program Coordinator may return the process to the DNA Technical Leader for revisions by clicking the “Returned for Reconsideration” button. A comment regarding the reason for the return may be recorded in the comment field.

C. Once the “Reviewed” button is selected, the workflow is considered complete and will be included in the DNA Contamination Log report.

7 General Considerations

A. If the Qualtrax system is not available for use, LAB-DNA-54 will be used to make a temporary record of contamination events. Once the Qualtrax system is available, entry of any contamination events will be made as soon as practicable and the copy of LAB-DNA-54 is not retained.

B. All documentation pertaining to the incident must be placed in the case record. The case record may also include a reference to the contamination log workflow.

C. A report may be run by any Qualtrax user at any time to view contamination occurrences in the DPS system by clicking on “Reports” then “Workflow” then “Run” under “DNA Contamination Log”. The report can be filtered based on the fields selected.

D. The contamination log workflow report will be posted on the external DPS website and updated on a monthly basis.

8 Records

LAB-DNA-54 DNA Contamination Log
DNA-02-07 CRITICAL REAGENTS

1 Scope
In order to maintain the quality of the work performed in the DNA section, it is necessary to identify certain reagents as critical. Critical reagents are those that affect the quality of the test and therefore require testing prior to use on evidentiary samples in order to prevent unnecessary loss of sample.

2 Related Chapters/Documents
Analytical Controls
Legacy Amplification Kit Data Reinterpretation
Male Screening Process – Plexor HY
DNA Quantification – Quantifiler
Total Human and Male DNA Quantification – Quantifiler Duo
CLS Manual: Laboratory Equipment
CLS Manual: Standards, Reference Materials/Collections, Databases, and Controls

3 Practice
3.1 General
A. If any new lot of critical reagent does not meet the guidelines stated below, it may not be utilized in casework.
   1. All inconsistencies will be documented and reported to the Technical Leader.
   2. Inconsistencies that cannot be resolved must be reported to the manufacturer.
B. If additional components are individually purchased separate from a kit, these components must be tested to the same extent as the original kit components prior to using them in casework.
   1. Examples of additional components that may be individually purchased include BTO from the 24plex kit, Taq polymerase from the Y23 and Yfiler kits, and Buffer AL from the QIAamp kit.

3.2 List of Critical Reagents
The following are considered critical reagents:
- Amplification Kits
- Quantification Kits
- Taq polymerase (individually purchased)
- Extraction Kits
- Extraction reagents (prepared in-house)
- Reagents used for Male Screening with Plexor HY
3.3 Quality Control

A. Each new lot of Investigator 24plex QS and MiniFiler kits must be subjected to an internal quality control test as outlined below:

1. All reagents in a kit that are used during processing of casework samples must be used to demonstrate efficacy.

2. The positive amplification control must be analyzed to determine activity. Acceptable performance is indicated if a complete profile with the expected alleles as listed in the Analytical Controls chapter is obtained.

3. A negative amplification control must be analyzed to determine purity. Acceptable performance is indicated if no contamination, as defined in the Analytical Controls chapter for 24plex QS samples or as defined either in the Legacy Amplification Kit Data Reinterpretation chapter or local policy for MiniFiler samples, is apparent.

4. The allelic ladder must be analyzed. Acceptable performance is indicated if all of the appropriate alleles are detected.

B. Each new lot of Yfiler/Y23 kits must be subjected to an internal quality control test as outlined below:

1. All reagents in a kit that are used during processing of casework samples must be used to demonstrate efficacy.

2. The male positive amplification control must be analyzed to determine activity. Acceptable performance is indicated if a complete profile with the expected alleles as listed in the Analytical Controls chapter is obtained.

3. The female amplification control must be analyzed to determine sex specificity for Yfiler. Acceptable performance is indicated if no female profile is observed. There is no female negative control for the Y23 kit.

4. A negative amplification control must be analyzed to determine purity. Acceptable performance is indicated if no contamination, as defined in either the Legacy Amplification Kit Data Reinterpretation chapter or local policy for Yfiler/Y23, is apparent.

5. The allelic ladder must be analyzed. Acceptable performance is indicated if all of the appropriate alleles are detected.

C. Each new lot of Quantifiler/Quantifiler Duo kits must be subjected to an internal quality control test as outlined below:

1. All reagents in a kit that are used during processing of casework samples must be used to demonstrate efficacy.

2. The DNA Standard must be diluted to concentrations ranging from 50 ng/µL to 0.023 ng/µL, according to the DNA SOP chapters on Quantifiler or Quantifiler Duo. A blank consisting of TE/dilution buffer must also be included. Acceptable performance is indicated if the lower limit of detectability is at least equal to the lowest standard.

3. The standard curve(s) must be evaluated according to the DNA SOP chapters on Quantifiler or Quantifiler Duo. Acceptable performance is indicated if the slope, R^2, and Y-intercept values are consistent with values obtained from the previous kit lot.
D. Each new lot of Plexor HY kits used for quantification separate from male screening must be subjected to an internal quality control test as outlined below:

1. All reagents in a kit that are used during processing of casework samples must be used to demonstrate efficacy.

2. The DNA Standard must be diluted to concentrations ranging from 50 ng/µL to 0.0032 ng/µL, according to the DNA SOP chapter on Male Screening Process – Plexor HY. A blank consisting of TE/amplification grade water must also be included. Acceptable performance is indicated if the lower limit of detectability is at least equal to the lowest standard.

3. The standard curve(s) must be evaluated according to the DNA SOP chapter on Male Screening Process – Plexor HY. Acceptable performance is indicated if the slope and R² values are consistent with values obtained from the previous kit lot.

E. Each new lot of an extraction kit must be subjected to an internal quality control test as outlined below:

1. All reagents in a kit that are used during processing of casework samples must be used to demonstrate efficacy.

2. Perform extraction, amplify and type according to relevant DNA SOP.

3. Acceptable performance is indicated if a full DNA profile consistent with previously typed DNA profile is obtained.

F. Each new lot of extraction reagents prepared in-house must be subjected to an internal quality control test as outlined below:

1. Perform extraction, amplify and type according to relevant DNA SOP.

2. Acceptable performance is indicated if a full DNA profile consistent with previously typed DNA profile is obtained.

G. Each new lot of reagents used for male screening with Plexor HY, including SwabSolution, 5x Amp Solution and the Plexor HY Quantification kit must be subjected to an internal quality control test as outlined below:

1. All reagents used for male screening with Plexor HY must be tested to demonstrate efficacy.

2. The lysis of a known semen sample and a known female sample must be performed using the SwabSolution to ascertain its potency in the lysis of cells and release of DNA for quantitation. Acceptable performance is indicated if quantification results consistent with sample type are obtained.

3. The quantitation of a known semen sample and a known female sample must be performed using the 5x Amp solution and the Plexor HY Quantification kit to ascertain the chemistry is working correctly. Acceptable performance is indicated if quantification results consistent with sample type are obtained.
4 Records

A. A record shall be maintained for the quality control of all critical reagents.

B. The record shall consist of the following worksheets (as relevant to the reagent being tested) along with the supporting documentation necessary to demonstrate the reagent met the relevant standards as listed above:

1. Extraction Kit Reagent Quality Control (LAB-DNA-43),
2. Amplification Kit Reagent Quality Control (LAB-DNA-11)
3. Quantification Kit Reagent Quality Control (LAB-DNA-12)
4. Extraction reagents prepared in-house shall include the appropriate reagent preparation and quality control record.
DNA-02-08 EQUIPMENT

1 Scope
Only validated, properly maintained, calibrated, and performance checked equipment will be used in casework analyses.

If the equipment is in proper working order for the function in which it is being used, the performance check intervals defined in this chapter are considered to be acceptable. If there is any question concerning the reliability of a piece of equipment, a performance check should be performed immediately.

All equipment-related documentation must be retained and be readily available for inspection. The LAB-405 is used in conjunction with DNA-specific forms to document significant equipment related activity.

2 Related Chapters/Documents
Validation
CLS Manual: Laboratory Equipment
Safety Manual: Ventilation Devices

3 Significant Equipment

3.1 List of Significant Equipment
A. Capillary Electrophoresis (CE) Instrument
B. Thermal Cycler
C. AB Prism 7500 Real-Time PCR system
D. Thermal Cycler temperature verification system
E. Robotic Workstation
F. NIST-traceable Thermometer
G. Wet or Dry Incubators/Thermomixers used for DNA extraction
H. Mechanical Pipettes used for DNA processes

3.2 CE Instrument
A. The capillary electrophoresis instrument is used to separate DNA fragments based upon size and fluorescent tags. All parts must be working properly to ensure accurate and usable results are obtained.
B. This equipment is located in the amplified DNA product area of the laboratory.
C. Refer to the Validation chapter for specific validation requirements for newly purchased CE instruments.
D. Quality Control Procedure
   1. After service engineer repair:
      a) An allelic ladder and amplification controls must be run using Investigator 24plex QS kit and analyzed under normal conditions.
      b) The CE instrument is considered to have acceptable performance if all peaks in the allelic ladder and positive amplification control are called correctly and if no peaks attributable to DNA are present in the negative amplification control.
c) **Documentation for performance verification after repair includes completion of CE Instrument Maintenance Form (LAB-DNA-67) and LAB-408.**

2. A new spectral for each dye set used must be created at least annually or more frequently as needed for each CE instrument in the laboratory.
   a) **Follow the manufacturer’s guidelines for making the spectral and verifying its accuracy.** A ladder, positive, and negative control for the corresponding amplification kit must be run, analyzed and documented in an appropriate log/record.
   b) **The CE instrument is considered to have acceptable performance if all peaks in the allelic ladder and positive amplification control are called correctly and if no peaks attributable to DNA are present in the negative amplification control.**

3. The documentation of the completion of the quality control procedure and the analyzed data must be recorded using the CE Instrument Maintenance Form (LAB-DNA-67). LAB-408 is not required if the spectral is made outside of a repair or planned maintenance event.

E. An annual planned maintenance visit by a qualified Service Engineer is required.

1. After planned maintenance, the following performance verification must be performed:
   a) A new spatial must be run (unless already performed by the service engineer)
   b) A new spectral must be run for each dye set used.
   c) An allelic ladder, positive and negative control appropriate to the dye set used must be run and analyzed with the new spectral.
   d) The analyzed data from this run must be documented using the CE Instrument Maintenance Form (LAB-DNA-67) and LAB-408 must be completed.
   e) The CE instrument is considered to have acceptable performance if all peaks in the allelic ladder and positive amplification control are called correctly and if no peaks attributable to DNA are present in the negative amplification control.

F. Repairs

1. If the CE instrument is damaged or not functioning, either the manufacturer or a qualified service technician may repair the instrument.
   a) **Prior to returning the equipment to service after repair, a performance verification consisting of the quality control procedure listed above under “after service engineer repair” must be completed.**
   b) **For equipment that is removed from service, follow the procedures in Validations and Performance Verifications chapter of CLS Manual.**

2. Record the event on LAB-405.

G. Preventive Measures

1. Analytical data must be archived once a year at a minimum. This must include raw data.
2. A water wash should be performed at a minimum with every polymer lot change.
3. When preventive measures are completed, they must be recorded on the 3130 Series Run Log (LAB-DNA-69) or the 3500 Series Run Log (LAB-DNA-70) as applicable. LAB-405 and LAB-408 are not required.

3.3 Thermal Cycler

A. Thermal cyclers automate the polymerase chain reaction (PCR) for amplifying DNA. PCR is a cyclic process that mimics the natural DNA replication process by exponentially increasing the amount of template DNA after each cycle.

B. This equipment is located in the amplified DNA product area of the laboratory.

C. Refer to the Validation chapter for specific validation requirements for newly purchased thermal cyclers.

D. Quality Control Procedure

1. A performance check consisting of temperature verification, temperature uniformity, and diagnostic tests must be performed annually. Follow manufacturer’s instructions for performing these tests and document the results on the Thermal Cycler Quality Control Worksheet (LAB-DNA-20 or LAB-DNA-20A).

2. The thermal cycler uses established parameters to determine if temperature verification, temperature uniformity and diagnostic tests pass or fail. The thermal cycler will alert the user of the test result.
   a) Any variations outside of established parameters will necessitate recalibration or repair of the equipment by the manufacturer or a qualified service technician.
   b) The thermal cycler is considered to have acceptable performance if all tests are indicated as passing.

E. Repairs

1. If the thermal cycler is damaged or not functioning, either the manufacturer or a qualified service technician may repair the equipment. Any repair must be documented.

2. For equipment that is removed from service, follow the procedures in Validations and Performance Verifications chapter of CLS Manual.
   a) Prior to returning the equipment to service after repair, a performance verification consisting of the quality control procedure listed above must be completed and recorded on LAB-408.

3. Record on LAB-405.

F. Preventive Measures

1. The sample block or exterior may be cleaned as needed. LAB-405 and LAB-408 are not required.

3.4 AB Prism 7500 Real-Time PCR System

A. The AB Prism 7500 Real-Time PCR system utilizes the PCR process in order to perform quantitation through the use of a halogen lamp which directs light through each well on the reaction plate. The fluorescence emission data from the wells is captured and the software applies data analysis algorithms to determine the amplifiable DNA quantity.

B. This equipment is located in the amplified DNA product area of the laboratory.
C. Refer to the *Validation* chapter for specific validation requirements for newly purchased 7500 Real-Time PCR systems.

D. Quality Control Procedure

1. After service engineer repair:
   a) The standard curve dilutions and a blank must be run using a quantification kit used by the laboratory.
   b) The 7500 Real Time PCR System is considered to have acceptable performance if the standard curve slope, Y-intercept, and \( R^2 \) values are within recommended parameters for the kit used and if a blank gives a value of undetermined or no value.
   c) Documentation for performance verification after repair includes completion of the Real Time PCR System Maintenance Form (LAB-DNA-66) and LAB-408.

2. Dye calibration for each dye set must be run at least annually or more frequently as needed for each Real Time PCR system in the laboratory.
   a) Follow the manufacturer’s guidelines for running the dye calibration and verifying its accuracy.
   b) The standard curve dilutions for the corresponding quantification kit and a blank must be run, analyzed and documented.
   c) The 7500 Real Time PCR System is considered to have acceptable performance if the standard curve, Y-intercept, and \( R^2 \) values are within recommended parameters for the kit used and if a blank gives a value of undetermined or no value.

3. A background plate must be run at least annually or more frequently to assess background signal.
   a) The 7500 Real Time PCR System will alert the user if the background calibration passed or failed.
   b) The block may be cleaned with 10% bleach or ethanol if elevated background is noted.
   c) This requirement may be met during the annual planned maintenance visit if the Service Engineer runs a background plate.
   d) The 7500 Real Time PCR system is considered to have acceptable performance if the background calibration status is pass, the values for filters 1-4 are less than 72000 and the value for filter 5 is less than 90000.

4. The documentation of the completion of the quality control procedure and the analyzed data must be recorded on the Real Time PCR System Maintenance Form (LAB-DNA-66). LAB-408 is not required if the dye calibration or background plate are performed outside of a repair or planned maintenance event.

E. Planned Maintenance

1. An annual planned maintenance visit by a qualified service engineer is required.

2. The planned maintenance visit will include the system’s diagnostic programs and the use of an appropriate certified temperature verification system or process.
3. After planned maintenance, the following performance verification must be performed:
   a) A dye calibration plate must be run for each dye set used in the laboratory. If the dye calibration was performed by the Service Engineer, it does not have to be rerun by the laboratory.
   b) The standard curve dilutions appropriate to each dye set used along with a blank must be run.
   c) The analyzed data from this run must be recorded on the Real Time PCR System Maintenance Form (LAB-DNA-66) and LAB-408 must be completed.
   d) The 7500 Real Time PCR system is considered to have acceptable performance if the standard curve, Y-intercept, and $R^2$ values are within recommended parameters for each kit used and if the blank gives a value of undetermined or no value.

F. Repairs

1. If the 7500 is damaged or not functioning, either the manufacturer or a qualified service technician may repair the equipment.
   a) For equipment that is removed from service, follow the procedures in Validations and Performance Verifications chapter of CLS Manual.
   b) Prior to returning the equipment to service after repair, a performance verification consisting of the quality control procedure listed above under “after service engineer repair” must be completed.

2. Record on LAB-405.

G. Routine Maintenance

1. The halogen lamp will be changed when burned out or when there is an indication that the bulb is approaching the end of its life.
   a) The halogen lamp is good for approximately 2000 hours.
   b) The bulb can be changed by laboratory personnel.
   c) Gloves must be worn when changing the bulb.
   d) Due to the short life of the bulb and the fact that the bulb is on up to four hours after the run has completed, the Real Time PCR system should only be turned on immediately prior to a run and turned off as soon as the run is finished.
   e) A drop in ROX values can indicate the necessity for bulb replacement.

2. Routine maintenance is recorded on the LAB-405.

H. Preventive Measures

1. Analytical data will be routinely archived once a year at a minimum.

2. Preventive measures taken should be recorded on a run log or equivalent when performed. LAB-405 and LAB-408 are not required.
3.5 Robotic Workstations

A. The robotic workstations are liquid handling robotic platforms capable of transferring liquids for a wide range of sample volumes and a large number of samples.

B. Refer to the Validation chapter for specific validation requirements for newly purchased robotic workstations.

C. Quality Control Procedure

1. Robotic workstations require calibration to enable them to ascertain the correct location of hardware/labware. Depending on the model of workstation, the software may allow the user to accomplish this function.
   
   a) A performance check appropriate to the extent of the adjustment performed will be done prior to use in casework.

   b) The robotic workstation is considered to have acceptable performance if the performance check ends in successful downstream results for the process being tested (e.g. extraction results in the correct DNA profile for the samples and no DNA profile for the blanks, water run is successful, for CE set-up the ladder, positive, and negative controls are placed in the expected wells, etcetera).

2. The documentation of the completion of the quality control procedure and the analyzed data must be recorded on the Robotic Workstation Maintenance Form (LAB-DNA-68). If the quality control procedure is performed outside of repair or annual planned maintenance, a LAB-408 is not required.

D. Planned Maintenance

1. An annual planned maintenance visit by a qualified service engineer is required.
   
   a) For the Tecan, the planned maintenance includes a precision verification test.

2. After planned maintenance, the following performance verification must be performed:
   
   a) A performance verification is done to the extent to which the robotic system is used by the laboratory to ensure the functionality of the workstation.

   b) The analyzed data from this run must be recorded on the Robotic Workstation Maintenance Form (LAB-DNA-68) and documented on LAB-408.

   c) The robotic workstation is considered to have acceptable performance if the performance verification ends in successful downstream results for the process being tested (e.g. a quantification plate set up results in a passing standard curve, quantification values for samples, and no values for blanks).

E. Repairs

1. For repairs made outside of an annual planned maintenance visit, it may be necessary to contact the manufacturer or a qualified service technician to repair the equipment.
   
   a) For equipment that is removed from service, follow the procedures in Validations and Performance Verifications chapter of CLS Manual.

   b) Prior to returning the equipment to service after repair, a performance verification consisting of the quality control procedure listed above must be completed.

2. Record on LAB-405.
F. Preventive Measures

1. The EZ1 workstation should have the o rings of the tip adapters greased at a minimum of once a year or more often as needed. This may be accomplished during the annual planned maintenance visit if the Service Engineer greases the o rings during the visit.

2. The QIAgility workstation should have the “UV” protocol run and the computer restarted weekly when in use.

3. Preventive measures should be recorded on a run log or equivalent when performed. Completion of LAB-405 and LAB-408 is not required.

3.6 NIST Traceable Thermometer

A. A NIST traceable thermometer is used to performance check significant equipment and also to verify the accuracy of thermometers listed in section 4.5.

B. The thermometer may be used for the duration of its certification. Once the certification has expired, the thermometer must be replaced or the certification renewed through external calibration.

C. If the thermometer leaves the control of the laboratory for calibration by an approved vendor, a performance verification using the quality control procedure is required prior to placing the thermometer back into service. Complete LAB-408.

D. Newly purchased thermometers require a performance verification using the quality control procedure prior to placing the thermometer into service. Complete LAB-408.

E. Quality Control Procedure

1. Set a wet/dry incubator or theromixer with a current performance/quality control check to 56°C.

2. Once the unit stabilizes, place the NIST Traceable thermometer into the unit and allow the thermometer to stabilize.

3. The thermometer is considered to have acceptable performance if the reading is within ± 2°C of the set point.

F. Preventive Measures

There are no recommended preventive measures for NIST traceable thermometers.

3.7 Thermal Cycler Temperature Verification System

A. A thermal cycler temperature verification system is used to verify the performance of thermal cyclers.

B. Calibration by an approved vendor is required annually.

C. If the thermal cycler temperature verification system leaves the control of the laboratory for calibration by an approved vendor or to be sent to another laboratory within the DPS system, a performance verification consisting of the quality control procedure is required prior to placing the thermal cycler temperature verification system back into service. Complete LAB-408.

D. Newly purchased thermal cycler temperature verification systems require a performance verification consisting of the quality control procedure prior to being placed into service. Complete LAB-408.
E. Quality Control Procedure
   1. Place the probe of the thermal cycler temperature verification system into the well of a currently quality control/performance checked thermal cycler.
   2. Close the heated cover and set the thermal cycler to 95°C hold.
   3. Once the unit stabilizes, take the reading from the thermal cycler temperature verification performance system.
   4. The thermal cycler temperature verification system is considered to have acceptable performance if the reading on the thermal cycler temperature verification performance system is within ± 1°C of the set point.

F. Preventive Measures
   There are no recommended preventive measures for thermal cycler temperature verification systems.

3.8 Wet or Dry Incubators/Thermomixers used for DNA extraction
   A. Wet or Dry Incubators/Thermomixers are used to heat samples during the lysis portion of DNA extraction.
   B. A performance check is required annually and consists of one of the options in the quality control procedure.
   C. Newly purchased wet or dry incubators/thermomixers must complete a performance verification using the quality control procedure outlined in either step 1 or 2 below prior to being placed into service. Complete LAB-408.
   D. Quality Control Procedure
      1. The thermometer used to monitor the temperature of the incubator will be checked with a NIST traceable thermometer annually at the appropriate temperature. If a thermometer is strictly used to monitor the incubator that comes with certification of NIST calibration, this thermometer does not need a performance check until the expiration of this certification. Refer to section 4.4.
      2. If the incubator is not monitored by an external thermometer and temperature readout of the incubator can be adjusted, it will be adjusted to match the reading on the NIST-traceable thermometer. If the temperature readout of the incubator cannot be adjusted, the amount of the deviation from the NIST thermometer will be recorded on the Dry Bath/Oven/Thermomixer/Incubator Quality Check Form (LAB-DNA-55) and the setting adjusted by the same amount, and in the same direction.
      3. The wet or dry incubator/thermomixer is considered to have acceptable performance for the year if the unit is being monitored by a NIST-traceable thermometer with a current certificate or if the readout has been adjusted or the deviation from the NIST thermometer noted.
      4. Completion of LAB-408 is necessary if the quality control procedure is performed due to newly purchased equipment or repaired equipment.
E. Repairs

1. When an incubator is not performing as expected, it will be removed from service and repaired.
   a) *For equipment that is removed from service, follow the procedures in Validations and Performance Verifications chapter of CLS Manual.*
   b) *After maintenance or repairs, the wet or dry incubator/thermomixer must complete a performance verification using the quality control procedure outlined in either step 1 or 2 above prior to being placed into service.*

2. Record on LAB-405.

F. Preventive Measures

1. For wet incubators, the water in the bath should be clear and clean with no evidence of bacterial/fungal growth or rust. If the water becomes dirty, discard and clean water bath. Replenish with water.
2. Incubators will be cleaned as needed.
3. When preventive measures are performed, they should be recorded on the Dry Bath/Oven/Thermomixer/Incubator Quality Check Form (LAB-DNA-55). Completion of LAB-405 and LAB-408 is not required.

G. If an external thermometer is performance checked against a NIST thermometer, documentation will be maintained on the Thermometer Quality Control worksheet (LAB-DNA-56).

H. Any adjustment of internal thermometers will be recorded on the Dry Bath/Oven/Thermomixer/Incubator Quality Check Form (LAB-DNA-55).

3.9 Mechanical Pipettes Used for DNA Processes

A. Some pipettes are dedicated to a specific function, such as those used in the post PCR amplification process.

B. Pipettes that are dedicated only to Serology/Biology screening are not considered significant equipment.

C. External calibration is required annually.

D. If a pipette leaves the control of the laboratory during calibration, a performance verification consisting of one of the quality control procedure options is required prior to placing the pipette back into service. Complete LAB-408.

E. Newly purchased pipettes must undergo performance verification using one of the quality control procedure options prior to being placed into service. Complete LAB-408.

F. Quality Control Procedure

1. Quality control data should include the type of pipette, volume range, model, and serial number.
2. Option 1: Delivery Volume by Comparison
   a) *Adjust a pipette with a current calibration certificate to a set volume. For example, a p10 pipette might be set to 5 µL.*
   b) *Use the pipette with the current calibration certificate to aspirate the set volume of water.*
c) Dispense this liquid into a microcentrifuge tube. For volumes less than 50 µL, it is recommended that the liquid be dispensed into a microcentrifuge tube containing at least 50 µL of water to ensure accurate delivery. For example, if using a p10 pipette set to 5 µL, 5 µL of water is dispensed into a tube containing at least 50 µL of water.

d) Use the pipette requiring the performance check to aspirate the tested volume of water from the microcentrifuge tube prepared in the previous step. For example, if using a p10 pipette set to 5 µL, 5 µL of water is aspirated from the tube.

e) Determine the difference in volume between the two pipettes by measuring any liquid remaining in the microcentrifuge tube or by dialing down the pipette to determine the amount of liquid that remains in the tip if an air gap is observed. For example, if using a p10 pipette set to 5 µL, the amount of liquid remaining in the microcentrifuge tube should be approximately 45 µL.

f) The pipette is considered to have acceptable performance if the measured volume is no more than the maximum permissible error allowed by the manufacturer.

3. Option 2: Application Test

a) Use the pipettes to complete an application for which they are intended for use. For example, the pipettes may be used to set up a quantification plate or to set up a CE plate containing a ladder, positive, and negative control.

b) The pipette is considered to have acceptable performance if the performance check ends in successful downstream results for the process being tested (e.g. quantification results in expected values for the standard curve slope, \(R^2\), intercept and quantification blank or CE plate results in expected allele calls from the ladder, positive, and negative control).

4. Option 3: Delivery Volume by Weight

a) Tare/zero an analytical balance with a piece of Parafilm or glassine paper on the balance pan.

b) Adjust the pipette needing the performance check to a set volume for that pipette. For example, a p10 pipette might be set to 5 µL.

c) Aspirate the set volume of water and dispense it onto the Parafilm/glassine paper on the balance pan.

d) Quickly record the reading on the balance and convert it to mg. If the water is allowed to sit on the balance for an extended period of time, it will evaporate.

e) The density of water is 1 mg/ µL. Use this information to determine the target value for the pipette. For example, a p10 pipette set to 5 µL would be expected to dispense a volume of water that weighed 5 mg.

f) The pipette is considered to have acceptable performance if the measured volume is no more than the maximum permissible error allowed by the manufacturer.

5. Completion of LAB-408 is necessary if the quality control procedure is performed due to newly purchased equipment or repaired equipment.
G. Repairs

1. When a pipette is determined to be performing improperly, it will be returned to the manufacturer, or another qualified repair technician, so that the problem may be identified and corrected.
   
a) *If at any time a pipettor has been disassembled, it should be taken out of service until it can be calibrated and/or verified.*

b) *For equipment that is removed from service, follow the procedures in Validations and Performance Verifications chapter of CLS Manual.*

c) *Prior to returning the equipment to service after repair, a performance verification consisting of one of the quality control procedures listed above must be completed.*

2. Record on LAB-405.

H. Preventive Measures

1. Pipette delivery volumes may be verified periodically through use of one of the quality control procedure options to ensure proper operation. LAB-405 and LAB-408 are not required.

4 Non-Significant Equipment

The following equipment must be maintained and are subject to quality control measures: balances, centrifuges, tachometers, refrigerators/freezers, thermometers used to monitor set points, mechanical pipettes not used for DNA processes, biohazard safety cabinets, chemical/biological hoods, and autoclaves.

4.1 Balances

A. Balances will be calibrated by an approved vendor annually.

B. Balances will be located in a suitable area that is not exposed to extreme heat, radiation, drafts, extreme vibration, or corrosive chemicals.

C. If the balance is moved, it must be performance checked using the quality control procedure prior to being used.

   1. Quality Control Procedure Use the 1g weight from a NIST-traceable weight set with current certificate to determine the performance of the balance.

   2. The balance is considered to have acceptable performance if the weight is within 10% of the target value.

D. Maintenance Procedure

   1. Balances shall be cleaned as needed.

   2. When a balance is not performing properly, it will be removed from service and either repaired or replaced.

4.2 Centrifuges

A. The relative centrifugal force can be determined by the following calculation:

   1. Ascertain the spinning radius by measuring the distance from the center of the drive shaft to the most outer part of the centrifuge tube when it is in spinning position.
2. Use the equation \( g = 1.118 \times 10^{-5} \times r \times n^2 \) where \( g \) = relative centrifugal force (RCF); \( r \) = spinning radius in centimeters; \( n \) = revolutions per minute (RPM).

3. From these relationships, RCF values (i.e., \( g \) values) can be easily converted into RPM.

B. The quality control procedure is performed annually and recorded on the Centrifuge Verification Record (LAB-DNA-57).

C. Quality Control Procedure
   1. The speed as measured by a tachometer must correspond to a predictable number on the speed control setting or the digital readout.
   2. The centrifuge is considered to have acceptable performance if the speed from the centrifuge corresponds within 10% of the speed on the tachometer.

D. Maintenance Procedure
   1. There is no scheduled maintenance for centrifuges.
   2. Centrifuge housing, rotor chamber, and rotor accessories will be cleaned as needed.

4.3 Tachometers

A. The tachometer is used to measure rotational speed. This equipment is used to performance check centrifuges in the laboratory.

B. A performance check is required annually by either certification through an approved vendor or by using ballast operated fluorescent lighting as indicated in the quality control procedure.

C. Quality Control Procedure
   1. Point the tachometer at a ballast operated fluorescent light which has been turned on. Take the reading on the tachometer.
   2. The tachometer is considered to have acceptable performance if the reading on the display is approximately 7200 rpm.

D. Maintenance Procedure
   1. There is no scheduled maintenance for tachometers.
   2. If a tachometer cannot pass the quality control procedure, it will be recalibrated by an external vendor.

4.4 Refrigerators/Freezers

A. Refrigerators/freezers used in the laboratory should be capable of maintaining the optimum temperature range required for storing reagents and samples.

B. Weekly records of temperature readings are maintained and recorded on the Temperature Monitoring Log (LAB-DNA-58).

C. Refrigerators and freezers should maintain the temperature storage requirements specified by the manufacturer for those items stored within.
D. Quality Control Procedure

1. A thermometer for monitoring the temperature is placed in the upper/lower compartments. The thermometer can sit in oil or water to buffer against rapid temperature swings.

2. If the temperature is not being maintained within ± 1°C of prescribed values, verify that the temperature regulation control is at the proper setting and adjust if necessary.

3. The temperature will be rechecked to ensure the temperature is within the acceptable range.

4. A refrigerator is considered to have acceptable performance if it maintains optimum temperature with the required range of 2 to 8°C. A freezer is considered to have acceptable performance if it maintains a temperature below 0°C.

E. Maintenance Procedures

1. If necessary, a technical representative may need to be called for service or the refrigerator/freezer may need to be replaced if acceptable performance cannot be achieved.

2. It may be necessary to reduce over-crowding in an effort to increase air circulation in all compartments.

3. Refrigerators/freezers are maintained in working order. There is no preventive maintenance requirement; the only requirement is for corrective maintenance.

4.5 Thermometers Used to Monitor Set Points

A. These types of thermometers are not used to performance check other thermometers. They may be used to monitor set points such as for refrigerators/freezers or to monitor temperatures that are critical to analytical procedures such as use with ovens/wet and dry baths or thermomixers during DNA extraction.

B. Thermometers used to monitor set points will be performance checked annually using the quality control procedure.

1. Alternatively, if a thermometer comes with certification of NIST calibration, it will not need to be performance checked against the NIST-traceable thermometer until the expiration of this certification.

C. Quality Control Procedure

1. Thermometers used to monitor set points will be checked with a NIST traceable thermometer at the appropriate temperature.

2. Documentation will be maintained on the Thermometer Quality Control worksheet (LAB-DNA-56).

3. If the thermometer varies greater than ± 1°C, then the amount of deviation will be noted and all other readings adjusted by the same amount, and in the same direction.

D. Maintenance Procedure

1. If the deviation of the thermometer is greater than 2°C, then the thermometer must be replaced or repaired by an external vendor.
2. If any discontinuities are detected in the liquid column on mercury/organic liquid thermometers, repair or replace the thermometer.

3. There is no scheduled maintenance for thermometers.

### 4.6 Mechanical Pipettes

A. These pipettes are not used in any DNA process (including male screening) and are dedicated for use in Biology Screening/Serology only.

B. Quality Control Procedure

Pipettes must undergo calibration annually by an approved vendor.

C. Maintenance Procedure

1. If at any time a pipettor has been disassembled, it should be taken out of service until it can be calibrated and/or verified.

2. When a pipette is determined to be performing improperly, it will be returned to the manufacturer, or another qualified repair technician, so that the problem may be identified and corrected.

### 4.7 Biohazard Safety Cabinets

A. These are hoods or cabinets that do not vent to the outside and have HEPA filters.

B. Quality Control Procedure

The hood must be certified at least once a year, after maintenance performed, and/or after every HEPA filter change by an external vendor.

C. Maintenance Procedure

There is no scheduled maintenance for biohazard safety cabinets.

### 4.8 Biological/Chemical Hoods

A. These are hoods that vent to the outside and may not have HEPA filters.

B. Quality Control Procedure

The hood must be certified at least once a year, after maintenance performed, and/or after every HEPA filter change by an external vendor.

C. Maintenance Procedure

There is no scheduled maintenance for biological/chemical hoods.

### 4.9 Autoclave

A. Autoclaves are used to sterilize solids, liquids, and equipment. Hot high-pressure steam is used to achieve sterilization.

B. Quality Control Procedure

1. Autoclave tape should be used to check the adequacy of the steam sterilization cycle.

2. The autoclave is considered to have acceptable performance if the indicator on the autoclave tape is activated (words appear, color changes, etcetera).
C. Maintenance Procedure
   1. If the autoclave is not functioning properly, a qualified service technician may repair it.
   2. Autoclaves will be cleaned as needed.
   3. There is no scheduled maintenance for autoclaves.

D. Document weight of any treated waste in a log.

5 Literature References and Supporting Documentation


EZ1 Advanced XL User Manual (current version).


Qiagen QIAgility User’s Manual (current version).

Tecan Application Manual HID EVOLution – Combination System (DNA Extraction and qPCR/STR Setup) (current version).

Tecan Freedom Evo Operating Manual (current version).

https://www.ccohs.ca/oshanswers/ergonomics/lighting_flicker.html

User’s Guides for equipment relevant to individual laboratories.
DNA-02-09 GUIDELINES FOR TECHNICAL REVIEW

1 Scope

The technical review is a documented review of the case file, biological testing, DNA profiles, reports, and other information, which forms the basis of the scientific conclusion. This chapter covers the requirements related to technical review.

2 Related Documents

CLS Manual: Review of Laboratory Records
CLS Manual: Laboratory Records

3 Practices

A. The individual conducting the technical review shall be an employee or contract employee of the Texas DPS Crime lab. They shall:

1. Be a current or previously qualified analyst in the methodologies (extraction method, quantification method, amplification kit, and platform) being reviewed.
2. Be issued a license by the Texas Forensic Science Commission
3. Pass a competency test administered by the laboratory prior to participating in technical review.
4. Participate in the proficiency testing program as a technical reviewer on the same technology, platform, and amplification kit used to generate the data they are reviewing to the full extent in which they participate in the review of the DNA data.

B. The reviewer shall thoroughly examine the case record to:

1. Assess whether proper technical procedures have been applied to the examination of test items;
2. Review the actual data for quality, validity, appropriately applied calculations, and successful data transfers or transcriptions where applicable;
3. Verify that sufficient documentation is in the case record, including batch records, evidence inventory, chain-of-custody, and disposition of evidence as appropriate;
4. Ensure conformance with approved methods and applicable management system documents;
5. Ensure that the case report contains all required elements and accurate information;
6. Ensure that results, interpretations, opinions, and conclusions in the case report are accurate, within the constraints of validated scientific knowledge, and supported by the technical records; and
7. Ensure that clearly communicated and properly qualified statements regarding significance of associations, exclusions, or reason(s) that no definitive conclusions could be reached are included in the test report.
8. Verify the DNA profile(s) selected for CODIS entry.
C. Individuals participating in review of data generated using legacy DNA amplification kits shall:

1. Have been a previously qualified analyst in the legacy amplification kit (including the most current interpretation procedures) or complete an initial training program related to interpretation and review of data generated using legacy DNA amplification kits prior to participating in data review.

2. Complete a review of the validation studies and standard operating procedures related to the legacy amplification kit they are reviewing once every two calendar years.

3. Have the review of the validation studies and standard operating procedures approved by the Technical Leader.

NOTE: if the analyst has participated in proficiency testing using the legacy DNA amplification kit within two years of performing legacy data review, they are not required to complete the review of the validation studies and standard operating procedures for the legacy amplification kit that was used to complete the proficiency test.

3.1 Substance of Technical Review

The following points should be examined during a technical review. A holistic approach should be taken during technical review so that the entire case record, including any past analysis, is assessed.

A. Case Record Documentation/Case Processing

1. Case notes and data in the record generated by an analyst/technician must include the case number, identification of the analyst/technician and the date generated. This information can be handwritten or in electronic format. This applies to any case notes and data generated and reviewed during processing of reinterpretation requests.

2. The contents of the case record must be prepared and organized in such a manner that another qualified analyst would be able to evaluate and interpret the data and ascertain that sufficient and relevant testing was correctly performed.

3. Case records for DNA analysis must contain:

a) A printout of the complete DNA analysis software project list for each run.

b) A printout of at least one electropherogram used in the final interpretation of each sample.

   i. If a sample is extracted more than once and processed through amplification, a printout of one electropherogram from each extraction and/or amplification kit is also required.

   ii. If samples are analyzed both with and without stutter filters, then both electropherograms for the samples used in the final interpretation are required to be present in the case record.

   iii. Document why a sample or ladder injection was not used. Reasons for not using these injections must be noted on the project list from the DNA analysis software. Alternatively, each sample may have all usable sample injections printed and the reason for not using an injection documented on the electropherogram(s) retained in the case record instead of the project list. If a
sample injection, ladder, control or reagent blank is unusable, the reason for not using these injections will be documented on the project list from the DNA analysis software.

iv. Statistical data relevant to the case.

v. Conclusions must be documented in the case record on the electropherograms, a Laboratory Information Sheet (LAB-403), or the STRmix Advanced Report.

4. The disposition of evidence stain(s)/cuttings must be communicated in the report.

5. If presumptive tests are performed during biological testing, reagent quality control must be documented for positive and negative controls on the day tests are performed.

6. Review all comparisons (i.e. inclusions, exclusions, uninformative) for compliance with interpretation guidelines.

7. If any samples require re-extraction, re-amplification, or re-injection, documentation must reflect the reasons for the additional analyses and the samples not used should be clearly denoted.

B. Review DNA Analytical Results/Conditions of Analysis

1. A quantitation system must be used by the laboratory such that DNA is quantitated for samples and that the appropriate amounts of target DNA are used for amplification.

2. Verify that documentation of data reanalysis is present in the case record. Data reanalysis may be performed at the time of the final technical review or can be performed on a run by run basis.

3. Data reanalysis will consist of the following:
   a) Evaluate the raw data for the presence of primer peaks and to ensure DNA types are supported by the raw data.
   b) Examine the concordance check performed by the DNA analysis software for all ladders, positive controls, negative controls and reagent blanks. Examine negative controls and reagent blanks for below threshold peaks and verification of primer peaks.
   c) Examine electropherograms to ensure any artifacts are correctly labeled.
   d) Examine known and questioned stain electropherograms for concordance with printed results.
   e) All appropriate internal size standard peaks must be present for all samples and controls.
   f) The Relative Fluorescence Units (RFUs) for a sample must not exceed the saturation point of the CCD camera of the instrument, except for Amelogenin.
   g) Review data for potential contamination issues. The profile comparison tool in the DNA analysis software must be used to assist the analyst in this assessment, and the analyst must visually examine any issues. In order for the profile comparison tool to function effectively, all “OL” designations must be removed from profiles and all samples extracted in the same extraction session and/or amplified at the same time must be included in the same project. This may necessitate creation of
a project and/or run folder containing samples from multiple runs that is used specifically for the purpose of running the profile comparison tool.

h) Document reanalysis in the case record.

i) For cases interpreted using STRmix the advanced run report will be reviewed to ensure a proper deconvolution was performed. The review must include the following components: case number and sample ID, the amplification kit used, number of contributors assigned, the propositions, mixture proportions, contributor specific degradation, total iterations, effective sample size, average log likelihood, the Gelman-Rubin Convergence Diagnostic, allelic and stutter variance and the input files. Refer to the STRmix Autosomal STR Interpretation Guidelines chapter for specific technical review requirements for any case interpreted using STRmix.

C. Review CODIS Profile(s)

1. Verify that all profiles entered into CODIS are eligible, have the correct DNA types and correct specimen category.
   a) Verification of eligibility must be performed by a current CODIS user.

2. Compare allele calls to appropriate electropherogram(s) or allele table for concordance.

3. Verify that if the case involved elimination samples that were not yet received, there is documentation that such samples were requested prior to CODIS entry.

D. Male Screening and DNA Batch Reviews

1. If samples are worked together as a batch, the laboratory may choose to review the associated analytical data as a batch.
   a) For Male Screening batches, the analytical elements reviewed are lysis and quantification data.
   b) For DNA batches, the analytical elements reviewed are extraction, quantification, amplification, and capillary electrophoresis run data.

2. If the analytical data is reviewed as a batch, this review will serve as the documented technical review of this data.

3. The technical review of analytical data in a batch of samples does not negate the need for technical review of the remaining interpretation data in the case record as outlined above.

3.2 Identification of Potential Technical Inconsistencies or Administrative Issues

A. If the reviewer has identified a potential issue that may require correction, the reviewer and the analyst will discuss it so that final resolution may be achieved.

B. Any corrections made will be documented in the case record.

C. If the issue is deemed as a significant technical problem, or if resolution cannot be reached, the issue must be discussed with the Technical Leader and Quality Manager of the laboratory.
4 Records

A. Technical reviewers will use the appropriate review checklist from the following when performing technical review:
   1. Forensic Biology Technical Review Worksheet (LAB-DNA-60)
   2. Male Screening Batch/Analytical Data Technical Review Sheet (LAB-DNA-61) and Male Screening Technical Review Worksheet (LAB-DNA-62)
   3. DNA Batch/Analytical Data Technical Review Sheet (LAB-DNA-63) and DNA Technical Review Worksheet (LAB-DNA-64).

B. The checklist(s) is required to be retained in the case record only if the reviewer has made handwritten or electronic markings on it.

C. Both technical and administrative reviews will be documented in the LIMS.
DNA-02-10 CASE DOCUMENTATION

1 Scope
These policies were established as minimum requirements for case documentation and record keeping for Forensic Biology, Male Screening and DNA cases.

2 Related Documents
CLS Manual: Laboratory Records

3 Content of Case Record
*These documents are only required if they are relevant to the case.

A. Male Screening
1. Laboratory Report
2. Laboratory Submission Form (LAB-201)
3. *Sexual Assault Evidence Submission Certification Form (LAB-206) if submitted
4. Biological Screening Worksheet (LAB-DNA-01) or LIMS Equivalent Worksheet
5. Extraction/Lysis Worksheet (LAB-DNA-53) or equivalent
6. Quantitation results
7. Male Screening Batch/Analytical Data Technical Review Sheet (LAB-DNA-61) (if altered by reviewer)
8. Male Screening Technical Review Worksheet (LAB-DNA-62) (if altered by reviewer)
9. Photos and notes if generated or submitted

B. Forensic Biology
1. Laboratory Report
2. Laboratory Submission Form (LAB-201)
3. *Sexual Assault Evidence Submission Certification Form (LAB-206) if submitted
4. Biological Screening Worksheet (LAB-DNA-01) or LIMS Equivalent Worksheet
5. *P30 and Hematrace lot numbers and expiration dates
6. Lot numbers and expiration dates of reagents used during screening
7. Forensic Biology Technical Review Worksheet (LAB-DNA-60) (if altered by reviewer)
8. Sketches, photos and notes if generated

C. DNA Analysis
1. Laboratory Report
2. DNA Extraction Worksheet (LAB-DNA-07) or Extraction/Lysis Worksheet (LAB-DNA-53)
   a) If LAB-DNA-07 is used, the quantification and amplification columns are not required to be completed as long as the information is contained in the case record.
b) If the laboratory verifies the sample order or the plate, this activity should be completed by an individual other than the person that is performing the extraction/lysis or running the robot.

3. Quantitation results

4. Amplification Worksheet (LAB-DNA-08) or equivalent

5. DNA Analysis Software Project List for every capillary electrophoresis run relevant to the case

6. Electropherograms used in the final interpretation of a sample
   a) If a sample is extracted more than once and processed through amplification, an electropherogram from each extraction and/or amplification kit is required.

7. Lot numbers and expiration dates of reagents used during DNA analysis.
   b) May record on DNA Reagent Lot Record (LAB-DNA-59) or worksheets that have dedicated spaces for recording reagents such as individual extraction worksheets (LAB-DNA-07, LAB-DNA-53), Quantitation Load Sheet (LAB-DNA-13), and Amplification Worksheet (LAB-DNA-08).
   c) Lot numbers are only required to be recorded once in the case record.

8. *Statistical data relevant to the case

9. *CODIS Data Worksheet (LAB-DNA-15, LAB-DNA-21 or LAB-DNA-23) or equivalent documentation

10. DNA Batch/Analytical Data Technical Review Sheet (LAB-DNA-63) (if altered by reviewer)

11. DNA Technical Review Worksheet (LAB-DNA-64) (if altered by reviewer)

4 Case-specific Records Maintained in Laboratory

The records listed below must be maintained in the laboratory. Examples may include keeping the documents in the case record, electronically on a shared drive, or in a binder.

- Quantitation Load Sheet (LAB-DNA-13)
- Quantitation Standards Curve Evaluation
- Printed (paper or electronic) IPC values/status for samples
DNA-02-11 FORENSIC BIOLOGY, MALE SCREENING AND DNA PROFICIENCY INSTRUCTIONS

1 Scope

Proficiency tests are designed and intended to be handled similar to casework in an effort to evaluate the system’s procedures and an analyst’s accuracy in applying those procedures and analysis methods. However, other guidance has been provided to analysts suggesting all tests should be applied to all samples to demonstrate their ability to perform all tests that they could potentially use in casework analysis (which is not a casework-based approach).

The intent of this document is to provide clarification and instruction on the appropriate testing to perform on Forensic Biology, Male Screening and/or DNA proficiency tests for standardization purposes. This guidance falls in between a strict casework approach and an all test-performance approach. Because non-blind proficiency tests are not presented and performed identical to casework, written guidance is warranted.

The intent of this document is also to provide clarification and instruction for the analysts and reviewers. This will add consistency among the DPS Crime Labs to ensure a quality system.

2 Related Chapters/Documents

Personnel

CLS Manual: Monitoring the Validity of Results

3 Forensic Biology (Body Fluid Identification)

3.1 Frequency

A. Forensic Biology proficiency tests are performed at least annually. The source of a Forensic Biology proficiency test should be an approved provider.

B. Each analyst is assigned their own test that is submitted to the provider for grading. There is no approved team approach for Forensic Biology (Body Fluid Identification).

3.2 Instructions

A. Perform the following tests on each evidentiary item:

1. An approved blood presumptive test used in casework.
   - a) If blood presumptive test(s) are positive, perform the presumptive test for species origin, if used in laboratory.
   - b) If the blood presumptive test(s) are negative, do not perform the species origin test.

2. Semen presumptive tests to include only:
   - a) Alternate Light Source
   - b) Acid Phosphatase Mapping

3. Perform the remainder of the semen detection tests based on the results of the ALS and AP tests:
   - a) If positive, perform a sperm search and p30 test.
   - b) If negative, no further semen testing is needed.
B. Identical to casework handling, no Forensic Biology testing is performed on the reference samples provided in a proficiency test.

C. No trace evidence is required to be collected from the evidentiary or reference samples.

4 Male Screening

4.1 Frequency and Instructions

Male screening proficiency tests are performed depending on the qualifications of the analyst/technician.

A. Because male screening is not directly graded by the provider, this test must be performed in conjunction with Forensic Biology (Body Fluid Identification) and/or DNA testing. The Forensic Biology (Body Fluid Identification) and/or DNA testing results are graded by the provider and male screening results are determined to be consistent with these results.

B. In general, each analyst is assigned their own test that is submitted to the provider for grading; however, multiple analysts/technicians may perform work on a single test if a team work approach is used.

C. Analysts/technicians whose sole responsibility is male screening sample preparation:

1. Frequency: complete a test at least annually

2. Instructions:
   a) Prepare samples as part of a team effort on another analyst’s assigned test. The analyst to whom the test is assigned will carry the male screening samples through manual or robotic processing and complete DNA testing.
   b) The DNA testing results are submitted to the provider for grading by the analyst to whom the test is assigned.
   c) If analysts/technicians proficiency test in biology (body fluid identification) or in DNA testing on the proficiency test, they are not required to complete a test in male screening preparation.

D. Analysts/technicians whose sole responsibility is male screening laboratory process:

1. Frequency: complete a test at least annually if also qualified in DNA analysis OR complete a test twice a year if not also qualified in DNA analysis.

2. Instructions (choose one option):
   a) Complete lysis and quantification of questioned samples using the male screening laboratory process and DNA testing on their own assigned test. Submit the test to the provider for grading. The DNA interpretation portion of the test may be completed or marked as “not applicable” or “no interpretation” depending on the qualifications of the assigned analyst.
   b) Complete lysis and quantification of questioned samples using the male screening laboratory process on another analyst or technician’s assigned test. The analyst/technician to whom the test is assigned will complete DNA testing. The DNA testing results are submitted to the provider for grading by the analyst/technician to whom the test is assigned. The DNA interpretation portion of the test may be completed or marked as “not applicable” or “no interpretation” depending on the qualifications of the assigned analyst/technician.
E. Analysts whose sole responsibility is male screening interpretation:

1. Frequency: complete a test at least annually if also qualified in DNA interpretation OR complete a test twice a year if not also qualified in DNA interpretation. Regardless, these analysts must be assigned their own test that is submitted to the provider for grading.

2. Instructions (choose one option):
   a) Complete lysis, quantification, and interpretation of questioned samples using the male screening laboratory process and DNA testing on their own assigned test. The DNA testing results are submitted to the provider for grading. The DNA interpretation portion of the test may be completed or marked as “not applicable” or “no interpretation” depending on the qualifications of the analyst.

   b) Complete interpretation of male screening results from questioned samples on their own assigned test. Another qualified analyst(s) will complete the lysis and quantification steps using male screening laboratory process and DNA testing. The DNA testing results are submitted to the provider for grading under the name of the analyst assigned to interpret male screening results. The DNA interpretation portion of the test is marked “not applicable” or “no interpretation”.

5 DNA Analysis

5.1 Frequency

Each DNA analyst and DNA technician is required to successfully complete an approved proficiency test in DNA analysis to the full extent in which they participate in casework semiannually from an approved provider.

A. In general, each analyst is assigned their own test that is submitted to the provider for grading; however, multiple analysts/technicians may perform work on a single test if a team work approach is used.

B. Qualified analysts/technicians are required to test using at least one method in each methodology (i.e. extraction, quantification, amplification, detection) at least once per year.

C. Analysts/technicians that are qualified in more than one amplification kit are required to test using each amplification kit at least once per calendar year.

D. Analysts/technicians that are qualified in more than one technology are required to test using each technology at least once per calendar year.

E. Analysts/technicians whose sole responsibility is generation of analytical DNA data may satisfy testing requirements through one of the following:

   1. Completion of their own assigned test that is submitted to the provider for grading; however, the interpretation portion is marked as “not applicable” or “no interpretation”.

   2. Participation through a team effort on another analyst’s assigned test which is submitted to the provider for grading.

F. Analysts whose sole responsibility is DNA interpretation are required to complete their own assigned test by interpreting DNA results twice per calendar year.
5.2 Methodologies

A. Extraction
1. Qualified analysts or technicians shall be proficiency tested on at least one of the extraction methods per year.
2. This method may be either manual or automated.

B. Quantification
1. Qualified analysts or technicians shall be proficiency tested on at least one of the quantification methods per year.
2. The set up for this method may be either manual or automated.

C. Amplification
1. Qualified analysts or technicians shall be proficiency tested on each technology (i.e. STR and Y-STR) at least once per year.
2. STR Typing of the 20 CODIS core loci must be attempted at least once per year.
3. Qualified analysts or technicians shall be proficiency tested on each amplification kit at least once per year.
   a) If qualified for Minifiler, a proficiency test must include amplification with Minifiler at least once a year on at least one sample.
   b) If qualified for Y-filer, a proficiency test must include amplification with Y-filer at least once a year on a sample that exhibits a Y component.
   c) If qualified for Y23, a proficiency test must include amplification with Y23 at least once a year on a sample that exhibits a Y component.
4. The set up for this method may be either manual or automated.

D. Capillary Electrophoresis (detection)
1. Qualified analysts or technicians shall be proficiency tested on at least one of the types of detection instruments per year.
2. The set up for this method may be either manual or automated.

5.3 Generation of Analytical DNA Data

A. Profiles containing artifacts, spikes, and pull-up may be used as long as artifacts have been labeled. Technical Leader approval is not required; however, analysts are encouraged to consult with the technical leader if the identity of the artifact is in question.

B. A full single source DNA profile shall be developed for all known reference samples.

C. For questioned, non-differential samples, all loci should be interpretable for comparison purposes. Every attempt shall be made to obtain interpretable data at every locus to include reinjection, re-amplification, and/or re-extraction.

D. For questioned differential samples:
   1. Epithelial Cell Fraction
      a) A full DNA profile should be developed. Every attempt shall be made to obtain interpretable data at every locus to include reinjection, re-amplification, and/or re-extraction.
b) The epithelial cell fraction should have a female contributor if the test synopsis indicates a potential female contribution.

2. Sperm Cell Fraction
   a) A full DNA profile should be developed. Every attempt shall be made to obtain interpretable data at every locus to include reinjection, re-amplification, and/or re-extraction.
   b) The sperm cell fraction shall have a male contributor.

5.4 Interpretation of Data

A. Analysts that are qualified in interpretation of data from more than one amplification kit are required to test by interpreting data from each amplification kit at least once per calendar year.

B. Analysts that are qualified in interpretation of data from more than one technology are required to test by interpreting data from each technology at least once per calendar year.

C. STRmix
   1. All evidentiary profiles that yield a mixture will be de-convoluted in STRmix to develop an advanced report. The Genotype Probability Distribution table from the STRmix Advanced Report will be used to complete the test provider’s results table for these samples.
   2. Single source evidentiary profiles will be interpreted consistent with what is done in casework. A manual interpretation will be used to complete the test provider’s results table for these samples.
   3. Propositions will be made in STRmix consistent with what is done in casework. Provided information from the test provider can be used to determine if co-contributor and conditional likelihood ratios may be needed.
   4. If stutter is present at DYS391, the stutter will be labeled as such and will not be included in the proficiency provider results table.

D. CODIS
   1. For STR profiles, a CODIS Entry Worksheet (LAB-DNA-15) will be completed for a CODIS eligible profile. The profile selected shall be complete enough to be NDIS eligible.
   2. For Y-STR profiles, a CODIS Entry Worksheet (LAB-DNA-21 or LAB-DNA-23) will be completed for a CODIS eligible profile.
   3. None of the profiles selected will be entered into CODIS.

6 Reporting

6.1 Information to Test Provider:

A. Comments must be added to the test provider’s form to explain results in an effort to assist review of the results by entities that may not have a complete record of the results (e.g. System Quality Assurance, accrediting body Proficiency Review Committee). Multiple comments may be added as dictated by the results or tests undertaken.
B. For Male screening: A comment must be provided to the test provider if male screening process was performed such as:

*Male screening was performed on [list items] using the Plexor HY quantification kit.*

C. For DNA:

1. All alleles from every contributor on the Genotype Probability Distribution table from a mixture profile shall be reported to the test provider. Alleles should not be repeated; however every allele given weight as a possible true allele in the table shall be reported. This includes any potential stutter peaks that may have been given weight during the development of the genotype probability distribution table.

2. Any allele calls for Amelogenin and DYS391 shall be reported to the test provider even though this information is not included on the genotype probability distribution table.
   a) If stutter peaks are observed for the DYS391 locus, do not include those in the report to the provider.

3. A comment must be provided to the test provider to explain reported stutter peaks such as:

   *Evidence profiles were interpreted using a probabilistic genotyping approach. STRmix software was used to aid interpretation, which includes biological modelling for forward and reverse stutter. Any allele included in a proposed, weighted genotype combination in the STRmix output was reported in the DNA Results tables of this form. This approach may result in at least one stutter peak being included in the DNA Results tables.*

4. A comment must be provided to the test provider to explain partial profiles such as:

   *A partial profile was obtained for item [X] in this test. The majority of the called alleles in the [profile] are within the stochastic region; therefore, dropout [is] OR [may] be occurring. However, despite multiple attempts, a complete profile was not obtained, indicating a potential problem with sample quality.*

5. A comment must be provided to the test provider to explain incomplete separation in sperm and epithelial cell fractions such as:

   *Item [X] in this test was extracted by a differential extraction method. Full profiles were obtained for the epithelial cell and sperm cell fractions; however, there was incomplete separation in one or both fractions, resulting in a mixture profile. Allele calls from both donors were observed in the profile due to the incomplete separation.*

6.2 Information To Laboratory System

A. Conclusions shall be reported in accordance with laboratory’s current practices. Refer to the *Report Writing Guidelines* chapter.

B. Conclusions may be reported in LIMS.

   1. If conclusions are reported in LIMS, requests should be added only for the categories of testing performed (i.e. Forensic Biology, Male Screening, or DNA).
2. If testing is performed on a DNA-only test, it is not necessary to complete a request Biology or Male Screening.

7 Technical Review

A. A qualified analyst who completes a proficiency test in a specific technology is qualified to serve as a technical reviewer without needing to take an additional proficiency test as a technical reviewer.

B. Persons whose sole responsibility is technical review shall complete semiannual proficiency testing in the technologies and amplification kits for which they have authorization to technical review through technical review of another analyst’s proficiency test prior to submission to the test provider.

1. For Male Screening process and/or interpretation, technical review must occur on a test where male screening with Plexor HY was used at least once per year.

2. For autosomal STR, technical review must occur on a test where 24plex was used at least once per year. If the reviewer is qualified to review Minifiler, technical review must also occur on a test where Minifiler was used at least once per year.

3. For Y-STR, technical review must occur on a test where Y-STRs were used at least once per year. If the reviewer is qualified to review both Yfiler and Y23, technical review must occur on a test where Yfiler was used at least once per year and on a test where Y23 was used at least once per year.
DNA-02-12 QUALITY ASSURANCE STANDARDS (QAS) AUDITS

1 Scope

In order to participate in NDIS, the DPS is required to comply with the FBI DNA audit document (FBI Quality Assurance Standards Audit for Forensic DNA Testing Laboratories) (QAS). Standard 15 of the QAS addresses audits and requires the laboratory to be audited annually in accordance with the FBI Quality Assurance Standards. An external QAS audit is required at least once every two years by an external agency. Internal QAS audits are not required to be performed during years when the laboratory has undergone an external QAS audit.

This document addresses how the internal QAS audit is to be conducted during the years when the external QAS audit is not performed. It also addresses post-audit activities for internal and external QAS audits.

2 Related Documents

CLS Manual: Audits

3 Practices

3.1 Audit Document, Frequency, and Team

A. The most current version of the FBI’s Quality Assurance Standards Audit for Forensic DNA Testing Laboratories will be used to conduct the internal audit.

B. The internal QAS audit shall occur at least biennially (on years when there has been no external QAS audit) and shall be at least 6 months but no more than 18 months apart from the previous QAS audit.

C. The internal audit team may consist of one or more individuals. At least one person on the team must be currently or previously qualified in each specific DNA technology performed in the laboratory (e.g. STR, and YSTR), and at least one person on the team must have successfully completed the FBI sponsored audit training.

3.2 Performing the Internal Audit

A. The laboratory that is being audited will complete the “Checklist of General Laboratory Information” located in the QAS and provide it to the audit team for inclusion in the final audit record.

B. The auditor(s), in conjunction with the laboratory that is being audited, will complete Appendix C, “Auditor Self-Certification for QAS Audits”. Appendix C will be signed by the auditor(s) and included in the final audit record.

C. All standards located in the QAS document will be assessed by the auditors as part of the audit.

D. The audit must include some type of case review in order to address Standards 11 and 12.

1. The annual system peer reviewed cases may be used by on-site auditors to meet this requirement.

2. Any potential non-conformity identified during the annual system peer review of cases should be addressed during the internal QAS for that year. This may require the on-site audit team to request more cases from the laboratory for review to determine if the non-conformity is a recurring or isolated event.
3. If the on-site audit team does not prefer to use the annual system peer review cases, the auditors may request that the laboratory provide additional cases for review on-site in order to address Standards 11 and 12.

E. The QAS audit document must be completed by the audit team. This includes ensuring all boxes are checked as “Yes”, “No”, or “N/A” for all standards, information for all appendices has been completed or listed as “N/A”, and the cover page of the audit document has been completed and signed by the auditors.

F. The lead auditor should meet with the DNA Technical Leader and DNA Section Supervisor as appropriate throughout the audit to apprise them of audit progress including any potential findings.

3.3 Post-Internal Audit Activities

A. The audit team will complete the QAS audit document and provide it to the Biology Program Coordinator within 10 business days of completion of the audit.

B. The Biology Program Coordinator will review the QAS audit document for completeness. The document may be returned to the audit team as necessary to ensure that it has been completed.

C. The completed QAS audit document will be provided to the laboratory being audited by the Biology Program Coordinator within 30 calendar days of the audit.

D. The Technical Leader and the Local CODIS Administrator of the laboratory being audited shall review the QAS audit document and provide responses to any findings to System Quality Assurance through the Biology Program Coordinator within 30 calendar days of the laboratory’s receipt of the final audit document.

   1. The date of receipt of the final audit document must be documented by the laboratory.

E. Findings may be contested by providing documentation to System Quality Assurance through the Biology Program Coordinator.

F. The laboratory will be notified by System Quality Assurance through the Biology Program Coordinator once the audit has been closed.

G. After the audit has been closed, the Technical Leader and Local CODIS Administrator will document review and acceptance of the audit by placing their initials and date on the cover page (signature page) of the audit document.

H. The finalized internal QAS audit document record consists of the audit document, responses to any findings, and Technical Leader and Local CODIS Administrator initials and date. This record will be forwarded to System Quality Assurance through the Biology Program Coordinator. Completed audit document records will be stored electronically.

I. Internal QAS audit records are not provided to NDIS.

3.4 Post-External Audit Activities

A. Once the audit is received, the Technical Leader will review the QAS audit document for accuracy and completeness. The audit will be provided to the Biology Program Coordinator within 10 business days of completion of this review.

B. The Biology Program Coordinator will review the QAS audit document for completeness within 5 business days of receipt after the review by the Technical Leader.
C. If corrections are required, the Biology Program Coordinator will provide System Quality Assurance with a list of corrections. System Quality Assurance will forward the request for corrections to the auditors.

D. Once the final (corrected) QAS audit is received, the Technical Leader and Local CODIS Administrator will prepare responses to any findings. The responses should include supporting documentation to show how the issue was remediated.

1. The date of receipt of the final audit document must be documented by the laboratory.

E. If the laboratory intends to contest findings, this may be done by providing supporting documentation, along with information as to why the finding is contested, to System QA through the Biology Program Coordinator for review. Once the review is complete, the information concerning the finding is provided to NDIS as part of the laboratory’s response to findings by including it in Appendix A or as a memo attached to the QAS document. All correspondence should be typed.

F. The final QAS audit along with any responses to findings or information concerning contested findings must be provided to NDIS within 30 days of receipt of the final audit by the local laboratory. The State CODIS Administrator should be informed when the audit has been submitted to NDIS for review.

G. If an acknowledgement of receipt of audit is provided by NDIS, this acknowledgement must be retained as part of the final audit record.

H. The laboratory will be notified by NDIS once the audit has been closed. This notification must be retained as part of the final audit record.

I. After the audit has been submitted to NDIS, the Technical Leader and Local CODIS Administrator will document review and acceptance of the audit by placing their initials and date on the cover page (signature page) of the audit document.

J. The finalized external QAS audit record consists of the audit document, Technical Leader and Local CODIS Administrator initials and date, responses to any findings (including records associated with the finding that demonstrate compliance), proof that the audit was provided to NDIS within 30 days of receipt, proof of acknowledgement of receipt by NDIS, and proof of notification by NDIS that the audit has been closed. This record will be forwarded to System Quality Assurance through the Biology Program Coordinator. Completed audit document records will be stored electronically.

4 Literature References and Supporting Documentation


FBI. National DNA Index System (NDIS) Operational Procedures Manual (most recent version).
03 CASEWORK PROCESSING

DNA-03-01 EVIDENCE HANDLING

1 Scope

The purpose of collection and packaging of biological evidence is to preserve it for future analysis, protect it from contamination, and maintain the integrity of the evidence.

2 Related Documents

CLS Manual:
- Receipt and Review of Laboratory Requests for Service
- Submission and Receipt of Evidence
- Evidence and Database Sample Integrity
- Evidence Processing
- Return of Evidence

3 Practices

3.1 Storage of Evidence

A. Biological evidence must be properly stored to preserve biological constituents.

B. Store sexual assault kits in the refrigerator or at room temperature. If the sexual assault kit is stored at room temperature, the liquid blood specimen must be removed and stored in the refrigerator, or a sample of the specimen must be dried on FTA paper or cloth substrate. Blood and urine specimens requiring toxicological screening will be stored in the refrigerator.

C. Small, dried evidentiary items may be stored frozen depending on available space.

D. Refrigerate, do not freeze, liquid whole blood specimens.

E. Store larger items such as clothing, bedding, weapons, and other physical evidence containing stains at room temperature until examined.

F. Collected cuttings or swabs are considered evidence. For long-term storage, this evidence may be stored either frozen or in a temperature-controlled environment. A portion of collected cuttings and swabs and DNA extracts will be retained by the laboratory whenever possible.

G. DNA extracts are considered evidence and will be retained by the laboratory whenever possible.

H. Amplification products, male screening lysates, and/or slides prepared by the laboratory are considered work product and not considered evidence. These items should be properly discarded after analysis has been completed.

I. Prior to June 29, 2018, amplification products were considered evidence if they were the only remaining sample from that piece of evidence. Any amplification product retained prior to June 29, 2018 must be handled according to the Evidence and Database Sample Integrity chapter of the CLS Manual.

J. All items considered evidence by the laboratory will be handled according to the Evidence and Database Sample Integrity chapter of the CLS manual.
3.2 Interlaboratory Transfer

A. For interlaboratory transfer procedures, refer to the Evidence and Database Sample Integrity chapter of the CLS manual.

B. If screening and evaluation of the evidence has been performed, the evidence will be forwarded according to these procedures:

1. All known and evidentiary samples should be submitted as dried stains. Known blood standards should be spotted onto FTA paper.
2. Select a sufficient portion of the evidentiary stain for submission.
3. All probative swabs and stains should be submitted.
4. In the event that hairs, bone, teeth, muscle, or other tissues are required for DNA analysis, arrangements should be made between laboratories before transfer. Mounted hairs should remain mounted until transfer to the laboratory to prevent possible contamination. Soft tissue samples should be transferred frozen.
DNA-03-02 PHYSICAL EVIDENCE EXAMINATION

1 Scope

The laboratory has established unifying documentation and collection procedures for physical evidence examinations. The initial examiner of an item is primarily responsible for the collection and preservation of evidentiary materials and relevant stains that may be on that item.

Persistence and transfer studies prove that debris on clothing surfaces is easily lost if garments are handled, shaken, or laid out. Items being submitted for trace evidence examination need to be handled as little as possible to minimize loss of the trace evidence and to limit exposure of the items to contaminants until the trace evidence has been collected and preserved.

The procedures presented are intended to assist the analyst in the inspection of physical evidence. They are to be used in conjunction with all applicable laboratory policies, good laboratory practice, and proper scientific techniques. The analyst will be given flexibility to determine an appropriate course of action in regard to the collection, preservation, and analysis of evidence to attain the ultimate goal of quality and efficiency.

Documentation must be in such a form that another qualified analyst or supervisor, in the absence of the primary examiner, would be able to evaluate what was done and interpret the data. The reviewer of the case should be able to determine from the notes that sufficient, relevant, and correct testing methods were used.

2 Related Documents

None

3 Safety

A. Use universal precautions when handling evidence. Use a particle mask and/or safety glasses when appropriate. Use appropriate safety measures when handling sharp objects. Appropriate personal protective equipment must be worn during testing. Clothing may protect unbroken skin; broken skin must be covered.

B. All firearms shall be treated as if loaded. All firearms must be rendered safe prior to handling, marking and/or packaging.

4 Equipment and Materials

Varies with the type of technique used to collect evidence, including the following:

- Trace evidence recovery materials
- Tape and examination paper
- Etching pencil, scribing tool, or permanent marker
- Stereomicroscope, microscope, or magnifying lens
- Camera
- Measuring devices
- Alternate light source
- Tweezers, scalpel, scissors, probe and other implements as necessary
- Plastic bags, paper envelopes, or appropriate evidence containers
5 Standards, Controls, and Calibration
Respective positive and negative controls will be conducted and documented as required by specific analytical procedures.

6 Procedure

6.1 Case/Evidence Evaluation

A. The results of the examinations may either implicate or exonerate a suspect from involvement in a crime. Because each case is different, only guidelines can be prescribed; the case evaluation should include consultation with the investigator/prosecutor and other DPS disciplines as necessary.

B. An offense report is very helpful in assessing the probative value of the evidentiary material.

C. All communications pertinent to the case evaluation must be documented.

D. Cases should be evaluated to:
   1. Determine the quality and quantity of the evidence to be analyzed
   2. Prevent the loss of potentially valuable information
   3. Maximize the meaningful information obtained from the evidence
   4. Determine if the requested examinations can be performed with the submitted evidence and with the available resources

E. When possible, cases with requests for post-conviction analysis should be worked by personnel other than the analyst or analysts that originally worked the case.

F. Some considerations in evaluating the evidence should include:
   1. The age of the evidence/case
   2. The storage conditions of the samples prior to submission
   3. Whether stain/smear samples, such as blood, semen, saliva were dried before submission
   4. Whether the evidence is moldy and/or putrefied
   5. Possible dilution of the samples
   6. Whether the evidence requires analysis by other disciplines or has already been analyzed by other disciplines

With the exception of porous items (paper, envelopes, cardboard, cigarette butts, etc.), any item submitted for both DNA and Latent Prints examinations (requested before the evidence is transferred) will be screened by the DNA Section of the laboratory where the request originated.

7. Whether all pertinent evidence has been submitted
8. Whether the victim(s), suspect(s), or potential witnesses were injured
9. The relationship between victim(s), suspect(s), and potential witnesses
10. The availability and adequacy of suspect and/or victim known samples
11. The extent of screening required to obtain a search warrant for suspect known samples
12. The analyses that should be run if sample is limited
13. Possibility of sample remaining after analysis
14. Possibility and effect of cross-contamination

6.2 Evidence Examination

A. For cases with a large volume of evidence (excluding sexual assault kits), a maximum of ten probative items of evidence should be screened.

B. It may be necessary to consult with another qualified analyst, the Technical Leader, and/or the supervisor to determine the appropriate analytical approach. How far the analyst proceeds in a particular case will depend on the available sample and what is necessary to answer the question(s) posed. If a limited amount of staining is observed on an item, the analyst should evaluate whether presumptive and/or confirmatory testing should be conducted in order to maximize the potential for future DNA testing.

C. Retrieve evidence from evidence storage, evidence custodian or another analyst. Verify that the Laboratory Submission Form (LAB-201) is appropriately completed and a chain of custody maintained. Any changes or additions to the form should be initialed and dated. Identify the pertinent forensic question(s). Plan the approach to the case. Evaluate the potential value of evidence relative to the items of evidence submitted for examination.

D. Wear a lab coat, disposable gloves, and mask as appropriate and change as necessary to ensure safety and to avoid contamination of evidentiary items. It is recommended to avoid talking when evidence is being examined.

E. Clean and then cover the work surface with clean, unused paper.

F. At all times during the examination, items from any suspect(s) are kept separate from those of associated victim(s). Whenever possible, items from suspect(s) and items from associated victim(s) are examined in different locations and/or times. Always keep known materials separated from the questioned materials to be examined.

G. No more than one item of evidence in an unpackaged state is allowed on an examination table at one time unless the items were submitted in the same package.

H. Note instances where packaging or handling of the evidence creates a potential for contamination. These instances should be brought to the attention of the supervisor, other involved analysts and the investigator. Such instances may preclude the examination of the evidence.

I. The case number, unique identifier and analyst initials should be labeled on the packaging. Open the outer container (avoid breaking previous seals, if possible). Mark inner evidence packages as encountered.

J. Label or tag each item with unique case number and analyst initials.
   1. If the evidence is too small to mark, place the evidence in a package then seal and mark the package.
   2. Any markings and notations on the evidence should not interfere or obstruct forensically significant areas (e.g. bloodstains).
3. Because it is not possible to determine where and if some types of evidence exist on an item until after it has been processed, markings and notations should not be placed directly on items that will be processed by the Latent Print or Questioned Documents sections.
   
a) As an alternative to directly marking the item, photographs or diagrams should be made and labeled to indicate areas of testing and stain collection.

b) It is also recommended to include a note in the case record explaining why markings and notations were not placed directly on the item.

K. Document the individual items of evidence. Note whether other items were packaged together with the selected item. Visually examine the evidence and document as appropriate:

1. A description of the outer evidence packaging and condition of the evidence, especially relevant factors to the preservation of the biological material
2. Physical description such as color, size, material, holes and tears, broken parts, missing parts, or other modifications that make the item appear unusual
3. Manufacturer’s identification, serial numbers, or other marks
4. The collection of trace evidence
5. Significant stains, patterned marks, or impressions should be documented in a manner which clearly indicates the location, physical characteristics, relationship to other stains, and results of screening tests
6. Use of microscopes or alternate light sources

L. Perform and record results of presumptive tests conducted based on the respective Analytical Approaches as described below.

M. A sufficient number of samples should be collected from an item to represent stains of probative value.

N. Collected samples must be protected from loss or contamination by individually packaging and labeling with case number, item number, unique identifier as applicable, and initials.

O. Any collected trace evidence may be packaged separately or with the original item, as long as it is uniquely labeled, sealed, initialed and dated. All original exhibits will be re-packaged in the original container, if possible. The evidence is re-sealed in a manner that would detect tampering.

P. Upon completion of screening, the evidence should be transferred to the submitting agency, evidence storage area, evidence custodian or appropriate analyst.

6.3 Analytical Approaches

The analyst will evaluate the case synopsis, scene, and collected evidence to determine the appropriate course of analysis that should be taken to address the request. The most probative evidence items will be examined first.

A. Body Fluid Identification – Blood Examinations

1. A typical analysis scheme for a suspected bloodstain may include:
   
a) visual examination

b) presumptive testing
2. How far the analyst proceeds in a particular case will depend on the available sample and on what is necessary to answer the question(s) posed.

B. Body Fluid Identification – Semen Examinations

1. When screening evidence for semen, an alternate light source can be helpful, especially for larger items of clothing or bedding. An alternate light source also aids in the discovery of some trace fibers. Refer to the Alternate Light Source chapter in the DNA SOP for further information.

2. A typical analysis scheme for a suspected semen stain may include:
   a) visual examination
   b) presumptive testing
   c) confirmatory testing
   d) preserve the stain, cutting or swab
   e) test for other body fluids if indicated
   f) Male screening can be used as an alternative to semen examinations
   g) Direct to DNA processing may follow a different analysis scheme than what is listed below.

3. Semen should be confirmed on at least one item. The only confirmatory test for semen is the microscopic identification of spermatozoa.

4. Once semen is confirmed on at least one item in the sexual assault kit, it is not necessary to examine other submitted items unless circumstances dictate the need for additional analysis (e.g., multiple assailants or blood).

5. If an orifice sample is AP tested, and a negative AP result is obtained, an attempt to confirm semen shall be done unless semen has been confirmed on another orifice sample or the sample is being forwarded to DNA extraction as a differential (refer to flowchart).

6. If an orifice sample is AP tested, and a positive AP result is obtained:
   a) A sperm search is not required provided the sample is forwarded for DNA extraction as a differential (refer to flowchart).
      i. The sample must be retained but is not required to be forwarded to DNA analysis if semen has been confirmed on another sample in the case.
   b) If an attempt to confirm semen was made and the sperm search results were negative, a p30 test must be performed or the sample forwarded to DNA extraction as a differential to account for potential differences in sampling. If the p30 test is negative, then the sample is not required to be forwarded for DNA extraction (refer to flowchart).
      i. If semen has been confirmed on another sample in the case, the sample must be retained but is not required to be p30 tested or forwarded to DNA analysis.
7. Flowchart for testing orifice swabs:

![Flowchart Image]

*Reminder: samples sent to DNA as differentials must include a sperm search as part of the differential process unless a slide was made during screening OR the sample was screened using Male Screening Process with Plexor HY.*

8. If an orifice swab has accompanying slides and the AP exam on the swab and sperm search from the corresponding slides are both negative, an appropriate conclusion is no semen detected.

9. Examination of the victim’s panties or other items of clothing with a crotch must include a qualitative acid phosphatase test of the crotch area and/or back panel as the case circumstances dictate.

10. When both presumptive tests for semen are positive, but spermatozoa are not detected to confirm the presence of semen, DNA should be attempted on case-appropriate samples.

C. Trace DNA Collection

D. If there is no identification of biological fluids and there are areas of the evidence which could be reasonable to contain probative DNA, the following should be considered when collecting this type of sample.

1. General Guidelines

   The choice of collection method and location should be made after careful evaluation of the case circumstances and with consideration of the requirements of other forensic disciplines.

   a) **Primary consideration should be given to collecting the most probative evidence from the evidentiary item.**

   b) **If the analyst believes that the technique they plan to use may hinder the ability of another discipline to collect evidence, the analyst should consult with a qualified analyst from that discipline prior to collection.**
2. When the probative value of a forensic discipline, collection method, or collection location is in question, analysts may need to consult with investigators prior to collection.

3. Swabs Collected Prior to Friction Ridge Development
   a) For non-porous items, care should be taken to avoid smooth areas if the submitting agency desires latent prints on the item(s), especially when possible ridge detail is present.
   b) For porous items, avoid possible latent print development areas or consult with the latent print section before swabbing.

E. Reference Samples
   1. As needed for comparison, record, collect, and uniquely label known sample(s).
   2. Liquid biological samples shall be stored refrigerated until a dried sample is prepared.

F. Hair Evidence
   1. The screening process includes the recognition, collection, and preservation of possible hair and trace evidence. Assessment of root material or suitability for DNA may be documented.
   2. A consultation with a qualified hair examiner must be performed prior to DNA analysis.
   3. With any attempt to DNA type a hair root, a result is not assured and, for telogen hairs, not expected. DNA typing of hair is necessarily destructive but may not yield results. Therefore, the evidentiary value of the hair must be carefully evaluated and the potential loss of information weighed before proceeding with DNA analysis. If it is evident that the hair will likely be consumed by analysis, it may be necessary to consult with the investigator and/or prosecutor. The approval from the investigator and/or prosecutor for the consumption of the hair in the analysis should be documented.

G. DNA Analysis
   1. Determining the number of items to be typed for a single case should be done with concern for both the probative value and the need for timely information. It is recommended that a maximum of five evidentiary stains be subjected to DNA analysis. Additional items/stains may be analyzed at a later date depending on circumstances of the case.
   2. Whenever possible, at least half of the evidence sample will be preserved for possible re-analysis. Sample consumption will be evaluated on a case by case basis at the discretion of the analyst. Sample will only be consumed to gain the most probative information. Total consumption of the evidence must be documented.

7 Interpretation
Respective interpretations will be conducted and documented as required by specific analytical procedures.
8 Additional Casework Processing Concerns

A. If insect activity is present, examine the item carefully on an isolated bench, if possible. The evidence may also be placed in a freezer to curtail the insect activity prior to examination.

B. If the analyst discovers an item is received in a wet condition, the container should be opened and the object allowed to air dry. This may entail spreading the object out on a cleaned, flat surface and exposing it to the air for a period of time. The wet items should not be heated or exposed to direct sunlight.

C. Care should be exercised not to lose evidentiary materials when attempting to dry the object.

D. Items that have become foul smelling due to decomposition should be placed in the hood with the exhaust fan running to remove odors and moisture.

E. Communication with the submitting agency may be in order to ensure evidence is properly submitted in the future.

F. If no sperm search was performed on an item during male screening/biology screening OR if there was a negative sperm search result on the item during screening, but a positive sperm search result was obtained during DNA extraction of that item, a statement indicating the results of the sperm search during extraction should be included in the DNA report.

G. As a part of working a current request for analysis, the analyst should assess any previous analysis (analyses) performed in the case. Requests for analysis should not be worked in isolation, and the case should be processed using a holistic approach.

9 Records

LAB-201 Laboratory Submission Form

LAB-DNA-01 Biological Screening Worksheet or LIMS equivalent
DNA-03-03 PHYSICAL EVIDENCE COLLECTION USING MINI-TAPES

1 Scope
Biological stains and/or cellular material (most commonly epithelial cells) may be found on many different types of evidence. Proper collection is imperative for downstream DNA testing. The following collection method may be used in lieu of common collection techniques including cutting or swabbing stain/material.

2 Related Chapters
Physical Evidence Examination

3 Safety
Use universal precautions during evidence handling. Appropriate personal protective equipment must be worn. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- 10% bleach solution
- Laminar flow biological hood
- Paper cutter or scissors
- Double sided tape
- Transparency sheet
- Ruler

5 Procedure
5.1 Mini-Tapes
A. Making Mini-tapes
1. Mini-tapes should be made in a sterile location, such as a laminar flow biological hood wiped down with a 10% bleach solution followed by sterile water. A cutting implement such as a guillotine style paper cutter or scissors should also be sterilized prior to use, and used only for the purpose of making mini-tapes.

2. Mini-tapes are made by placing two parallel rows of half inch double sided tape across the width of a clear, uncoated transparency sheet. The protective paper backing should remain on the other side of the tape. Multiple parallel rows can be placed on each transparency, with approximately 2 inches of empty space between the next parallel rows of tape. The transparencies are cut into half inch wide strips, creating a half inch wide by 3 inch long strip, with two half inch square pieces of tape on one end. The strips can then be stored in zip-lock bags until used.

B. Using Mini-tapes
1. Remove the paper backing from both squares on the mini-tape. Mini-tape collection is extremely sensitive. Gloves should be changed immediately prior to removing the paper backing. Remove the paper backing from only one mini-tape at a time, and change gloves often to minimize the risk of contamination.

2. Using light pressure, apply the exposed tape to the surface to be collected. As the tape becomes saturated, the amount of pressure should be increased. The analyst
should be able to feel the difference in stickiness as the mini-tape becomes saturated.

3. Once a mini-tape is completely saturated with debris and loses its stickiness, place it in a labeled zip-lock bag for storage. Multiple mini-tapes may be used to collect from the same area as needed. The number of mini-tapes required to collect from a given area is dependent upon the substrate and the amount of debris present on the item. Multiple mini-tapes from the same area of collection may be stored in a single bag and extracted together.

4. Mini-tapes may be extracted while still attached to the clear plastic backing by cutting the half-inch squares from the rest of the strip.
   a) Extraction efficiency may be increased by bending the squares slightly so that they do not lie flat against each other in the tube.
   b) Generally, 10-12 half-inch squares can be extracted in a single 1.5 mL tube. The analyst should take care to balance the number of mini-tapes used for collection between the necessity to obtain probative evidence and the difficulty of extracting a sample in multiple tubes.

5. These samples are ready for extraction using the methods outlined in the DNA Extraction with Stain Extraction Buffer – Organic, DNA Extraction with Digest Buffer – Organic, and DNA Extraction and Purification with Chelex chapters.

6. Records
   Laboratory Submission Form (LAB-201)
   Biological Screening Worksheet or LIMS equivalent (LAB-DNA-01)

7. Literature References and Supporting Documentation
   Valid-Method-LUB-DNA-MiniTapeCollection-2010-0407
   Valid-Method-HOU-DNA-2014-MiniTape Implementation Validation-Summary
   Fairley, Martin. DNA Mini Tapes, Scottish Police Services Authority, presentation.
DNA-03-04 OUTSOURCING TO A CONTRACT LABORATORY

1 Scope

The guidelines contained within this document are for the DNA analysis of casework samples by a contract laboratory for which validated procedures exist within the DPS DNA SOP as well as for the acceptance of profiles generated by non-CODIS laboratories or non-CODIS contract laboratories performing forensic DNA analysis on behalf of law enforcement agencies for entry into CODIS. The FBI DNA Audit document and NDIS procedures shall be followed.

2 Practices

2.1 Acceptance of Data from Non-CODIS Laboratories or Non-CODIS Contract Laboratories Performing Forensic DNA Analysis on Behalf of a Law Enforcement Agency

A. An agreement in the form of an MOU must be reached between this Agency on behalf of the DPS Laboratory accepting the profiles for upload to CODIS and the non-CODIS laboratory that generates the DNA data or the law enforcement agency that uses a non-CODIS contract laboratory for performing forensic DNA analysis.

1. This agreement must be approved by the Technical Leader of the DPS Laboratory accepting the profiles.

2. The agreement must be fully executed prior to acceptance of DNA profiles or site visits.

B. The non-CODIS laboratory or non-CODIS contract laboratory generating the DNA data must provide documentation that the laboratory is in compliance with the Quality Assurance Standards for Forensic DNA Testing Laboratories and the accreditation requirements of federal and state law.

C. The Technical Leader of the DPS laboratory accepting the profiles must review and approve the technical specifications of the outsourcing agreement between a non-CODIS contract laboratory and the law enforcement agency prior to accepting ownership of the data. It is standard practice that this approval take place prior to initiation of testing by the non-CODIS contract laboratory. This documentation must be maintained by the DPS laboratory.

D. An on-site visit of the non-CODIS laboratory or non-CODIS contract laboratory must be conducted prior to beginning DNA analysis. The Technical Leader of the DPS Laboratory accepting the profiles or a designated employee of the DPS Laboratory accepting the profiles may perform the on-site visit. Alternatively, the Technical Leader of the DPS Laboratory accepting the profiles may accept an on-site visit conducted by a designated FBI employee.

E. Only profiles analyzed after the date of the agreement will be accepted.

F. If the agreement extends beyond one year, annual on-site visits must be performed. Alternatively, on-site visits performed by other NDIS participating laboratories may be accepted, with documented approval of the DPS Laboratory’s Technical Leader, if the NDIS participating laboratory conducting the review utilizes the same technology, platform, and typing amplification kit for the generation of DNA data.

G. If an agreement is in place, the laboratory must maintain proof of compliance with the practices listed above, even if no data was accepted during the term of the agreement.
2.2 Outsourcing DPS Cases to Contract Laboratories

The following applies to DNA analysis by a contract laboratory of casework samples originally submitted to a Texas DPS Laboratory when validated procedures exist within the DPS DNA SOP. Cases outsourced prior to the revision of this document will follow the guidelines with effective date October 16, 2012.

A. Outsourcing Agreement

1. The Technical Leader shall document approval of the technical specifications of the outsourcing agreement with the contract laboratory before the agreement is awarded. This documentation must be maintained by the DPS laboratory.

2. The contract laboratory must provide documentation that they are in compliance with the Quality Assurance Standards for Forensic DNA Testing Laboratories and the accreditation requirements of federal and state law.

3. An on-site visit of the contract laboratory must be conducted prior to initiation of DNA analysis. Either the Technical Leader or a designated employee of the DPS Laboratory that is outsourcing the cases may perform the on-site visit. Alternatively, the Technical Leader may accept an on-site visit conducted by a designated FBI employee.

4. If the agreement extends beyond one year, annual on-site visits must be performed. Alternatively, on-site visits performed by other NDIS participating laboratories may be accepted, with documented approval of the DPS Laboratory’s Technical Leader, provided the NDIS participating laboratory conducting the review utilizes the same technology, platform, and typing amplification kit for the generation of DNA data.

5. If an agreement is in place, the laboratory must maintain proof of compliance with the practices listed above, even if no cases were outsourced during the term of the agreement.

B. Case evaluation

C. Case evaluation of samples for outsourcing shall be equivalent to that used for samples analyzed in-house.

D. Shipment

1. Samples/batches shall be sent by overnight carrier or submitted in person to the contract laboratory and individual cases shall be tracked by barcoding in LIMS.

2. A letter will be generated by DPS and sent to the submitting agency informing them that their case has been outsourced to a contract laboratory.

3. Samples, when received after analysis from the contract laboratory, shall be tracked by barcoding in LIMS.

E. Analysis of Outsourced Samples

The contract laboratory shall use DPS approved analysis procedures and analysis methods as stated in the contract.

F. Testimony

Testimony will be provided by the contract laboratory for all analyses performed by the contract laboratory.
2.3 Performing an On-site Visit

A. The Technical Leader or designated employee of the laboratory that is a qualified or previously qualified analyst in the same technology, platform and typing amplification test kit as the contract laboratory should conduct the on-site visit.

B. A brief audit of the laboratory should be conducted using the FBI QAS audit document as a guide.

C. A limited review of cases from the laboratory where data is generated should be performed. Cases that include data developed using the same technologies as profiles that are being considered for CODIS entry should be selected for this review.

D. A review of validations for the technologies that are being used to generate data for CODIS entry should be performed.

E. Documentation of the on-site visit will be approved by the Technical Leader and retained by the laboratory.

2.4 Review of Data

A. DNA profiles developed by a non-CODIS/contract laboratory shall not be entered or uploaded to CODIS until a documented technical review has been conducted of the data record by a qualified DPS DNA analyst or an approved contract employee.

B. The analyst conducting the review must be qualified or previously qualified in the technology, platform, and typing amplification kit used to generate the data and participate in the laboratory’s proficiency testing program.

2.5 Guidelines for Technical Review of Data from Non-CODIS Laboratories or Data from Outsourced Casework

The following points should be examined during technical review of data from non-CODIS laboratories or contract laboratories that have processed outsource cases.

A. Data Record Documentation

1. The reviewer must ensure that all notes and analytical data in the data record and any notes generated through review are labeled with the non-CODIS/contract laboratory case number or other appropriately related number, reviewer’s initials and the date of the review. This may be accomplished through handwriting any missing information on the reviewed pages or through electronic means such as placing the non-CODIS/contract laboratory paperwork in LIMS or by placing a case activity in LIMS stating that the entire data record was reviewed.

2. Data record documentation received from the non-CODIS/contract laboratory may include:
   a) data obtained through the analytical process (standards and controls used, analytical conditions used, allele tables, reagent lot numbers, observations made, results of test performed, charts, graphs photographs, sketches, electropherograms, etc.),
   b) a non-CODIS/contract laboratory report with conclusions supported by the data record notes and analytical data,
   c) information to assist in determination of CODIS eligibility and
d) all communications pertaining to the case.
3. The data record must be prepared in such a manner that another qualified analyst would be able to evaluate and interpret the data and ascertain that sufficient and relevant testing was correctly performed.

B. Review DNA Analytical Results/Conditions of Analysis

1. A quantitation system must be used by the non-CODIS/contract laboratory such that human DNA is quantified for unknowns and that the appropriate amounts of target DNA are used for amplification.

2. Slope, R² values, and IPC values must be reviewed to ensure quality of the analysis.

3. Reanalysis of Raw Data and Data Review
   a) Select the appropriate analysis method for the non-CODIS/contract lab data being reviewed and reanalyze the raw data.
      i. If provided to the laboratory, the project must contain all samples extracted in the same extraction session and all samples amplified at the same time regardless of if the data is being reviewed as a batch or on a case-by-case basis.
      ii. An injection list or electropherograms from the reanalysis project are included in the retained data record as proof of reanalysis of raw data.
   b) Examine internal lane size standards, ladders, positive controls, negative controls, and any associated reagent blanks using the guidelines set forth in the Guidelines for Technical Review chapter.
   c) Examine all relevant known and questioned stain electropherograms to verify they are supported by the raw and/or analyzed data. Compare to allele call table or chart, as applicable.
   d) Review data for potential contamination issues.
      i. The profile comparison tool in the DNA analysis software must be used to assist the analyst in this assessment, and the analyst must visually examine any issues.
      ii. All samples extracted on the same plate and all samples amplified on the same plate must be included in the same project to the extent that these samples are provided to the laboratory. This may necessitate creation of a project and/or run folder containing samples from multiple runs that is used specifically for the purpose of running the profile comparison tool.
      iii. All "OL" designations must be removed from the profiles prior to running the tool in order for it to function effectively.
   e) At a minimum, review non-CODIS/contract laboratory report to verify that each item (or its probative fraction) was addressed, the results/conclusions are supported by the data for profiles being uploaded into CODIS and that profiles considered for CODIS upload have been reported as suitable for comparison by the non-CODIS/contract laboratory.
   f) Ensure completeness of the DNA results is as expected in light of the screening results and sample quantitation information.
      i. The profile that was obtained is expected based on the amount of input DNA concentration targeted during amplification.
ii. If a partial profile was obtained, it could be explained by the presence of low template DNA.

iii. Assess the need for sample concentration and cleanup and contact the vendor lab to request if necessary.

iv. If possible, evaluate whether additional analysis of samples is needed.

g) At a minimum, include a paper or electronic copy of an electropherogram for all samples that will be uploaded to CODIS. These electropherograms may be from the reanalysis project or the non-CODIS/contract laboratory’s provided data record.

h) Electronically archive raw data.

4. CODIS Entry

a) The profiles will be reviewed using the guidelines set forth in the Guidelines for Technical Review chapter and the CODIS chapter for case and profile evaluation.

b) A CODIS Entry Worksheet (LAB-DNA-15, LAB-DNA-21, or LAB-DNA-23) may be filled out for each CODIS entry. If the CODIS Entry Worksheet is not used, the LDIS Specimen Details Report for the sample must be included in the data record.

c) Each CODIS Entry Worksheet will be reviewed by a second reviewer prior to entry into the CODIS database. If the LDIS Specimen Details Report is used, the profile will not be uploaded or searched until a review by a second reviewer is complete and documented in the data record.

d) Verification of CODIS eligibility must be performed by a current CODIS user.

5. Identification of Potential Technical Inconsistencies or Administrative Issues

a) If the reviewer has identified a potential issue (typographical errors, contamination, missing data, etc...) that may require correction and/or remediation, it will be documented in the outsource data record.

b) The reviewer and the original non-CODIS/contract laboratory case analyst or non-CODIS/contract laboratory representative may discuss the situation so that final resolution may be achieved.

c) If the issue is deemed as a significant technical problem the issue will be discussed with the Technical Leader and/or Section Supervisor.

d) Any profile generated from data which, upon reanalysis, is not reproducible in comparison to the non-CODIS/contract laboratory’s electropherograms will not be uploaded into CODIS.

6. CODIS Reporting

a) The requesting client must be notified of any CODIS entries

   i. The CODIS Upload request in LIMS may be used to generate and distribute a report to the requesting client (laboratory and/or law enforcement agency) informing them of CODIS entry.

   ii. Alternatively, notification may be accomplished by written communication such as email. Documentation of the communication must be retained by the laboratory.

   iii. If no CODIS profiles are entered, requesting client notification is optional.
b) The requesting client must be notified of deletion of a profile from CODIS
   
   i. Deletion of a profile is at the discretion of the CODIS Administrator.
   
   ii. Notification of deletion can be accomplished by written communication such as email. Documentation of the communication must be retained by the laboratory.

3 Data Records Retained by DPS

A. Data records retained by DPS from non-CODIS laboratories or contract laboratories that have processed outsource cases will be identified using the non-CODIS/contract laboratory case number or another appropriately related number, such as the non-CODIS/contract laboratory case number with an added prefix.

   If the case file is assigned a LIMS case number then both the non-CODIS/contract laboratory case number and the original submitting agency case number should be related to the record.

B. Data records can be retained as paper documents or as electronic documents and will include the following:

1. The Outsource Data Technical Review Checklist (LAB-DNA-65) will be used as a guide during review of data. This form is not required to be retained in the outsource data record unless the reviewer places handwritten or electronic information on it.

2. CODIS Entry Worksheet(s) (LAB-DNA-15, LAB-DNA-21, LAB-DNA-23) with appropriate signatures or the LDIS Specimen Details report if CODIS profiles are selected for upload.

3. Proof of second review of raw data such as an injection list or electropherograms from the reanalysis project.

4. Electropherograms of any samples to be uploaded into CODIS (may be from the reanalysis project or the non-CODIS/contract laboratory’s provided data record.

5. Table of allele calls from all samples being technically reviewed, if provided by the non-CODIS/contract laboratory.

6. Non-CODIS/contract laboratory reports associated with relevant samples.

7. Submitting agency reports (if provided).

8. Any relevant data record documentation as outlined in section 2.5.A (Data Record Documentation).
DNA-03-05 CAPITAL OFFENSE CASE PROCESSING GUIDELINES

1 Scope

Article 38.43, subsections (i)-(m) of the Texas Code of Criminal Procedure relates to DNA testing of biological evidence in capital offenses in which the death penalty is being sought. This law went into effect on September 1, 2013 and states that the court shall order the state and the defendant to meet and confer about which biological materials collected as part of the investigation qualify as biological evidence. If the state and defendant agree on which biological materials constitute biological evidence, the biological evidence shall be tested. If testing is not conducted on an item, the defendant may seek a writ of mandamus at any time.

A. It is in the Texas DPS Crime Laboratory's best interest to be involved in this process as early as possible to insure that our resources are being utilized to their fullest extent and as efficiently as possible. Depending upon the situation, the Crime Laboratory may receive court orders prior to signature by the judge presiding over the case in question.

B. Forensic Biology analysis may proceed without a signed court order as this preliminary information may be valuable to determine the presence of biological material on an item.

C. The Texas Department of Public Safety requires a signed court order prior to proceeding with DNA analysis in Capital Offense cases where the death penalty is being sought unless the submission already follows the current DPS case acceptance policy for homicide cases (i.e. 10 items for Forensic Biology analysis with 5 items proceeding for DNA analysis).

2 Practices

A. Receiving a court order signed by the judge presiding over the case:
   1. The analyst who received the court order will mark the case as SB1292 within LIMS.
   2. The analyst will meet with their section supervisor and/or technical leader to determine the best way to approach the analysis.

B. Receiving a court order prior to signature by the judge presiding over the case:
   1. Schedule a meeting with the prosecution and defense teams, and possibly the judge if they are willing, to examine crime scene photos and determine what questions need to be answered via biological testing.
   2. Inform the prosecution, defense, and judge what items have already been tested and the results of that testing.
   3. Request elimination samples as appropriate. From this meeting, create a prioritized list of what items have been agreed upon to be tested, including stains to be tested from each item.
   4. Notify the prosecution, defense, and judge which items will most likely be consumed during testing so that a decision can be made as to what laboratory (TXDPS or another accredited laboratory) will process the evidence for DNA analysis.
   5. Based upon the number of items requested for testing, develop a timeline for when the testing will be completed. The timeline starts when the evidence has been submitted to the laboratory. Advise the attorneys and court of the timeline with any
appropriate qualifiers (i.e., this is a time estimate that is subject to change if unusual difficulties are experienced with DNA testing of samples).

6. The TXDPS laboratory processing the case may choose to test all of the items at once or test and report the items in sets. Testing procedures will follow the testing protocols outlined in the TXDPS DNA SOP and the Crime Laboratory Service manual.
   a) This includes evaluating samples of “touch” DNA on a case by case basis. In general labs will not analyze items where there has been a minimal amount of contact such as swabs of steering wheels, shift knobs, door handles, switches, counters, key/locks, ammunition/cartridge cases, prints/smudges, etc as directed by the Crime Laboratory Service manual.
   b) This includes screening items of evidence as directed by our SOP.

7. If testing is requested that falls outside of our normal testing procedures, recommend a private laboratory that can conduct that testing. TXDPS is not responsible for the payment of testing performed by other laboratories.

8. It is important that all parties involved communicate their progress on a regular basis. Regular email updates to all parties involved will accomplish this.
DNA-03-06  GUIDELINES FOR WORK AND TESTIMONY PREPARATION

1  Scope

This document provides guidelines for how an analyst or technician can prepare to work supplemental requests for analysis and requests for expedited analysis, as well as how to prepare for court testimony either directly or on behalf of another analyst/technician. It is recognized that not every situation can be anticipated or addressed; however, general guidelines are set forth here to assist analysts and technicians with preparation prior to beginning a supplemental analysis, a request for expedited analysis or for testifying in court.

2  Related Chapters

Guidelines for Technical Review
Physical Evidence Examination

3  Practices

3.1 Preparation to Work Supplemental Requests and Expedited Requests for Analysis

A. Review the request to determine:
   1. Has new evidence been submitted for analysis that was not previously submitted to the laboratory;
   2. Is the request for reinterpretation or recalculation of statistics;
   3. Does the request include evidence that was previously analyzed by the laboratory using old technology;
   4. Is the request related to a request for post-conviction analysis;
      a) When possible, cases with requests for post-conviction analysis should be worked by personnel other than the analyst or analysts that originally worked the case.

B. Review of the case record should occur prior to the start of supplemental analysis or expedited requests for analysis in order to determine:
   1. What previous testing (if any) was performed in the case and what methods were used;
      a) At a minimum, this review should include previous testing done in biological evidence screening, male screening, and DNA analysis.
      b) It is not necessary to repeat previous testing on items of evidence if previous testing was performed using current technology (e.g. if testing for blood was previously performed using TMB/LMG/or PHT, it is not necessary to repeat this testing).
   2. If there have been updates to procedures since the previous testing that could be applied to the current testing request;
   3. If it is possible to make comparisons between the previous testing and the current testing or if it is necessary to retest previous evidence outside of the current request
      a) Determine if known DNA samples should be reamplified
b) Determine if evidentiary samples should be reamplified so that probabilistic genotyping can be used

c) Determine if reinterpretation is sufficient without reamplification of samples

d) Determine if the original reporting analyst is qualified to interpret the data or if another analyst will need to work the case.

4. If all necessary evidence has been submitted to the laboratory.

5. If all necessary information has been communicated to the laboratory so that evidence processing may proceed.

C. Based on the assessment of the request for supplemental analysis or expedited analysts request and the review of the case record, a plan for analysis should be developed.

1. It may be necessary to consult with another qualified analyst, the Technical Leader, and/or the Section Supervisor to determine the appropriate approach to the case.

D. When developing an approach to case analysis an analyst should consider:

1. How much evidence is left for testing;

2. What evidence will be tested;

3. Prioritization of tests (what order testing will occur and which tests precede others is especially important when there is limited sample);

4. How previous testing may affect the ability to perform new tests;

5. Laboratory capabilities to perform the testing needed;

6. The need to retest evidence unrelated to the current request;

7. Who will perform what types of tests;

8. The time needed to complete testing, reporting, and technical review;

9. Considerations for DNA analysis such as:

   a) Conditioning

   b) Reamplification of knowns or evidence samples

   c) Co-contributors

   d) Number of contributors

   e) Updating reporting for inconclusives

   f) Updating reporting for mixture interpretation

   g) Updating statistics

E. Once testing and reporting is complete, both the analyst and technical reviewer should compare the results from any previous testing to the results from current testing so that they are aware of any gaps in analysis or potentially conflicting results.

1. This comparison should include review of any previously entered CODIS profiles to determine if they need to be updated or removed based on additional results and information.
F. Regular communication with the customer is recommended to apprise them of progress of analysis and any additional laboratory needs that may be encountered as analysis proceeds.

G. Quality analysis is paramount and is prioritized over all other considerations.

3.2 Preparation for Court Testimony

A. It is not expected or advised that an analyst/technician testify from memory. Preparation for court testimony is a necessity and time must be provided to allow for this type of preparation.

B. The analyst/technician must obtain a copy of the case record pertaining to any biological evidence screening, male screening, or DNA analysis performed in the case including the following documentation as applicable:

1. Submission forms
2. Chain of custody
3. Case activities (phone logs, emails)
4. Information regarding case milestones (dates and names of reviewers)
5. Any Quality Incidents, Quality Action Plans, or Contamination Logs related to the analysis
6. Copy of SOP and any deviations that were in use during the range of analysis for the case
7. Notes and report from biological evidence screening including photos or diagrams even if the analysis was performed by another employee
8. Notes and report from male screening even if the analysis was performed by another employee
9. Notes and report from DNA including electropherograms, Popstats/statistical reports, and STRmix results even if the analysis was performed by another employee

C. The analyst/technician should review the case record as relates to the DNA discipline in its entirety. The focus of the review should include the following information as relates to their testimony:

1. What items were screened and how were these items chosen for screening;
2. What screening exams were used;
3. Any results for screening of items;
4. Condition of items that might impact the results (e.g. were the items moldy or packaged improperly);
5. What items were forwarded to DNA analysis and how were these items chosen;
6. What extraction methods, quantification methods, and amplification methods were used;
7. Were robotics used and if so at what steps in the process;
8. Were there indications of inhibition or contamination and if so, what was done to remedy the situation;
9. How were known samples processed in relation to evidence samples;
10. Any results for DNA analysis of items;
11. What statistics were calculated and what programs/databases were used in the calculations;
12. Was the quality of the DNA profile expected based on the quantification results (i.e. was inhibition present or low quantity of DNA that resulted in a partial or no profile);
13. What types of assumptions were made during analysis to include known contributors, conditioning, and co-contributors and do these assumptions make sense;
14. If any type of reinterpretation or reanalysis was done in the case and if so why it was done;
15. How the results compare between different analyses and explanations for any difference noted;
16. Any service notices issued by laboratory management that might have an impact on the case;
17. If a team approach is used, which staff performed which parts of the analysis;
18. Were there any deviations in place that affected the analysis of the case;
19. Have there been any changes in interpretation protocol since the case report was issued and would they affect the case outcome?

D. The analyst/technician should complete a review of the scientific basis for any methods used in the case relevant to their testimony.
   1. They should be able to explain how these methods work, how the methods were tested during validation prior to use by DPS, and how they were used in the analysis of the case. The explanation should be in a manner that could be understood by a jury of laypersons.
   2. Suggested sources of information that can be used in completion of this review include:
      a) Exams or notes taken during training on the method
      b) Textbooks and scientific papers related to the method
      c) Validation studies performed using the method
      d) Manufacturer manuals about the method
      e) Standard Operating Procedures and any deviations related to the method

E. The analyst/technician must complete a review of any Quality Incidents, Quality Action Plans, or Contamination logs associated with the case relevant to their testimony. They must be able to explain what happened, how it impacted the case, and actions taken to remedy the situation.
F. It is suggested that the analyst/technician prepare a court notebook to take with them to court. The court notebook is an aide that can be used to study prior to testimony or during testimony to assist in answering questions that may be asked by the court. The notebook should be reviewed and updated periodically if it is used.

1. Be aware that if a court notebook is prepared and taken to court, it may be examined by the officers of the court during trial.

2. The following items may be included in a court notebook:
   a) Current Statement of Qualifications and or Curriculum Vitae
   b) Disclosure Form and any related documents
   c) College transcripts
   d) Certificates of Attendance from Continuing Education/Training
   e) Copy of current License and any Certifications
   f) Copy of Authorizations to conduct work (memos, LAB-309, congratulatory emails, etcetera)
   g) Scientific papers relevant to methods that the analyst/technician will testify about (suggest having a copy of any developmental validation papers)
   h) Analyst/technician notes regarding any methods on which they will testify (e.g. the chemical formula for TMB and how the test works)
04 CASE SCREENING

DNA-04-01 ALTERNATE LIGHT SOURCE

1 Scope

The Polilight, Luma-Lite, Forensic PAL, Crime-Lite or equivalent lamps are specially designed for detection of forensic stains, fibers, and fingerprints. Commonly called alternate light sources (ALS), these lamps provide intense light of specific wavelengths.

Typically, dry untreated bloodstains do not fluoresce with ALS. However, ALS can enhance the contrast between the bloodstain and the substratum.

Most dried semen stains on cloth are detectable visually because their color, off-white or yellow, is different from that of the material on which the semen has been deposited. On many substrates, however, semen stains are not readily visible. Under ultraviolet (UV, <400 nm) and blue light (420-510 nm), semen stains fluoresce.

Most biological stains (such as saliva and sweat) also fluoresce under ultraviolet and blue light. An alternate light source can be used to indicate such stains.

2 Related Chapters

Physical Evidence Examination

3 Safety

A. Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling.

B. Appropriate personal protective equipment must be worn during evidence handling. Clothing may protect unbroken skin; broken skin must be covered.

C. Alternate light sources produce intense light, and some are capable of producing light in the ultraviolet range (below 400 nm). Do not look into the light wand. Wear UV glasses when using wavelengths below 400 nm.

4 Equipment and Materials

- Alternate light source (ALS) - e.g., Polilight, Luma-Lite, Forensic PAL, Crime-Lite or equivalent lamp
- Amber glasses
- Yellow glasses

5 Standards, Controls, and Calibration

A known semen stain (positive control) and an unstained non-fluorescent substrate (negative control) shall be examined using the ALS prior to use each day.

6 Procedure

1. Follow the manufacturer’s instructions for setting the wavelength, turning on, and warming up the lamp.
2. Put on the glasses or insert filter.
3. Direct the ALS at the evidence.
4. Photograph the fluorescence, if desired, using an orange KV-550 barrier filter.
5. Document observations.
7 Interpretation

Semen stains typically appear fluorescent under ALS.

8 Records

Observations and controls shall be recorded on the Biological Screening Worksheet (LAB-DNA-01) or LIMS equivalent and, as necessary, in data notes.

9 Literature References and Supporting Documentation

Polilight PL10A Instruction Manual (version 7, 10/92)

Forensic PAL Personal Alternate Light Operating Instructions (current version).


CrimeLite Information Sheet: QCL/80S-NB.(current version).

Mini-CrimeScope Operation and Maintenance Instructions (current version).
DNA-04-02 INFRARED CAMERA

1 Scope
The infrared camera is intended to be used as a screening tool. All typical screening processes for blood (i.e. visual examination, oblique lighting, etc.) should be used in conjunction with this tool. Caution should be used if it is suspected the deposited blood may have been diluted.

2 Related Chapters
Physical Evidence Examination

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Appropriate personal protective equipment must be worn during evidence handling. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Infrared Light Source
- Camera
- Computer with CrimeLite Cam software
- Green glasses or filter

5 Standards, Controls, and Calibration
A known bloodstain on dark material or substrate (positive control) and an unstained dark material or substrate (negative control) shall be examined using the infrared light source prior to use each day.

6 Procedure
1. Login to computer.
2. Double click CrimeLite Cam icon.
3. Once open, the camera should already be on and working. If the error message “camera not found” is displayed, please make sure all wires are attached to camera.
4. Change the settings on the left side of the screen to appropriate settings for device, light source, filter and camera filter.
5. The case details box can be used to add case information to the photos which will be stored in a text file.
6. Turn off overhead lights, put on green glasses or insert filter and turn on IR light. A difference in contrast on computer screen should be observed.
7. Move camera arm around to acquire area of interest.
8. Camera can be focused by clicking “auto” focus button in software or right and left buttons on remote.
9. When an area of interest is within the camera picture area, either click grab picture in the software or use remote to capture picture, if desired. The picture will show up on the bottom of the software screen.

Note: If image will not capture, make sure the “LIVE” button in the top left of the task bar is pressed.
10. When finished, turn off CrimeLite and exit out of camera program.
11. Photos are located in “CLC Grab Folder”.

7 Interpretation
Bloodstains typically appear dark in color in contrast to a lighter background.

8 Records
A. Biological Screening Worksheet or LIMS equivalent (LAB-DNA-01)
B. All probative photos should be uploaded to LIMS.
C. Observations and controls shall be recorded in data notes or on the uploaded photo.

9 Literature References and Supporting Documentation
DNA-04-03 PRESumptive Blood Tests – PHT, TMB, AND LMG

1 Scope
Catalytic tests for blood are based on the peroxidase-like activity exhibited by the heme group of hemoglobin. For example, colorless phenolphthalein is oxidized to phenolphthalein in the presence of heme and hydrogen peroxide. In a basic solution such as the test reagent, the phenolphthalein is pink. The test is exceedingly sensitive to minute traces of hemoglobin and its derivatives but will produce a false positive reaction in the presence of any of a number of oxidizing substances. Should a color reaction take place, the result only suggests the presence of blood; the test is therefore a presumptive test.

2 Related Chapters
Physical Evidence Examination
Leucomalachite Green (LMG) Solution
Phenolphthalein (PHT) Solution
Tetramethylbenzidine (TMB) Solution

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Deionized H₂O
- Cotton swab(s) and/or filter paper
- Test reagent
  - Phenolphthalein (PHT) solution
  - Tetramethylbenzidine (TMB) solution
  - Leucomalachite Green (LMG) solution
- 3-10% hydrogen peroxide – purchased

5 Standards, Controls, and Calibration
A. A positive and negative control must be tested prior to use each day.
B. Positive controls:
   1. An appropriate positive control is a small neat or diluted bloodstain prepared in-house.
   2. Appearance of a rapid color change is an appropriate positive control result.
C. Negative controls:
   1. Appropriate negative controls include a cotton swab or small fabric swatch tested in the same manner as the unknown.
   2. Absence of a rapid color change is an appropriate negative control result.
D. If appropriate control results are not obtained, contact the Technical Leader.
6 Procedure

1. Rub or scrape a moistened cotton swab or dry folded filter paper over the suspected bloodstain.
2. Apply the test reagent (PHT, TMB, or LMG solution).
3. Observe briefly to identify color change, which is indicative of some false positive reactions.
4. Apply 3-10% hydrogen peroxide.
5. Document observations.

7 Interpretation

A. A reaction prior to the addition of hydrogen peroxide may be indicative of a false positive reaction and should be documented as uninterpretable.

B. Appearance of a rapid color change is a presumptive positive result for blood.
   1. Phenolphthalin: colorless \(\rightarrow\) bright pink
   2. Tetramethylbenzidine: colorless \(\rightarrow\) blue-green
   3. Leucomalachite Green: colorless \(\rightarrow\) blue-green

C. Absence of rapid color change indicates the blood is absent or is below the detection threshold.

8 Records

Test results and controls shall be recorded on the Biological Screening Worksheet or LIMS equivalent and, as necessary, in data notes.

- Biological Screening Worksheet or LIMS equivalent (LAB-DNA-01)
- Phenolphthalin (PHT) Reagent Preparation Form (LAB-DNA-30)
- Leucomalachite Green (LMG) Reagent Preparation Form (LAB-DNA-31)
- Tetramethylbenzidine (TMB) Reagent Preparation Form (LAB-DNA-33)

9 Literature References and Supporting Documentation

DNA-04-04  PRESUMPTIVE BLOOD TEST – LUMINOL

1 Scope
Catalytic tests for blood are based on the peroxidase-like activity exhibited by the heme group of hemoglobin. In the case of luminol, the catalytic oxidation of the substrate compound produces light. Because the reagents are applied as a spray over a large area, the test is primarily used in the visualization of bloodstain patterns. The test is sensitive to at least 100 ppm and perhaps to 0.1 ppm of blood but will produce a false positive reaction in the presence of any of a number of oxidizing substances. Should a positive reaction take place, the result only suggests the presence of occult blood; the stain is therefore tested further with another presumptive test prior to reporting.

2 Related Chapters
Physical Evidence Examination

3 Safety
A. Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling.
B. Luminol is an irritant.
C. Sodium perborate and sodium carbonate are toxic and irritating.
D. Avoid breathing dust; do not get in eyes, on skin, or on clothing. Avoid breathing sprayed solution. Spray will deposit a light white film on surfaces.
E. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Sodium carbonate
- Luminol: 5-amino-2,3-dihydro-1,4-phthalazinedione free acid
- Sodium perborate
- Deionized H₂O
- Spray bottle(s)
- 3% H₂O₂ or 30% H₂O₂

5 Standards, Controls, and Calibration
A. A positive and negative control must be tested prior to use each day.
B. Positive Controls:
   1. An appropriate positive control is a small neat or diluted bloodstain prepared in-house.
   2. Appearance of a faint to strong luminescence is an appropriate positive control result.
   3. A penny should not be substituted for a known blood control.
C. Negative Controls:
   1. Appropriate negative controls include a cotton swab or small fabric swatch tested in the same manner as the unknown.
   2. Negative luminescence is an appropriate negative control result.

D. If appropriate control results are not obtained, contact the Technical Leader.

6 Procedure

6.1 Reagent Preparation

A. The prepared reagents are unstable and must be prepared fresh immediately before use.

B. The components can be measured for transport to the crime scene for preparation prior to use.

C. Reagents A and B can be stored separately at 2-8°C for up to 8 weeks. If stored, the label must include at a minimum, “Luminol, Reagent A” or “Luminol, Reagent B” as appropriate, initials, and the date prepared.

D. Method 1
   1. Reagent A - Dissolve 25 g sodium carbonate in 250 mL deionized H$_2$O then dissolve 0.5 g luminol.
   2. Reagent B - Dissolve 3.5 g sodium perborate in 250 mL deionized H$_2$O.
   3. Working solution – When ready to use, combine equal volumes of A and B. This mixture is stable for about one hour.

E. Method 2
   1. Reagent A - Dissolve 25 g sodium carbonate in 450 mL deionized H$_2$O and then dissolve 0.5 g luminol.
   2. Reagent B - 50 mL 3% H$_2$O$_2$ or 5 mL 30% H$_2$O$_2$ added to 45 mL deionized H$_2$O.
   3. Working Solution – When ready to use, combine A and B. This mixture is stable for about one hour.

6.2 Application of Test

1. Darken the examination room or crime scene area completely.
2. Spray working solution.
3. Document or photograph luminescent areas.
4. Scale can be established by taking advantage of the false positive reaction produced by copper.
5. Re-spray as necessary. The ability to further test the stain decreases with increased or repeated spraying. Excessive spraying will cause stains to run. Repeated spraying of non-porous surfaces is not suggested.
6. If photographing: Mount camera to a tripod and connect cable release.
7. Take an overall image of the scene.
8. **TURN THE AUTO-FOCUS OFF.** Manually focus prior to darkening the examination room or crime scene area. Set ISO to 500 or higher.
9. Open shutter to “B” or bulb setting for the duration of the luminescence. If the stain is sprayed throughout the exposure, do not expect to further test the stain.
   a) Suggested lenses
      i. f/1.2, 50 mm
      ii. f/1.4, 50 mm
   b) Exposure time
      i. duration of the luminescence
      ii. Luminescence usually lasts about 30 seconds, so the stain must be re-sprayed periodically to make longer exposures effective. Shutter can be left open during re-spraying. The first spraying typically produces the most luminescence.

10. Properly dispose of excess solutions in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.

7 Interpretation
A. Faint to strong luminescence shows oxidation of the luminol reagent and represents a positive presumptive result for blood, however, there must be further testing with another presumptive test prior to reporting.
B. Negative luminescence indicates blood is absent or below the detection threshold.

8 Records
A. Lot numbers of chemicals used to prepare the reagent, date of preparation, and preparer’s initials are recorded in the Luminol Reagent Preparation Form (LAB-DNA-32) and placed in appropriate reagent logs.
B. Test results and controls shall be recorded on the Biological Screening Worksheet (LAB-DNA-01) or LIMS equivalent and, as necessary, in data notes.

9 Literature References and Supporting Documentation
DNA-04-05  PRESUMPTIVE SEMEN TEST – ACID PHOSPHATASE (AP)

1 Scope

Acid phosphatase is found in relatively large quantities in semen and its detection is reason to suspect the presence of semen in a body fluid stain. In the following procedure, acid phosphatase is detected by a color-change reaction. Acid phosphatase liberates the phosphate from α-naphthyl phosphate, and the released naphthol combines with tetrazotized o-dianisidine to form a purple azo dye.

The AP test is semi-quantitative. A stronger reaction is more likely to indicate semen. However, because acid phosphatase occurs in other body fluids, most notably vaginal secretions, this is only a presumptive test. The presence of semen in the sample can subsequently be confirmed by identifying spermatozoa.

2 Related Chapters

Physical Evidence Examination
AP Test Reagent

3 Safety

A. Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling.

B. AP test reagent contains a dye that is a suspected carcinogen. The components of the AP test reagent are irritants. Avoid contact and inhalation. Spray only in a chemical fume hood.

C. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials

- Deionized H₂O
- Cotton swab(s) and/or filter paper
- Non-porous support and tacks (optional, mapping only)
- AP test reagent
- Chemical fume hood with paper hanger (mapping only)

5 Standards, Controls, and Calibration

A. A positive and negative control must be tested prior to use each day.

B. Positive Control:
   1. An appropriate positive control is a neat semen stain prepared in-house.
   2. An appropriate positive control result is AP 4+ with a dark purple color change.

C. Negative Control:
   1. Appropriate negative controls include a cotton swab or small fabric swatch.
   2. An appropriate negative control result is no color change within 60 seconds.

D. If appropriate control results are not obtained, contact the Technical Leader.
6 Procedure

6.1 AP Spot Test Procedure
1. Take a small cutting, blotting, or swabbing of the unknown stain.
2. Apply the AP test reagent.
3. Observe the cutting, blotting, or swabbing for up to 60 seconds for a color change.

6.2 AP Map Procedure
1. Spread the garment, sheet, shirt, panties, towel or other item flat.
2. Moisten a sheet or swatch of filter paper with deionized H$_2$O. Remove excess water by blotting the moistened paper onto a dry sheet of filter paper.
3. Lay the moist filter paper over the item or area of the item to be tested. Press the paper firmly against the item at all points of the item's surface. Allow at least 30 seconds to approximately 1 minute for any possible acid phosphatase to transfer to the paper.
4. Mark the position of the paper on the item.
5. Remove the sheet of filter paper.
6. Apply the AP reagent from the spot test procedure to the filter paper.
7. Observe the treated filter paper for up to 60 seconds for a color change.
9. Discard used filter paper in plastic bag and place in regular trash.

7 Interpretation
A. The appearance of a purple color within 60 seconds indicates the presence of acid phosphatase and is a positive presumptive test for semen.
B. The reaction is graded as 4+, 3+, 2+, 1+, or negative based on the following:
   - 4+ = color change at 1-15 seconds
   - 3+ = color change at 16-30 seconds
   - 2+ = color change at 31-45 seconds
   - 1+ = color change at 46-60 seconds
   - Negative = appearance of no color within 60 seconds.
C. A note regarding the intensity of the color change may be added as necessary.
D. The AP map procedure reflects the location, size and shape of the acid-phosphatase-containing stains.
E. A negative result indicates acid phosphatase is absent or below the detection threshold.

8 Records
Test results and controls shall be recorded on the Biological Screening Worksheet (LAB-DNA-01) or LIMS equivalent and, as necessary, in data notes.
9 Literature References and Supporting Documentation

Gaensslen RE. Sourcebook in Forensic Serology, Immunology, and Biochemistry. US Department of Justice, National Institute of Justice. 1983. Sections 10.3.2 and 10.3.3.


DNA-04-06 PRESUMPTIVE SPECIES ORIGIN DETERMINATION – HEMATRACE

1 Scope
This test is designed to be used as a presumptive test for human (primate) origin. This rapid immunochromatographic test offers extremely high sensitivity and specificity and is capable of detecting trace levels of human hemoglobin. Due to the extreme sensitivity of this test, trace levels of hemoglobin might be detected occasionally in body fluid samples other than blood (e.g. urine, semen, stool, saliva, vaginal fluid, perspiration). The detection limit has been shown to be 0.05 µg/mL in 10 minutes. All primate bloods produce positive results. Positive results may be obtained from whole blood from the domestic ferret.

2 Related Chapters
Physical Evidence Examination

3 Safety
A. Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling.

B. Follow instructions for test execution.

C. Appropriate personal protective equipment must be worn during testing. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- OneStep ABAcard HemaTrace test kit
- Extraction tubes containing extraction buffer (included in kit)
- Timer

5 Standards, Controls, and Calibration
A. The lot of ABAcard test devices must be quality control tested using a known blood sample and reagent blank (extraction buffer) prior to first use of the lot.

B. Each individual test card contains a control line “C”. If the control line “C” does not appear, that test is invalid.

6 Procedure
1. Allow the sample(s) and control(s) to warm to room temperature if they have been refrigerated.

2. Add bloodstained specimen to a volume of buffer. The buffer volume may range from 300 µL up to the entire buffer volume in the extraction tube included in the kit depending on stain quantity and quality.

3. Soak the specimen for at least 1-5 minutes.

4. Aged bloodstains may be allowed to soak for 30 minutes in the buffer in the extraction tube or soaked in 2-3 drops of 5% NH₄OH for 2-5 minutes to extract the hemoglobin.
   a) Allow the NH₄OH to evaporate and add 2-3 drops of the buffer.
   b) The pH of the sample must be between 1 and 9 (do not adjust the pH using sodium hydroxide gel).
5. Add 4-7 drops or 150-200 µL sample extract to the sample well “S” of the test device.

6. Read test result at 10 minutes. Positive results may be seen as early as 2 minutes. Negative results may not be called without waiting the full 10 minutes.

7 Interpretation

A. Two pink lines, one each in the test area “T” and in the control area “C” indicate a positive test and indicates that the human hemoglobin level is at or above 0.05 µg/mL.

B. If there is only one pink line in the control area “C”, the test result is negative and may indicate that insufficient human hemoglobin is present or the presence of “high dose hook effect”.
   1. The presence of “high dose hook effect” may give false negative results due to the presence of high concentration of human hemoglobin in the sample: i.e. undiluted fluid.
   2. In such cases the sample may be retested using a 1:100 dilution.

C. If there is no pink line visible in the control area “C”, that test is uninterpretable.
   1. If sufficient sample remains, the test may be repeated with a subsequent card(s).
   2. If the “C” line appears for the subsequent card(s), then the test may be interpreted and the results reported accordingly.

8 Records

A. The lot of ABAcard test devices and the quality control test results shall be recorded on the HemaTrace Reagent Quality Control Form (LAB-DNA-09).

B. Test results along with lot number and expiration of cards shall be recorded on the Biological Screening Worksheet (LAB-DNA-01) or LIMS equivalent and, as necessary, in data notes.

C. A photocopy or photograph of the test card device at the final reading may be prepared.

9 Literature References and Supporting Documentation

OneStep ABAcard HemaTrace for the forensic identification of human blood product insert.
DNA-04-07 BODY FLUID STAIN EXTRACTION

1 Scope
Possible body fluid stains are removed from the substrate and extracted for testing. The supernatant is used for presumptive testing. It is also used to collect cellular debris from a stain for a spermatozoa examination. Extraction of DNA is described in different protocols and should not be confused with a body fluid stain extraction.

2 Related Chapters
Physical Evidence Examination
Spermatozoa Examination
P30 Identification

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during testing. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Microcentrifuge tube
- Spin basket
- Microcentrifuge
- Vortex – optional
- Sonicator – optional
- dH2O

5 Standards, Controls, and Calibration
None – see related procedures.

6 Procedure
1. In a microcentrifuge tube, soak a portion of the dried body fluid, stain, or swab in an appropriate volume of extraction solution for at least 10 minutes to overnight.
   a) The extraction solution may be dH2O or an extraction buffer as supplied in p30 test kit.
   b) The amount of extraction solution is dependent on the number of analyses to be conducted and the apparent concentration of the stain.
   c) The p30 assay requires 200 µL of extract supernatant, thus extract volume may be greater than or equal to 200 µL.
   d) Substrate may be agitation by vortexing, sonicating, or twirling with a sterile toothpick to increase dissolution of the stain.
   e) Degraded stains or stains fixed to fabrics can become increasingly insoluble and may require longer extraction times, e.g., 24 hours.
   f) Long extractions should be performed at 2-8 ºC.
2. Remove the substrate from the extraction solution. Return as much solution as possible from the substrate to the extract.
3. Collect the cellular debris by centrifugation at up to 15,000 x g for 2 to 5 minutes.
4. Store extract at 2-8°C or <0°C for long term storage.

7 Interpretation
None

8 Records
None

9 Literature References and Supporting Documentation
None
DNA-04-08 SPERMATOZOA EXAMINATION

1 Scope
Spermatozoa detected in a stain or on a swab confirm the presence of semen. For spermatozoa examination, material from the stain or swab is fixed to a microscope slide, stained (usually), and examined under the microscope for cells with spermatozoa characteristics. This procedure uses nuclear fast red (also known as Kernechtrot) and picroindigocarmine. This stain is also called “Christmas tree” because it stains the spermatozoa red and green.

2 Related Documents
Physical Evidence Examination
Body Fluid Stain Extraction
Nuclear Fast Red (NFR) Solution
Picroindigocarmine (PIC) Solution

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Microscope slide
- Incubator oven, hot plate, or other heating device
- Microscope
- Known spermatozoa slides – optional, prepared in-house
- Nuclear fast red (NFR)
- Picroindigocarmine (PIC)
- Denatured ethanol (non-organic denaturants), absolute ethanol, or methanol
- Sterile Water

5 Standards, Controls, and Calibration
The analyst may refer to known spermatozoa slides for comparison.

6 Procedure
A. Use a smear provided in the sexual assault evidence kit or prepare a smear from a stain or swab using one of the following:
   1. Put a drop of sterile water on the slide and smear the sample on the slide to transfer cellular material; or
   2. Perform Body Fluid Stain Extraction procedure (DNA SOP). Collect the cellular debris from the extract by centrifugation and pipet at least 1 to 10 μL of the cellular debris from the body fluid extract onto a glass slide.

B. A thorough search of a stained slide at a minimum of 400X is required before negative results for spermatozoa can be reported.
C. For staining, follow the procedure below.
   1. Fix smear by heating the slide at approximately 60°C or dry then pass through a flame.
   2. Cover the sample area on the slide with NFR for approximately 15 minutes. The slide may be placed in a moisture chamber.
   3. Wash with distilled water by gentle flooding.
   4. Cover the sample area with PIC for up to 15 seconds.
   5. Wash and fix by gently flooding the slide with denatured ethanol, absolute ethanol or methanol.
   6. Allow to dry.

D. Confirm the presence or absence of spermatozoa at a minimum of 400X.

E. Xylene or xylene substitute may be added to slide during microscopic examination to facilitate sperm searches.

7 Interpretation

A. Record the number of spermatozoa per slide or the average number of spermatozoa per field, the magnification used, and indicate whether stained or unstained. Alternatively, score the number of spermatozoa as the following:
   - 0 = none
   - 1+ = few in some fields, hard to find
   - 2+ = some in some fields, easy to find
   - 3+ = many or some in most fields
   - 4+ = many in every field

B. Human spermatozoa are flagellated with a total length of about 50 µm. The spermatozoa cell head generally is oval, flattened at the anterior end, with dimensions about 4.6 µm × 2.6 µm × 1.5 µm. The head shape varies from species to species and may be diagnostic.

C. Typical appearance of cellular components after staining
   1. Spermatozoa
      - acrosome (anterior head) – pink
      - posterior head – red
      - midpiece – blue-green
      - tail – yellowish green
   2. Epithelial cells
      - nucleus - pink to purple
      - cytoplasm – light green to blue green

8 Records

A. Test results shall be recorded on the Biological Screening Worksheet (LAB-DNA-01) or LIMS equivalent and, as necessary, in data notes.

B. A photograph of the spermatozoa may be prepared.
9 Literature References and Supporting Documentation


**DNA-04-09 P30 IDENTIFICATION**

1 **Scope**

The cells that line the ducts of the prostate make a protein known as p30 or prostate-specific antigen (PSA). The protein is secreted into seminal fluid to a concentration of approximately 0.24-5.5 mg/mL. p30 has been detected in non-prostatic sources such as normal and abnormal breast tissue, various breast fluids (milk, nipple aspirate, cyst fluid), amniotic fluid, and female serum.

p30 is considered to be a presumptive test for semen. The presence of p30 indicates, but does not confirm the presence of semen. Semen can only be confirmed by the presence of spermatozoa.

The ABAcard p30 test is a qualitative detection method specifically designed for forensic presumptive identification of semen. Sample is added to a sample well where any detectable p30 present in the sample will bind with mobile p30 antibody. The resultant mobile antigen-antibody complex migrates through an absorbent strip to an area where immobile p30 antibody is bound. The mobile antigen-antibody complex binds to the immobile antibody creating an antibody-antigen-antibody sandwich. When the p30 concentration in the sample exceeds 4 ng/mL, pink dye particles become visible in the area of immobilized antibody. The resultant pink band indicates a positive result.

2 **Related Chapters**

Physical Evidence Examination
Body Fluid Stain Extraction

3 **Safety**

Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Appropriate personal protective equipment must be worn during use. Clothing may protect unbroken skin; broken skin must be covered.

4 **Equipment and Materials**

- OneStep ABAcard p30 test strips and extraction buffer
- Deionized H₂O
- Timer

5 **Standards, Controls, and Calibration**

A. The lot of ABAcard test devices must be quality control tested using a known semen sample and reagent blank (deionized H₂O or extraction buffer) prior to the first use of the lot.

B. The examiner may choose to also run a substrate control extracted in the same manner as the suspected semen stain.

C. Each individual test card contains a control line “C”. If the control line “C” does not appear, that test is invalid.
6 Procedure

A. Allow the sample(s) and control(s) to warm to room temperature if they have been refrigerated.

B. For each sample and control:
   1. Perform DNA-04-07 Body Fluid Stain Extraction procedure.
   2. Unwrap an ABAcard device and dropper.
   3. Add 200 µL (6-7 drops with provided dropper) of sample to the sample well “S” of the device.
   4. Positive results may be called at any time within 10 minutes. Negative results may not be called without waiting a full 10 minutes.

C. Samples that produce a negative result but that show 4+ acid phosphatase activity must be diluted 1:100 and re-tested.

7 Interpretation

A. The appearance of a pink line at the “C” (control) area is expected for all tests and must be present.
   1. Absence of the “C” line is an uninterpretable result for that test.
   2. If sufficient sample remains, the test may be repeated with a subsequent card(s). If the “C” line appears for the subsequent card(s), then the test may be interpreted and the results reported accordingly.

B. The appearance of a pink line at the “T” (test) area is a positive result and indicates, but does not confirm the presence of semen. The p30 concentration in the applied solution is at least 4 ng/mL, equivalent to approximately a 1:1,000,000 dilution of semen.

C. The absence of a pink line at the “T” area after 10 minutes is a negative result and indicates that semen is absent, below the detection threshold, or above the high dose threshold.

8 Records

A. The lot of ABAcard test devices and the quality control test results shall be recorded on the ABAcard Reagent Quality Control Form (LAB-DNA-10).

B. Evidence and test observations along with card lot number and expiration date shall be recorded on the Biological Screening Worksheet (LAB-DNA-01) or LIMS equivalent and, as necessary, in data notes.

C. A photocopy or photograph of the test card device at the final reading may be prepared.

9 Literature References and Supporting Documentation


DNA-04-10 MALE SCREENING PROCESS – PLEXOR HY

1 Scope

Male screening is an alternative screening method to traditional serological testing of sexual assault samples. Swabs are screened for the presence of male DNA by performing a quick lysis and subjecting them to a quantification system that can detect the presence of amplifiable human (and higher primate) and male-specific DNA. This process is not the same as using male quantification values determined during traditional DNA extraction to make a decision on whether or not to proceed with sample amplification. The process described within this document is only used to determine whether or not to proceed with traditional DNA extraction of samples.

The validation of this process mainly applies to cotton swabs. Clothing, bedding, etc. will typically be screened using traditional serological techniques.

Due to the screening method relying on the determination if male DNA is present, sexual assault cases that involve male victims and sexual assault cases that involve female suspects are not recommended for processing using this method. In general, traditional serological testing is recommended for these types of cases.

2 Related Chapters/Documents

Evidence Processing (CLS Manual)
Evidence Handling (DNA SOP)
Physical Evidence Examination (DNA SOP)
Physical Evidence Collection (DNA SOP)
Report Writing Guidelines (DNA SOP)
DTT 0.39M/1M (DNA SOP)
TE-4 Buffer (DNA SOP)

3 Safety

A. Use universal precautions when handling evidence. Use a particle mask and/or safety glasses when appropriate. Use appropriate safety measures when handling sharp objects. Appropriate personal protective equipment must be worn during testing. Clothing may protect unbroken skin; broken skin must be covered.

B. Body fluids and extracts (including male screening lysate) may contain infective agents. Beyond mild irritation of the skin or eyes, contact with the Plexor HY reagents does not cause acute health effects and are not known to cause any significant chronic health effects after prolonged exposure. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during testing.

4 Equipment and Materials

- Tweezers, scalpel, scissors, probe and other implements as necessary
- Plastic bags, paper envelopes, or appropriate evidence containers
- Tecan Freedom EVO 150 Platform Combination System (optional)
- Promega reagent blocks
- Quantifiler reagent block
• 1000 μL disposable tips with filter (DiTis)
• 50 μL disposable tips with filter (DiTis)
• 200 μL disposable tips with filter (DiTis)
• 100mL disposable trough
• 5mL graduated transport tube
• AB Prism 7500 using HID RealTime PCR Software v1.2 or newer
• *96 well optical reaction plates
• 96 well reaction plates
• *Optical adhesive covers/ applicator
• Pipettors and pipette tips
• Vortex
• Microcentrifuge tubes
• Microcentrifuge
• Plate centrifuge or salad spinner
• Water bath/dry incubator (70°C +/- 1°C)
• SwabSolution
• 1M Dithiothreitol
• Plexor HY DNA Quantification Kit
• Promega 5x AmpSolution
• TE-4 buffer
• Precision plate holder 7500
• 96-well base

*Only approved vendors for AB 7500, Promega and Tecan consumables may be used.

5 Standards, Controls, and Calibration

A. At least one reagent blank consisting of swab solution and DTT will be placed into a microcentrifuge tube in the appropriate location on the carrier for each extraction batch.

B. One microcentrifuge tube consisting of TE-4 buffer will be placed in the appropriate location on the reagent block to be used in the preparation of the standard dilution series and as the non-template controls.

C. The quantification of reagent blanks is not optional for this method (either manual or robotic set up).

D. Human DNA Standard in a five-fold dilution series with seven concentration points ranging from 50 ng/μl to 0.0032 ng/µl must be applied in duplicate. These dilution standards will be used to generate a standard curve in order to calculate the concentration of human DNA applied to each sample well.

E. Each new lot of reagents used for male screening, including SwabSolution, 5x Amp Solution and the Plexor HY Quantification kit must be subjected to an internal quality control test.
6 Procedures

A. Sample Preparation

1. Sexual assault kits will be itemized in the LIM system.
2. Swab sticks may be labeled with hash marks (I, II, III, etc).
3. Cut swab tips for all swabs in the kit including body swabs.
   a) Fingernail scraping swabs/fingernail swabs will not typically go through the male screening process.
   b) Hand swabs are different from fingernail scraping swabs/fingernail swabs and may be sent through the male screening process.
4. Place the sample(s) into a labeled microcentrifuge tube. Return the remaining swabs to the kit.
   a) Any panties, clothing etc. in the kit may be processed after the evaluation of the male screening results of the swabs. Other items may be processed through male screening depending on case scenario.
   b) Evidence samples may be in limited supply. Always use clean scalpel, scissors, or forceps with each sample.
      i. Blood samples: approximately 2 mm²
      ii. Saliva samples including but not limited to oral contact swab: a small portion of the swab tip (up to 4 swab tips may be tested simultaneously)
      iii. Orifice samples including but not limited to items that may contain trace amounts of DNA or other body fluids that may contain sufficient quantities of recoverable DNA: a small portion of the swab tip (up to 4 swab tips may be tested simultaneously)
   c) Add the sample name to the current appropriate worksheet in the position in which the sample is placed in the tube rack.
   d) Suspect reference samples may be prep for DNA extraction at this time. Reference samples from victim(s) and elimination(s) will only be processed if evidentiary items are sent forward for DNA analysis. Suspect reference samples will be sent forward for DNA analysis even if evidentiary items are not being sent forward.
   e) Collect and retain all samples that are reported as positive or inconclusive for male DNA.
   f) For cases in which there is a consensual partner and/or multiple assailants, more than one item, orifice swabs and/or body swabs will be taken forward for DNA analysis if available and eligible for processing.

B. DNA Processing – Lysis

1. Prepare Reagents
   a) Upon arrival or prior to first use, thaw the SwabSolution completely at 37°C and mix by gentle inversion.
   b) After thawing, store at 2-10°C.
2. For lysis solution added robotically:
   a) Open the tubes and load them onto the robotic deck appropriately. Depending on the validated method use one of the following lysis solution volumes:
      i. Prepare the SwabSolution/DTT master mix and place it in the appropriate spot on the robotic deck. The solution consists of 200 μL of SwabSolution plus 10 μL 1M DTT per sample. For a full plate you will require 24.5 mL SwabSolution and 1225 μL 1M DTT. The overfill volume is 8.5 mL of this master mix and is included in the 24.5 mL/1225 μL solution.
      ii. Prepare the SwabSolution/DTT master mix and place it in the appropriate spot on the robotic deck. The solution consists of 200 μL of SwabSolution plus 20 μL 1M DTT per sample. For a full plate you will require 24.5 mL SwabSolution and 2450 μL 1M DTT. The overfill volume is 8.5 mL of this master mix and is included in the 24.5 mL/2450 μL solution.
      iii. When adding master mix to the trough, pipette it down the side of the trough to avoid introducing bubbles into the solution.

   b) Set up the Tecan deck according to the appropriate script.
      i. Ensure adequate tips are present on the deck.
      ii. Fill the disposable reagent trough with the appropriate amounts of reagent and load onto robot to the left of the DiTi Waste in site 3 position.
      iii. Place the tubes with the sample cuttings into the tube carriers in the appropriate place on the deck. Tubes must be placed back to front starting at position one of the carrier.

   c) Run the script. Cap and vortex the samples. Proceed to Step 4.

3. For lysis solution added manually:
   a) Add 200 μL of SwabSolution and 10-20 μL 1M DTT, as validated, to each sample tube.
   b) Cap and vortex the samples.

4. Incubate samples at approximately 70°C for approximately 1 hour.

5. After digestion, vortex and spin tubes. The substrate is left in the tube and spun at approximately 10,000-15,000 x g for 5 minutes to force the substrate to the bottom of the tube.

C. Reagent Preparation

1. 5X AmpSolution
   Upon arrival or prior to first use, thaw the 5X AmpSolution completely either at 37°C or at ambient temperature and mix by vortexing. After thawing, may be stored at 2-10°C. If the reagent appears turbid, warm briefly at 37°C and vortex until clear before use.

2. Plexor HY Male Genomic DNA Standard
   Place the DNA standard at 2-10°C overnight before using it for the first time. Vortex prior to use. Store at 2-10°C. Multiple freeze-thaw cycles can increase variability in the standard curve.
3. **2X Master Mix and 20X Primer/IPC Mix**

   *Thaw at room temperature and vortex prior to use. Do not centrifuge after vortexing as this may cause the primers to be concentrated at the bottom of the tube.*

   **NOTE:** It is important that the optical 96 well reaction plate not come into contact with the counter or any other surface. It should always be placed into a base plate until loaded into the AB Prism 7500. This is to minimize the interference caused by dust or other debris adhering to the bottom of the wells and interfering with the optical reading of the wells.

   Thaw the 2X Master Mix and 20X Primer/IPC Mix before each set-up.

D. To set up the quantification plate robotically:

   1. **Prepare the robot.**
      
      a) *Ensure there is sufficient degassed DI water for the system.*
      
      b) *Run appropriate scripts to prime the system and to eliminate bubbles in the pressure lines.*
      
      c) *A system flush must be run on the day of use prior to casework.*
      
      d) *If the robot was subsequently shut off on the same day daily startup was run, perform the system flush with at least 40 mL flush volume.*
      
      e) *Select the appropriate script.*
      
      f) *Prepare the worktable following the layout provided by the software in the Extended View. Labware necessary includes two racks of 200 µL DiTis, four racks of 50 µL DiTis, and two 96 well PCR plates (one must be optical).*
      
      g) *Ensure reagents in the Plexor HY kit contain sufficient volume for the run. The full 80-sample run requires 1026 µL Master Mix (this will require combining master mix from two tubes), 112 µL Primer Mix, 416 µL 5X AmpSolution, 400 µL sterile or amplification grade water, and 400 µL TE^-4. If standards are being created there must be 70 µL of male standard. Vortex and place these reagents into the appropriate Promega reagent block locations.*

      i. *If running a partial (less than 80 samples) plate, the script will provide the volumes necessary for each reagent in order for the quantification reaction to perform properly. It may be necessary to combine two or more tubes in order to meet the volume requirements of the run. This can only be done with reagents from the same kit lot number.*

      ii. *The analyst may choose to make the master mix manually and place it on the robot deck prior to starting the script.*

      iii. *The analyst may choose to pause the script (either manually or by adding a pause to the script) in order to mix the master mix by hand and replace it on the robot deck prior to continuing the script.*
h) The Promega reagent blocks will each be placed in an empty reagent trough. Place the troughs and blocks to the right of the DiTi waste- one in the back (site 1) and one in the front (site 3). The blocks will be positioned with open caps to the right.

i) Using the AB Reagent block specific for the Quantifiler Kit, load the block with an empty 5 mL graduated transport tube into left-hand hole and place the block at the appropriate site on the deck.

j) DNA Standards -

i. The Tecan Freedom EVO is capable of creating the standard dilution series for the quantification kit. Automated standard dilution series creation is the default setting in the software but an option is available to allow the user to use a manually or prior created series.

ii. If standard dilutions are previously made then vortex and quick spin the standards and place them at the appropriate site on the deck, ensuring that the standards are in the wells on the left hand side of the plate. Dilution standards are good for up to 5 days.

iii. If the robot is to prepare the standard dilution series, place the Human DNA Standard (stock) and empty 96 well reaction plate on the deck at the appropriate site. The dilution series consists of seven points and is added in duplicate to column 11 and 12 of the quantification plate. Non-template controls are added to wells H11 and H12 by the robot.

k) Place an empty optical 96-well plate on the appropriate site on the deck.

l) Place the male screening lysate in the appropriate locations in the tube carriers.

m) Open all sample tubes and secure the caps (as appropriate).

n) Ensure the safety front panel is closed by pressing on each of the locks on the front of the robot.

2. Verify that all prepared reagents and labware are ready and in the appropriate locations. Ensure that all tubes are open and that all obstacles are removed from the deck.

3. Click the “Run” button. Respond to query boxes as necessary.

i. Define the number of samples and starting labware. The maximum number of samples that can be processed is 80. (This number excludes the standards and NTCs, and includes reagent blanks.)

ii. Answer “Y” if the robot will be preparing the standard dilution series. (Answer “N” if using previously made standards.)

iii. Verify that all labware was loaded on the worktable and all reagents have appropriate volumes.

4. Click “Run” to begin the liquid handling procedure.

5. Upon completion, exit the software (click Exit, unload drivers) first then shut down the robot by pressing the ON button (if needed).

6. Remove and properly store all remaining reagents and male screening lysate. Male screening lysate is considered work product.
7. Male screening lysate that has been stored frozen for no more than 1 month is suitable for use in the Male Screening Process. If using lysate that has been stored frozen, thaw completely at room temperature, vortex and spin the tube at approximately 10,000-15,000 x g for 5 minutes to force the substrate to the bottom of the tube prior to using the lysate.

8. Verify that liquid transfer steps were completed successfully by inspecting the 96-well reaction plate.

9. Seal the reaction plate with the optical adhesive cover using the applicator. Do not touch the portion of the adhesive cover that comes into contact with the reaction wells except with the applicator.

10. Centrifuge or spin the plate to remove any bubbles in the bottom of the wells.

E. To set-up the quantification plate manually

1. Prepare the DNA Standard Dilution Series

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Volume of DNA</th>
<th>Volume of TE-4 Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>50ng/µl</td>
<td>Use undiluted DNA</td>
<td>0µl</td>
</tr>
<tr>
<td>10ng/µl</td>
<td>10µl of undiluted DNA</td>
<td>40µl</td>
</tr>
<tr>
<td>2ng/µl</td>
<td>10µl of 10ng/µl dilution</td>
<td>40µl</td>
</tr>
<tr>
<td>0.4ng/µl</td>
<td>10µl of 2ng/µl dilution</td>
<td>40µl</td>
</tr>
<tr>
<td>0.08ng/µl</td>
<td>10µl of 0.4ng/µl dilution</td>
<td>40µl</td>
</tr>
<tr>
<td>0.016ng/µl</td>
<td>10µl of 0.08ng/µl dilution</td>
<td>40µl</td>
</tr>
<tr>
<td>0.0032ng/µl</td>
<td>10µl of 0.016ng/µl dilution</td>
<td>40µl</td>
</tr>
</tbody>
</table>

2. Prepare sufficient Master Mix by using the following amounts per reaction:
   a) 2X Master Mix            10µL
   b) Water, Amplification Grade 3µL
   c) 20X Primer/IPC Mix       1µL
   d) 5X Amplification Solution 4µL

   Note: Additional reactions may be included in your calculations in order to provide excess volume to make up for pipetting loss.

3. Dispense 18µL of the Master mix to each of the appropriate wells of an optical-grade PCR plate.

4. Add 2µL of the sample, standard, or TE-4 to the appropriate well. (At least one non-template control is required).

5. Properly store all remaining reagents and male screening lysate.

6. Seal the reaction plate with the optical adhesive cover using the applicator. Do not touch the portion of the adhesive cover that comes into contact with the reaction wells except with the applicator.

7. Centrifuge or spin the plate to remove any bubbles in the bottom of the wells.
F. 7500
   1. Load the plate onto the AB Prism 7500 and run appropriate experiment.
   2. After the run is complete, export appropriate data.
   3. Launch the Plexor Analysis Software. Import appropriate data and generate appropriate report for the case record.

7 Interpretation

7.1 Quantification Cycle
   A. The quantification cycle (C\textsubscript{q}) is when the fluorescent signal from the amplification product for each sample decreases beyond the value of the threshold setting (which defines the level of detectable fluorescence).
   B. The quantification cycle depends upon the starting template copy number and efficiency of the DNA amplification.
   C. The number of cycles required for a sample to reach the threshold allows the software to determine DNA quantity by comparison to the standard curve. Therefore the lower the C\textsubscript{q} value, the greater the amount of template DNA in the original sample.

7.2 Standard Curve
   The standard curve plots the C\textsubscript{q} of the standard dilutions versus the known quantity of the standards. This produces a regression line by calculating the best fit with the quantification standard data points. This information is used to determine the quantitation values of the casework samples.
   A. Slope
      1. The slope of this regression line indicates the PCR amplification efficiency for the assay.
      2. A slope of -3.3 indicates 100% amplification efficiency.
      3. Slope values typically fall between –3.2 to –4.0 for the autosomal target.
      4. Slope values typically fall between -3.0 and -3.6 for the Y target.
      5. Values outside of these ranges may be used with discretion.
   B. R\textsuperscript{2} value
      1. The R\textsuperscript{2} value indicates the fit between the standard curve regression line and the individual C\textsubscript{q} data points of the quantification standard reactions.
      2. An R\textsuperscript{2} value of 1 indicates a perfect fit between the regression line and the C\textsubscript{q} data points.
      3. The R\textsuperscript{2} value should be ≥ 0.98.
      4. If the R\textsuperscript{2} value is less than 0.98, further investigation should be undertaken to determine if the values entered for quantification standards in the during plate document setup are correct, the serial dilutions are correct, the loading of reactions for quantification standards occurred, or if there was a failure of reactions for the quantification standards.
      5. If, after confirming the values, one or more of the standards falls significantly outside of the standard curve, it may be omitted from the standard curve analysis.
7.3 Interpretation – Quant Results

A. Quantification results in the extraction reagent blank(s) must be evaluated.

1. If values for male quantification are observed in the inconclusive range (<0.001 ng/μL), the male screening batch can be reported with Technical Leader approval.

2. If a value for male quantification observed is >0.001 ng/μL for any reagent blank in the batch that cannot be attributed to non-specific amplification, the affected reagent blank(s) must be requantified to determine reproducibility. If reproducible at the >0.001 ng/μL the male screening batch will not be interpretable and will be reported as such. The evidentiary samples will be processed through conventional serology and reported.

3. If a check melts message was indicated, the raw data must be reviewed. If due to non-specific amplification, the batch may proceed with Technical Leader approval.

4. If an autosomal value is observed in the reagent blank in the absence of a male quantification, the male screening batch may proceed as if no values were observed in the reagent blank.

B. Quantification results in the non-template control wells need to be evaluated to determine if they are actual DNA quantification readings or an invalid result. Results in the NTC wells do not preclude the use of data for that male screening plate.

C. Sample Evaluation

1. A “N/A” quantification value was obtained the reported result is no male DNA was detected.

2. The sample has a male quant value ≥0.01 ng/μL or a male quant value ≥0.001 ng/μL with a low autosomal/male DNA ratio (less than approximately 200) the reported result is male DNA was detected and the sample may proceed through DNA analysis.

3. The sample has a male quant value <0.01 ng/μL, but ≥0.001 ng/μL, with a high autosomal/male DNA ratio (greater than approximately 200) the reported result is male DNA was detected, but the sample will not generally proceed to autosomal STR analysis.

4. Samples exhibiting a check melts curve status should be evaluated and may proceed through DNA analysis.
a) If a melt curve is present within the window that crosses the threshold (even if it is shifted) and the male quantification value is ≥0.01ng/μL or ≥0.001 ng/μL with a low autosomal/male DNA ratio (less than approximately 200): the sample will be reported as male DNA was detected. (see example above)

![Melt Curve Example](image1.png)

b) If a melt curve is present within the window (even if it is shifted, regardless of the presence of other curves outside the window) and the male quantification value is <0.01ng/μL, but ≥0.001ng/μL with a high autosomal/male DNA ratio (greater than approximately 200), the samples will be reported in the same manner as samples not exhibiting a check melts curve status. (see examples above)

![Melt Curve Example](image2.png)

c) If a melt curve is present within the window (regardless of the presence of other curves outside the window), the threshold may or may not be crossed, and the male quantification value is <0.001 ng/μL, the samples will be reported as inconclusive due to low male quantification value. (see examples above)

![Melt Curve Example](image3.png)

d) If non-specific amplification is indicated (curve is outside of the melt curve window), the sample will be reported as negative for male DNA regardless of any male quantification value given by the software. (see examples above)

5. Samples exhibiting a check IPC reading should be evaluated. Inhibition is noted if the IPC Cq of an unknown sample is delayed by more than two cycles. If this occurs, then the IPC amplification and melt curves should be reviewed. Any sample that appears to be displaying partial inhibition based on the melt curve
status may need to be processed further (with either presumptive testing or DNA analysis) if there are no positive samples in the case.

a) If the sample gives a reading $\geq 0.01 \text{ng/}$μL in the [Y] ng/μL column the result is positive.

b) If most of the melt curve is outside of the window, the threshold is not crossed, and the male quantification value is <.001 ng/μL, the sample will be reported as inconclusive due to possible inhibition and/or non-specific amplification. (see example above)

c) If check melts and check IPC are flagged and the melt curve is in the window (regardless of if it crosses the threshold or not), interpret the results as if there is no check melts indicator. (see example above)

d) If no reading is obtained in the [auto] and/or the [Y] ng/μL column or if the sample has a male quantitation value <0.01ng/μL, the sample may be displaying partial or complete inhibition.

6. The sample has a male quant value <0.001ng/μL the reported result is inconclusive.

7. Samples with any male quant value (including inconclusive) and no indication that non-specific amplification is the cause of this quant value are appropriate for Y-STR analysis regardless of the autosomal/male ratio, if a known sample from a male contributor is available for testing.

a) Laboratories may choose to establish a threshold through validation to assist with determining when samples will be considered for Y-STR analysis.
8 Identification of Semen during DNA Analysis

A. It is not necessary to complete microscopic examination for spermatozoa during differential extraction if cases are analyzed using the Male Screening Process described in this document unless a sample will be depleted during DNA extraction.

**Note:** If a sample will be depleted during DNA extraction, a sperm search must be performed during the differential extraction process.

B. If microscopic examination for spermatozoa is performed during differential extraction, the results of this examination will be reported as part of the DNA analysis report.

C. If no microscopic examination for spermatozoa is performed during screening or differential extraction, a statement must be included in the Investigative Leads section of the report informing the customer that no sperm search was performed.

D. The customer may request in writing on a case-by-case basis that a sperm search be performed. If such a request is received, the following steps must be taken:

1. Assess the quantification results obtained during the Male Screening Process with Plexor HY.
   
   a) **Select the remaining swabs that gave the highest male quantification value and use a portion of those swabs to search for spermatozoa as indicated in the Body Fluid Stain Extraction chapter and the Spermatozoa Examination chapter.**

   b) **P30 testing is not required for negative sperm search results.**

2. Report the results of the sperm search using a Biology request.

3. Results of this sperm search must be released to the customer in the form of a final report within 5 business days of receipt of the written request.

9 Records

A. Samples for lysis must be recorded on the Extraction/Lysis Worksheet (LAB-DNA-53) or equivalent.

B. Quantification results (including LAB-DNA-13) must be retained in the case file.

C. The standard curve evaluation must be documented either by printing the standard curve or documenting on the results summary table generated by the software.

D. Other forms

   - Laboratory Submission Form (LAB-201)
   - Biological Screening Worksheet (LAB-DNA-01) or LIMS equivalent

10 Literature References and Supporting Documentation


Plexor HY Technical Manual, current version

SWGDAM Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits in a Laboratory (most recent version)

Valid-Method-SYS-DNA-MaleScreening-2015-0522
Valid-Method-SYS-DNA-MaleScreening-Cutoff-2017-0404
05 DNA EXTRACTION AND PURIFICATION
DNA-05-01 DNA EXTRACTION WITH STAIN EXTRACTION BUFFER – ORGANIC

1 Scope
The procedure uses stain extraction buffer, along with other reagents to digest and extract DNA from a variety of forensic samples, such as, blood, saliva, hair, tissue, bone, and teeth. Extracts are purified using phenol-chloroform-isoamyl alcohol and followed by dialysis and concentration using membrane filtration devices.

2 Related Chapters
Concentration and Purification of DNA with Membrane Filtration Devices
DTT 0.39M/1M
Proteinase K Solution – 10 mg/mL
Stain Extraction Buffer

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Perform phenol-chloroform extractions in a chemical fume hood. Appropriate personal protective equipment must be worn during testing. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Scissors, scalpel, tweezers
- Pipettors and tips
- Microcentrifuge tubes or spin basket tube
- Microcentrifuge
- Vortex
- Water bath/dry bath incubator (56°C +/- 1°C)
- Sterile toothpicks
- Sterile tissue pulverizer
- Stain Extraction Buffer
- Proteinase K (10mg/mL)
- 0.39M Dithiothreitol
- Phenol:chloroform:isoamyl alcohol (v/v 25:24:1)

5 Standards, Controls, and Calibration
At least one reagent blank must be processed for each extraction batch as a negative control.

6 Procedure
Note: The following are recommended amounts for reference samples. Evidence samples may be in limited supply. The user should add as much evidentiary sample to the tube as necessary, retaining sufficient sample for additional analysis if possible. Examples of possible quantities to add to the tube are listed below. Always use clean scalpel, scissors, or forceps with each sample.
6.1 **Blood samples – stains or liquid**

1. Add 10 to 50 µL of whole blood or add a bloodstain approximately 3 mm² to 1 cm² to a labeled microcentrifuge tube.

2. Pipette 0.5 mL Stain Extraction Buffer into a microcentrifuge tube or spin basket tube containing the sample.

   **Note:** If 0.39M DTT is not added to Stain Extraction Buffer, then add 50 µL of 0.39M DTT to 450 µL of Stain Extraction Buffer.

   Alternatively, the reagents may be placed into microcentrifuge tubes prior to samples being added.

3. Add 10 µL Proteinase K solution. Mix gently.

4. Incubate at approximately 56°C for
   
   a) *at least 1 hour for known samples.*

   b) *a minimum of 6 hours for questioned samples (recommended). Digestion may be performed overnight, but more than 24 hours is not recommended.*

5. After digestion, briefly spin tubes.

6. Remove any substrate with clean forceps or with a sterile toothpick.
   
   a) *The substrate may be discarded, or if necessary, placed in a spin basket and spun at 10,000-15,000 x g for 5 minutes.*

   b) *Alternatively, the substrate may be left in the tube and spun at 10,000-15,000 x g for 1 minute to force the substrate to the bottom of the tube.*

7. Add 0.5 mL phenol-chloroform-isoamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.

8. Spin in centrifuge for 3 to 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.

9. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.

10. Proceed with cleaning and concentrating the DNA sample using the *Concentration and Purification of DNA with Membrane Filtration Devices* chapter.

6.2 **Saliva samples**

Including but not limited to, oral swabs, filter paper, stamps, envelope flaps, cigarette butts, oral contact swab.

1. Add approximately 1 cm² stain, 1 cm strip of paper covering end of cigarette butt, 1/3 of a swab up to one whole swab to a labeled microcentrifuge tube.

2. Pipette 0.5 mL Stain Extraction Buffer into a microcentrifuge tube or spin basket tube containing the sample.

   **Note:** If 0.39M DTT is not added to Stain Extraction Buffer, then add 50 µL of 0.39M DTT to 450 µL of Stain Extraction Buffer.

   Alternatively, the reagents may be placed into microcentrifuge tubes prior to samples being added.
3. Add 10 μL Proteinase K solution. Mix gently.

4. Incubate at approximately 56°C for
   a) at least 1 hour for known samples.
   b) a minimum of 6 hours for questioned samples (recommended). Digestion may be performed overnight, but more than 24 hours is not recommended.

5. After digestion, briefly spin tubes.

6. Remove any substrate with clean forceps or with a sterile toothpick.
   a) The substrate may be discarded, or if necessary, placed in a spin basket and spun at 10,000-15,000 x g for 5 minutes.
   b) Alternatively, the substrate may be left in the tube and spun at 10,000-15,000 x g for 1 minute to force the substrate to the bottom of the tube.

7. Add 0.5 mL phenol-chloroform-isoamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.

8. Spin in centrifuge for 3 to 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.

9. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.

10. Proceed with cleaning and concentrating the DNA sample using the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

6.3 Hair samples

1. Hairs should be cleaned in sterile dH₂O prior to extraction.

2. Add approximately 1 cm of root end of hair to one labeled microcentrifuge tube and, optionally, a separate tube for a 1 cm portion of the adjacent shaft of each hair may be used as a control.

3. Pipette 0.5 mL Stain Extraction Buffer into a microcentrifuge tube or spin basket tube containing the sample.

   Note: If 0.39M DTT is not added to Stain Extraction Buffer, then add 50 μL of 0.39M DTT to 450 μL of Stain Extraction Buffer.

   Alternatively, the reagents may be placed into microcentrifuge tubes prior to samples being added.

4. Add 10 μL Proteinase K solution and 20 μL 0.39M DTT. Mix gently.

5. Incubate at approximately 56°C for a minimum of 6 hours to allow hair samples to digest.

6. Perform a second digestion after the initial 6 hour digestion by adding 15 μL Proteinase K solution and 20 μL 0.39M DTT.

7. Incubate at approximately 56°C overnight to complete the second digest.

8. After digestion, briefly spin tubes.

9. Remove any substrate with clean forceps or with a sterile toothpick.
a) The substrate may be discarded, or if necessary, placed in a spin basket and spun at 10,000-15,000 x g for 5 minutes.

b) Alternatively, the substrate may be left in the tube and spun at 10,000-15,000 x g for 1 minute to force the substrate to the bottom of the tube.

10. Add 0.5 mL phenol-chloroform-isoamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.

11. Spin in centrifuge for 3 to 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.

12. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.

13. Proceed with cleaning and concentrating the DNA sample using the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

6.4 Tissue samples
Including but not limited to skin, muscle, and body organs.

1. Add approximately 3-5 mm² to a labeled microcentrifuge tube. It is helpful to mince the tissue prior to adding stain extraction buffer.

2. Pipette 0.5 mL Stain Extraction Buffer into a microcentrifuge tube or spin basket tube containing the sample.

Note: If 0.39M DTT is not added to Stain Extraction Buffer, then add 50 µL of 0.39M DTT to 450 µL of Stain Extraction Buffer.

Alternatively, the reagents may be placed into microcentrifuge tubes prior to samples being added.

3. Add 10 µL Proteinase K solution. Mix gently.

4. Incubate at approximately 56°C for
   a) at least 1 hour for known samples.
   b) a minimum of 6 hours for questioned samples (recommended). Digestion may be performed overnight, but more than 24 hours is not recommended.

5. After digestion, briefly spin tubes.

6. Remove any substrate with clean forceps or with a sterile toothpick.
   a) The substrate may be discarded, or if necessary, placed in a spin basket and spun at 10,000-15,000 x g for 5 minutes.
   b) Alternatively, the substrate may be left in the tube and spun at 10,000-15,000 x g for 1 minute to force the substrate to the bottom of the tube.

7. Add 0.5 mL phenol-chloroform-isoamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.

8. Spin in centrifuge for 3 to 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.
9. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.

10. Proceed with cleaning and concentrating the DNA sample using the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

6.5 Bone and Teeth samples

1. Prepare the sample
   a) One tooth or approximately 2 cm³ of bone (preferably flat bone in adults, e.g. pelvis, sternum, ribs).
   b) The exterior surface of each sample should be cleaned thoroughly of all debris. Bones may be sanded using a Dremel tool to remove debris. Teeth may be cleaned with sterile water and bleach unless there are surface fractures. In which case, only use sterile water to clean the tooth.
   c) Bone and teeth samples are prepared by crushing them into a fine powder. This can be achieved by using a hydraulic press with a stainless steel chamber that is cleaned with bleach between each sample, or a tissue pulverizer.

2. Place approximately 0.5 g sample into each microcentrifuge tube (this may take several tubes).

3. Pipette 0.5 mL Stain Extraction Buffer into a microcentrifuge tube or spin basket tube containing the sample.
   Note: If 0.39M DTT is not added to Stain Extraction Buffer, then add 50 µL of 0.39M DTT to 450 µL of Stain Extraction Buffer.
   a) Alternatively, the reagents may be placed into microcentrifuge tubes prior to samples being added.


5. Incubate at approximately 56°C for
   a) at least 1 hour for known samples.
   b) a minimum of 6 hours for questioned samples (recommended). Digestion may be performed overnight, but more than 24 hours is not recommended.

6. After digestion, briefly spin tubes.

7. Remove any substrate with clean forceps or with a sterile toothpick.
   a) The substrate may be discarded, or if necessary, placed in a spin basket and spun at 10,000-15,000 x g for 5 minutes.
   b) Alternatively, the substrate may be left in the tube and spun at 10,000-15,000 x g for 1 minute to force the substrate to the bottom of the tube.

8. Add 0.5 mL phenol-chloroform-isoamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.

9. Spin in centrifuge for 3 to 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.
10. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.

11. Proceed with cleaning and concentrating the DNA sample using the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

6.6 Miscellaneous samples

Including but not limited to, items that may contain trace amounts of DNA, such as shed skin cells, sweat, or other body fluids which may contain sufficient quantities of recoverable DNA.

Note: Amounts to be used will be at the analyst's discretion.

1. Add sample to a labeled microcentrifuge tube.
2. Pipette 0.5 mL Stain Extraction Buffer into a microcentrifuge tube or spin basket tube containing the sample.

Note: If 0.39M DTT is not added to Stain Extraction Buffer, then add 50 µL of 0.39M DTT to 450 µL of Stain Extraction Buffer.

Alternatively, the reagents may be placed into microcentrifuge tubes prior to samples being added.

3. Add 10 µL Proteinase K solution. Mix gently.
4. Incubate at approximately 56°C for
   a) at least 1 hour for known samples.
   b) a minimum of 6 hours for questioned samples (recommended). Digestion may be performed overnight, but more than 24 hours is not recommended.
5. After digestion, briefly spin tubes.
6. Remove any substrate with clean forceps or with a sterile toothpick.
   a) The substrate may be discarded, or if necessary, placed in a spin basket and spun at 10,000-15,000 x g for 5 minutes.
   b) Alternatively, the substrate may be left in the tube and spun at 10,000-15,000 x g for 1 minute to force the substrate to the bottom of the tube.
7. Add 0.5 mL phenol-chloroform-isoamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.
8. Spin in centrifuge for 3 to 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.
9. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.
10. Proceed with cleaning and concentrating the DNA sample using the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

7 Interpretation

None
8 Records

This extraction method (Stain Extraction Buffer or SEB), samples and control(s) shall be recorded in the case record.

- DNA Extraction Worksheet (LAB-DNA-07) [optional]
- Extraction/Lysis Worksheet (LAB-DNA-53) [optional]

9 Literature References and Supporting Documentation

DNA-05-02 DNA EXTRACTION WITH DIGEST BUFFER – ORGANIC

1 Scope
The procedure uses digest buffer along with other reagents to digest and extract DNA from a variety of forensic samples, such as blood, saliva, hair, tissue, bone, and teeth. Extracts are purified using phenol-chloroform-isoamyl alcohol and followed by dialysis and concentration using membrane filtration devices.

2 Related Chapters
Concentration and Purification of DNA with Membrane Filtration Devices
Digest Buffer
DTT 0.39M/1M
Proteinase K Solution – 10 mg/mL

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Perform phenol-chloroform extractions in a chemical fume hood. Appropriate personal protective equipment must be worn during testing. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Scissors, scalpels, tweezers
- Pipettors and tips
- Microcentrifuge tubes or spin basket tubes
- Microcentrifuge
- Vortex
- Water bath/dry bath incubator (56°C +/- 1°C)
- Sterile toothpicks
- Sterile tissue pulverizer
- Digest Buffer
- Proteinase K (10mg/mL)
- 1M DTT
- Phenol:chloroform:isoamyl alcohol (v/v 25:24:1)

5 Standards, Controls, and Calibration
At least one reagent blank must be processed for each extraction batch as a negative control.

6 Procedure
Note: The following are recommended amounts for reference samples. Evidence samples may be in limited supply. The user should add as much evidentiary sample to the tube as necessary, retaining sufficient sample for additional analysis if possible. Examples of quantities to add to the tube are listed below. Always use clean scalpel, scissors, or forceps with each sample.

6.1 Blood samples – stains or liquid
1. Add 10 to 50 µL of whole blood or add a bloodstain approximately 3 mm² to 1 cm² to a labeled microcentrifuge tube.
2. Pipette 0.5 mL Digest Buffer into a microcentrifuge tube or spin basket tube containing the sample. 
   Alternatively, Digest Buffer may be placed into microcentrifuge tubes prior to samples being added.

3. Add 15 µL Proteinase K solution. Mix gently.

4. Incubate at approximately 56°C for
   a) at least 1 hour for known samples.
   b) a minimum of 6 hours for questioned samples (recommended). Digestion may be performed overnight, but more than 24 hours is not recommended.

5. After digestion, briefly spin tubes.

6. Remove any substrate with clean forceps or with a sterile toothpick.
   a) The substrate may be discarded, or if necessary, placed in a spin basket and spun at 10,000-15,000 x g for 5 minutes.
   b) Alternatively, the substrate may be left in the tube and spun at 10,000-15,000 x g for 1 minute to force the substrate to the bottom of the tube.

7. Add 0.5 mL phenol-chloroform-isoamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.

8. Spin in centrifuge for 3 to 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.

9. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.

10. Proceed with cleaning and concentrating the DNA sample using the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

6.2 Saliva samples
Including but not limited to, oral swabs, filter paper, stamps, envelope flaps, cigarette butts, oral contact swab.

1. Add approximately 1 cm² stain (including filter paper, stamps, and envelope flaps), 1 cm strip of paper covering end of cigarette butt, 1/3 of a swab up to one whole swab to a labeled microcentrifuge tube.

2. Pipette 0.5 mL Digest Buffer into a microcentrifuge tube or spin basket tube containing the sample. 
   Alternatively, Digest Buffer may be placed into microcentrifuge tubes prior to samples being added.

3. Add 15 µL Proteinase K solution. Mix gently.

4. Incubate at approximately 56°C for
   a) at least 1 hour for known samples.
   b) a minimum of 6 hours for questioned samples (recommended). Digestion may be performed overnight, but more than 24 hours is not recommended.

5. After digestion, briefly spin tubes.
6. Remove any substrate with clean forceps or with a sterile toothpick.
   a) The substrate may be discarded, or if necessary, placed in a spin basket and spun at 10,000-15,000 x g for 5 minutes.
   b) Alternatively, the substrate may be left in the tube and spun at 10,000-15,000 x g for 1 minute to force the substrate to the bottom of the tube.

7. Add 0.5 mL phenol-chloroform-isoamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.

8. Spin in centrifuge for 3 to 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.

9. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.

10. Proceed with cleaning and concentrating the DNA sample using the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

### 6.3 Hair samples

1. Hairs should be cleaned in sterile dIH₂O prior to extraction.

2. Add approximately 1 cm of root end of hair to one labeled microcentrifuge tube and, optionally, a separate tube for a 1 cm portion of the adjacent shaft of each hair may be used as a control.

3. Pipette 0.5 mL Digest Buffer into a microcentrifuge tube or spin basket tube containing the sample.

   *Alternatively, Digest Buffer may be placed into microcentrifuge tubes prior to samples being added.*

4. Add 15 µL Proteinase K solution and 20 µL 1M DTT. Mix gently.

5. Incubate at approximately 56°C for a minimum of 6 hours to allow hair samples to digest.

6. Perform a second digestion after the initial 6 hour digestion by adding 15 µL Proteinase K solution and 20 µL 1 M DTT.

7. Incubate at approximately 56°C overnight to complete the second digest.

8. After digestion, briefly spin tubes.

9. Remove any substrate with clean forceps or with a sterile toothpick.
   a) The substrate may be discarded, or if necessary, placed in a spin basket and spun at 10,000-15,000 x g for 5 minutes.
   b) Alternatively, the substrate may be left in the tube and spun at 10,000-15,000 x g for 1 minute to force the substrate to the bottom of the tube.

10. Add 0.5 mL phenol-chloroform-isoamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.

11. Spin in centrifuge for 3 to 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.
12. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.

13. Proceed with cleaning and concentrating the DNA sample using the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

### 6.4 Tissue samples

Including but not limited to skin, muscle, and body organs.

1. Add approximately 3-5 mm\(^2\) to a labeled microcentrifuge tube. It is helpful to mince the tissue prior to adding digest buffer.

2. Pipette 0.5 mL Digest Buffer into a microcentrifuge tube or spin basket tube containing the sample. Alternatively, Digest Buffer may be placed into microcentrifuge tubes prior to samples being added.

3. Add 15 µL Proteinase K solution. Mix gently.

4. Incubate at approximately 56°C for
   a) at least 1 hour for known samples.
   b) a minimum of 6 hours for questioned samples (recommended). Digestion may be performed overnight, but more than 24 hours is not recommended.

5. After digestion, briefly spin tubes.

6. Remove any substrate with clean forceps or with a sterile toothpick.
   a) The substrate may be discarded, or if necessary, placed in a spin basket and spun at 10,000-15,000 x g for 5 minutes.
   b) Alternatively, the substrate may be left in the tube and spun at 10,000-15,000 x g for 1 minute to force the substrate to the bottom of the tube.

7. Add 0.5 mL phenol-chloroform-isooamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.

8. Spin in centrifuge for 3 to 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.

9. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.

10. Proceed with cleaning and concentrating the DNA sample using the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

### 6.5 Bone and Teeth samples

1. Prepare the sample
   a) One tooth or approximately 2 cm\(^3\) of bone (preferably flat bone in adults, e.g. pelvis, sternum, ribs).
   b) The exterior surface of each sample should be cleaned thoroughly of all debris. Bones may be sanded using a Dremel tool to remove debris. Teeth may be
cleaned with sterile water and bleach unless there are surface fractures. In which case, only use sterile water to clean the tooth.

c) Bone and teeth samples are prepared by crushing them into a fine powder. This can be achieved by using a hydraulic press with a stainless steel chamber that is cleaned with bleach between each sample, or a tissue pulverizer.

2. Place approximately 0.5 g sample into each microcentrifuge tube (this may take several tubes).

3. Pipette 0.5 mL Digest Buffer into a microcentrifuge tube or spin basket tube containing the sample.
   Alternatively, Digest Buffer may be placed into microcentrifuge tubes prior to samples being added.


5. Incubate at approximately 56°C for
   a) at least 1 hour for known samples.
   b) a minimum of 6 hours for questioned samples (recommended). Digestion may be performed overnight, but more than 24 hours is not recommended.

6. After digestion, briefly spin tubes.

7. Remove any substrate with clean forceps or with a sterile toothpick.
   a) The substrate may be discarded, or if necessary, placed in a spin basket and spun at 10,000-15,000 x g for 5 minutes.
   b) Alternatively, the substrate may be left in the tube and spun at 10,000-15,000 x g for 1 minute to force the substrate to the bottom of the tube.

8. Add 0.5 mL phenol-chloroform-isoamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.

9. Spin in centrifuge for 3 to 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.

10. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.

11. Proceed with cleaning and concentrating the DNA sample using the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

6.6 Miscellaneous

Including but not limited to items containing shed skin cells, sweat or other body fluids that may contain sufficient quantities of recoverable DNA.

Note: Amounts to be used will be at the analyst’s discretion.

1. Add sample to a labeled microcentrifuge tube.

2. Pipette 0.5 mL Digest Buffer into a microcentrifuge tube or spin basket tube containing the sample.
   Alternatively, Digest Buffer may be placed into microcentrifuge tubes prior to samples being added.
3. Add 15 µL Proteinase K solution. Mix gently.

4. Incubate at approximately 56°C for
   a) at least 1 hour for known samples.
   b) a minimum of 6 hours for questioned samples (recommended). Digestion may be performed overnight, but more than 24 hours is not recommended.

5. After digestion, briefly spin tubes.

6. Remove any substrate with clean forceps or with a sterile toothpick.
   a) The substrate may be discarded, or if necessary, placed in a spin basket and spun at 10,000-15,000 x g for 5 minutes.
   b) Alternatively, the substrate may be left in the tube and spun at 10,000-15,000 x g for 1 minute to force the substrate to the bottom of the tube.

7. Add 0.5 mL phenol-chloroform-isoamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.

8. Spin in centrifuge for 3 to 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.

9. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.

10. Proceed with cleaning and concentrating the DNA sample using the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

7 Interpretation

None

8 Records

This extraction method (Digest Buffer or DB), samples, and control(s) shall be recorded in the case record.

- DNA Extraction Worksheet (LAB-DNA-07) [optional]
- Extraction/Lysis Worksheet (LAB-DNA-53) [optional]

9 Literature References and Supporting Documentation

PE Applied Biosystems AmpF/STR ProfilerPlus PCR Amplification Kit User’s Manual
DNA-05-03 DNA EXTRACTION AND PURIFICATION WITH CHELEX

1 Scope
The procedure uses Chelex 100 chelating resin to extract DNA from forensic samples such as blood, saliva, and hair. The Chelex method of DNA extraction is rapid and may involve fewer steps than organic methods. This method produces single-stranded DNA that is suitable for PCR amplification.

The Chelex extraction consists of boiling the sample in a solution containing minute beads of the Chelex chemical. The boiling breaks open the evidentiary cells, releasing the DNA, and the Chelex binds up most other extraneous materials in the evidentiary sample that might interfere in subsequent analysis. This procedure may also be used to remove suspected PCR inhibitors or purify the DNA sample following other extraction or isolation procedures.

2 Related Chapters
Concentration and Purification of DNA with Membrane Filtration Devices
Chelex Solution
Proteinase K Solution – 10 mg/mL
TE\(^{-1}\) Buffer

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during testing. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Scissors, scalpel, tweezers
- Microcentrifuge tubes or spin basket tubes
- Microcentrifuge
- Vortex
- Water bath/dry bath incubator (56°C +/- 1°C)
- Sterile toothpicks
- Sterile tissue pulverizer
- Microtube rack, i.e. floating rack with lid clamp
- Large bore pipette tips
- Pipettors and tips
- Boiling water bath
- Hot plate
- Magnetic stir plate
- Boileezers
- 1L beaker
- 5% Chelex
- Sterile dIH\(_2\)O
- Proteinase K (10 mg/mL)
5 Standards, Controls, and Calibration

A. At least one reagent blank must be processed for each extraction batch as a negative control.

B. If previously extracted or inhibited samples are being cleaned up, the corresponding reagent blank must also be processed.

6 Procedure

Note: The following are recommended amounts for reference samples. Evidence samples may be in limited supply. The user should add as much evidentiary sample to the tube as necessary, retaining sufficient sample for additional analysis if possible. Always use clean scalpel, scissors, or forceps with each sample.

6.1 Blood samples - stains or liquid

1. Add 3 µL of whole blood or add a bloodstain approximately 3 mm² to a labeled microcentrifuge tube.

2. Add 1 mL sterile dH₂O or TE⁻⁴ Buffer to the tube. Vortex tubes briefly.
   *Alternatively, dH₂O or TE⁻⁴ Buffer may be placed into microcentrifuge tubes prior to samples being added.*

3. Incubate at room temperature 15 to 30 minutes. Vortex tubes for approximately 5 seconds.

4. Spin in microcentrifuge for 2 to 3 minutes at 10,000-15,000 x g.

5. Without disturbing the pellet, carefully remove and discard the supernatant, leaving enough behind to cover the pellet without disturbing it. If the sample is a bloodstain, leave the fabric substrate in the tube with the pellet. Steps 2-5 may be repeated to remove excess heme.

6. Add 200 µL 5% Chelex using a large bore pipet tip.

7. Incubate at approximately 56°C for 30 minutes to 2 hours.

8. Vortex tubes for 5 to 10 seconds.

9. Incubate in a boiling water bath for 8 minutes.

10. Vortex tubes for 5 to 10 seconds.

11. Spin in a microcentrifuge for 2 to 3 minutes at 10,000-15,000 x g.

12. Store extracts at 2-8°C or frozen. Prior to reuse of these samples repeat steps 10 and 11.

6.2 Hair samples

1. Hairs should be cleaned in sterile dH₂O prior to extraction.

2. Cut approximately 1 cm portion from the root end of the hair and place into a labeled microcentrifuge tube.
   *Optional:* In a separate labeled tube, add approximately 1 cm of the shaft adjacent to the root of each hair as a control.
3. To each tube, add 200 µL 5% Chelex using a large bore pipet tip and 2 µL Proteinase K.

   *Alternatively, 5% Chelex and Proteinase K may be placed into microcentrifuge tubes prior to samples being added.*

4. Incubate at approximately 56°C at least 6 to 8 hours or overnight.

5. Vortex tubes for 5 to 10 seconds.

6. Incubate in a boiling water bath for 8 minutes. Check that the hair root is completely immersed in the Chelex solution before boiling.

7. Vortex tubes for 5 to 10 seconds.

8. Spin in a microcentrifuge for 2 to 3 minutes at 10,000-15,000 x g.

9. Store extracts at 2-8°C or frozen. Prior to reuse of these samples repeat steps 7 and 8.

**6.3 Saliva - swabs, filter paper, gauze**

1. Cut approximately 3 mm² portion of the sample, or dissect into sections, and place into a labeled microcentrifuge tube.

   *(steps 2 - 6 are optional)*

2. Add 1 mL sterile dH₂O or TE-d Buffer to tube with sample.

3. Incubate at room temperature for 15 to 30 minutes.

4. Twirl the swab with a sterile toothpick for at least 2 minutes to agitate the cells off the substrate. Remove the swab and toothpick.

5. Spin in a microcentrifuge for approximately 2 minutes at 10,000-15,000 x g.

6. Without disturbing the pellet, remove and discard all but ~50 µL of the supernatant. Re-suspend the pellet in the remaining 50 µL by stirring-a sterile pipet tip may be used.

   *(steps 7 - 12 are required)*

7. Add 200 µL 5% Chelex using a large bore pipet tip. Vortex tubes for approximately 10 seconds.

   *Alternatively, 5% Chelex may be placed into microcentrifuge tubes prior to samples being added.*

8. Incubate at approximately 56°C for 30 minutes to 2 hours. Vortex tubes for approximately 10 seconds.

9. Incubate in a boiling water bath for 8 minutes.

10. Vortex tubes for approximately 10 seconds.

11. Spin in a microcentrifuge at 10,000-15,000 x g for 2 to 3 minutes.

12. Store extracts at 2-8°C or frozen. Prior to reuse of these samples repeat steps 10 and 11.
6.4 Saliva - cigarette butts

1. Using a sterile scalpel blade or scissors, slice an approximately 5 mm wide strip from the paper covering the cigarette butt in the area which would have been in contact with the mouth.

2. Pull paper slice from cigarette butt, cut into smaller pieces, and put pieces into a labeled microcentrifuge tube.

3. Add 1 mL 5% Chelex to tube using a large bore pipet tip. Vortex for approximately 30 seconds.

   Alternatively, 5% Chelex may be placed into microcentrifuge tubes prior to samples being added.

4. Incubate at approximately 56°C for 30 minutes to 2 hours.

5. Vortex tubes for approximately 30 seconds.

6. Incubate in a boiling water bath for 8 minutes.

7. Vortex tubes for approximately 30 seconds.

8. Spin tubes in a microcentrifuge at 10,000-15,000 x g for 30 seconds.

9. Store extracts at 2-8°C or frozen. Prior to reuse of these samples repeat steps 7 and 8.

6.5 Saliva - envelope flaps and stamps

1. Carefully slit open envelope flap with a sterile scalpel blade or carefully remove stamp from the envelope with a sterile scalpel blade.

   Proceed to step 2 or, alternatively, proceed to step 5.

2. Add 50 µL sterile dH₂O onto a 1 cm² area of gummed flap or stamp. Pipet water up and down on this area a few times and transfer fluid to a labeled microcentrifuge tube.

3. Repeat step 2 for two additional 1 cm² areas adjacent to area of first extraction. Add each extract to tube containing first extract.

4. Repeat steps 2 through 3 for adjacent 3 cm² areas if necessary to consume sufficient sample area for testing. Go to step 6.

5. Alternatively, cut a portion of the gummed flap or stamp and place into a labeled microcentrifuge tube.

6. Add 450 µL 5% Chelex to the pooled extracts using a large bore pipet tip.

   Alternatively, 5% Chelex may be placed into microcentrifuge tubes prior to pooled extracts being added.

7. Add 30 µL Proteinase K to each tube. Vortex tubes for approximately 2 seconds.

8. Incubate at approximately 56°C for approximately 90 minutes.

9. Vortex tubes for approximately 10 seconds.

10. Incubate samples in a boiling water bath for 8 minutes.

11. Vortex tubes for approximately 10 seconds.
12. Spin in a microcentrifuge at 10,000-15,000 x g for 2-3 minutes.
13. Store extracts at 2-8°C or frozen. Prior to reuse of these samples repeat steps 11 and 12.

6.6 Purification of previously purified DNA extracts
1. Add 200 μL 5% Chelex using a large bore pipet tip.
   Alternatively, 5% Chelex may be placed into microcentrifuge tubes prior to samples being added.
2. Vortex tubes for approximately 10 seconds.
3. Incubate in a boiling water bath for 8 minutes.
4. Vortex tubes for approximately 10 seconds.
5. Spin in a microcentrifuge at 10,000-15,000 x g for 2 to 3 minutes.
6. Using a 200ul pipette (small-bore tip), remove all liquid from the tube leaving the Chelex resin behind.
7. Transfer the liquid to a membrane filtration device and concentrate using the instructions in the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

6.7 Sample concentration and purification
Instructions in the Concentration and Purification of DNA with Membrane Filtration Devices chapter may be used to concentrate and clean the DNA sample.

7 Records
The extraction method, samples and control(s) shall be recorded in the case record.
- DNA Extraction Worksheet (LAB-DNA-07) [optional]
- Extraction/Lysis Worksheet (LAB-DNA-53) [optional]

8 Literature References and Supporting Documentation
DNA-05-04 DIFFERENTIAL EXTRACTION WITH TNE

1 Scope
This method of DNA extraction is appropriate for use on stains or swabs mixed with or originating from seminal fluid possibly containing spermatozoa. The differential lysis procedure separates the stain components into a sperm fraction and a non-sperm (epithelial cell) fraction. The DNA fraction extracts are either purified using phenol-chloroform-isoamyl alcohol followed by dialysis and concentration using membrane filtration devices or using QIAamp/PrepFiler procedures.

2 Related Documents
Spermatozoa Examination
DNA Extraction and Purification with QIAamp
Concentration and Purification of DNA with Membrane Filtration Devices
PrepFiler DNA Extraction
Digest Buffer
DTT 0.39M/1M
Nuclear Fast Red (NFR) Solution
Picroindigocarmine (PIC) Solution
Proteinase K Solution – 10 mg/mL
Sarcosyl Solution 20%
TNE Solution

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Perform phenol-chloroform extractions in a chemical fume hood. Appropriate personal protective equipment must be worn during testing. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Scissors, scalpel, tweezers
- Pipettors and tips
- Microcentrifuge tubes or spin basket tubes
- Microcentrifuge
- Vortex
- Water bath/dry bath incubator (37°C +/- 1°C)
- Microscope slides
- Microscope
- Sterile toothpicks
- Nuclear fast red (NFR)
- Picroindigocarmine (PIC)
- Tris/NaCl/EDTA (TNE)
- 20% Sarcosyl
5 Standards, Controls, and Calibration

At least one reagent blank for the epithelial cell fraction and at least one reagent blank for the sperm cell fraction must be processed for each extraction batch as a negative control.

6 Procedure

6.1 Differential Cell Lysis

1. Swabs should be dissected into pieces of appropriate size and placed into a labeled microcentrifuge tube.

**Note:** The following are recommended amounts. Evidence samples may be in limited supply. The user should add as much evidentiary sample to the tube as necessary, retaining sufficient sample for additional analysis if possible. Always use clean scalpel, scissors, or forceps with each sample.

- ¼ to a whole swab
- 0.1 - 0.5 cm² dried stain

2. Add the following reagents to the sample to obtain 505 µL total volume:

- 400 µL Tris/NaCl/EDTA (TNE)
- 25 µL 20% Sarcosyl
- 75 µL sterile deionized H₂O
- 5 µL Proteinase K

**Note:** If you are using the PrepFiler extraction procedure use the following volumes to begin the epithelial cell lysis step:

- 240 µL Tris/NaCl/EDTA (TNE)
- 15 µL 20% Sarcosyl
- 45 µL sterile deionized H₂O
- 3 µL Proteinase K

3. Mix tube contents and incubate at approximately 37°C for at least 2 hours. Ultrasonic agitation for no more than 10 minutes, vortexing, or twirling the substrate with a sterile toothpick for 2 minutes may aid in recovery of cellular material.

4. After digestion, briefly spin tubes. Remove any substrate with clean forceps or with a sterile toothpick while retaining as much liquid as possible in the tube. The substrate may be discarded, or if necessary, placed in a spin basket.

5. Centrifuge for 5 minutes at 10,000-15,000 x g.

6. Transfer the supernatant fluid (epithelial cell DNA) to a new labeled microcentrifuge tube.
Note: Take care not to pipet too near the cell debris in the bottom of the tube to avoid pulling up sperm cells and insure a cleaner epithelial cell fraction.

7. The epithelial cell DNA fraction can be spun an additional time to remove any sperm heads or particles that may not have pelleted during the first spin.
   a) Centrifuge supernatant for an additional 5 minutes at 10,000-15,000 x g. A pellet may be visible on the bottom of the tube.
   b) Carefully remove liquid and place in the epithelial cell microcentrifuge tube from step 6 above.
   c) The residual pellet from this step may be combined with the pellet from the first centrifugation step.

6.2 Sperm Cell Fraction

Note: The preparation of the microscope slide is optional if sperm have been identified on the sample during screening or if the sample was processed using Male Screening with Plexor HY.

1. Resuspend sperm pellet in TNE, digest buffer, or sterile deionized H₂O (recommended 500 µL each wash). Vortex and centrifuge for 5 minutes at 10,000-15,000 x g. Carefully remove the supernatant and discard. This wash process may be repeated three to five times.

2. For PrepFiler cleanup: resuspend sperm pellet in 50 µL TNE;

3. For all other cleanup: resuspend sperm pellet in 160 µL TNE;

4. Aliquot up to 10 µL on microscope slide. Fix slide and stain with NFR/PIC (Christmas tree stain) and examine and record number of spermatozoa per field, the total per slide, or score the slide.

5. If undigested epithelial cells are present, it may be necessary to repeat the Differential Cell Lysis steps for no more than 45 minutes.

6. For PrepFiler cleanup: refer to section 6.2 E.2 of the PrepFiler DNA Extraction chapter for resuspension volumes.

7. For all other cleanup: to the sperm pellet add the following:
   - 50 µL 20% Sarcosyl
   - 40 µL 0.39 M DTT
   - 150 µL sterile water
   - 10 µL Proteinase K

8. Mix tube contents and incubate at approximately 37°C for at least 2 hours. Samples may be incubated overnight.

9. Purification of DNA in the sperm and epithelial fraction can be accomplished by utilizing
   a) Phenol-chloroform-isoamyl alcohol described below,
   b) QIAamp spin columns (DNA Extraction and Purification with QIAamp chapter), or
   c) PrepFiler DNA extraction (Prepfiler DNA Extraction chapter).
6.3 Phenol-Chloroform-Isoamyl Alcohol Clean-Up of Epithelial and Sperm Cell Fractions

1. After digestion, briefly spin tubes.

2. Add 0.5 mL phenol-chloroform-isooamyl alcohol to tube. Cap tube and vortex for approximately 15 seconds until an emulsion forms.

3. Spin in centrifuge for 5 minutes at 10,000-15,000 x g at room temperature to separate the two phases.

4. If necessary, repeat the previous two steps, using the aqueous layer (after it has been transferred to a fresh microcentrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.

5. Proceed with cleaning and concentrating the DNA sample using the procedure in the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

7 Interpretation

None

8 Records

A. This extraction method (TNE), samples, and control(s) shall be recorded in the case record.
   - DNA Extraction Worksheet (LAB-DNA-07) [optional]
   - Extraction/Lysis Worksheet (LAB-DNA-53) [optional]

B. For epithelial cell fractions, the number of epithelial cells per field may be recorded in the case record.

C. For sperm fractions, the number of spermatozoa shall be recorded in the case record if a microscope slide is prepared. This can be recorded as an approximate number per slide, approximate number per field, or by scoring.

9 Literature References and Supporting Documentation


DNA-05-05 DIFFERENTIAL EXTRACTION WITH DIGEST BUFFER

1 Scope
This method of DNA extraction is appropriate for use on stains or swabs mixed with or originating from seminal fluid containing spermatozoa. The differential lysis procedure separates the stain components into a sperm fraction and a non-sperm (epithelial cell) fraction. The DNA fraction extracts are either purified using phenol-chloroform-isoamyl alcohol followed by dialysis and concentration using membrane filtration devices or using QIAamp/Prepfiler procedures.

2 Related Chapters
Spermatozoa Examination
DNA Extraction and Purification with QIAamp
Concentration and Purification of DNA with Membrane Filtration Devices
Prepfiler DNA Extraction
Digest Buffer
DTT 0.39M/1M
Nuclear Fast Red (NFR) Solution
Picroindigocarmine (PIC) Solution
Proteinase K Solution – 10 mg/mL
TE⁻¹ Buffer

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Perform phenol-chloroform extractions in a chemical fume hood. Appropriate personal protective equipment must be worn during testing. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Scissors, scalpel, tweezers
- Pipettors and tips
- Microcentrifuge tubes or spin basket tubes
- Microcentrifuge
- Vortex
- Water baths/ovens/dry bath incubators (56°C +/- 1°C)
- Sonicator
- Sterile toothpicks
- Nuclear fast red (NFR)
- Picroindigocarmine (PIC)
- Digest Buffer
- Proteinase K (10 mg/mL)
- 1M DTT
- Sterile deionized H₂O
5 Standards, Controls, and Calibration

At least one reagent blank for the epithelial cell fraction and at least one reagent blank for the sperm cell fraction must be processed for each extraction batch as a negative control.

6 Procedure

6.1 Body Fluid Extraction

1. Swabs should be dissected into pieces of appropriate size and placed into a labeled microcentrifuge tube. Sections which are not to be analyzed immediately should be stored frozen.

   **Note:** The following are recommended amounts. Evidence samples may be in limited supply. The user should add as much evidentiary sample to the tube as necessary, retaining sufficient sample for additional analysis if possible. Always use clean scalpel, scissors, or forceps with each sample.

   - ¼ to a whole swab
   - 36B0.1 - 0.5 cm² dried stain

2. Suspend the swab or stain in 1 mL sterile TE\(^{-4}\) or sterile deionized H\(_2\)O. Incubate at room temperature for at least 30 minutes. Ultrasonic agitation for no more than 10 minutes or vortexing may aid in recovery of cellular material.

   *Alternatively, TE\(^{-4}\) or H\(_2\)O may be placed into microcentrifuge tubes prior to samples being added.*

3. Twirl the swab or fabric with a sterile toothpick for at least 2 minutes to agitate the cells off of the substrate.

4. Remove the swab or fabric and toothpick. It is advisable to retain the substrate until microscopic analysis shows that the supernatant contains spermatozoa. Store swab or fabric in a tube. If spermatozoa are not visible microscopically, it may be necessary to re-extract substrate more vigorously.

5. Centrifuge the sample for 1 minute at 10,000-15,000 x g.

6. Without disturbing the pellet, remove and discard all but approximately 50 µL (or twice the volume of the pellet, whichever is greater) of the supernatant. Resuspend the pellet in the remaining 50 µL by stirring it with a pipette tip.

   **Note:** This pellet contains epithelial cells and sperm cells and is called the cell debris pellet.

7. Remove 3 µL of the resuspended sample using a sterile pipette tip and spot on a glass microscope slide for examination using NFR/PIC (Christmas tree) stain. If epithelial cells are detected, record the number of epithelial cells on the DNA extraction worksheet and proceed with the differential cell lysis. This can be recorded as an approximate number per slide, approximate number per field, or by scoring. If no epithelial cells are observed, the differential cell lysis procedure may be skipped and proceed with sperm cell lysis.

   **Note:** The preparation of the microscope slide is optional.
### 6.2 Differential Cell Lysis

1. To the approximately 50 µL resuspended cell debris pellet, add the following reagents and mix gently.
   - 0.5 mL Digest Buffer
   - 15 µL Proteinase K

   **Note:** If you are using the PrepFiler extraction procedure use the following volumes:
   - 300 µL Digest Buffer
   - 9 µL Proteinase K

2. Incubate at approximately 56°C for at least 1 hour to lyse epithelial cells, but for no more than two hours, to reduce lysis of sperm.

3. Spin the sample in a microcentrifuge for 5 minutes at 10,000-15,000 x g.

4. Remove the supernatant to a new microcentrifuge tube to proceed with epithelial DNA isolation.

   **Note:** Take care not to pipet too near the cell debris in the bottom of the tube to avoid pulling up sperm cells and insure a cleaner epithelial cell fraction.

### 6.3 Sperm Cell Fraction

1. Wash the pellet as follows: Resuspend the pellet in 0.5 mL digest buffer by vortexing briefly. Spin the sample in a microcentrifuge for 5 minutes at 10,000-15,000 x g. Using a sterile pipette tip, remove all but approximately 50 µL of the supernatant and discard.

2. Repeat wash step 1 an additional 1 to 2 times.

   **Note:** Additional wash steps are recommended when the ratio of sperm to epithelial cells is low.

3. Resuspend the pellet in 0.5 mL sterile deionized H₂O by vortexing briefly. Spin the sample in a microcentrifuge for 5 minutes at 10,000-15,000 x g. Using a sterile pipette tip, remove and discard all but approximately 50 µL of the supernatant. Resuspend the pellet in the remaining 50 µL by stirring it with a sterile pipette tip.

4. Remove 3 µL of the resuspended sample using a sterile disposable pipette tip and spot on a glass microscope slide for examination using NFR/PIC (Christmas tree) stain. Verify the digestion of the epithelial cells and recovery of spermatozoa. Record the number of spermatozoa on the DNA extraction worksheet as an approximate number per slide, approximate number per field, or by scoring. If undigested epithelial cells are present, it may be necessary to repeat the Differential Cell Lysis steps for no more than 45 minutes. At the end of the second differential cell lysis, centrifuge at 10,000-15,000 x g and discard supernatant. Continue with step 1 for Sperm Cell Fraction.

   **Note:** The preparation of the microscope slide is **optional** if sperm have been identified on the sample during screening or if the case was processed using Male Screening with Plexor HY.

5. **For PrepFiler cleanup:** refer to the PrepFiler DNA Extraction chapter in the DNA SOP, section 6.2 E.2 to determine resuspension volume.
6. For other cleanup methods, add 0.5 mL digest buffer to the approximately 50 µL resuspended sperm cell pellet. Add 20 µL 1M DTT and 15 µL Proteinase K. Incubate at 56°C for at least 1 hour.

Note: It is recommended that digestion continue for a minimum of 6 hours. It is appropriate at this point to allow samples to incubate overnight.

7. Purification of DNA in the sperm and epithelial fraction can be accomplished by utilizing
   a) Phenol-chloroform-isooamyl alcohol described below,
   b) QIAamp spin columns (DNA Extraction and Purification with QIAamp chapter in the DNA SOP), or
   c) Prepfilr DNA extraction (Prepfilr DNA Extraction chapter in the DNA SOP).

6.4 Phenol-Chloroform-Isooamyl Alcohol Clean-Up of Epithelial and Sperm Cell Fractions
1. Add 0.5 mL phenol-chloroform-isooamyl alcohol to tube. Cap tube and vortex approximately 15 seconds until an emulsion forms.
2. Spin in centrifuge for 3-5 minutes at 10,000-15,000 x g to separate the two phases.
3. If necessary, repeat steps 1 and 2, using the aqueous layer (after it has been transferred to a fresh centrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.
4. Proceed with cleaning and concentrating the DNA sample using the procedure in the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

7 Interpretation
None

8 Records
A. This extraction method (Digest Buffer or DB), samples, and control(s) shall be recorded in the case record.
   • DNA Extraction Worksheet (LAB-DNA-07) [optional]
   • Extraction/Lysis Worksheet (LAB-DNA-53) [optional]
B. For epithelial cell fractions, the number of epithelial cells may be recorded in the case record as an approximate number per slide, an approximate number per field or by scoring.
C. For sperm fractions, the number of spermatozoa shall be recorded in the case record as an approximate number per slide, an approximate number per field or by scoring if microscope slide prepared.

9 Literature References and Supporting Documentation
DNA-05-06 DNA EXTRACTION AND PURIFICATION WITH QIAAMP

1 Scope
This procedure uses the QIAamp DNA Mini Kit to produce a single extract of all nucleic acids from a dried body fluid stain or swab.

The QIAamp silica-gel membrane has an affinity for nucleic acids under certain buffer and temperature conditions. The membrane is supported in a microcentrifuge tube, which simplifies handling. A body fluid extract is centrifuged through the membrane. The adsorbed nucleic acids are washed and then eluted. DNA purified using QIAamp ranges in size up to 50 kilobases, with fragments of approximately 20–30 kilobases predominating.

This procedure may also be used to remove suspected PCR inhibitors or purify the DNA sample following other extraction or isolation procedures.

2 Related Chapters
Differential Extraction with TNE
Differential Extraction with Digest Buffer
Concentration and Purification of DNA with Membrane Filtration Devices
DTT 0.39M/1M
Stain Extraction Buffer
TE-4 Buffer

3 Safety
Buffers AL and AW1 are irritants and are incompatible with bleach. Buffer AW2 contains sodium azide, which is highly toxic and may react explosively with lead and copper drainpipes. Ethanol is an irritant and is flammable. Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Scissors, scalpel, tweezers
- Pipettors and tips
- Microcentrifuge tubes or spin basket tubes
- Spin baskets
- Investigator Lyse&Spin tubes
- Microcentrifuge
- Vortex
- Water baths/ovens/dry bath incubators (56°C +/- 1°C and 70°C +/- 1°C)
- TE-4 buffer
- Ethanol, denatured or pure
- For hair extraction only: Stain Extraction Buffer
- QIAamp® DNA Mini Kit, QIAGEN catalog number 51304, 51306, or 51308 for 50, 250, and 1000 preparations respectively. Contains the following:
5 Standards, Controls, and Calibration

A. At least one reagent blank must be processed for each extraction batch as a negative control.

B. If previously extracted or inhibited samples are being cleaned up, the corresponding reagent blank must also be processed.

6 Procedure

6.1 General

A. Do not wet the rim of the spin column when transferring liquid.

B. Do not touch the membrane with the pipette tip.

C. Prepare reagents and samples.

   1. Equilibrate samples and elution solution to room temperature (20–25°C).
   2. Dissolve any precipitate in Buffer ATL by incubating at approximately 56°C.
   3. Preheat water baths/ovens/dry bath incubators to approximately 56°C and approximately 70°C.

6.2 DNA Extraction and Cell Lysis

Note: The following are recommended amounts for reference samples. Evidence samples may be in limited supply. The user should add as much evidentiary sample to the tube as necessary, retaining sufficient sample for replicate analysis if possible. Always use clean scalpel, scissors, or forceps with each sample.

A. Blood and saliva samples

   1. Add to a labeled microcentrifuge tube or labeled Lyse&Spin tube

      • 0.1–0.25 cm² dried bloodstain
      • ¼ to a whole saliva swab (buccal, suspected oral contact, etc.)
      • approximately 1 cm² saliva stain (filter paper, stamp, envelope flap)
      • Approximately 1 cm strip of paper from outside of cigarette butt

   2. Add 200 µL Buffer ATL (approximately 56°C), 8 µL 1M DTT, and 20 µL Proteinase K.

      Alternatively, extraction solutions may be placed into tubes prior to samples being added.

   3. Incubate overnight at approximately 56°C or, alternatively, at least one hour for reference samples.
4. **If using Lyse& Spin tubes**: spin at 10,000 to 20,000 x g for 1 minute. Remove and discard the basket containing the substrate.

5. If using microcentrifuge tubes:
   a) *Briefly spin tubes.*
   b) *Remove and discard the swab or cutting while retaining as much liquid as possible in the tube. Alternatively, the substrate may be placed in a spin basket and spun at 10,000 - 15,000 x g for 5 minutes.*

B. Hair samples

1. Add to a labeled microcentrifuge tube or a labeled Lyse&Spin tube approximately 1 cm root end of hair
   a) *Rinse hair in sterile dH₂O prior to removing root end. (This step is optional when there is no visible debris)*
   b) *Optional: 1 cm of adjacent hair shaft may also be extracted as a negative control if available.*

2. Add 300 µL stain extraction buffer and 7.5 µL Proteinase K. Alternatively, extraction solutions may be placed into tubes prior to samples being added.

3. Incubate 18–24 hours at approximately 56°C.

4. If using Lyse&Spin tubes: spin at 10,000 to 20,000 x g for 1 minute. Remove and discard the basket containing the substrate.

5. If using microcentrifuge tubes:
   a) *Briefly spin tubes.*
   b) *Remove and discard any substrate while retaining as much liquid as possible in the tube. Alternatively, any substrate may be placed in a spin basket and spun at 10,000 - 15,000 x g for 5 minutes.*

C. Bone and teeth samples

1. Prepare the sample
   a) *One tooth or approximately 2 cm³ of bone (preferably flat bone in adults- i.e. pelvis, sternum, ribs).*
   b) *The exterior surface of each sample should be cleaned thoroughly of all debris. Bones may be sanded using a Dremel tool to remove debris. Teeth may be cleaned with sterile water and bleach unless there are surface fractures. In which case, only use sterile water to clean the tooth.*
   c) *Bone and teeth samples are prepared by crushing them into a fine powder. This can be achieved by using a hydraulic press with a stainless steel chamber that is cleaned with bleach between each sample, or a tissue pulverizer.*

2. Place approximately 0.5 g sample into each microcentrifuge tube (this may take several tubes).

3. Add 200 µL buffer ATL (approximately 56°C), 8 µL 1M DTT, and 20 µL Proteinase K.
   *Alternatively, extraction solutions may be placed into microcentrifuge tubes prior to samples being added.*
4. Incubate overnight at approximately 56°C or, alternatively, at least one hour for reference samples.
5. Briefly spin tubes.
6. Remove and discard any substrate while retaining as much liquid as possible in the tube. Alternatively, any substrate may be placed in a spin basket and spun at 10,000 - 15,000 x g for 5 minutes.

6.3 QIAamp® DNA Purification

A. Purification of QIAamp extracted samples begins here.
   1. Shake Buffer AL.
   2. Add 200 µL Buffer AL to liquid in sample tube.
   3. Incubate at approximately 70°C for 10 minutes. Briefly spin tubes.
   5. Proceed to step D-1.

B. Purification of previously extracted or inhibited samples begins here.
   1. Add 200 µL ATL Buffer to DNA extract.
   2. Incubate at approximately 56°C for a minimum of 1 hour. Briefly spin tubes.
   3. Shake Buffer AL.
   4. Add 200 µL Buffer AL to liquid in sample tube.
   5. Incubate at approximately 70°C for 10 minutes. Briefly spin tubes.
   7. Proceed to step D-1.

C. Purification of differential extractions begins here.
   1. Shake Buffer AL.
   2. Add 500 µL Buffer AL to liquid in sample tube.
   3. Incubate at approximately 70°C for 10 minutes. Briefly spin tubes.
   5. Proceed to step D-1.

D. Adsorb nucleic acids to membrane.
   1. Transfer tube contents (including any precipitate) to a labeled QIAamp spin column in a collection tube.
   2. Centrifuge at 6000 x g for 1 minute or until all solution has passed through the membrane.
   3. If the entire volume cannot be loaded on the QIAamp spin column in one application, the sample should be transferred and centrifuged as above for the entire sample. This may require that the collection tube be emptied between spins.
E. Wash membrane.
   1. Before first use, add denatured or pure ethanol to Buffers AW1 and AW2 according to the directions on the buffer bottles.
   2. Transfer the column to a new collection tube. Add 250 µL Buffer AW1 to the column.
   3. Centrifuge at 6000 x g for 1 minute or until all solution has passed through the membrane.
   4. Add 250 µL Buffer AW2 to the column and centrifuge at 6000 x g for 1 minute.
   5. (Optional) Transfer the column to a new collection tube.
   6. Centrifuge at 20,000 x g (or maximum speed) for an additional 2 minutes or until all solution has passed through the membrane. No Buffer AW2 should remain in or on the spin column.

F. Elute nucleic acids from membrane.
   1. Transfer the column to a new, labeled collection tube.
   2. Add 50–200 µL TE-4 or Buffer AE to the column depending on the expected quantity of DNA. Incubate for 10 minutes at room temperature.
   3. Centrifuge at 6000 x g for 1 minute.
   4. Transfer liquid from collection tube to a labeled storage tube. The elution step may be repeated with the same recovered sample to increase yield or concentrate the sample with centrifugal microfiltration.
   5. Store DNA extracts at approximately 2–8°C or frozen.

6.4 Concentration and Purification of DNA Extract
Concentration and cleaning of DNA extract may be performed using the procedure in the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

7 Records
The extraction method, samples, and control(s) shall be recorded in the case record.
   • DNA Extraction Worksheet (LAB-DNA-07) [optional]
   • Extraction/Lysis Worksheet (LAB-DNA-53) [optional]

8 Literature References and Supporting Documentation


DNA-05-07 CONCENTRATION AND PURIFICATION OF DNA WITH MEMBRANE FILTRATION DEVICES

1 Scope
Approved membrane filtration devices may be used to concentrate, desalt, and clean-up the DNA sample. The selection of the type of device to be used typically depends on the volume of the sample to be concentrated, the nature/amount of sample, or the extraction procedure used.

2 Related Chapters
DNA Extraction with Stain Extraction Buffer
DNA Extraction with Digest Buffer
DNA Extraction and Purification with Chelex
Differential Extraction with TNE
Differential Extraction with Digest Buffer
DNA Extraction and Purification with QIAamp
Prepfiler DNA Extraction
Qiagen EZ1 Investigator Kit Extraction with Qiagen EZ1 Purification
TE-4 Buffer

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Appropriate personal protective equipment must be worn during testing. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Amicon/Microcon microconcentrators consist of two components:
  - filtration unit (sample reservoir)
  - filtrate/recovery tube (Amicon Ultra 10K /YM-30 and Microcon DNA Fast Flow)
- Microcentrifuge
- Centricon/Vivacon consists of three components:
  - filtration unit (sample reservoir)
  - filtrate tube
  - conical collection/retentate cup (Centricon-100/Vivacon-100)
- Variable speed centrifuge with fixed-angle rotor
- Pipettors and tips
- Vortex
- TE-4 buffer

5 Standards, Controls, and Calibration
Extraction reagent blanks must be concentrated as a negative control.
6 Procedure

6.1 General

A. Do not touch filtration membrane with pipette tip or other object.
B. The membrane filtration devices are sensitive to centrifugal force. Excessive g-force may result in leakage or damage to the membrane or device.
C. The recovery volume should be approximately 10-40 µL.

6.2 Centricon Device

1. Assemble the Centricon unit according to the manufacturer’s directions and label each unit.
2. Add 1.5 mL TE-$4$ buffer to the upper Centricon reservoir.
3. Carefully transfer the entire supernatant of the sample without debris and/or resin (approximately 0.5 mL) to the TE-$4$ in the upper reservoir. Cap with the retentate cup.
4. Centrifuge at approximately 2,000 x g in a fixed-angle rotor for 10-20 minutes. The effluent in the lower reservoir may be discarded at this time. The centrifugation time may be increased to reduce the volume if an insufficient amount of filtrate has passed through filter.
5. If further clean-up is necessary, add another 2 mL TE-$4$ to the upper reservoir and repeat step 4.
6. Repeat step 5 an additional time, if necessary.
7. Collect the concentrated DNA sample by inverting the upper reservoir into the retentate cup and centrifuge at 500 x g for 2-5 minutes.
8. The filtrate should be transferred to a labeled microcentrifuge tube. Store the DNA extract at 2-8 °C or frozen.

6.3 Microcon Device

1. Assemble the Microcon unit according to the manufacturer’s directions and label each unit.
2. Add up to 500 µL sample to upper reservoir. Seal with attached cap.
   
   **Optional**: Sample volume may be adjusted by adding TE-$4$ buffer up to a final volume of 500 µL.

3. Centrifugation rate should be between 9,300 x g and 14,000 x g for the YM 30 and 500 x g for the Microcon DNA Fast Flow. Centrifugation time for either unit is approximately 8 minutes. The centrifugation time may be adjusted to ensure an appropriate amount of filtrate has passed through the filter. The effluent in the lower reservoir may be discarded at this time.
4. Optional: Add up to 500 µL TE-$4$ buffer to the sample reservoir to wash DNA and repeat centrifugation.
5. Collect the concentrated DNA sample by inverting the sample reservoir into a labeled, new sample recovery tube. Centrifuge for approximately 2-3 minutes at 1000 x g.
6. Extended centrifugation can lead to dryness. If this should occur, add at least 10 µL TE-4 buffer to the sample reservoir, agitate gently for 30 seconds, proceed with recovery.

7. Store the DNA extract at 2-8 °C or frozen.

6.4 Vivacon Device
1. Label each Vivacon unit.
2. Add 1.5 mL TE-4 buffer to the upper Vivacon reservoir.
3. Transfer the entire supernatant of the sample without debris and/or resin (approximately 0.5 mL) to the TE-4 in the upper reservoir. Cap with the retentate cup.
4. Centrifuge at approximately 2,500 x g in a fixed-angle rotor for 10-20 minutes. The effluent in the lower reservoir may be discarded at this time. The centrifugation time may be increased to reduce the volume if an insufficient amount of filtrate has passed through the filter.
5. If further clean-up is necessary, add another 2 mL TE-4 to the upper reservoir and repeat step 4.
6. Collect the concentrated DNA sample by inverting the upper reservoir into the retentate cup and centrifuge at 500 x g for 2-5 minutes.
7. The filtrate should be transferred to a labeled microcentrifuge tube. Store the DNA extract at 2-8 °C or frozen.

6.5 Amicon Ultra
1. Assemble the Amicon Ultra unit according to the manufacturer directions and label each unit.
2. Add the sample to the Amicon Ultra 0.5 mL concentrator filter device. Up to 500 µL can be added. TE-4 may be added to the sample to increase the starting volume if desired.
3. Place the device into a centrifuge rotor and spin the device at 14,000 x g for approximately 10 minutes. The centrifugation time may be adjusted to ensure an appropriate amount of filtrate has passed through the filter.

Optional: An additional 500 µL of TE-4 may be added to the device at this point for an additional wash of the membrane. If this additional wash is completed place the device into a new centrifuge tube or decant the original filtrate prior to centrifugation.
4. Remove the assembled device from the centrifuge and separate the Amicon Ultra filter from the tube.
5. Place the Amicon Ultra filter device upside down into a clean centrifuge tube.
6. Spin for 2 minutes at 1,000 x g.
7. Transfer the filtrate to a labeled microcentrifuge tube. Store the DNA extract at 2-8 °C or frozen.
7 Interpretation

If there is a significant volume of recovered sample, it may be necessary to repeat the procedure and/or perform the procedures listed in the DNA Extraction and Purification with QIAamp chapter or Extraction and Purification with Chelex chapter to further clean up sample.

8 Records

DNA Extraction Worksheet (LAB-DNA-07) [optional]
Extraction/Lysis Worksheet (LAB-DNA-53) [optional]

9 Literature References and Supporting Documentation

Centricon Centrifugal Filter Devices User Guide. Amicon. Revision Y. 03/05
Microcon Centrifugal Filter Devices User Guide. Millipore Corporation. Revision M. 06/18
Vivacon 2mL Technical data and operating instructions. Sartorius Stedim Biotech. 09/12
Amicon Ultra-0.5 Centrifugal Filter Devices User Guide. Millipore Corporation, Revision A, 11/11
DNA-05-08 CONCENTRATION OF DNA EXTRACTS WITH SPEED VACUUM EVAPORATION

1 Scope
Approved Speed Vacuum devices may be used to further concentrate DNA extracts that have been purified with a membrane filtration device. The duration and temperature of centrifugation may be varied to partially or fully dehydrate extracts.

2 Related Chapters
DNA Extraction with Stain Extraction Buffer
DNA Extraction with Digest Buffer
DNA Extraction and Purification with Chelex
Differential Extraction with TNE
Differential Extraction with Digest Buffer
DNA Extraction and Purification with QIAamp
Prepfiler DNA Extraction
Qiagen EZ1 Investigator Kit extraction with Qiagen EZ1 Purification
TE-4 Buffer

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Appropriate personal protective equipment must be worn during the handling of samples. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Thermo Scientific Savant DNA 120 Speed Vacuum Concentrator
- Pipettors and tips
- Microcentrifuge tubes with extract
- TE-4 buffer

5 Standards, Controls, and Calibration
Extraction reagent blanks must be processed in the same manner as evidentiary extracts.

6 Procedure
6.1 General
A. Rotors for 0.5 mL and 1.5 mL microcentrifuge tubes can be used.
B. The lids of the microcentrifuge tubes must be open during vacuum centrifugation to allow for evaporation.
C. Rotor Installation:
   1. Open the lid of the rotor chamber.
   2. Carefully lower the rotor onto the drive shaft.
3. Secure the assembly by screwing the retaining knob into the drive shaft above the rotor. Tighten it firmly but not excessively.

4. Load the rotor and close the lid. Always balance rotor loads.

D. The recovery volume should be approximately 0-40 µL. If the samples are evaporated to dryness, TE\(^{-4}\) buffer can be used to rehydrate them to 20-40 µL.

6.2 **Operation of the Thermo Scientific Savant DNA 120 SpeedVac\(^{®}\) Concentrator**

1. Turn on the speed vacuum with the switch at the back, right side and the cover latch mechanism releases automatically.

2. Place sample tubes into the SpeedVac\(^{®}\).

3. Set the drying rate to “low” for ambient room temperature centrifugation.

4. To centrifuge the samples on manual mode, push the switch on the panel to “Manual”. The SpeedVac\(^{®}\) will run until it is switched to off.

5. To centrifuge the samples on automatic mode, set switch B to RUN, set switch C to the desired time, set switch A to Auto to start the run. The SpeedVac\(^{®}\) will run until the designated time expires.

6. The lid cannot be opened until the vacuum pressure has been released. When there is no longer a vacuum on the chamber, the lid latch will release and the lid can be opened.

7. When the samples are vacuum evaporated to dryness or the desired volume, remove them from the SpeedVac\(^{®}\) and store them with the lids closed at 2-8 °C or frozen.

7 **Interpretation**

None

8 **Records**

A record of the evaporation of samples must be documented in the case record.

- DNA Extraction Worksheet (LAB-DNA-07) [optional]
- Extraction/Lysis Worksheet (LAB-DNA-53) [optional]

9 **Literature References and Supporting Documentation**

Instruction Manual for the Savant\(^{®}\) DNA120, DNA120 OP SpeedVac\(^{®}\) Concentrator- 104-3003-00 Rev.C.
DNA-05-09 REMOVAL OF MOUNTED HAIRS FOR DNA ANALYSIS

1 Scope
Hair samples that have been mounted on slides are viable sources of DNA for forensic analysis. This procedure describes the removal of mounted hairs from slides prior to DNA analysis.

2 Related Documents
None

3 Safety
Tissue samples may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves must be worn during testing. Clothing may protect unbroken skin; broken skin must be covered. Xylene is flammable and should be used under a chemical fume hood.

4 Equipment and Materials
- Freezer, liquid nitrogen, or other material used for freezing
- Scalpel
- Sterile diH₂O
- Absolute ethanol
- Centrifuge tubes or 15 mL conical tubes
- Pasteur pipette
- Xylene

5 Standards, Controls, and Calibration
None

6 Procedure
1. Freeze the slide.
2. Remove the cover slip using a scalpel blade. 
   Alternatively, crack the coverslip and soak slide in xylene.
3. Once the coverslip is removed, wash the mounting media away from the hair using a Pasteur pipette filled with xylene.
4. Wash hair, either in a centrifuge tube or a 15 mL conical tube with absolute ethanol.
5. Rinse hair with sterile water.
6. Continue with selected extraction procedure.

7 Interpretation
None

8 Records
Document in the case record the removal of the hair from the slide.

9 Literature References and Supporting Documentation
None
DNA-05-10 PREPFILER DNA EXTRACTION

1 Scope

This procedure uses the PrepFiler Automated Forensic DNA Extraction Kit to produce a single extract of nucleic acids from a variety of forensic samples, such as, blood, saliva, hair, tissue, bone, and teeth. The procedure may also be used to complete extractions of both fractions of differential extractions.

The PrepFiler Magnetic Particles have an affinity for nucleic acids under certain buffer and temperature conditions. The adsorbed nucleic acids are washed and then eluted.

This procedure may be performed manually or using the Tecan Freedom Evo robotic platform.

2 Related Chapters

Differential Extraction with TNE
Differential Extraction with Digest Buffer
Concentration and Purification of DNA with Membrane Filtration Devices
Differential Extraction with Erase Sperm Isolation Kit
DTT 0.39M/1M
TE-4 Buffer

3 Safety

Magnetic Particles and Lysis buffer contain guanidine thiocyanate, are incompatible with acids or bases including bleach, and must be disposed of properly. Wash Buffer is an irritant. Ethanol and isopropanol are irritants and flammable. Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials

- Scissors, scalpel, tweezers
- Pipettors and pipette tips
- Microcentrifuge tubes or spin basket tubes
- Spin baskets
- Investigator Lyse&Spin tubes
- Microcentrifuge
- Vortex
- 1M Dithiothreitol
- Shaking incubator
- Benchtop centrifuge with rotor for 96-deep-well plates
- Isopropanol (ACS reagent grade, >= 99.5%)
- Ethanol (Molecular biology grade 95%)
- Liquid Handling (LiHa) disposable tips (DiTis) with filters for Tecan (1000 and 200 µL)
- 100 mL disposable troughs for reagents
96-well reaction plate
96-well Magnetic Ring Stand
96-well deep-well plate
PrepFiler Automated Forensic DNA Extraction Kit
Tecan Freedom Evo robotic platform
TE-4 buffer

5 Standards, Controls, and Calibration
At least one reagent blank must be processed for each extraction batch as a negative control. An extraction batch is defined as a set of samples processed during one robotic run.

6 Procedure
6.1 Prepare Reagents
A. Magnetic Particles
   1. Magnetic particles must be incubated at approximately 37°C for approximately 30 minutes, vortexed thoroughly (at least 5 seconds) then centrifuged prior to use.
   2. Once heated, these particles may be stored at room temperature for up to 3 months.
   3. It is recommended that this procedure be performed at least one day prior to use, to allow all bubbles to settle. If bubbles are present, they may be removed with a pipette tip or with a Kimwipe.
B. 1.0 M DTT – thaw aliquot or prepare fresh
C. Lysis Buffer – if buffer contains precipitate heat the solution to approximately 37°C, then shake bottle to ensure mixing.
D. Wash Buffer A
   1. Wash Buffer A may be prepared fresh before each extraction using a mixture of 26% Wash Buffer Concentrate and 74% ethanol (e.g. mix 260 mL Wash Buffer Concentrate with 740 mL ethanol).
   2. Prepared wash buffer may be stored at room temperature for up to six months.
E. Wash Buffer B
   1. If not using the wash buffer concentrate provided with the kit, Wash Buffer B may be prepared fresh before each extraction using a mixture of 40% TE-4 and 60% ethanol for the 4x4 workstation or 30% TE-4 and 70% ethanol for the 1x8 workstation.
   2. If using the wash buffer concentrate provided with the kit, Wash Buffer B may be prepared fresh before each extraction using a mixture of 40% Wash Buffer B concentrate and 60% ethanol for the 4x4 workstation or 30% Wash Buffer B concentrate and 70% ethanol for the 1x8 workstation.
   3. Prepared wash buffer may be stored at room temperature for up to six months.
6.2 DNA Extraction and Cell Lysis

**Note:** The following are recommended amounts for reference samples. Evidence samples may be in limited supply. The user should add as much evidentiary sample to the tube as necessary, retaining sufficient sample for additional analysis if possible. Always use clean scalpel, scissors, or forceps with each sample.

A. Blood samples
   1. Add 0.1 - 0.25 cm² dried bloodstain to a labeled microcentrifuge tube or a labeled Lyse&Spin tube (this may be performed at time of collection or immediately prior to extraction).
   2. Pipette 300 µL lysis buffer and 3 µL 1M DTT into the Lyse&Spin tube, microcentrifuge tube or spin basket tube containing the sample.  
      Alternatively, the reagents may be placed into tubes prior to the samples being added.
   3. Cap tubes.
   4. If using microcentrifuge tubes: vortex for 5 seconds and briefly centrifuge.
   5. Place samples in a shaking incubator. Incubate at approximately 70°C with shaking at a minimum of 200 RPM for approximately 60 minutes.
   6. If using Lyse&Spin tubes: spin at 10,000 to 20,000 x g for 1 minute. Remove and discard the basket containing the substrate.
   7. If using microcentrifuge tubes:
      a) Briefly centrifuge tubes to remove any liquid from inside of caps.
      b) Transfer substrate into spin basket.
      c) Place spin basket into tube containing liquid.  
         Alternatively, transfer liquid into new microcentrifuge tube and use this tube for remainder of process.
      d) Cap and centrifuge for two minutes at 13,000 to 16,000 x g.
      e) Remove the spin basket from the microcentrifuge tube.
   8. The lysate can be stored for up to one week in the refrigerator prior to clean up using the PrepFiler extraction kit. If stored refrigerated, ensure that lysate has returned to room temperature before proceeding to automated run.

B. Saliva samples
   1. Add approximately 1/4 to whole saliva swab (buccal, suspected oral contact, etc.), 1 cm² saliva stain (filter paper, stamp, envelope flap), 1 cm strip of paper from outside of cigarette butt to a labeled microcentrifuge tube or labeled Lyse&Spin tube (this may be performed at time of collection or immediately prior to extraction).
   2. Pipette 300 µL lysis buffer and 3 µL 1M DTT into a Lyse&Spin tube, microcentrifuge tube or spin basket tube containing the sample.  
      Alternatively, the reagents may be placed into tubes prior to the samples being added.
   3. Cap tubes.
4. **If using microcentrifuge tubes**, vortex for 5 seconds and briefly centrifuge.

5. Place samples in a shaking incubator. Incubate at approximately 70°C with shaking at a minimum of 200 RPM for approximately 60 minutes.

6. **If using Lyse&Spin tubes**: spin at 10,000 to 20,000 x g for 1 minute. Remove and discard the basket containing the substrate.

7. **If using microcentrifuge tubes**:
   a) Briefly centrifuge tubes to remove any liquid from inside of caps.
   b) Transfer substrate into spin basket.
   c) Place spin basket into tube containing liquid.
   Alternatively, transfer liquid into new microcentrifuge tube and use this tube for remainder of process.
   d) Cap and centrifuge for two minutes at 13,000 to 16,000 x g.
   e) Remove the spin basket from the microcentrifuge tube.

8. The lysate can be stored for up to one week in the refrigerator prior to clean up using the PrepFiler extraction kit. If stored refrigerated, ensure that lysate has returned to room temperature before proceeding to automated run.

C. **Hair Samples**

1. Hairs should be cleaned in sterile diH$_2$O prior to extraction.

2. Add approximately 1 cm root end of hair to one labeled microcentrifuge tube or labeled Lyse&Spin tube and, optionally, a separate tube for a 1 cm portion of the adjacent shaft of each hair may be used as a control.

3. Pipette 300 µL lysis buffer and 9 µL 1M DTT into a Lyse&Spin tube, microcentrifuge tube or spin basket tube containing the sample.
   Alternatively, the reagents may be placed into tubes prior to the samples being added.


5. **If using microcentrifuge tubes**, vortex for 5 seconds and briefly centrifuge.

6. Place samples in a shaking incubator. Incubate at approximately 70°C with shaking at a minimum of 200 RPM for approximately 60 minutes.

7. **If using Lyse&Spin tubes**: spin at 10,000 to 20,000 x g for 1 minute. Remove and discard the basket containing the substrate.

8. **If using microcentrifuge tubes**:
   a) Briefly centrifuge tubes to remove any liquid from inside of caps.
   b) Transfer any substrate into spin basket.
   c) Place spin basket into tube containing liquid.
   Alternatively, transfer liquid into new microcentrifuge tube and use this tube for remainder of process.
   d) Cap and centrifuge for two minutes at 13,000 to 16,000 x g.
   e) Remove the spin basket from the microcentrifuge tube.
9. The lysate can be stored for up to one week in the refrigerator prior to clean up using the PrepFiler extraction kit. If stored refrigerated, ensure that lysate has returned to room temperature before proceeding to automated run.

D. Bone and Teeth samples

1. Prepare the sample
   a) One tooth or approximately 2 cm³ of bone (preferably flat bone in adults- i.e. pelvis, sternum, ribs).
   b) The exterior surface of each sample should be cleaned thoroughly of all debris. Bones may be sanded using a Dremel tool to remove debris. Teeth may be cleaned with sterile water and bleach unless there are surface fractures. In which case, only use sterile water to clean the tooth.
   c) Bone and teeth samples are prepared by crushing them into a fine powder. This can be achieved by using a hydraulic press with a stainless steel chamber that is cleaned with bleach between each sample, or a tissue pulverizer.

2. Place approximately 0.5 g sample into each microcentrifuge tube (this may take several tubes).

3. Pipette 300 µL lysis buffer and 3 µL 1M DTT into the microcentrifuge tube or spin basket container containing the sample.

   Alternatively, the reagents may be placed into microcentrifuge tubes prior to the samples being added.

4. Cap tubes, vortex for 5 seconds, and briefly centrifuge

5. Place samples in a shaking incubator. Incubate at approximately 70°C with shaking at a minimum of 200 RPM for approximately 60 minutes.

6. Briefly centrifuge tubes to remove any liquid from inside of caps.

7. Transfer any substrate into spin basket.

8. Place spin basket into tube containing liquid.

   Alternatively, transfer liquid into new microcentrifuge tube and use this tube for remainder of process.

9. Cap and centrifuge for two minutes at 13,000 to 16,000 x g.

10. Remove the spin basket from the microcentrifuge tube.

11. The lysate can be stored for up to one week in the refrigerator prior to clean up using the PrepFiler extraction kit. If stored refrigerated, ensure that lysate has returned to room temperature before proceeding to automated run.

E. Differential Samples

1. Differential Lysis
   a) Follow the Differential Extraction with TNE chapter for TNE differential lysis using 240 µL TNE, 15 µL 20% sarcosyl, 45 µL sterile water, and 3 µL ProK to begin the epithelial cell lysis step.

   b) Follow the Differential Extraction with Digest Buffer chapter for Digest Buffer differential lysis using 300 µL Digest Buffer and 9 µL ProK to begin the epithelial cell lysis step.
c) Proceed with step 6.4 (Automated DNA Extraction) or 6.5 (Manual DNA Extraction) after differential lysis is complete to purify the epithelial cell fraction DNA.

2. Sperm Cell Fraction
   a) The unlysed sperm cell fraction can be stored up to 1 week in the refrigerator prior to lysing and clean up using the PrepFiler extraction kit.
   b) Prior to lysing and clean up using the PrepFiler extraction kit, resuspend the sperm pellet in the remaining 50 µL of supernatant by stirring it with a sterile pipette tip and remove 3 µL of the resuspended sample to spot on a glass microscope slide for examination using NFR/PIC (Christmas tree) stain.
      Note: The preparation of the microscope slide is optional if sperm have been identified on the sample during screening or if the samples were processed using Male Screening with Plexor HY.
   c) If using 90 minute lysis procedure: add 300 µL lysis buffer and 5 µL 1M DTT to the tube containing the sperm cell fraction.
   d) If using 30 minute lysis procedure: add 300 µL lysis buffer and 20 µL 1M DTT to the tube containing the sperm cell fraction.
   e) Cap tubes, vortex for 5 seconds, and briefly centrifuge.
   f) Place samples in a shaking incubator.
   g) If using 90 minute lysis procedure (5 µL 1M DTT added), incubate at approximately 70°C with shaking at a minimum of 200 RPM for approximately 90 minutes.
   h) If using 30 minute lysis procedure (20 µL 1M DTT added), incubate at approximately 70°C with shaking at a minimum of 200 RPM for approximately 30 minutes.
   i) Briefly centrifuge tubes to remove any liquid from inside of caps.
   j) The lysate can be stored for up to one week in the refrigerator prior to clean up using the PrepFiler extraction kit. If storing refrigerated, ensure that lysate has returned to room temperature before proceeding to automated run.

6.3 Tecan Robotic Setup

A. Labware and consumables
   1. Ensure deck layout is set up appropriately.
   2. Place tubes of magnetic particles in back two wells of column one of the Magnetic particle holder.
   3. All DiTi racks must be full at start of script.
   4. Three additional full 1000 µL DiTi racks should be placed in rear shelf positions 5, 6, and 7.
   5. Position 4 must be empty at start of this script.

B. Reagent Volumes as listed in table
<table>
<thead>
<tr>
<th>Reagent</th>
<th>Reagent volume per sample (µL)</th>
<th>Overfill volume per run (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropanol</td>
<td>180</td>
<td>8</td>
</tr>
<tr>
<td>Prepared Wash Buffer A</td>
<td>600 (1x8 workstation)</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>900 (4x4 workstation)</td>
<td></td>
</tr>
<tr>
<td>Prepared Wash Buffer B</td>
<td>300</td>
<td>13</td>
</tr>
<tr>
<td>Elution Buffer or TE-4 Buffer</td>
<td>55</td>
<td>6</td>
</tr>
<tr>
<td>Magnetic particles</td>
<td>Two vials. One full, one with at least 850µL of particles</td>
<td></td>
</tr>
</tbody>
</table>

### 6.4 Automated DNA Extraction using Tecan Freedom Evo

1. Run appropriate script using EvoWare.
2. If necessary, enter sample names and information (this may be entered manually or imported) if the Scan labware feature is not enabled.
3. Ensure that “Scan labware” box is not checked if this feature is not being utilized. (If scan labware feature has been disabled within the script, box does not need to be unchecked.)
4. Confirm that the worktable is setup correctly.
   a) Confirm that labware is in the correct location and positioned correctly on the robot.
   b) After confirmation, mark each consumable on check-list as loaded or click loaded all.
   c) If desired, select Liquid Level Detection.
5. Begin automated extraction.

### 6.5 Manual Extraction

Some or all of these steps may be done in place of the robotic extraction.

1. Add 15 µL Prepfilier magnetic particles to each sample.
2. Vortex for 10 seconds at low speed and centrifuge briefly.
3. Add 180 µL Isopropanol to each well of the 96 deep well plate.
4. Transfer samples (~320 µL) into corresponding wells on the 96 deep well plate.
5. Shake the plate at ~1000 rpm for 10 minutes at room temperature.
6. Place the plate on the magnetic well plate for 6 minutes.
7. Without disturbing the magnetic particles, remove and discard the liquid from each sample.
8. Add 600 µL of Wash Buffer A to each sample and shake plate at 1000 rpm for 2 minutes.
9. Place the plate on the magnetic well plate for 2 minutes.
10. Without disturbing the magnetic particles, remove and discard the liquid from each sample.

11. Repeat steps 8-10 using 300 μL of Wash Buffer A.

12. Repeat steps 8-10 using 300 μL of Wash Buffer B.

13. Let the samples air dry for 7-10 minutes at room temperature. Do not exceed 10 minutes.

14. Add 50 μL Elution buffer or TE-4 Buffer to each sample and place plate on thermal shaker at 65°C and ~900 rpm for 5 minutes, pause for 8 minutes, and shake for 3 minutes.

15. Place the plate on the magnetic well plate for 7 minutes.

16. Without disturbing the magnetic particles, transfer the samples into labeled microcentrifuge tubes and store samples frozen.

6.6 Post Extraction Activities

1. Verify that liquid transfer steps were completed successfully prior to discarding any used labware by checking the tubes, the deep well plate, and the 96 well plate.

2. Either proceed to quantification or seal sample plate/tubes.

3. Store samples at 2-8°C or frozen.

4. Concentration and cleaning of the DNA sample may be performed using the procedure in the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

7 Records

A. The extraction method, samples, unique equipment identification, and control(s) shall be recorded in the case record.
   • DNA Extraction Worksheet (LAB-DNA-07) [optional]
   • Extraction/Lysis Worksheet (LAB-DNA-53) [optional]

B. For sperm cell fractions, the number of sperm shall be recorded in the case record as an approximate number per slide, an approximate number per field, or by scoring if microscope slide prepared.

8 Literature References and Supporting Documentation


DNA-05-11 QIAGEN EZ1 INVESTIGATOR KIT EXTRACTION WITH QIAGEN EZ1 PURIFICATION

1 Scope
This procedure uses the Qiagen EZ1 Investigator Kit for the extraction of DNA from a variety of forensic samples, such as blood, saliva, hair, and semen, and automated DNA purification using the Qiagen EZ1 Advanced XL robotic workstation.

2 Related Chapters
Concentration and Purification of DNA with Membrane Filtration Devices
DTT 0.39M/1M
Nuclear Fast Red (NFR) Solution
Picroindigocarmine (PIC) Solution
TE-4 Buffer

3 Safety
The reagent cartridge contains ethanol, guanidine hydrochloride, and guanidine thiocyanate and is therefore flammable, harmful, and an irritant. The guanidine salts are not compatible with disinfecting reagents containing bleach. Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Scissors, scalpel, tweezers
- Pipettors and pipette tips
- Microcentrifuge tubes or spin basket tubes
- Spin baskets
- Microcentrifuge
- Vortex
- 1.0 M Dithiothreitol
- Wet/dry incubator
- TE-4 or sterile water
- NFR (Nuclear Fast Red)
- PIC (Picroindigocarmine)
- Microscope slides
- Qiagen Buffer ATL
- Qiagen Buffer MTL
- Qiagen EZ1 Investigator Kit which contains reagent cartridges, disposable tip holders, disposable filter tips, sample tubes, elution tubes, Buffer G2, Proteinase K (20mg/mL), and Carrier RNA
- Qiagen EZ1 Advanced XL robotic workstation
- Flip cap rack
5 Standards, Controls, and Calibration

At least one reagent blank must be processed for each extraction batch as a negative control. An extraction batch is defined as a set of samples processed during one robotic run.

6 Procedure

6.1 Prepare Reagents

1. If necessary, dissolve any precipitate in well 1 of the reagent cartridge by incubating at 37°C.
2. For all extraction types except liquid blood, thaw aliquot of 1.0 M DTT or prepare fresh.
3. Pre-heat incubators to appropriate lysis temperatures.
4. If extracting semen or liquid blood, prepare a 1:1 dilution of Buffer G2 using equal parts Buffer G2 and distilled water for use with absorbent sample types (e.g. fabrics or swabs. Use of diluted Buffer G2 does not influence DNA yield or quality.) Samples require 290 µL each.
5. For Sperm washes, prepare a 1:5 dilution of Buffer G2 using dH2O. Each sample requires 1500-2000 µL/sample (i.e. 3-4 washes).
6. Purification of low amounts of DNA (<100 ng) may be assisted by the use of carrier RNA to enhance binding of DNA to the silica surface of the magnetic particles. Lyophilized carrier RNA is provided in the EZ1 Investigator Kit. Before first use, add 310 µL of nuclease-free water or TE-4 buffer to obtain a solution of 1 µg/µL, divide into single-use aliquots, and store frozen.
7. For samples to be purified using the Large Volume Protocol, make a master mix by adding 1 µL carrier RNA to 200 µL MTL buffer for each sample.

6.2 DNA Extraction and Cell Lysis

Note: The following are recommended amounts for reference samples. Evidence samples may be in limited supply. The user should add as much evidentiary sample to the tube as necessary, retaining sufficient sample for additional analysis if possible. Always use clean scalpel, scissors, or forceps with each sample.

A. Bone and Teeth samples

1. Prepare the sample
   a) One tooth or approximately 2 cm³ of bone (preferably flat bone in adults - i.e. pelvis, sternum, ribs).
   b) The exterior surface of each sample should be cleaned thoroughly of all debris. Bones may be sanded using a Dremel tool to remove debris. Teeth may be cleaned with sterile water and bleach unless there are surface fractures. In which case, only use sterile water to clean the tooth.
   c) Bone and teeth samples are prepared by crushing them into a fine powder. This can be achieved by using a hydraulic press with a stainless steel chamber that is cleaned with bleach between each sample, or a tissue pulverizer.

2. Place approximately 0.5 g sample into each microcentrifuge tube (this may take several tubes).
3. Add 200 µL Buffer ATL, 8 µL 1 M DTT and 20 µL Proteinase K. 
   *Alternatively, ATL/Proteinase K/DTT may be placed into microcentrifuge tubes 
   prior to samples being added.*
4. Incubate at least one hour to overnight at 56°C or alternatively, at least 15 minutes for reference samples.
5. Incubate at 70°C for 5 minutes.
6. Centrifuge the tube briefly.
7. Remove and discard the swab or cutting while retaining as much liquid as possible in the tube. Sample volume should be at least 200 µL. 
   *Alternatively, the substrate may be placed in a spin basket and spun at 10,000 - 15,000 x g for 5 minutes.*
8. Transfer the sample to an appropriate sample tube. Proceed to section 6.3 for EZ1 purification.

B. Semen stains-swabs or fabric
1. Place ¼ to a whole swab or 0.1 - 1.0 cm² of dried semen stain in a labeled microcentrifuge tube (this may be performed at the time of collection or immediately prior to extraction).
2. Add 290 µL 1:1 Buffer G2 to the sample.
3. Add 10 µL Proteinase K and vortex tubes for 10 seconds. 
   *Alternatively, 1:1 G2 buffer/Proteinase K may be placed into microcentrifuge tubes 
   prior to samples being added.*
4. Incubate at 56°C for at least 15 minutes to one hour, then vortex for 10 seconds.
5. Centrifuge the tube briefly to remove drops from inside the lid.
6. Remove any solid substrate from the tube. Spin baskets may be used.
7. Centrifuge the tube at 10,000-15,000 x g for 5 minutes. Carefully transfer the supernatant (epithelial cell fraction) to an appropriate sample tube without disturbing the sperm cell pellet.
8. Optional Second Epithelial Digest: If the ratio of sperm to epithelial cells is low, a second digest of the epithelial cells may be necessary. Repeat steps 1-4 with the sperm cell fraction with another 15 minute lysis. Following the second digestion, centrifuge the tube at 10,000-15,000 x g for 5 minutes and carefully remove and discard the supernatant.
9. Proceed to section 6.3 for EZ1 purification of the epithelial cell fraction.
10. Wash the sperm pellet by resuspending the pellet in 500 µL 1:5 diluted Buffer G2 and vortexing briefly. Centrifuge the tube at 10,000-15,000 x g for 5 minutes and discard the supernatant.
11. Repeat wash step 10 an additional one to two times.
12. Resuspend the pellet in 0.5 mL sterile ddH₂O by vortexing briefly. Spin the sample in a microcentrifuge for 5 minutes at 10,000-15,000 x g. Remove all but approximately 50 µL of the supernatant and discard. Resuspend the pellet in the remaining 50 µL by stirring it with a sterile pipette tip.
**Note:** The preparation of the microscope slide is optional if sperm have been identified on the sample during screening or if the sample was processed using Male Screening with Plexor HY.

13. Remove 3 µL of the resuspended sample using a sterile disposable pipette tip and spot on a glass microscope slide for examination using NFR/PIC (Christmas tree) stain. Verify the digestion of the epithelial cells and the recovery of spermatozoa. Record the number of spermatozoa on the DNA extraction worksheet by indicating the approximate number per slide, approximate number per field, or by scoring.

14. Add 170 µL Buffer ATL to the approximately 50 µL resuspended sperm cell pellet. Add 10 µL Proteinase K and 20 µL DTT. Incubate the samples at 70°C for 10 minutes.

15. Centrifuge the tube briefly to remove drops from inside the lid. Transfer the sample to an appropriate sample tube.

16. Proceed to section 6.3 for EZ1 purification.

**C. Hairs**

1. Hairs should be cleaned in sterile diH₂O prior to extraction.

2. Add approximately 1 cm root end of hair to one labeled microcentrifuge tube and, optionally, a separate tube for a 1 cm portion of the adjacent shaft of each hair may be used as a control.

3. Add 170 µL Buffer ATL to the sample.

4. Add 10 µL Proteinase K and 20 µL 1M DTT; vortex briefly.

   *Alternatively, ATL buffer/Proteinase K may be placed into microcentrifuge tubes prior to samples being added.*

5. Incubate at 56°C for at least 1 hour.

6. Vortex sample for 10 seconds and centrifuge briefly.

7. Transfer the sample to an appropriate sample tube. Proceed to section 6.3 for EZ1 purification.

**D. Blood-Liquid**

1. Transfer 200 µL of whole blood into labeled appropriate sample tube (for samples <200 µL, bring the volume up to 200 µL with Buffer G2).

2. Place samples on the EZ1 Advanced XL for purification using the Trace Protocol. Proceed to section 6.3 for EZ1 purification.

**E. Blood – dried stains**

1. Place 0.1 – 1.0 cm² dried bloodstain in a labeled microcentrifuge tube (this may be performed at the time of collection or immediately prior to extraction).

2. Add 200 µL Buffer ATL, 8 µL 1 M DTT and 20 µL Proteinase K.

   *Alternatively, ATL/Proteinase K/DTT may be placed into microcentrifuge tubes prior to samples being added.*

3. Incubate at least one hour to overnight at 56°C or alternatively, at least 15 minutes for reference samples.
4. Incubate at 70°C for 5 minutes.
5. Centrifuge the tube briefly.
6. Remove and discard the swab or cutting while retaining as much liquid as possible in the tube. Sample volume should be at least 200 µL. 
   *Alternatively, the substrate may be placed in a spin basket and spun at 10,000 - 15,000 x g for 5 minutes.*
7. Transfer the sample to an appropriate sample tube. Proceed to section 6.3 for EZ1 purification.

F. Saliva Samples
1. Place 1/4 to a whole saliva swab (buccal, suspected oral contact, etc.), approximately 1 cm² saliva stain (filter paper, envelope, stamp), approximately 1 cm strip of paper from outside of cigarette butt, or up to 40 mg of chewing gum cut into small pieces into a labeled microcentrifuge tube.
2. Add 200 µL Buffer ATL, 8 µL 1 M DTT and 20 µL Proteinase K. 
   *Alternatively, ATL/Proteinase K/DTT may be placed into microcentrifuge tubes prior to samples being added.*
3. Incubate at least one hour to overnight at 56°C or alternatively, at least 15 minutes for reference samples.
4. Incubate at 70°C for 5 minutes.
5. Centrifuge the tube briefly.
6. Remove and discard the swab or cutting while retaining as much liquid as possible in the tube. Sample volume should be at least 200 µL. 
   *Alternatively, the substrate may be placed in a spin basket and spun at 10,000 - 15,000 x g for 5 minutes.*
7. Transfer the sample to an appropriate sample tube. Proceed to section 6.3 for EZ1 purification.

G. Forensic Surface and Contact Swabs and Stains
1. Place 1/4 to a whole swab for forensic surface and contact swabs into a labeled microcentrifuge tube.
2. Add 200 µL Buffer ATL, 8 µL 1 M DTT and 20 µL Proteinase K. 
   *Alternatively, ATL/Proteinase K/DTT may be placed into microcentrifuge tubes prior to samples being added.*
3. Incubate at least one hour to overnight at 56°C.
4. Incubate at 70°C for 5 minutes.
5. Centrifuge the tube briefly.
6. Remove and discard the swab or cutting while retaining as much liquid as possible in the tube. Sample volume should be at least 200 µL. 
   *Alternatively, the substrate may be placed in a spin basket and spun at 10,000 - 15,000 x g for 5 minutes.*
7. Transfer the sample to an appropriate sample tube. Proceed to section 6.3 for EZ1 purification.

6.3 Purification using the EZ1 Advanced XL

A. Purification

1. For purification using the Trace protocol or Tip Dance protocol add 1 µL of carrier RNA to the sample tube.
2. For purification using the Large Volume protocol add 200 µL MTL Buffer/1 µL carrier RNA.
3. A precipitate may form with hair and sperm extracts, this is normal.

B. Set-up/Prepare equipment

1. Switch on the EZ1 Advanced XL.
2. Press START to start protocol setup.

C. Purification

1. Press 1 for Trace protocol, press 2 for Tip Dance protocol, or press 3 for Large Volume protocol.
2. Choose elution buffer (TE-4 or water).
3. Choose elution volume (40 µL, 50 µL, 100 µL, or 200 µL).
4. Press any key to proceed through the text shown on the display and start worktable setup.
5. Open the workstation door.
6. Invert reagent cartridges to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.
7. Load the reagent cartridges into the cartridge rack. Press down on the reagent cartridge once fully in the reagent rack until it clicks into place.
8. Load opened elution tubes into row 1 of the tip rack or flip cap rack.
9. Load tip holders containing filter-tips into row 2 of the tip rack or flip cap rack.
10. Load the opened sample tubes containing digested samples into row 4 (the back row) of the tip rack. (Row 3 will remain empty.) If using flip cap rack load the opened sample tubes containing digested samples into row 3 (the back row).
11. Press START and follow directions to start the purification.
12. When the protocol ends, the display shows Protocol Finished. Press ENT.
13. Press ESC twice to exit to main menu.
14. Open the workstation door and retrieve the elution tubes containing the purified DNA. Transfer samples to labeled storage tubes. Appropriately discard the sample-preparation waste.
15. Lower piercing units using the manual menu and clean piercing units and interior surfaces with EtOH.
16. Follow the onscreen instructions to perform an approximately 25 minute UV decontamination of the worktable surfaces after each use or at the end of the day.

17. Turn off workstation.

6.4 Post Extraction Activities

1. Verify that liquid transfer steps were completed successfully prior to discarding any used labware.

2. Proceed to quantification.

3. Store samples at 2-8°C or frozen.

4. Concentration and cleaning of the DNA samples may be performed using the procedure in the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

7 Records

A. The unique equipment identification, extraction method, samples, and control(s) shall be recorded in the case record.

   - DNA Extraction Worksheet (LAB-DNA-07) [optional]
   - Extraction/Lysis Worksheet (LAB-DNA-53) [optional]

B. For the sperm cell fractions of the semen stains, the number of spermatozoa shall be recorded in the case record if microscope slide prepared. The number can be recorded as an approximate number per slide, an approximate number per field, or by scoring.

8 Literature References and Supporting Documentation


DNA-05-12 DIFFERENTIAL EXTRACTION WITH ERASE SPERM ISOLATION KIT

1 Scope

This method of DNA extraction is appropriate for use on stains or swabs mixed with or originating from seminal fluid containing spermatozoa. This procedure uses selective enzymatic degradation of soluble DNA to separate the stain components into a sperm fraction and a non-sperm (epithelial cell) fraction. The DNA fraction extracts are purified using phenol-chloroform-isoamyl alcohol followed by dialysis and concentration using membrane filtration, using QIAamp, or PrepFiler Automated DNA extraction with a Tecan robotic platform.

2 Related Chapters

Spermatozoa Examination
DNA Extraction and Purification with QIAamp
Concentration and Purification of DNA with Membrane Filtration Devices
PrepFiler DNA Extraction
Nuclear Fast Red (NFR) Solution
Picroindigocarmine (PIC) Solution
TE-4 Buffer

3 Safety

Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Perform phenol-chloroform extractions in a chemical fume hood. Appropriate personal protective equipment must be worn during testing. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials

- Scissors, scalpel, tweezers
- Pipettors and tips
- Microcentrifuge
- Vortex
- Water baths/ovens/dry bath incubators (37°C, 56°C, and 70°C +/- 1°C)
- Sterile toothpicks
- Nuclear fast red (NFR)
- Picroindigocarmine (PIC)
- Erase Sperm Isolation kit
- QIAamp kit
- Ethanol, denatured or pure (to use with QIAamp kit)
- TE-4 buffer
- phenol:chloroform:isoamyl alcohol (v/v 25:24:1)
- PrepFiler Automated Forensic DNA Extraction Kit
- Tecan Freedom Evo robotic platform
5 Standards, Controls, and Calibration
At least one reagent blank for the epithelial cell fraction and at least one reagent blank for the sperm cell fraction must be processed for each extraction batch as a negative control.

6 Procedure

6.1 Body Fluid Extraction

1. Swabs should be dissected into pieces of appropriate size and placed into Tube A. Sections which are not to be analyzed immediately should be stored frozen.

   Note: Evidence samples may be in limited supply. The user should add as much evidentiary sample to the tube as necessary, retaining sufficient sample for additional analysis if possible. Always use clean scalpel, scissors, or forceps with each sample.
   - ¼ to a whole swab
   - 0.1 - 0.5 cm² dried stain

2. Add the following reagents to the sample for a total volume of 407 µL:
   - 400 µL Extraction Buffer
   - m7 µL Proteinase K

   Alternatively, Master mix may be placed into microcentrifuge tubes prior to samples being added.

3. Vortex at full speed for 20 seconds.

4. Centrifuge the sample at less than 6000 x g for 2 seconds to remove liquid from the sides and cap of the tube.

5. Incubate at approximately 56°C for about 1 hour to complete Proteinase K digestion.

6.2 Differential Cell Lysis

1. Centrifuge samples briefly to remove condensation from caps.

2. Remove the substrate and place it in a spin basket. Put the basket back into the SAME Tube A.

3. Cap and centrifuge Tube A at 10,000 to 15,000 x g for 5 minutes.

4. Remove spin basket from Tube A.

5. Using the lower line on Tube A as a guide, SLOWLY pipette approximately 350 µL of liquid from Tube A into Tube B until approximately 50 µL of liquid remains in Tube A.
6. If performing clean-up using QIAamp or organic, add 100 µL TE-4 to Tube B.
7. If performing clean-up using PrepFiler automated extraction, combine 150 µL of liquid from Tube B with 150 µL of PrepFiler lysis buffer for a total of 300 µL. The remainder of the liquid from Tube B can be saved refrigerated until it can be verified that a sufficient quantity of DNA was obtained from this fraction to produce a complete DNA profile.
8. Tube B is the non-sperm (epithelial cell) fraction and is ready for DNA purification.

6.3 Sperm Cell Fraction

1. Mix the sperm pellet in Tube A and remove 3 µL of the re-suspended sample using a sterile disposable pipette tip and spot on a glass microscope slide for examination using NFR/PIC (Christmas tree) stain. Record the number of spermatozoa on the DNA Extraction Worksheet (LAB-DNA-07) by indicating an average per slide, average per field, or scoring. This step is optional if spermatozoa were observed during screening or if the samples were processed using Male Screening with Plexor HY.

***Note: the order in which solutions are added in the following steps is CRITICAL

2. Mix the red cap tube containing Solution #1 by vortexing and add 10 µL of Solution #1 to Tube A. (discard any unused Solution #1 after use)
3. Mix the white cap tube containing Solution #2 by pipetting up and down then add 10 µL of Solution #2 to Tube A. (discard any unused Solution #2 after use)
4. Mix the liquid in Tube A thoroughly but GENTLY by pipetting up and down several times until the sperm pellet has been fully re-suspended. AVOID bubbles and DO NOT vortex.
5. Transfer the liquid to Tube C.
6. Incubate the samples in Tube C at approximately 37°C for about 15 minutes.
7. Briefly centrifuge samples to remove any condensation from the caps prior to opening them.
8. Thaw and vortex the blue cap tube containing Solution #3 to solubilize any precipitate.
9. Add 10 µL of Solution #3 to Tube C and mix by vortexing. (discard any unused Solution #3 after use)
10. Incubate the samples in Tube C at approximately 56°C for about 15 minutes.
11. Briefly centrifuge samples to remove any condensation from the caps prior to opening them.
12. If performing clean-up using QIAamp or organic, add 370 µL TE-4 to the sperm fraction in Tube C.
13. If performing clean-up using PrepFiler automated DNA extraction, add 225 µL PrepFiler Lysis buffer to the liquid in Tube C.
14. Tube C is the sperm cell fraction and is ready for DNA purification. Due to the presence of DNase in the sample at this point, the purification steps must be undertaken the day of extraction in order to limit digestion of DNA present in the sperm cell fraction.
6.4 Clean-Up of Epithelial and Sperm Cell Fractions Using QIAamp

1. Shake Buffer AL.
2. Add 200 μL Buffer AL to liquid in sample tube.
3. Incubate at approximately 70°C for 10 minutes. Briefly spin tubes.
5. Transfer tube contents (including any precipitate) to a labeled QIAamp spin column in a collection tube.
6. Centrifuge at 6000 x g for 1 minute or until all solution has passed through the membrane.
7. If the entire volume cannot be loaded on the QIAamp spin column in one application, the sample should be transferred and centrifuged as above for the entire sample. This may require that the collection tube be emptied between spins.
8. Before first use, add denatured ethanol to Buffers AW1 and AW2 according to the directions on the buffer bottles.
9. Transfer the column to a new collection tube. Add 250 μL Buffer AW1 to the column.
10. Centrifuge at 6000 x g for 1 minute or until all solution has passed through the membrane.
11. Add 250 μL Buffer AW2 to the column and centrifuge at 6000 x g for 1 minute.
12. (Optional) Transfer the column to a new collection tube.
13. Centrifuge at 20,000 x g (or maximum speed) for an additional 2 minutes or until all solution has passed through the membrane. No Buffer AW2 should remain in or on the spin column.
14. Transfer the column to a new, labeled collection tube.
15. Add 50–200 μL TE-4 or Buffer AE to the column depending on the expected quantity of DNA. Incubate for 10 minutes at room temperature.
16. Centrifuge at 6000 x g for 1 minute.
17. Transfer liquid from collection tube to a labeled storage tube. The elution step may be repeated with the same recovered sample to increase yield or concentrate the sample with centrifugal microfiltration.

6.5 Phenol-Chloroform-Isoamyl Alcohol Clean-Up of Epithelial and Sperm Cell Fractions

1. Add 0.5 mL phenol-chloroform-isooamyl alcohol to tube. Cap tube and vortex approximately 15 seconds until an emulsion forms.
2. Spin in centrifuge for 3-5 minutes at 10,000-15,000 x g to separate the two phases.
3. If necessary, repeat steps 1 and 2, using the aqueous layer (after it has been transferred to a fresh centrifuge tube) an additional 2-3 times, until the interface is clean and the aqueous layer is clear.
4. Proceed with cleaning and concentrating the DNA sample using the procedure in the Concentration and Purification of DNA with Membrane Filtration Devices chapter.

6.6 PrepFiler Automated DNA extraction Clean-Up of Epithelial and Sperm Cell Fractions

A. Setup Robot

1. Labware and consumables
   a) Ensure deck layout is set up appropriately.
   b) Place tubes of magnetic particles in back two wells of column one of the Magnetic particle holder.
   c) All DiTi racks must be full at start of script.
   d) Three additional full 1000 μL DiTi racks should be placed in rear shelf positions 5, 6, and 7.
   e) Position 4 must be empty at start of this script.

2. Reagent Volumes as listed – refer to the PrepFiler DNA Extraction chapter in the DNA SOP for appropriate volumes.

B. Automated DNA Extraction

1. Run appropriate script using EvoWare.
2. If necessary, enter sample names and information (this may be entered manually or imported) if the Scan labware feature is not enabled.
3. Ensure that “Scan labware” box is not checked if this feature is not being utilized. (If scan labware feature has been disabled within the script, box does not need to be unchecked.)
4. Confirm that the worktable is setup correctly.
   a) Confirm that labware is in the correct location and positioned correctly on the robot.
   b) After confirmation, mark each consumable on check-list as loaded or click loaded all.
   c) If desired, select Liquid Level Detection.
5. Begin automated extraction.

7 Records

A. This extraction method (Erase), samples, and control(s) shall be recorded in the case record.
   • DNA Extraction Worksheet (LAB-DNA-07) [optional]
   • Extraction/Lysis Worksheet (LAB-DNA-53) [optional]

B. For sperm fractions, the number of spermatozoa shall be recorded in the case record if a slide is made during extraction. This may be recorded as average per slide, average per field, or by scoring.
8 Literature References and Supporting Documentation


Valid-Method-WES-DNA-EraseSpermIsolation-2013-0510
Valid-Method-SYS-DNA-EraseSpermIsolation-PrepFiler-2015-0207
06 DNA QUANTIFICATION

DNA-06-01 DNA QUANTIFICATION – QUANTIFILER

1 Scope
The Quantifiler Human DNA Quantification Kit is designed to quantify the concentration of amplifiable human (and higher primate) DNA in a sample. The results from the quantification can aid in determining if sufficient amplifiable human DNA is present to proceed with STR analysis and how much sample to use in STR analysis. The DNA quantification assay combines two 5’ nuclease assays which are a target-specific human DNA assay and an internal PCR control (IPC) assay.

Note: This procedure may be performed using an approved robotic platform. Please refer to DNA Quantification – Quantifiler Using Tecan in the DNA SOP for instructions.

2 Related Chapters
DNA Quantification – Quantifier Using Tecan

3 Safety
Body fluids and extracts may contain infective agents. Beyond mild irritation of the skin or eyes, contact with the Quantifiler reagents does not usually cause acute health effects and are not known to cause any significant chronic health effects after prolonged exposure. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- AB Prism 7500
- *96 well optical reaction plates
- *optical adhesive covers/applicator
- Pipettors and pipette tips
- Vortex
- Microtubes – 0.5 mL
- Microcentrifuge
- Plate centrifuge or salad spinner
- Quantifiler Human DNA Quantification kit
- TE-4 buffer
- Glycogen (optional)
- Precision plate holder 7500
- 96-well base

*Only approved vendors for AB 7500 consumables may be used.

5 Standards, Controls, and Calibration
   A. Human DNA Standard in a three-fold dilution series with eight concentration points ranging from 50 ng/µL to 0.023 ng/µL must be applied in duplicate. These dilution
standards will be used to generate a standard curve in order to calculate the concentration of human DNA applied to each sample well.

B. One quantification blank consisting of 2 µL TE⁻⁴ instead of sample will be applied to one well of the plate as a background control for the quantification procedure.
   1. If a result is obtained in this well, it may indicate the need to run a background plate, check the assay setup, software setup, reagents or Real Time PCR system.
   2. The information from this plate may be used if a true negative is present.

C. The quantification of reagent blanks is optional.
   1. If a decision is made based on quantification as to whether or not samples are carried forward to DNA analysis, the reagent blank must undergo quantification or DNA typing must be completed on the reagent blank.

D. A quality control check is performed on new lots of Quantifiler Kits prior to use on casework samples.

6 Procedure

NOTE: It is important that the 96 well reaction plate not come into contact with the counter or any other surface. It should always be placed into a base plate until loaded into the AB Prism 7500. This is to minimize the interference caused by dust or other debris adhering to the bottom of the wells and interfering with the optical reading of the wells.

1. Prepare the Human DNA Standard dilution series:

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration (ng/µL)</th>
<th>Example Amounts</th>
<th>Minimum Amounts</th>
<th>Dilution Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std. 1</td>
<td>50.000</td>
<td>50 µL [200 ng/ µL stock] + 150 µL T₁₀E₀,1/glycogen buffer</td>
<td>10 µL [200 ng/ µL stock] + 30 µL T₁₀E₀,1 buffer</td>
<td>4X</td>
</tr>
<tr>
<td>Std. 2</td>
<td>16.700</td>
<td>50 µL [Std. 1] + 100 µL T₁₀E₀,1/glycogen buffer</td>
<td>10 µL [Std. 1] + 20 µL T₁₀E₀,1 buffer</td>
<td>3X</td>
</tr>
<tr>
<td>Std. 3</td>
<td>5.560</td>
<td>50 µL [Std. 2] + 100 µL T₁₀E₀,1/glycogen buffer</td>
<td>10 µL [Std. 2] + 20 µL T₁₀E₀,1 buffer</td>
<td>3X</td>
</tr>
<tr>
<td>Std. 4</td>
<td>1.850</td>
<td>50 µL [Std. 3] + 100 µL T₁₀E₀,1/glycogen buffer</td>
<td>10 µL [Std. 3] + 20 µL T₁₀E₀,1 buffer</td>
<td>3X</td>
</tr>
<tr>
<td>Std. 5</td>
<td>0.620</td>
<td>50 µL [Std. 4] + 100 µL T₁₀E₀,1/glycogen buffer</td>
<td>10 µL [Std. 4] + 20 µL T₁₀E₀,1 buffer</td>
<td>3X</td>
</tr>
<tr>
<td>Std. 6</td>
<td>0.210</td>
<td>50 µL [Std. 5] + 100 µL T₁₀E₀,1/glycogen buffer</td>
<td>10 µL [Std. 5] + 20 µL T₁₀E₀,1 buffer</td>
<td>3X</td>
</tr>
<tr>
<td>Std. 7</td>
<td>0.068</td>
<td>50 µL [Std. 6] + 100 µL T₁₀E₀,1/glycogen buffer</td>
<td>10 µL [Std. 6] + 20 µL T₁₀E₀,1 buffer</td>
<td>3X</td>
</tr>
<tr>
<td>Std. 8</td>
<td>0.023</td>
<td>50 µL [Std. 7] + 100 µL T₁₀E₀,1/glycogen buffer</td>
<td>10 µL [Std. 7] + 20 µL T₁₀E₀,1 buffer</td>
<td>3X</td>
</tr>
</tbody>
</table>

2. Prepare sufficient Master Mix by using the following amounts:
   a) Human Primer Mix 10.5 µL per reaction X # of samples
   b) PCR Reaction Mix 12.5 µL per reaction X # of samples

Additional reactions may be included in your calculations in order to provide excess volume to make up for pipetting loss.
3. Dispense 23 µL of the master mix into each reaction well.

4. Add 2 µL of the sample, standard, or TE to the appropriate well.

5. Seal the reaction plate with the optical adhesive cover using the applicator. Do not touch the portion of the adhesive cover that comes into contact with the reaction wells except with the applicator.

6. Centrifuge or spin the plate to remove any bubbles in the bottom of the wells.

7. Place the plate inside the AB Prism 7500 so that well A1 is in the upper-left corner.

8. Close the AB Prism 7500 tray.

9. On the plate tab/plate set up tab within the AB Prism 7500 software, verify correct detectors/targets and well identification for all wells to be quantified.

10. Confirm the thermal cycler conditions as a two-step cycle with the following parameters:
    a) 1 cycle of 95°C for 10 minutes (Stage 1)
    b) 40 cycles of 95°C for 15 seconds and 60°C for 1 minute (Stage 2)
    c) The 9600 emulation mode must be selected and the sample volume must be 25 µL, if applicable.

11. Save the plate document and click the Start/Start Run button. The plate will take approximately 1.5 hours to complete.

12. Analyze plate results.

13. Turn off AB Prism 7500.

7 Interpretation

7.1 Threshold cycle (C<sub>T</sub>)

A. The threshold cycle (C<sub>T</sub>) is when the fluorescent signal from the amplification product for each sample increases beyond the value of the threshold setting (which defines the level of detectable fluorescence).

B. The threshold cycle depends upon the starting template copy number and efficiency of the DNA amplification.

C. The number of cycles required for a sample to reach the threshold allows the software to determine DNA quantity by comparison to the standard curve. Therefore the lower the C<sub>T</sub> value, the greater the amount of template DNA in the original sample.

7.2 Standard Curve

A. The standard curve plots the C<sub>T</sub> of the standard dilutions versus the known quantity of the standards. This produces a regression line by calculating the best fit with the quantification standard data points.

1. The slope of this regression line indicates the PCR amplification efficiency for the assay. A slope of -3.3 indicates 100% amplification efficiency.

2. The R<sup>2</sup> value indicates the fit between the standard curve regression line and the individual C<sub>T</sub> data points of the quantification standard reactions. An R<sup>2</sup> value of 1 indicates a perfect fit between the regression line and the C<sub>T</sub> data points.
3. The Y-intercept value indicates the expected C_{T} values for a sample that is 1 ng/µL. This value can fluctuate over time and can be dependent on the instrument used and the reagent lot used.

B. The standard curve must be evaluated. Slope values typically fall between -2.9 and -3.3. Values outside of this range may be used with discretion.

C. The R^{2} value should be \geq 0.98. If the R^{2} value is less than 0.98, further investigation should be undertaken to determine:
   1. If the quantity values entered for quantification standards during plate document setup are correct.
   2. The serial dilutions of the quantification standards are correct.
   3. The loading of reactions for quantification standards is correct.
   4. If failure of reactions for the quantification standards occurred.

D. The range of Y-intercept values is specific to each laboratory and is determined by taking an average of Y-intercept values over time. If the Y-intercept value falls significantly outside of the range determined to be acceptable, the standard curve should be remade and samples should be requantified prior to amplification.

E. If, after confirming the values, one or more of the standards falls significantly outside of the standard curve, it may be omitted from the standard curve analysis. Up to 3 standards may be omitted without Technical Leader approval. Omitting more than 3 standards in a curve or both points of a single standard requires Technical Leader approval.

7.3 Internal PCR Control (IPC) amplification results

The purpose of the IPC (Internal PCR Control) is to distinguish between true negative sample results and reactions affected by inhibitors, assay setup and chemistry/equipment failures.

<table>
<thead>
<tr>
<th>Quantifiler Human (FAM)</th>
<th>Internal Positive Control (VIC)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplification</td>
<td>Amplification</td>
<td>Valid test result</td>
</tr>
<tr>
<td>No amplification</td>
<td>Amplification</td>
<td>True negative</td>
</tr>
<tr>
<td>No amplification</td>
<td>No amplification</td>
<td>Invalid test result</td>
</tr>
<tr>
<td>Amplification (low C_{T} and high ΔR_{n})</td>
<td>No amplification</td>
<td>Disregard IPC result</td>
</tr>
<tr>
<td>Amplification (high C_{T} and low ΔR_{n})</td>
<td>No amplification</td>
<td>Partial PCR inhibition</td>
</tr>
</tbody>
</table>

7.4 Quantification Values

A. Quantification values > 0 and < 50 ng/µL, as determined by Quantifiler, should be used to determine the amount of template DNA for STR amplification.

B. Quantification values that are \geq 50 ng/µL should be interpreted with discretion or diluted and requantified.
C. Quantification results for samples listed as “undetermined” will fall into two categories, an invalid test or a true negative.
   1. An invalid test is typically caused by complete inhibition. If an invalid test is obtained, the analyst may choose to do the following: proceed directly to STR analysis of the sample or perform additional clean-up of the sample. If the analyst chooses to proceed straight to STR analysis and no profile is obtained, then additional clean-up is required.
   2. A true negative contains no amplifiable DNA and therefore further analysis is not required as long as the extraction volume is \( \leq 50 \, \mu L \). True negative samples must have printed documentation in the case record to verify the sample is a true negative and not subject to inhibition. This does not apply to reagent blanks.

8 Records

A. Quantification results must be retained in the case record. A copy of the Quantitation Load Sheet (LAB-DNA-13) must be maintained in the laboratory. The standard curve evaluation must be documented.

B. DNA sample concentration shall be recorded in the case record.

9 Literature References and Supporting Documentation

Quantifiler Human DNA Quantification Kit User’s Manual. Applied Biosystems PN 4343895

DNA-06-02 DNA QUANTIFICATION – QUANTIFILER USING TECAN

1 Scope
The Quantifiler Human DNA Quantification Kit is designed to quantify the concentration of amplifiable human (and higher primate) DNA in a sample. The results from the quantification can aid in determining if sufficient amplifiable human DNA is present to proceed with STR analysis and how much sample to use in STR analysis.

2 Related Chapters
DNA Quantification – Quantifiler
DNA Amplification – Investigator 24plex QS Tecan
DNA Amplification - Investigator 24plex QS

TE-4 Buffer

3 Safety
Body fluids and extracts may contain infective agents. Beyond mild irritation of the skin or eyes, contact with the Quantifiler reagents does not usually cause acute health effects and are not known to cause any significant chronic health effects after prolonged exposure. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Tecan Freedom EVO Platform Combination System
- Quantifiler reagent block
- *50µL Disposable tips with filter (DiTis)
- *200µL DiTis with filter
- 100mL disposable trough
- VWR 5mL graduated transport tube
- AB Prism 7500
- *96 well optical reaction plates
- *Optical adhesive covers/ applicator
- Pipettors and pipette tips
- Vortex
- Microtubes
- Microcentrifuge
- Plate centrifuge or salad spinner
- Quantifiler Human DNA Quantification kit
- TE-4 buffer
- Precision plate holder 7500
- 96-well base

*Only approved vendors for AB 7500 and Tecan consumables may be used.
5 Standards, Controls, and Calibration

A. One quantification blank consisting of TE^-4 buffer will be placed into a microcentrifuge tube in the appropriate location on the carrier.

B. The quantification of reagent blanks is not optional when using the robotic platform.

C. A quality control check is performed on new lots of Quantifiler kits prior to use on casework.

D. Human DNA Standard in a three-fold dilution series with eight concentration points ranging from 50 ng/µL to 0.023 ng/µL must be applied in duplicate. These dilution standards will be used to generate a standard curve in order to calculate the concentration of human DNA applied to each sample well.

6 Procedure

NOTE: It is important that the 96 well reaction plate not come into contact with the counter or any other surface. It should always be placed into a base plate until loaded into the AB Prism 7500. This is to minimize the interference caused by dust or other debris adhering to the bottom of the wells and interfering with the optical reading of the wells.

6.1 Pre-Run Procedure

1. Ensure there is sufficient degassed DI water for the system.

2. Run Maintenance scripts as appropriate to prime the system and to eliminate bubbles in the pressure lines.
   a) A system flush must be run on the day of use prior to casework.
   b) If the robot was subsequently shut off on the same day daily startup was run, perform the Combination_system_Flush with a flush volume of at least 40mL.
   c) If in between applications, perform the Combination_system_Flush2 with a flush volume of at least 40 mL.

6.2 Deck Set Up

1. Select the appropriate script (such as QuantifilerHuman_tubes Combo).

2. Prepare the worktable following the layout provided by the software in the Extended View. Labware necessary includes two racks of 200µL DiTis, four racks of 50ul DiTis, and a 96 well PCR plate.

3. Ensure reagents in the Quantifiler kit contain sufficient volume for the run.

4. Using the AB Reagent block (at 2-8°C or as validated) specific for the Quantifiler Kit, follow the layout provided by the software and load the block with the corresponding reagents and tubes (Human Primer Mix vial, PCR Reaction Mix vial, and empty VWR vial for preparation of the master mix).

5. The Tecan Freedom EVO is capable of creating the standard dilution series for Quantifiler. Automated standard dilution series creation is the default setting in the software but an option is available to allow the user to use a manually or prior created series and place it in the appropriate locations on the block.

6. If previously prepared standards are being used or if preparing the standards by hand (refer to the DNA Quantification – Quantifiler chapter in the DNA SOP), place the standard dilutions into the appropriate block locations.
7. If the robot is to prepare the standard dilution series, place the Human DNA Standard (stock) and 8 empty 1.5 mL tubes into the appropriate block locations.

8. Add appropriate volume of TE4 to the TE4 trough (minimum volume of 6mL) and place it into the appropriate location on the worktable. (This step can be skipped if a previously created standard dilution series is being used.)

9. Place the DNA extracts in the appropriate locations. Remember to include a NTC (no template control) as a sample.

10. Open all tubes and vials and secure the caps (as appropriate). Label caps or keep track of which caps go with which tubes.

11. Ensure the safety front panel is closed by pressing on each of the locks on the front of the robot.

12. Verify that all prepared reagents and labware are ready and in the appropriate locations. Ensure that all tubes are open and that all obstacles are removed from the deck.

6.3 Running the Script

1. Click the “Run” button. Respond to query boxes as necessary.
   a) If necessary, define the number of samples plus the TE4 blank and starting labware. The start position should be 1 and the maximum depends on the number of samples being processed with a limit of 80. (This number excludes the standards.)
   b) Record reagent information in the software if necessary. Ensure the Prepare Standards box is checked if the robot will be preparing the standard dilution series. (Uncheck the box if using previously made standards.)
   c) Verify that all labware was loaded on the worktable and confirm by clicking Loaded for each item or Loaded All. Highlight any particular item to locate it on the worktable figure displayed.
   d) The PosID will scan the labware barcodes. If not using barcodes, click Ignore.
   e) Import a sample file or click Edit to enter the sample information.

2. Click “Run” to begin the liquid handling procedure.

6.4 Post-Run Procedure

1. Upon completion, click “Run” to end the process. Exit the software first then shut down the robot by pressing the ON button (if needed).

2. Remove and properly store all remaining reagents and DNA extracts.

3. Verify that liquid transfer steps were completed successfully by inspecting the 96-well plate.

4. Seal the reaction plate with the optical adhesive cover using the applicator. Do not touch the portion of the adhesive cover that comes into contact with the reaction wells except with the applicator.

5. Centrifuge or spin the plate to remove any bubbles in the bottom of the wells.

6. Follow the remainder of the procedure for the Quantifiler kit in the DNA Quantification – Quantifiler chapter.
7. The data generated by the 7500 may be used by the robot during the Normalization and PCR setup program (refer to the DNA Amplification – Investigator 24plex QS Using Tecan chapter).

7 Records

A. Quantification results must be retained in the case record. A copy of an appropriate Quantitation Load Sheet (LAB-DNA-13) must be maintained in the laboratory. The standard curve evaluation must be documented.

B. DNA sample concentration shall be recorded in the case record.

8 Literature References and Supporting Documentation


Freedom EVO Operating Manual (Tecan PN 30018631.03 version 4.0).

HID EVOlution – Combination System Application Manual (DNA Extraction and qPCR/STR Setup) (Tecan, 395967 current version).
DNA-06-03 TOTAL HUMAN AND MALE DNA QUANTIFICATION – QUANTIFILER DUO

1 Scope

Forensic Scientists routinely encounter a variety of challenging biological samples, many of which contain mixtures of male and female DNA, and/or have been exposed to environmental insults. Determining the ratio of male to female DNA present in a mixture, and determining the presence of inhibitors helps the analysts select the most appropriate STR chemistry. The Quantifiler Duo DNA Quantification Kit provides a quantitative and qualitative assessment of total human and human male DNA in a single assay. This can help guide selection of the optimal STR chemistry: Investigator 24plex QS (autosomal STR), Yfiler/Y23 (Y-STR), or MiniFiler (optimal for degraded or inhibited samples), and provides the appropriate amount of DNA to amplify in STR analysis.

The Quantifiler Duo Primer Mix contains primer pairs and probes that simultaneously amplify the ribonuclease P RNA component H1(RPPH1) human target and the sex determining region Y (SRY) male-specific target. A third primer and probe system along with an Internal PCR Control (IPC) template has been formulated into this reagent creating a triplex reaction. By amplifying all reactions with the IPC in a single well, the Quantifiler Duo kit enables detection of PCR inhibitors (samples may require additional purification procedures) and allows for the identification of samples that do not contain human DNA. This system also enables detection of minute quantities of human male DNA and provides a reliable estimate of the male to female DNA mixture ratio.

Note: This procedure may be performed using an approved robotic platform.

2 Related Chapters

TE-4 Buffer

3 Safety

Body fluids and extracts may contain infective agents. Beyond mild irritation of the skin or eyes, contact with the Quantifiler Duo reagents does not usually cause acute health effects and are not known to cause any significant chronic health effects after prolonged exposure. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials

- Applied Biosystems 7500 Real-Time PCR System and Sequence Detection System software
- *96 well optical reaction plates
- *Optical adhesive covers/applicator
- Pipettors and pipette tips
- Vortex
- Microtubes
- Microcentrifuge
- Plate centrifuge or salad spinner
- Quantifiler Duo DNA Quantification kit
- TE-4 buffer
- Precision plate holder 7500
- 96-well base

*Only approved vendors for AB 7500 consumables may be used.

5 Standards, Controls, and Calibration

A. Quantitation Duo DNA Standard in a three-fold dilution series with eight concentration points ranging from 50 ng/µL to 0.023 ng/µL must be applied in duplicate. These dilution standards are prepared using the Quantifiler Duo Dilution Buffer or TE-4 as diluent, and are used to generate standard curves for total human and male DNA. The total human and male standard curves allow for the calculation of total human and male component in each sample well.

B. One quantification blank consisting of 2 µL of Duo Dilution Buffer or TE-4 instead of sample will be applied to one well of the plate as a background control for the quantification procedure.
   1. If a result is obtained in this well, it may indicate the need to run a background plate, check the assay setup, software setup, reagents or Real Time PCR system.
   2. The information from this plate may be used if a true negative is present.

C. The quantification of reagent blanks is optional.
   1. If a decision is made based on quantification as to whether or not samples are carried forward to DNA analysis, the reagent blank must undergo quantification or DNA typing must be completed on the reagent blank.

D. A quality control check is performed on new lots of Quantifiler Duo prior to use on casework samples.

6 Procedure

NOTE: It is important that the 96 well reaction plate not come into contact with the counter or any other surface. It should always be placed into a base plate until loaded into the AB Prism 7500. This is to minimize the interference caused by dust or other debris adhering to the bottom of the wells and interfering with the optical reading of the wells.

1. Prepare the Quantitation Duo DNA Standard dilution series.

   The following table shows an example of the standards dilution series with concentrations ranging from 50 ng/µL to 0.023 ng/µL (23 pg/µL).

   a) When 2.0 µL of a sample at the lowest concentration (23 pg/µL) is loaded in a reaction, the well contains approximately 7 diploid human genome equivalents.

   b) These equivalents correspond to approximately 14 copies of the Duo Human target locus and approximately 7 copies of the Duo Male target locus (Y chromosome loci are haploid):
2. Prepare sufficient Master Mix by using the following amounts:
   a) *Duo Primer Mix* 10.5 µL per reaction X # of samples
   b) *Duo PCR Reaction Mix* 12.5 µL per reaction X # of samples

   *Additional reactions may be included in your calculations in order to provide excess volume to make up for pipetting loss.*

3. Dispense 23 µL of the master mix into each reaction well.

4. Add 2 µL of the sample, standard, or Duo Dilution buffer/TE-4 to the appropriate well.

5. Seal the reaction plate with the optical adhesive cover using the applicator. Do not touch the portion of the adhesive cover that comes into contact with the reaction wells except with the applicator.

6. Centrifuge or spin the plate to remove any bubbles in the bottom of the wells.

7. Place the plate inside the AB Prism 7500 so that well A1 is in the upper-left corner.

8. Close the AB Prism 7500 door.

9. On the plate tab/plate set up tab within the AB Prism 7500 software, verify correct detectors/targets and well identification for all wells to be quantified.
10. Confirm the thermal cycler conditions as a three-step cycle (Note: a hold step of 50°C for 2 minutes has been added for the Duo Kit) with the following parameters:
   a) 1 cycle of 50°C for 2 minutes (Stage 1)
   b) 1 cycle of 95°C for 10 minutes (Stage 2)
   c) 40 cycles of 95°C for 15 seconds and 60°C for 1 minute (Stage 3)
   d) The sample volume should be 25 µL and 9600 emulation mode must be selected, if applicable.

11. Save the plate document and click the Start/Start Run button. The plate will take approximately 1.5 hours to complete.

12. Analyze plate results.

13. Turn off AB Prism 7500.

7 Interpretation

7.1 Threshold cycle (Cₜ)

A. The threshold cycle (Cₜ) is when the fluorescent signal from the amplification product for each sample increases beyond the value of the threshold setting (which defines the level of detectable fluorescence).

B. The threshold cycle depends upon the starting template copy number and efficiency of the DNA amplification.

C. The number of cycles required for a sample to reach the threshold allows the software to determine DNA quantity by comparison to the standard curve. Therefore the lower the Cₜ value, the greater the amount of template DNA in the original sample.

7.2 Standard Curves

A. The standard curve is a graph of the Cₜ of quantification standard reactions plotted against the starting quantity of the standards. The software calculates the regression line by calculating the best fit with the quantification standard data points.

1. The slope of this regression line indicates the PCR amplification efficiency for the assay. A slope of close to -3.3 indicates 100% amplification efficiency.

2. The R² value indicates the fit between the standard curve regression line and the individual Cₜ data points of the quantification standard reactions. An R² value of 1 indicates a perfect fit between the regression line and the Cₜ data points.

3. The Y-intercept value indicates the expected Cₜ values for a sample that is 1ng/µL. This value can fluctuate over time and can be dependent on the instrument used and the reagent lot used.

B. The standard curves must be evaluated. Slope values typically fall between -3.0 and -3.6 with the Quantifiler Duo kit. If the slope varies beyond this range, check the assay setup, software setup, reagents or Real Time PCR system. Values outside of this range may be used with discretion.

C. The R² value should be ≥ 0.98. If the R² value is less than 0.98, further investigation should be undertaken to determine:

1. If the quantity values entered for quantification standards during plate document setup are correct.
2. The serial dilutions of the quantification standards are correct.
3. The loading of reactions for quantification standards is correct.
4. If failure of reactions for the quantification standards occurred.

D. The range of Y-intercept values is specific to each laboratory and is determined by taking an average of Y-intercept values over time. If the Y-intercept value falls significantly outside of the range determined to be acceptable, the standard curve should be remade and samples should be requantified prior to amplification.

E. If, after confirming the values, one or more of the standards falls significantly outside of the standard curve, it may be omitted from the standard curve analysis. Up to 3 standards may be omitted without Technical Leader approval. Omitting more than 3 standards in a curve or both points of a single standard requires Technical Leader approval.

### 7.3 Internal PCR Control (IPC) amplification results

**Valid results:** obtained when amplification is observed in both the Duo Human/Duo Male and the Duo IPC detectors.

<table>
<thead>
<tr>
<th>Duo Human (VIC dye) and/or Duo Male (FAM Dye)</th>
<th>Duo IPC (NED Dye)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No amplification</td>
<td>Amplification</td>
<td>Negative result – no human DNA detected</td>
</tr>
<tr>
<td>No amplification</td>
<td>No amplification</td>
<td>Invalid result</td>
</tr>
<tr>
<td>Amplification (low CT and high ΔRn)</td>
<td>No amplification or CT higher than 31</td>
<td>IPC result inconclusive</td>
</tr>
<tr>
<td>Amplification (high CT and low ΔRn)</td>
<td>No amplification or CT higher than 31</td>
<td>PCR inhibition</td>
</tr>
</tbody>
</table>

### 7.4 Quantification values

A. Quantification values > 0 and < 50 ng/µL, as determined by Quantifiler Duo, should be used to determine the amount of template DNA for STR amplification.

B. Quantification values that are ≥50 ng/µL should be interpreted with discretion or diluted and requantified.

C. Quantification results for samples listed as “undetermined” will fall into two categories, an invalid test or a true negative.

1. An invalid test is typically caused by complete inhibition. If an invalid test is obtained, the analyst may choose to do the following: proceed directly to STR analysis of the sample or perform additional clean-up of the sample. If the analyst chooses to proceed straight to STR analysis and no profile is obtained, then additional clean-up is required.

2. True negative samples must have printed documentation in the case record to verify the sample is a true negative and not subject to inhibition. True negative samples will not need to be analyzed using autosomal STR analysis. Y-STR analysis may be performed as appropriate.
7.5 Calculating Male:Female DNA Ratio

A. The Quantifiler Duo Quantification Kit determines the total human and male DNA in a sample. From these values the female contribution can be deduced and the male to female ratio can be calculated using the following equation:

\[
\text{Male:Female Ratio} = 1: \frac{\text{Human DNA} - \text{Male DNA}}{\text{Male DNA}}
\]

All quantities in the above equation are ng/µL.

B. The ratio determines the extent of the mixture and is useful in determining whether to proceed with autosomal STR (Investigator 24plex QS), or Y-STR (Yfiler/Y23) analysis. The maximum ratio at which meaningful autosomal STR analysis can be expected is approximately 1:30 for Investigator 24plex QS kit. Male:Female ratios beyond established thresholds are usually unable to detect the male component using autosomal STR analysis.

8 Records

A. Quantification data must be retained in the case record. A copy of the Quantitation Load Sheet (LAB-DNA-13) must be maintained in the laboratory. The standard curve (total human and male) evaluation must be documented.

B. DNA sample concentrations shall be recorded in the case record.

9 Literature References and Supporting Documentation


07  PCR AMPLIFICATION SYSTEM

DNA-07-01  DNA AMPLIFICATION – INVESTIGATOR 24PLEX QS USING TECAN

1  Scope
This protocol uses Investigator 24plex QS PCR Amplification Kit. This kit contains primers that are labeled with a light sensitive dye.

2  Related Chapters
DNA Amplification - Investigator 24plex QS

3  Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4  Equipment and Materials
- Tecan Freedom EVO Platform Combination System
- Amplification reagent block
- *50µL Disposable tips with filter (DiTis)
- *200µL DiTis with filter
- 100mL disposable trough
- VWR 5ml graduated transport tube
- *96-well reaction plates
- *Plate cover
- Calculator
- Microcentrifuge
- Plate centrifuge or salad spinner
- Microcentrifuge tubes, 1.5 mL
- Pipette tips
- Pipettors, adjustable
- Tube decapper
- Vortex
- Thermal cycler
- *Investigator 24plex QS Amplification Kit
- TE\(^{-4}\) buffer

*Only approved vendors for consumables may be used.
5 Standards, Controls, and Calibration

A. Qiagen Control DNA 9948 must be included with each amplification set. This amplification positive control is supplied with the associated kit.

B. An amplification negative control must be included with each amplification set. This negative control will consist of all amplification reagents with TE$^{-4}$ added in place of sample DNA.

6 Procedure

Note: Almost every step in the Normalization procedure is based on the information in the export file created from the Quantifiler data, so verify that it is completely correct before proceeding. The procedure is also directly linked to the specific rows and columns in this file as originally created. Extreme caution must be used if the layout of this file is changed in any way.

Refer to the DNA Amplification – Investigator 24plex QS chapter in the DNA SOP for further information on Investigator 24plex QS.

6.1 Pre-Run Procedure

1. Ensure there is sufficient degassed DI water for the system.

2. Run Maintenance scripts as appropriate to prime the system and to eliminate bubbles in the pressure lines.
   a) ComboDaily_Startup must be run on the day of use.
   b) If the robot was subsequently shut off on the same day daily startup was run or if bubbles are observed in the lines, perform the ComboFlush with a flush volume of at least 40mL.

6.2 Deck Set Up

1. Select the appropriate script.

2. Prepare the worktable following the layout provided by the software in the Extended View. Labware necessary includes two racks of 200µL DiTis, four racks of 50µl DiTis, and 96-well PCR plates.

3. Ensure the AB Reagent block (2-8°C or as validated) specific for the Amplification Kit is used. Following the layout provided by the software, load the block with the corresponding reagents and tubes (Primer Set, Master Mix, and VWR vial). If necessary, prepare the amplification master mix by hand according to the script and place it in the VWR vial after thorough mixing. If necessary, dilute the Positive Control and place the dilution on the block in the appropriate location. Otherwise, place the 9948 stock solution provided in the kit onto the workstation.

4. Add a minimum volume of 60 µL TE$^{-4}$ to a 1.5 mL tube (negative control) and place it in the corresponding location on the block.

5. Add appropriate volume of TE$^{-4}$ to the TE$^{-4}$ trough (minimum volume of 25mL) and place it into the appropriate location on the worktable.

6. Place the DNA extracts in the appropriate locations.

7. Open all reagent tubes and vials and secure the caps (as appropriate). Label caps or keep track of which caps go with which tubes.

8. Ensure the safety front panel is closed by pressing on each of the locks on the front of the robot.
6.3 Running the Script

1. Verify that all prepared reagents and labware are ready and in the appropriate locations. Ensure that all tubes are open and that all obstacles are removed from the deck.

2. Click the “Run” button. Respond to query boxes as necessary.
   a) Define the number of samples. The maximum depends on the number of samples being processed with a limit of 88. (This number excludes the controls.)
   b) Record reagent information in the software if necessary.
   c) Verify that all labware was loaded on the worktable and confirm by clicking Loaded for each item or Loaded All. Highlight any particular item to locate it on the worktable figure displayed.
   d) The PosID will scan the labware barcodes if the scan labware box is checked. If not using barcodes, un-check the scan labware box.
   e) Import a sample file or click Edit to enter the sample information. (The sample name must match the sample name in the quantification export file generated by the 7500 software.)
   f) Import the results file(s) generated by the 7500 software using the […] button.
   g) The sample normalization window will show how many samples require processing. Click on View to see the processing information and make adjustments if necessary. Here the DNA template amount may be adjusted depending on the CE instrument to be used and its validation data.

3. Click “Run” to begin the liquid handling procedure.

6.4 Post-Run Procedure

1. Upon completion, click “Run” to end the process. Exit the software first then shut down the robot (if needed) by pressing the ON button.

2. Remove and properly store all remaining reagents and DNA extracts.

3. Verify that liquid transfer steps were completed successfully by inspecting the 96-well plate.

4. Seal the amplification plate with an appropriate cover.

5. Vortex the plate to ensure adequate mixing of sample then centrifuge or spin the plate to remove any bubbles in the bottom of the wells.

6. Place the amplification plate into the thermal cycler with a compression pad (if needed) and start the appropriate program.

7. After amplification, remove the plate from the thermal cycler and store away from light. Store amplified samples at 2-8°C for short periods. For longer periods, store amplified samples frozen.

7 Interpretation

None
8 **Records**

A. The amount of DNA extract amplified and/or the dilution scheme shall be recorded in the case record.

B. Sample and control locations on the thermal cycler shall be recorded on the appropriate amplification worksheet.
   - Amplification Worksheet (LAB-DNA-08) [optional]

C. Amplification kit lot number and expiration date, robot identification, as well as the thermal cycler identification number shall be recorded on the appropriate amplification worksheet.

9 **Literature References and Supporting Documentation**


Freedom EVO Operating Manual (current version).

DNA-07-02 DNA AMPLIFICATION – YFILER USING TECAN

1 Scope
This protocol uses the AmpF/STR Yfiler PCR Amplification Kit. This kit contains primers that are labeled with a light sensitive dye.

2 Related Chapters
DNA Amplification – Yfiler

TE-4 Buffer

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Tecan Freedom EVO
- Amplification reagent block
- *50µL Disposable tips with filter (DiTis)
- *200µL DiTis with filter
- 100mL disposable trough
- VWR 5mL graduated transport tube
- *96-well reaction plates
- *Plate cover
- Calculator
- Microcentrifuge
- Plate centrifuge or salad spinner
- Microcentrifuge tubes, 1.5 mL
- Pipette tips
- Pipettors, adjustable
- Tube decapper
- Vortex
- Thermal cycler
- *AmpF/STR Yfiler PCR Amplification kit
- TE-4 buffer
*Only approved vendors for consumables may be used.

5 Standards, Controls, and Calibration
A. AmpF/STR Control DNA 007 must be included with each amplification set. This amplification positive control is supplied with the kit.
B. AmpF/STR Control DNA 9947A is a female negative control supplied with the kit. This control needs to be run only with the Quality Control of the kit-not with each amplification
set. The robotic script utilized requires that this control also be placed in the reagent block with each amplification set to avoid an error.

C. An amplification negative control must be included with each amplification set. This negative control will consist of all amplification reagents with TE\(^{-4}\) added in place of sample DNA.

### 6 Procedure

**Note:** Almost every step in the Normalization procedure is based on the information in the export file created from the Quantification data, so verify that it is completely correct before proceeding. The procedure is also directly linked to the specific rows and columns in this file as originally created. Extreme caution must be used if the layout of this file is changed in any way.

Refer to the *DNA Amplification – Yfiler* chapter for further information on Yfiler amplification.

#### 6.1 Pre-Run Procedure

1. Ensure there is sufficient degassed DI water for the system.
2. Run Maintenance scripts as appropriate to prime the system and to eliminate bubbles in the pressure lines. Perform the ComboFlush with a volume of at least 40 mL.

#### 6.2 Deck Set Up

1. Select the appropriate script.
2. Prepare the worktable following the layout provided by the software in the Extended View. Labware necessary includes two racks of 200µL DiTis, four racks of 50µl DiTis, and 96 well PCR plates.
3. Ensure the amplification reagent block is used. Following the layout provided by the software, load the block with the corresponding reagents and tubes. If necessary, dilute the Positive Control and place the dilution on the block in the appropriate location. Otherwise, place the 9947A stock solution and 007 provided in the kit onto the amplification reagent block.
4. Add a minimum volume of 60µL TE\(^{-4}\) to a 1.5mL tube (negative control) and place it in the corresponding location on the block.
5. Add appropriate volume of TE\(^{-4}\) to the TE\(^{-4}\) trough (minimum volume of 25mL) and place it into the appropriate location on the worktable.
6. Place the DNA extracts in the appropriate locations.
7. Open all reagent tubes and vials and secure the caps (as appropriate). Label caps or keep track of which caps go with which tubes.
8. Ensure the safety front panel is closed by pressing on each of the locks on the front of the robot.

#### 6.3 Running the Script

1. Verify that all prepared reagents and labware are ready and in the appropriate locations. Ensure that all tubes are open and that all obstacles are removed from the deck.
2. Click the “Run” button. Respond to query boxes as necessary.
   a) Define the number of samples. The maximum depends on the number of samples being processed with a limit of 88. (This number excludes the controls.)
   b) Record reagent information in the software if necessary.
   c) Verify that all labware was loaded on the worktable and confirm by clicking Loaded for each item or Loaded All. Highlight any particular item to locate it on the worktable figure displayed.
   d) The PosID will scan the labware barcodes if the scan labware box is checked. If not using barcodes, un-check the scan labware box.
   e) Import a sample file or click Edit to enter the sample information. (The sample name must match the sample name in the quantification export file generated by the 7500 software.)
   f) Import the results file(s) generated by the 7500 software using the [...] button.
   g) The sample normalization window will show how many samples require processing. Click on View to see the processing information and make adjustments if necessary. Here the DNA template amount may be adjusted depending on the CE instrument to be used and its validation data.

3. Click “Run” to begin the liquid handling procedure.

6.4 Post-Run Procedure

1. Upon completion, click “Run” to end the process. Exit the software first then shut down the robot (if needed) by pressing the ON button.
2. Remove and properly store all remaining reagents and DNA extracts.
3. Verify that liquid transfer steps were completed successfully by inspecting the 96-well plate.
4. Seal the amplification plate with an appropriate cover.
5. Centrifuge or spin the plate to remove any bubbles in the bottom of the wells.
6. Place the amplification plate into the thermal cycler with a compression pad (if needed) and start the appropriate program. (Refer to the DNA Amplification – Yfiler chapter.)
7. After amplification, remove the plate from the thermal cycler and store away from light. Store amplified samples at 2-8°C for short periods. For longer periods, store amplified samples frozen.

7 Interpretation

None

8 Records

A. The amount of DNA extract amplified and/or the dilution scheme shall be recorded in the case record.

B. Sample and control locations on the thermal cycler shall be recorded on the appropriate amplification worksheet.
   • Amplification Worksheet (LAB-DNA-08) [optional]
C. Amplification kit lot number and expiration date, unique equipment identification, as well as the thermal cycler identification number shall be recorded on the appropriate amplification worksheet.

9 Literature References and Supporting Documentation

Freedom EVO Operating Manual (current version).
DNA-07-03 DNA AMPLIFICATION – MINIFILER

1 Scope
This protocol uses the AmpF/STR MiniFiler PCR Amplification and Typing Kit. This kit contains primers that are labeled with a light sensitive dye.

2 Related Chapters

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Calculator
- Microcentrifuge
- Microcentrifuge tubes
- Pipette tips
- Pipettors, adjustable
- Tube decapper
- Vortex
- Thermal cycler
- Amplification tubes, 0.2 mL
- Tube rack
- *AmpF/STR MiniFiler PCR Amplification and Typing Kit
- TE-4 buffer
- 96 well amplification tray

*Only approved vendors for consumables may be used.

5 Standards, Controls, and Calibration

A. AmpF/STR Control DNA 007 must be included with each amplification set. This amplification positive control is supplied with the kit.

B. An amplification negative control must be included with each amplification set. This negative control will consist of all amplification reagents with TE-4 added in place of sample DNA.
## 6 Procedure

1. Thermal cycler parameters shall be used as described in the chart below.

   ### Times and Temperatures for MiniFiler kit

<table>
<thead>
<tr>
<th>Initial Incubation Step</th>
<th>30 cycles each</th>
<th>Final Extension</th>
<th>Final Step</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Melt</td>
<td>Anneal</td>
<td>Extend</td>
</tr>
<tr>
<td>95°C</td>
<td>94°C</td>
<td>59°C</td>
<td>72°C</td>
</tr>
<tr>
<td>11 min. hold</td>
<td>20 sec.</td>
<td>2 min.</td>
<td>1 min.</td>
</tr>
</tbody>
</table>

2. Label PCR amplification tubes/plates with appropriate information.

3. Vortex PCR master mix and primer set and spin tubes briefly in a microcentrifuge to remove any liquid from the caps.

4. Prepare Reaction Mix by adding the following volumes:
   a) **Master Mix** 10 µL \( \times \) # of samples
   b) **Primer Set** 5 µL \( \times \) # of samples

   **Note:** If necessary include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers.

5. Mix by vortexing for approximately 5 seconds

6. Spin briefly in microcentrifuge to remove liquid from cap

7. Dispense 15 µL of master mix into each amplification tube/well.

8. Add appropriate amount of sample DNA to the appropriate amplification tubes/wells not to exceed 10 µL in total sample volume added. For extracts with high concentrations of DNA, dilute with TE\(^{-4}\) buffer to attain the appropriate concentration.

9. For each amplification set, dilute an appropriately validated amount of positive control (0.1 – 1.0 ng/µL) with TE\(^{-4}\) buffer and add to one of the amplification tubes/wells containing the reaction mix.

10. For each amplification set, include a negative control by adding 10 µL of TE\(^{-4}\) to one of the amplification tubes/wells containing the reaction mix. For manual preparation, the negative control should be the last tube set up.

11. Add a compression pad (if needed), place the amplification tubes/plate into the thermal cycler and start the appropriate program.

12. After amplification, remove the tubes/plate from the thermal cycler and store away from light. Store amplified samples at 2-8°C for short periods. For longer periods, store amplified samples frozen.

## 7 Records

A. The amount of DNA extract amplified shall be recorded in the case record.

B. Sample and control locations on the thermal cycler shall be recorded on the Amplification Worksheet (LAB-DNA-08).
C. Amplification kit lot number and expiration date, as well as the thermal cycler identification number shall be recorded on the Amplification Worksheet (LAB-DNA-08).

8 Literature References and Supporting Documentation


DNA-07-04 DNA AMPLIFICATION – YFILER

1 Scope
This protocol uses the AmpF/STR Yfiler PCR Amplification Kit. This kit contains primers that are labeled with a light sensitive dye. This procedure may be performed using an approved robotic platform.

2 Related Chapters
DNA Amplification – Yfiler Using Tecan

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Calculator
- Microcentrifuge
- Microcentrifuge tubes
- Pipette tips
- Pipettors, adjustable
- Tube decapper
- Vortex
- Thermal cycler
- Amplification tubes, 0.2 mL
- Compression pad
- *96 well PCR plate
- Tube rack
- *AmpF/STR Yfiler PCR Amplification Kit
- TE-4 buffer

*Only approved vendors for consumables may be used.

5 Standards, Controls, and Calibration
A. AmpF/STR Control DNA 007 must be included with each amplification set. This amplification positive control is supplied with the kit. The amount of positive control template amplified should be determined by internal validation studies for each instrument.

B. AmpF/STR Control DNA 9947A is a female negative control supplied with the kit. This control needs to be run only with the Quality Control of the kit - not with each amplification set.

C. An amplification negative control must be included with each amplification set. This negative control will consist of all amplification reagents with TE-4 added in place of sample DNA.
6 Procedure

1. Thermal cycler parameters shall be used as described in the chart below.

<table>
<thead>
<tr>
<th>Initial Incubation Step</th>
<th>30 cycles each</th>
<th>Final Extension</th>
<th>Final Step</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Melt</td>
<td>Anneal</td>
<td>Extend</td>
</tr>
<tr>
<td>95°C</td>
<td>94°C</td>
<td>61°C</td>
<td>72°C</td>
</tr>
<tr>
<td>11 min. hold</td>
<td>1 min.</td>
<td>1 min.</td>
<td>1 min.</td>
</tr>
</tbody>
</table>

2. Label PCR amplification tubes/plates with appropriate information.

3. Vortex PCR reaction mix, primer set, and AmpliTaq Gold and spin tubes briefly in a microcentrifuge to remove any liquid from the caps.

4. Prepare master mix by adding the following volumes to a microcentrifuge tube:
   a) Reaction Mix 9.2 µL X # of samples
   b) AmpliTaq Gold 0.8 µL X # of samples
   c) Primer Set 5.0 µL X # of samples

   **Note:** If necessary include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers.

5. Mix by vortexing for approximately 5 seconds

6. Spin briefly in microcentrifuge to remove liquid from cap

7. Dispense 15 µL of master mix into each amplification tube/well.

8. Add appropriate amount of sample DNA to the appropriate amplification tubes/wells not to exceed 10 µL in total sample volume added. For samples with high concentrations of DNA, dilute with TE-4 buffer to attain the appropriate concentration.

9. For each amplification set, include a positive control by adding 0.5 ng to 1.0 ng of control DNA 007 to one of the amplification tubes/wells containing the master mix. TE-4 will be used to attain the appropriate dilution if 1.0 ng is not amplified.

10. For each amplification set, include a negative control by adding 10 µL of TE-4 to one of the amplification tubes/wells containing the master mix. The negative control should be the last tube set up.

11. Add compression pad (if needed) and place the amplification tubes/plates into the thermal cycler and start the appropriate program.

12. After amplification, remove the tubes/plate from the thermal cycler and store away from light. Store amplified samples at 2-8°C for short periods. For longer periods, store amplified samples frozen.

7 Interpretation

None
8 Records

A. The amount of DNA extract amplified shall be recorded in the case record.

B. Sample and control locations on the thermal cycler shall be recorded on the Amplification Worksheet (LAB-DNA-08).

C. Amplification kit lot number and expiration date, as well as the thermal cycler identification number shall be recorded on the Amplification Worksheet (LAB-DNA-08).

9 Literature References and Supporting Documentation


DNA-07-05 DNA AMPLIFICATION – Y23

1 Scope
This protocol uses the Promega Y23 PCR Amplification Kit. This kit contains primers that are labeled with a light sensitive dye.

2 Related Chapters
TE-4 Buffer

3 Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Calculator
- Microcentrifuge
- Microcentrifuge tubes
- Pipette tips
- Pipettors, adjustable
- Tube decapper
- Vortex
- Thermal cycler
- Amplification tubes, 0.2 mL
- Compression pad
- Strip caps or film
- *96 well PCR plate
- Tube rack
- *Promega PowerPlex Y23 amplification kit
- TE-4 buffer

*Only approved vendors for consumables may be used.

5 Standards, Controls, and Calibration
A. 2800M Control DNA must be included with each amplification set. This amplification positive control is supplied with the kit. The amount of positive control template amplified should be determined by internal validation studies for each instrument.

B. An amplification negative control must be included with each amplification set. This negative control will consist of all amplification reagents with TE-4/amplification grade water added in place of sample DNA.
6  Procedure

1. Thermal cycler parameters shall be used as described in the chart below.

<table>
<thead>
<tr>
<th>Initial Incubation Step</th>
<th>30 cycles each</th>
<th>Final Extension</th>
<th>Final Step</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Melt</td>
<td>Anneal</td>
<td>Extend</td>
</tr>
<tr>
<td>96°C</td>
<td>94°C</td>
<td>61°C</td>
<td>72°C</td>
</tr>
<tr>
<td>2 min. hold</td>
<td>10 sec.</td>
<td>1 min.</td>
<td>30 sec.</td>
</tr>
<tr>
<td></td>
<td>60°C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Label PCR amplification tubes/plates with appropriate information.

3. Thaw the PowerPlex Y23 5X Master Mix, PowerPlex Y23 10X Primer Pair Mix, and water (amplification grade).

4. Vortex PCR 5X Master Mix and 10X Primer Pair set for 15 seconds. Do not centrifuge after vortexing.

5. Prepare master mix by adding the following volumes to a microcentrifuge tube:
   
   a) 5X Master Mix 5.0 µL X # of samples
   b) 10X Primer Pair 2.5 µL X # of samples
   c) **Optional:** Amplification grade water or TE-4 can be used for a final reaction volume of 25.0 µL X # of samples

   **Note:** If necessary include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers.

6. Mix by vortexing for approximately 5 seconds.

7. Dispense appropriate amount of master mix into each amplification tube/well. This amount will be dependent upon the amount of sample you are adding.

8. Add appropriate amount of sample DNA to the appropriate amplification tubes/wells up to a maximum volume of 17.5 µL. For extracts with high concentrations of DNA, dilute with TE-4 buffer to attain the appropriate concentration.

9. For each amplification set, include a positive control by adding an appropriate validated amount of control 2800M DNA to one of the amplification tubes/wells containing the master mix. TE-4 will be used to attain the appropriate dilution if 0.5 ng is not amplified. 2800M DNA must be diluted prior to adding it to the plate as it is a 10 ng/µL stock solution.

10. For each amplification set, include a negative control by adding TE-4/amplification grade water to one of the amplification tubes/wells containing the master mix. The negative control should be the last tube set up.

   **Note:** If both TE-4 and amplification grade water are used in the amplification set up, a negative control must be made for each reagent used.

11. Seal plate using either strip caps or film.

12. Add compression pad (if needed) and place the amplification tubes/plates into the thermal cycler and start the appropriate program.
13. After amplification, remove the tubes from the thermal cycler and store away from light.
   a) Store amplified samples at 2-8 °C for short periods. For longer periods, store amplified samples frozen.
   b) It is recommended that samples not be left in the thermal cycler overnight.

7 Interpretation
None

8 Records
A. The amount of DNA extract amplified shall be recorded in the case record.
B. Sample and control locations on the thermal cycler shall be recorded on the Amplification Worksheet (LAB-DNA-08).
C. Amplification kit lot number and expiration date, as well as the thermal cycler identification number shall be recorded on the Amplification Worksheet (LAB-DNA-08).

9 Literature References and Supporting Documentation
DNA-07-06  DNA AMPLIFICATION – INVESTIGATOR 24PLEX QS

1  Scope
This protocol uses the Investigator 24plex QS PCR Amplification Kit. This kit contains primers that are labeled with a light sensitive dye. This procedure may be performed using an approved robotic platform.

2  Related Chapters
DNA Amplification – Investigator 24plex QS Tecan

TE\textsuperscript{-4} Buffer

3  Safety
Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4  Equipment and Materials
- Calculator
- Microcentrifuge
- Microcentrifuge tubes
- Pipette tips
- Pipettors, adjustable
- Tube decapper
- Vortex
- Thermal cycler
- Amplification tubes, 0.2 mL
- Amplification plates
- Strip caps or film
- Compression pad
- Tube rack
- *Investigator 24plex QS PCR Amplification Kit
- TE\textsuperscript{-4} buffer

*Only approved vendors for consumables may be used.

5  Standards, Controls, and Calibration
A. Control DNA 9948 must be included with each amplification set. This amplification positive control is supplied with the kit.

B. An amplification negative control must be included with each amplification set. This negative control will consist of all amplification reagents with TE\textsuperscript{-4} or nuclease free water added in place of sample DNA.
6 Procedure

1. Thermal cycler parameters shall be used as described in the chart below.

<table>
<thead>
<tr>
<th>Times and Temperatures for Investigator 24plex QS Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 cycles</td>
</tr>
<tr>
<td>Denature</td>
</tr>
<tr>
<td>98°C</td>
</tr>
<tr>
<td>30 sec.</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

2. Label PCR amplification tubes/plates with appropriate information.

3. Vortex master mix and primer set and spin tubes briefly in a microcentrifuge to remove any liquid from the caps.

4. Prepare reaction mix by adding the following volumes to a microcentrifuge tube:
   a) **Fast Reaction Mix 2.0** 7.5 µL X # of samples
   b) **Primer Mix** 2.5 µL X # of samples

   **Note:** If necessary include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers.

5. Mix by vortexing for approximately 10 seconds.

6. Spin briefly in microcentrifuge to remove liquid from cap.

7. Dispense 10 µL of reaction mix into each amplification tube/well.

8. Add appropriate amount of sample DNA to the appropriate amplification tubes/wells not to exceed 15 µL in total sample volume added. For extracts with high concentrations of DNA, dilute with TE-4 buffer to attain the appropriate concentration.

9. For each amplification set, include an appropriately validated amount of positive control (approximately 0.5 ng) in one of the amplification tubes/wells containing the reaction mix. TE-4 or nuclease free water will be used to attain the appropriate dilution.

10. For each amplification set, include a negative control by adding 15 µL of TE-4 or nuclease free water to one of the amplification tubes/wells containing the reaction mix. The negative control should be the last tube set up. Refer to the **DNA Amplification – Investigator 24plex QS Using Tecan** chapter in the DNA SOP for automated processing.

   **Note:** If both TE-4 and nuclease free water are used for amplification set up, a negative control must be made for each reagent used.

11. Seal plate using either strip caps or film and vortex.

12. Add compression pad (if needed) and place the amplification tubes/plate into the thermal cycler and start the appropriate program.
13. After amplification, remove the tubes/plate from the thermal cycler and store away from light. Store amplified samples at 2-8°C for short periods. For longer periods, store amplified samples frozen.

7 Interpretation
None

8 Records
A. The amount of DNA extract amplified shall be recorded in the case record.
B. Sample and control locations on the thermal cycler shall be recorded on the Amplification Worksheet (LAB-DNA-08).
C. Amplification kit lot number and expiration date, as well as the thermal cycler identification number shall be recorded on the Amplification Worksheet (LAB-DNA-08).

9 Literature References and Supporting Documentation
08 GENETIC ANALYSIS PLATFORM

DNA-08-01 SAMPLE SETUP FOR CAPILLARY ELECTROPHORESIS USING QIAGILITY ROBOT

1 Scope
Amplified samples are analyzed by injection on a capillary electrophoresis instrument. Prior to injection, the amplified sample is prepared by adding it to a mixture of formamide and internal lane size standard. Preparation of samples for injection on the capillary electrophoresis instrument can be automated through use of the Qiagen Qiagility robotic platform.

2 Related Chapters
DNA Amplification – Investigator 24plex QS
Capillary Electrophoresis

3 Safety
A. Use universal precautions during evidence handling.
B. Follow instructions for reagent preparation.
   1. Formamide is harmful if absorbed through the skin and is considered an irritant.
   2. POP polymers are considered irritants.
   3. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Qiagen QIAgility robotic platform
- *Qiagen liquid handling disposable tips (50 ul and 200 ul)
- *AB Prism Genetic Analyzer sample tubes/septa/plates
- Freezer or ice bath
- Heat block or thermal cycler
- Tip/reagent carriers
- *BTO size standard
- *Hi-Di formamide
- Vortex
- Microcentrifuge tubes
- Pipette tips
- Pipettes
- 1.5 mL tubes
- Decapper
- Plate centrifuge/microcentrifuge
- 5 mL master mix tubes

*Only approved vendors for consumables may be used
5 Standards and Controls

A. Appropriate ladders and positive and negative amplification controls must be included with each CE instrument run.

B. An appropriate size standard must be added to each sample.

6 Procedure

6.1 Pre-Run Procedure

1. Turn on the computer
2. Turn on the robot and launch QIAgility software.

6.2 Deck Set Up

1. Select the appropriate script: Automated CE Setup or Individual Sample Setup
2. Ensure deck layout is set up appropriately, including pipette tips, amplification plate, labeled CE plate, and necessary reagents.
   a) Spin down the amplification/sample plate, remove strip caps, and place amplification plate on the bottom right area on the deck. Position C2 is used for Automated CE Setup and positions B1, B2, and C2 may be used for Individual Sample Setup.
   b) Label and place an empty capillary electrophoresis (CE) plate on the bottom left area (C1: CE Plate/Reaction Plate) on the deck.
   c) Thaw a tube of allelic ladder, spin it down, remove the cap, and place the tube in the appropriate slot in the left area of the deck (R1: Reagent block).
   d) Thaw a tube of HiDi Formamide, spin it down, open cap, and place the tube in the appropriate slot in the left area of the work table (R1: Reagent block).
   e) Make master mix for n + 2 where n is the number of samples to be set up and place in the appropriate slot on the appropriate reagent block (R1 or M1) in the left area of the work table

Master mix for Qiagen chemistries consists of
   i. deionized formamide 12 µL x # of samples (including controls and ladders) and
   ii. BTO ILS standard 0.5 µL x # of samples (including controls and ladders)

6.3 Running the Script

1. Verify that all prepared reagents and labware are ready and in the appropriate locations. Ensure that all tubes/plates are open and that all obstacles are removed from the deck.
2. Import a sample file or add sample file names individually in the QIAgility software.
3. Add or remove ladders as necessary in the QIAgility software.
4. Add formamide blanks as necessary in the QIAgility software.
5. Verify that the samples in the sample plate correspond to the samples in the reaction/CE plate (C1 on the deck).
6. Confirm tips on the deck are correctly represented in the software.
7. Click to start the liquid handling procedure.
6.4 Post-Run Procedure

1. Remove and properly store all remaining reagents and PCR plates.
2. Seal the Reaction/CE plate with a plate septa and continue with capillary electrophoresis as outlined in the *Capillary Electrophoresis* chapter.
3. Review the run report.
4. An export file can be generated for in the QIAgility software that can be used to make the injection list in the CE collection software.

7 Literature References and Supporting Documentation

Qiagen QIAgility User’s Manual, most recent applicable version
DNA-08-02 CAPILLARY ELECTROPHORESIS

1 Scope
Amplified samples can be analyzed by injection into a CE (Capillary Electrophoresis) Instrument. An internal lane size standard is loaded with each sample to allow for automatic sizing of the PCR products and to normalize differences in electrophoretic mobility between injections. The DNA analysis software automatically analyzes the collected data, and assigns all allele designations.

2 Related Chapters
Autosomal STR Data Evaluation and Profile Characterization Guidelines
Manual Autosomal STR Interpretation Guidelines
STRmix
DNA Amplification- Investigator 24plex QS Using Tecan
DNA Amplification – MiniFiler
DNA Amplification – Yfiler
DNA Amplification—Y23
DNA Amplification – Investigator 24plex QS

3 Safety
A. Use universal precautions during evidence handling.
B. Follow instructions for reagent preparation.
   1. Formamide is harmful if absorbed through the skin and is considered an irritant.
   2. POP polymers are considered irritants.
   3. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
The following items or their equivalent may be used:
- AB Prism Genetic Analyzer (instrument, computer and appropriate software)
- *AB Prism Genetic Analyzer capillaries/array
- *AB Prism Genetic Analyzer sample tubes/septa /plates
- *AB Genetic Analyzer 3500/3500xl pre-filled Cathode Buffer Container (CBC) and septa
- *AB Genetic Analyzer 3500/3500xl pre-filled Anode Buffer Container (ABC)
- *AB Genetic Analyzer 3500/3500xl Conditioning Reagent
- *Genetic Analyzer buffer vials/reservoirs/reservoir septa
- Freezer or ice bath
- Heat block or thermal cycler
- *LIZ size standard
- *BTO size standard
- *AB Prism 10X Genetic Analyzer Buffer w/ EDTA
5 Standards, Controls, and Calibration

A. Appropriate ladders and positive and negative amplification controls must be included with each CE instrument run.

B. An appropriate internal size standard must be added to each sample.

6 Procedure

6.1 Sample Preparation

A. Formamide, stabilizer reagent, internal size standard addition should be as follows:

1. **For AB chemistries**, prepare a master mix of deionized formamide and LIZ size standard as follows and mix well:
   
   a) deionized formamide 8.7 µL x # of samples (include controls and ladders)
   
   b) LIZ size standard 0.3 µL x # of samples (include controls and ladders)

2. **For Promega chemistries**, prepare a master mix of deionized formamide, stabilizer reagent and WEN ILS as follows and mix well:

   a) deionized formamide 9.5 µL – 10 µL x # of samples (include controls and ladders)
   
   b) WEN ILS standard 0.5 µL - 1.5 µL x # of samples (include controls and ladders)
   
   c) Optional: stabilizer reagent 0.5 µL x # of samples (include controls and ladders)

   **Note**: These ratios may be adjusted as necessary, based on validation, to accommodate instrument sensitivity.

3. **For Qiagen chemistries**, prepare a master mix of deionized formamide and BTO ILS as follows and mix well:

   a) Deionized formamide 12 µL x # of samples (include controls and ladders)
   
   b) BTO ILS standard 0.5 µL x # of samples (include controls and ladders)

   **Note**: These ratios may be adjusted as necessary, based on validation, to accommodate instrument sensitivity.
B. Label sample tubes/tray with appropriate information.

C. Master mix aliquots:
   1. **For AB chemistries**, aliquot 9 µL of the deionized formamide/ LIZ size standard master mix into each well in the 96-well reaction plate.
   2. **For Promega chemistries**, aliquot 10-11 µL of the deionized formamide/WEN size standard/stabilizer reagent master mix into each well in the 96-well reaction plate.
   3. **For Qiagen chemistries**, aliquot 12 µL of the deionized formamide/ BTO ILS size standard master mix into each well in the 96-well reaction plate.

   **Note:** These ratios may be adjusted as necessary, based on validation, to accommodate instrument sensitivity.

D. For all chemistries, add 1.0 µL of PCR product or allelic ladder to the appropriate well.

E. Cover the samples with appropriate septa.

F. Centrifuge or spin the samples to remove bubbles.

G. Denature all of the samples at approximately 95°C for approximately 3 minutes in a heat block or thermal cycler. Do not exceed 5 minutes. Immediately chill all of the samples for at least 3 minutes in the freezer or in an ice-water bath. **Note:** This step is optional for Minifiler.

6.2 **Instrument setup**

A. All appropriate controls and ladders will be required for each run. Injection times for controls and ladders shall be consistent with validation studies for genetic analyzer model and chemistry set.

B. **For 3130/3130xl Genetic Analyzer:** Complete the plate editor spreadsheet for the wells you have loaded, type a name for the plate and click OK. For each of the columns enter the appropriate data (sample name, dye module).
   1. The initial injection of each sample can be either 2, 5, 10, or 15 seconds depending on the chemistry, sample type and/or quantitation value.
   2. Most sensitive validated injection time should be initially attempted.

C. **For 3500 Genetic Analyzer:** Complete the plate editor spreadsheet for the wells you have loaded, type a name for the plate and click OK. For each of the columns enter the appropriate data (sample name, dye module).
   1. The initial injection of each sample can be either 10 or 15 seconds or another validated injection time depending on the chemistry, sample type and/or quantitation value.
   2. Most sensitive validated injection time should be initially attempted.

D. **For 3500xl Genetic Analyzer:** Complete the plate editor spreadsheet for the wells you have loaded, type a name for the plate and click OK. For each of the columns enter the appropriate data (sample name, dye module).
   1. The initial injection of each sample can be either 15 or 20 seconds or another validated injection time depending on the chemistry, sample type and/or quantitation value.
   2. Most sensitive validated injection time should be initially attempted.
6.3 Sample Analysis

Analyze collected data using the DNA analysis software.

A. Analysis range should be set to exclude the primer peak.

B. The analysis smoothing option will be set to light for all samples.

7 Interpretation

Proceed to the *Autosomal STR Data Evaluation and Profile Characterization Guidelines* chapter, the *Manual STR Autosomal Interpretation Guidelines* chapter or the *STRmix Autosomal STR Interpretation Guidelines* chapter in the DNA SOP.

8 Records

A. At a minimum, case record documentation will consist of the following:

1. A printout of the DNA analysis software project list for each run.

2. A printout of one electropherogram used in the final interpretation of each sample. If a sample is extracted more than once, a printout of one electropherogram from each extraction is also required.

3. Document why a sample, amplification control, reagent blank, or ladder injection was not used. Reasons for not using these injections must be noted on the project list from the DNA analysis software. Alternatively, for automated workflow processes, each sample may have all usable sample injections printed and the reason for not using an injection documented on the electropherogram(s) retained in the case record instead of the project list. If a sample injection, ladder, control or reagent blank is unusable, the reason for not using these injections will be documented on the project list from the DNA analysis software.

B. Documentation will be kept for each CE instrument on the following:

1. A copy of the spectral for each capillary of the array for the 3130/3130xl/3500/3500xl (note: 3130/3130xl/3500/3500xl sample files are stored with the spectral applied)

2. A record of capillary changes (use LAB-DNA-69 or LAB-DNA-70)

3. A record of Performance Optimized Polymer 4 (POP-4) lot numbers and expiration dates (use LAB-DNA-60 or LAB-DNA-70)

4. A record of LIZ/WEN/BTO size standard lot numbers (use LAB-DNA-69 or LAB-DNA-70)

5. A copy of the analysis parameters

C. Run Documentation

1. All raw data will be electronically archived. A copy of all archived data will be retained off-site.

2. Per run, a complete DNA analysis software project list will be maintained in a notebook until archived. Alternatively, a copy of the project list will be contained in the case record of each case associated with that project.
3. DNA analysis software project list will contain at a minimum the following:
   a) Sample Name or Sample File
   b) Sample Type
   c) Analysis Method
   d) Injection Time
   e) Reasons an injection was not used

4. Instrument designation will be maintained in the case record.

9 Literature References and Supporting Documentation

09 INTERPRETATION AND REPORTING GUIDELINES
DNA-09-01 AUTOSOMAL STR DATA EVALUATION AND PROFILE CHARACTERIZATION GUIDELINES

1 Scope
Data evaluation is the first step in the analysis process. This step is then followed by profile characterization. Once the profile has been characterized, further analysis may proceed through either manual interpretation or interpretation with probabilistic genotyping. Data evaluation and profile characterizations are made as objectively and consistently as possible from analyst to analyst.

2 Related Chapters
Glossary of Terms
Analytical Controls
Guidelines for DNA Technical Review
Capillary Electrophoresis
Manual Autosomal STR Interpretation Guidelines
Report Writing Guidelines
STRmix

3 Preliminary Evaluation of Data
Data evaluation is necessary to determine whether the results are of sufficient quality for characterization and ultimately interpretation. The following criteria have been determined by evaluating data generated by the laboratory.

3.1 DNA Analysis Software
A. GeneMapper IDX must be used for all final data analysis and printouts.
B. The DNA analysis software will be set to the default settings and/or validated in-house methods for all kit dependent values.
C. Process Quality Values (PQVs) will not be used to replace individual data analysis completed by the analyst. Any flags or color designations provided in the software will not be used during analysis of sample data.
D. The DNA analysis software includes a profile comparison tool which must be used to aid in comparing profiles within a project to each other as well as comparing lab reference profiles to all samples within a project.
E. The DNA analysis software includes a mixture analysis tool which can aid in two person mixture deconvolution; however, this will not be used in lieu of analyst interpretation.

3.2 Thresholds
A. For 3130/3130xl Genetic Analyzer: All data shall be analyzed at an analytical threshold established by system validation. This threshold is 50 RFU (relative fluorescence units) for MiniFiler data and 60 RFU for Investigator 24plex QS data.
B. For 3130/3130xl Genetic Analyzer: An instrument specific validated stochastic threshold will be utilized for determination of whether or not single source profiles are partial or full, manual mixture interpretation, and determining if the STRmix output assessed dropout.

C. For 3500/3500xl Genetic Analyzer: All MiniFiler data shall be analyzed at the validated analytical thresholds established by each laboratory. All Investigator 24plex QS data shall be analyzed at an analytical threshold of 60 RFU unless a validated higher analytical threshold has been established by the laboratory.

D. For 3500/3500xl Genetic Analyzer: An instrument specific validated stochastic threshold will be utilized for determination of whether or not single source profiles are partial or full, manual mixture interpretation, and determining if the STRmix output assessed dropout.

E. The maximum analytical RFU for evaluating STR profiles is the saturation point of the CCD camera of the instrument in the raw data. Saturation is the point at which there is no longer a linear correlation between RFU and DNA template.
   1. The saturation point has been set at 7,000 RFU for 3130 series instruments and 30,000 RFU for 3500 series instruments.
   2. If data for the Amelogenin locus exceeds the maximum analytical RFU, this data may still be used for manual interpretation or probabilistic genotyping.
   3. Data exceeding saturation points should not be imported into the probabilistic genotyping software.
   4. If data exceeds the maximum analytical RFU at any locus except for Amelogenin, the off scale locus will not be used.
      a) This will necessitate the locus being ignored in the probabilistic genotyping software through use of the Run Settings Sample Summary window.
      b) Alternatively if the data exceeds the maximum analytical RFU at any locus except for Amelogenin, the sample may be reanalyzed using any of the following methods:
         i. Inject the sample for less time; as supported in validations for instrument being used.
         ii. Dilute the amplified product in dIH₂O or TE⁻⁴ buffer and add the diluted amplified product to the formamide and size standard mixture.
         iii. Re-amplify the sample with less template DNA.

F. If the maximum RFU is exceeded for the size standard, the analyst may use the data as long as any associated pull-up does not interfere with data interpretation.
   If using probabilistic genotyping software, any associated pull-up should be below analytical threshold or labeled as an artifact prior to importing the data into the software.

G. To increase peak height, the sample may be reanalyzed using the following method:
   1. Inject the sample for more time; as supported in validations for instrument being used.
   2. Re-amplify the sample with more or less template DNA.
3.3 Internal Size Standard Evaluation

A. Internal size standards must be run with every sample.

B. The LIZ size standard is used in conjunction with MiniFiler. MiniFiler requires an analysis range of 65 to 400 bp for proper sizing.

C. The BTO size standard is used in conjunction with Investigator 24plex QS. Investigator 24plex QS requires an analysis range of 60 to 500 bp for proper sizing.

3.4 Allelic Ladder Evaluation

A. When interpreting amplification results, allele calls are assigned to sample peaks by comparing their sizes to those obtained for the known alleles in the allelic ladders.

B. At a minimum, one acceptable allelic ladder must be present within each run for each respective kit.

C. Each ladder used for analysis must have the appropriate number of properly labeled alleles present for each locus when analyzed.

3.5 Analytical Controls

Analytical controls must be evaluated according to the Analytical Controls chapter.

4 Data Analysis

4.1 Allele Identification

A. An allele is defined as any peak that meets or exceeds the established analytical threshold values, has Gaussian morphology, and is not an artifact. Allelic data may be present below analytical threshold.

B. If no peaks for a given locus are detected above analytical threshold, that locus is designated as no result (NR).

4.2 Artifacts

A. General Guidelines

1. Generally, artifacts are easily characterized and do not pose a problem in identifying true allele peaks.

2. Artifacts or anomalies (including elevated baseline) may be detected on the electropherogram. Profiles which contain artifacts that occur above analytical threshold within the analysis range and do not interfere with interpretation may be used providing the artifacts are identified and labeled.

3. It is important to try to identify and document the cause for the appearance of an artifact.

4. Kit-associated artifacts (e.g. dye artifacts) may be documented and loci may be used.

5. Analysts are encouraged to consult with the technical leader if the identity of an artifact is in question.

B. Spikes

If a spike occurs and/or is labeled by the DNA analysis software the profile may be used or the sample may be re-injected.
C. Stutter

1. Stutter is a biological artifact that is known to occur during PCR due to slippage of DNA strands. Stutter occurs most commonly at the N-4 position, but is also known to occur at the N+4 position, and rarely at N-2, N-8 and N-12.

2. Stutter peaks are evaluated by the ratio of the stutter peak height to the height of the appropriate allele.

3. When determining the most appropriate designation for a peak that is only minimally above the expected stutter ratio, the overall profile must be carefully evaluated, taking into account number and ratio of donors. Reamplification of the sample may help clarify whether a peak is stutter or allelic.

4. An analyst may designate a peak as stutter outside of existing thresholds in single source or mixture samples by labeling the peak to that effect.

D. Non-Template Addition (Minus A)

1. Minus A may occur when too much amplification product is generated due to incomplete adenylation during PCR. If this occurs, the analyst may re-analyze the sample using any of the following methods:
   a) Re-inject the sample for a shorter period of time as supported in validations for instrument being used.
   b) Dilute the amplified product in dH₂O or TE⁻⁴ buffer and add the diluted amplified product to the formamide and size standard mixture and reinject.
   c) Re-amplify the sample with less template DNA.

2. After steps have been taken to minimize/remove minus A, the minus A artifacts may be labeled and the remaining data used.

E. Spectral Pull-up

1. If pull-up occurs in a sample, but there is no significant occurrence of minus A peaks, larger allele drop out and/or increased stutter, the pull-up peak(s) may be labeled “pull-up.” The remaining information from that sample may be used. Caution should be exercised when applying this to pull-up peaks that fall within an allelic bin.

2. One of the following must be performed on samples that exhibit significant occurrence of minus A peaks, larger allele drop out and/or increased stutter in addition to pull-up due to amplification of excessive template DNA:
   a) Re-inject for a shorter period of time; as supported in validations for instrument being used.
   b) Dilute the amplified product in dH₂O or TE⁻⁴ buffer and add the diluted amplified product to the formamide and size standard mixture and reinject.
   c) Re-amplify the sample with less template DNA.

4.3 Off-ladder and/or Out of Marker Range Alleles

A. The number designation for alleles containing an incomplete repeat unit, falling within the range spanned by the ladder alleles, should include the number of complete repeats, and separated by a decimal point, the number of base pairs in the incomplete repeat (i.e. 9.3 for TH01).
B. If an allele falls outside the smallest or largest allele at a locus and does not fall into a virtual bin, it will be designated as greater than or less than the appropriate ladder allele (i.e., >13.3 for TH01). No calculations are required for these alleles.

If an allele falls between two loci so that the analyst is unable to determine to which locus the allele belongs, both affected loci will be marked as uninterpretable.

C. Off-ladder alleles must be verified by re-injection of the sample or by confirmation in multiple samples.
   1. In general, known samples should not be used to confirm off-ladder alleles observed in questioned samples.
   2. An exception would be using a known sample from an assumed donor to confirm an off-ladder allele observed in an associated intimate or indigenous questioned sample.
   3. If an off-ladder allele is determined to be a stutter peak, it is not necessary to reinject the sample. Refer to 4.2C above for information on stutter peaks.

D. Calculate off-ladder alleles using the following formulas and document in the case record.
   1. If off-ladder alleles are homozygous, the allele from the nearest locus will be used as the sister allele.
   2. If the locus is a mixture, either the assumed sister allele or the perceived nearest allele will be used as the sister allele.

\[
\delta_1 = \text{the difference between the ladder allele } x (L_x) \text{ and the sample sister allele } (S_x) \text{ to the OL allele at any given allele } x
\]

\[
\delta_2 = \text{The difference between the perceived nearest ladder allele } y (L_y) \text{ and the sample OL allele } (S_{OL})
\]

\[
C = \text{The relative peak shift of the OL allele from the nearest ladder allele}
\]

\[
\delta_1 = S_x - L_x
\]

\[
\delta_2 = S_{OL} - L_y
\]

\[
C = \left| \delta_1 - \delta_2 \right| \text{ in basepairs}
\]

4.4 Tri-allelic Loci
   A. Tri-allelic patterns must be verified by re-injection and/or re-amplification of the sample or by confirmation in multiple samples. It is recommended that tri-allelic patterns that appear as unequal peaks be confirmed through re-amplification if possible.

B. Tri-allelic loci will be used for comparisons, but may not be used for single-source statistical calculations.

4.5 Incomplete Peak Resolution
   A. Incomplete peak resolution can occur between alleles that are 1 basepair apart such that one allele is not called by the analysis software.

B. The possibility of incomplete peak resolution should be considered when characterizing profiles and assigning number of contributors.
C. If incomplete peak resolution occurs, the profile may still be run in STRmix.
   1. If STRmix models the unlabeled allele as dropout, the interpretation may be used and no further action is needed.
   2. If STRmix does not model the unlabeled allele as dropout, the deconvolution should be rerun with the locus omitted.
   3. If an assumed contributor profile contains the unlabeled allele, then the locus does not need to be omitted.

4.6 Composite Data: Single Amplification Kit
   A. Composite profiles (i.e., generated by combining typing results from multiple amplifications from the same kit and/or multiple injections of the same amplicon) will not be used in manual STR interpretation. They may be used for probabilistic interpretation. Refer to the STRmix Autosomal STR Interpretation Guidelines chapter for more information.
   B. If a sample is subjected to additional amplifications or injected at different injection times, the profile with the most informative data will be used for interpretation provided it meets the guidelines concerning the presence of artifacts as set forth in section 4.2 (Artifacts).

4.7 Combined Data: Multiple Amplification Kits
   A. Investigator 24plex QS and Minifiler results from the same sample extract may be combined. For loci that are shared between the kits, the locus with the most information from either kit will be used to create the combined profile. Combined profiles must use the kit specific stochastic threshold for the respective loci.
   B. When results are discordant between Investigator 24plex QS and MiniFiler, the Technical Leader must be consulted.

5 Profile Characterization
5.1 General Characterization
   A. The evidentiary profile is characterized prior to comparison to known samples. Characterizations will be recorded in the case record. Refer to the flow chart below.
B. If no allelic peaks are present at or above the analytical threshold, the profile is interpreted as no result.

C. When allelic peaks are present at or above the analytical threshold, not including Amelogenin, an attempt should be made to characterize the profile. Possible characterizations are single source or mixture and partial or full.
   1. When considering the data, it may be impossible to characterize a profile. This type of profile would be considered uninterpretable.
   2. Allelic data may be present below analytical threshold. Although this data would not have the proper correlation between template DNA and RFU necessary for mixture interpretation, this data may be used for characterization of a profile. The number of sub-threshold peaks, baseline noise and amplification artifacts should be taken into consideration.

D. Any characterizations will be made prior to entry into probabilistic genotyping software and/or manual mixture deconvolution. The characterizations will be recorded in the case record.

E. The origin of the profile should be evaluated in order to determine if the profile can be classified as intimate, indigenous, or environmental. Any classification will be recorded in the case record.
   1. Intimate samples include those items which have been collected directly from an individual's body such as swabbings of any skin surface or orifice, fingernail scrapings/clippings, underwear (male or female), or feminine hygiene products.
   2. Indigenous samples include those items known to have been in direct contact with the individual's body, such as clothing, his/her own bedding or shoes.
   3. All other samples can be classified as environmental.

F. Amelogenin and DYS391 may be used for profile evaluation.

5.2 **Indicators of Single Source Profile**:
A. No more than two alleles per locus (with the exception of one tri-allelic locus)
B. Balanced peak heights for heterozygous alleles (typically ≥ 70%)
   1. Peak height imbalance may be present due to stochastic effects and/or primer binding site mutations
   2. Peak height imbalance may also be present due to the molecular weight of the loci (e.g. larger loci may display peak height ratios less than 70%)
C. Sample origin

5.3 **Indicators of Mixture Profiles**:
A. More than two alleles at one or more loci
B. Peaks in stutter position that exceed the expected stutter ratio
C. Significantly imbalanced alleles
D. Sample origin
5.4 Partial or Full Profile Status:
   A. Single source profiles are considered partial if there is allelic dropout at a locus.
   B. Mixture profiles are considered partial if there is complete locus dropout.

5.5 Uninterpretable Profiles
   A. Uninterpretable profiles may result from but are not limited to the following causes:
      1. Insufficient amounts of template DNA which can result in observation of stochastic effects at one or more loci for the sample tested.
      2. Degradation due to environmental or chemical exposure.
      3. Preferential amplification due to the presence of inhibitors or other factors that limit the amplification of larger fragments.
      4. Data is too complex for meaningful interpretation.
      5. No complete genotype is present.
   B. The nature of the sample, case synopsis, relatedness of individuals and/or case screening results may indicate the possibility of encountering complex profiles. Caution should be exercised if further interpretation is made to these types of profiles.

6 Records
All characterizations and classifications (intimate, indigenous, and environmental) will be documented in the case record.

7 Literature References and Supporting Documentation
Qiagen. Investigator 24plex QS Handbook (current version)
Applied Biosystems. DNA analysis software user's manual (current version).
SWGDAM. Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories. www.swgdam.org (current version)
DNA-09-02 MANUAL AUTOSOMAL STR INTERPRETATION GUIDELINES

1 Scope

These guidelines are in place to ensure that conclusions are scientifically supported by the analytical data with appropriate standards and controls. Interpretations are made as objectively and consistently as possible from analyst to analyst. Interpretation guidelines are based upon validation studies, literature, instrumentation and casework experience. Not every situation can nor should be covered by a rule; consultation with the Technical Leader and further documentation is required for scenarios not addressed in this chapter.

2 Related Chapters

Glossary of Terms
Analytical Controls
Guidelines for DNA Technical Review
Capillary Electrophoresis
Autosomal STR Data Evaluation and Profile Characterization Guidelines
Statistical Significance Estimation
Report Writing Guidelines
CODIS Guidelines

3 Interpretation of Results

3.1 Single Source Evidentiary Profile Interpretation and Comparison

A. Interpretation

Determine if there is enough data present to call a single source profile. (Refer to, section 1 of the Interpretation Flowcharts chapter).

1. A profile can be designated as single source with the observation of one or more complete genotypes. Without a complete genotype at one locus, the profile should be designated as uninterpretable.

2. Determine if allelic drop-out is reasonably suspected at any locus. If this is the case, the RMP statistic tool can be used to calculate the profile statistic, which incorporates the use of $2p^2$.

   a) Heterozygous loci with alleles in the stochastic region are considered complete genotypes.

   b) An apparent homozygote with an allele under stochastic threshold may be exhibiting allelic drop-out.

B. Comparison to Reference Samples

1. Comparisons will be made between the evidentiary profile and known profiles of possible contributors. The conclusions will be recorded in the case record.

2. Once an association to a full single source profile has been established, no further comparisons are required for that profile.

3. Known reference sample profiles developed by an external, accredited laboratory can be used with the approval of the Technical Leader.
3.2  Mixture Evidentiary Profile Interpretation and Comparison

A. Interpretation

Refer to section 2 of the Interpretation Flowcharts chapter.

1. Mixture Deconvolution

   a) Mixed profiles should be resolved whenever possible. When ambiguity persists at a particular locus, multiple combinations can be included in the final resolution for a contributor.

   b) Considerations for mixture deconvolution include:

      i. Evaluation of the profiles moving from the smaller loci to the larger loci.

      ii. Base line quality (e.g. low noise, no pull-up, spikes etc.)

      iii. Profile quality (e.g. degradation, low level, inhibition, etc.)

      iv. Mixture ratio consistency across the profile.

2. Assign the minimum number of contributors (e.g. at least 3 contributors) and document in the case record.

   a) Evaluating the number of unambiguous alleles per locus and peak height ratios will aid in the determination of the minimum number of potential contributors.

      i. All alleles at or above the analytical threshold may be used in this assessment. As an example, if at most five alleles are detected per locus, then the DNA typing results are consistent with having arisen from at least three individuals.

      ii. Allelic data may be present below analytical threshold and may be used to determine the number of contributors.

   b) It should not be assumed that the number of alleles present is always a definitive method for determining the minimum number of potential contributors, especially in low level template samples. As an example, some 3 or 4 person mixtures may not have more than four alleles present at any given locus, especially among related individuals.

   c) The possibility of degradation, drop out, additive effects, peak height ratio, baseline noise, and increased stutter should be considered when determining the number of contributors.

   d) To determine number of contributors for complex mixtures,

      i. Find the locus with the maximum number of alleles.

      ii. Round up this number to the nearest even number and divide by 2.

      iii. With this number try to pair the alleles at that locus. If there are significant peak height imbalances then you may have to account for an additional contributor.

      iv. Assess the entire profile to ensure the number of contributors makes sense across the profile.

   e) If the mixture profile is determined to have at least four contributors, it will be considered too complex for manual interpretation and comparison purposes unless approved by the Technical Leader. Approval will be documented in the case record.

   f) The assumption of two contributors is necessary to use mathematical comparisons to resolve a mixture. This assumption must be stated in the report. It
is reasonable to assume two individuals in a profile when there is no indication of a third contributor.

1. Possible peaks may be below analytical threshold that could potentially pair with an allele in the stochastic region. This indicates the possibility of a mixture of greater than two people, so the assumption should not be made.
2. Loci with moderate RFU values and a disparate ratio of contributors are more likely to be experiencing drop out.
3. Peak height ratios could also be used in determining when a low level contributor may be exhibiting drop-out. Observation of peaks below the analytical threshold may be used to determine whether drop-out is probable at a locus.

3. Determine if drop out is probable by evaluating the entire profile. (Inter-locus rule)
   a) Allele drop out can still occur in loci with alleles that minimally exceed the stochastic threshold due to additive and stochastic effects.
   b) Loci on either side of a locus and/or in a different dye channel can suggest that there could be potential contributor drop out.
   c) Alternatively, if alleles in the stochastic region can be seen at other loci, starting at the smallest molecular weight locus extend a predicted degradation curve (straight line) across the loci and assess if potential drop out has occurred at a locus.
   d) Reinstate loci for statistical analysis if it is determined that drop-out is not likely due to the number of contributors and/or peak height ratios.
   e) Allele sharing is a more likely assumption for loci with high RFU values and a close ratio of contributors as they are unlikely to be experiencing dropout.

4. Determine the mixture ratios
   a) This can be calculated by dividing the sum of the RFU values of the major contributor’s alleles by the sum of the value of the minor contributor’s alleles at a locus. The specific calculation will change based on number of contributors, allele sharing possibilities, and potential allelic drop-out. Some possibilities are listed here.

   i. 2 contributors; Mixture ratio = \((A+B)/(C+D)\)
   ii. 2 contributors; Mixture ratio = \((A+B-C)/(C*2)\)–
   iii. 2 contributors; Mixture ratio = \((B)/(C+D)\)
iv. 2 contributors; Mixture ratio = (B-C)/(C×2)

b) For three or more contributors, an evaluation of potential allelic sharing will determine the correct calculation. If an additional contributor does not appear to significantly affect peak heights, the above equations may be appropriate. However, if two additional contributors may be sharing with the major, calculations should reflect that assessment.

c) Mixture ratios can be utilized to justify the resolution of a mixture component, such as a major, minor, mixed major, or foreign.

d) Mixture ratios should be utilized during resolution to select reasonable genotype combinations and eliminate improbable genotype combinations, for use in comparison and statistical analysis.

e) To restrict possible genotypes for a single contributor, using math calculated by hand or an approved worksheet, the following must be considered:

   i. Any allele below the stochastic threshold indicates possible allelic drop-out and cannot be restricted to possible genotypes unless peak height ratios and assumption of contributors demonstrate the alternative. It will be considered [allele, any] and the 2p-p² formula will be used for a calculation of genotype possibilities.

   ii. Peak height ratios (usually ≥ 50% in mixture samples) can be utilized to eliminate possible genotype combinations. Caution should be taken when doing this in the stochastic region, as peak height ratios are known to fluctuate more at that level.

   iii. The observed ratio of contributors based on evaluation of the entire profile can be used to determine reasonable genotype restrictions.

5. Foreign Contributor Resolution

For each intimate or indigenous item, the reference profile of any known contributor may be used to assist the analyst in determining possible allele combinations of foreign contributors.

a) The known profile of an individual may be compared to a mixture obtained from his/her intimate or indigenous sample in order to resolve the contribution of the foreign contributor(s). Only the foreign profile is required for statistical analysis. This assumption must be stated in the report.

   i. If information is obtained that consensual sexual activity occurred within a reasonable time frame of the alleged assault and the partner’s alleles are observed, the reference standard from the consensual partner may be assumed to be present. This assumption must be stated in the report. Caution should be taken when assuming both the victim and the consensual partner are present. It may be possible that a mixture statistic is more suitable than a partially resolved foreign profile statistic.

   ii. If either the epithelial or sperm fraction from a differentially extracted semen stain is shown to be single source or is resolvable, that profile can be used to resolve the mixture in the corresponding fraction.
b) If the DNA profile does not demonstrate data to intuitively support an assumption of the known individual, the known profile will not be used to resolve a foreign profile.

6. Major Component Resolution

a) If the evaluation of the mixture profile results in a clear determination of a major and minor contributor, then the major component of the mixture profile is considered a single source for the purposes of comparison and statistical calculations (see single source interpretation).

b) A major contributor can be reliably distinguished from additional contributors when there is an approximately 3:1 ratio of contributors across the profile. This does not require that every locus be conclusively called in the resolution, nor does it require that each locus meet exactly 3:1 mathematically. Whether or not a major contributor is resolved should be based on evaluation of the profile as a whole. In addition, loci where the additional contributor(s) to the major does not appear may be used to reliably demonstrate a 3:1 ratio to justify calling the major contributor, by assuming drop-out of the additional contributor(s).

c) Consideration of number and relatedness of contributors due to the possibility of allele sharing is necessary to avoid calling a false major profile.

d) It is possible that multiple donor profiles to a mixture will degrade at different rates. This could result in a mixture that exhibits a major/minor pattern at some loci (typically the lowest molecular weight loci) but relatively equal intensities at other loci (typically the highest molecular weight loci). Caution should be exercised when identifying a major component to such a mixture. Alleles in the stochastic region can be designated as major contributor alleles as long as careful evaluation of the entire profile has been performed. This would include consideration for allele stacking, degradation and stochastic amplification effects.

e) Loci that failed the inter-locus rule assessment but exhibit a major contributor(s) may be used for RMP statistics.

f) Documentation of the mixture ratios at each appropriate locus is required to support a major component determination.

g) Loci where a major cannot be resolved will be deemed uninterpretable for the major contributor statistic calculation.

7. Minor Component Resolution

a) If an assumption of 2 donors to the mixture has been made, a minor component may be resolved. Minor profiles should only be resolved if at least one complete genotype can be determined.

b) Loci where a minor cannot be resolved will be deemed uninterpretable for the minor contributor statistic calculation.

c) Caution must be taken to clarify the difference between restricting genotype possibilities and resolving a contributor. For a resolved contributor, an obligate allele must be present for a locus to be included in the RMP statistic. If an obligate allele is not able to be resolved, that locus cannot be used in the statistic.

d) If a minor contributor cannot be resolved, the remaining contributor(s) in the mixture will typically be classified as uninterpretable.
8. Mixed Major Resolution
   a) Occasionally, a mixed major of two or more donors may be addressed separately from an additional low level contribution. This mixed major may be interpreted separately from the additional contributor if there is approximately a 3:1 ratio from the lowest possible contributor allele pair of the mixed major compared to the additional contributor(s) observed.
   b) This does not require that every locus be conclusively called in the resolution, nor does it require that each locus meet exactly 3:1 mathematically. Whether or not a mixed major contributor is resolved should be based on evaluation of the profile as a whole. In addition, loci where the additional contributor(s) to the mixed major does not appear to be called due to drop-out may be used to reliably demonstrate a 3:1 ratio to justify calling the major contributor.
   c) This mixed major may potentially be able to be further resolved under the assumption of two donors or the CPI approach may be applied for an inclusion.
   d) Loci where a mixed major cannot be resolved will be deemed uninterpretable for the mixed major contributor statistic calculation.

9. Indistinguishable mixtures and uninterpretable minor components of distinguishable mixtures
   a) When the criteria for resolving a specific component are not met, those profiles are considered indistinguishable mixtures.
   b) Determine which loci are conclusive (suitable) and which are uninterpretable (unsuitable) for statistical purposes.
   c) Based on the assumed number of contributors, loci with all allelic representation above the stochastic threshold are considered conclusive and can be used for statistical significance estimation.
   d) The minor component may not have enough information above threshold to use for meaningful interpretation. If this occurs, the analyst may choose to call the minor component uninterpretable, and no comparisons will be made to the minor component.

B. Comparison
   1. Deduced profiles (major, minor, foreign) resolved from a mixture, will be treated as single source samples. Reference profiles will be compared to each locus resolved, with inclusions and exclusions made based on the possible genotypes determined.
   2. For indistinguishable mixtures, reference profiles will first be compared to the loci of evidence profiles determined to be suitable for statistics. An initial inclusion and exclusion will be based on this comparison.
   3. The analyst may use the data in the stochastic region of the remaining loci to determine if sufficient information exists to exclude the reference profile. Justification for the exclusion must be documented in the case record.
   4. If a locus was determined to be interpretable (i.e a component of a distinguishable mixture was resolved or all alleles were determined to be present) and subsequently during comparison with references, a true genotype combination was eliminated and/or allelic dropout is indicated, the profile will be deemed uninterpretable and no comparisons will be reported.
a) If complete allelic drop out is observed, additional testing of the sample (extraction and/or amplification) may be attempted to determine if further information may be obtained for comparisons with Technical Leader approval.

b) If the comparison was to a sample previously entered into CODIS, consult with the Technical Leader.

3.3 Possible Conclusions for Single Source Evidentiary and Mixture Evidentiary Profiles

1. **Exclusion/Excluded** – The alleles obtained for the reference sample are not included in the alleles obtained for the evidentiary sample. The reference sample is excluded as a possible contributor of the DNA profile obtained from the evidentiary sample.

2. **Inclusion/Included/Not Excluded** – The alleles obtained for the reference sample are included in the alleles obtained for the evidentiary sample. The reference sample cannot be excluded as a possible contributor of the DNA profile obtained from the evidentiary sample.

3. **Uninterpretable** – The DNA data cannot be interpreted and may not be able to be fully characterized; therefore, the data is not suitable for comparisons. This could be due to poor or limited data quality, data that is too complex, or data that fails to meet laboratory quality requirements.

4. **No results** – No alleles above analytical threshold at any locus.

3.4 Profiles for CODIS Entry

A. The analyst may use the data in the stochastic region to develop profiles for CODIS entry if at least one locus used for CODIS entry is above stochastic threshold and suitable for statistics.

B. Any new or additional data obtained for CODIS eligible profiles through reamplification or reanalysis will be uploaded to CODIS.

C. If a profile has been determined to be unsuitable for comparison there will be no attempt to develop a profile for CODIS.

4 Records

A. All conclusions and characterizations (intimate, indigenous, environmental) will be documented in the case record.

B. All calculated mixture ratios will be documented in the case record.

C. The stochastic threshold will be documented on the project list or the electropherograms.

5 Literature References and Supporting Documentation


SWGDAM. Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories. www.swgdam.org (current version)


Estimating the number of contributors to a mixture powerpoint, Texas Forensic Science Commission sponsored training Fort Worth, Texas. 18-20 November 2015.

Mixture Interpretation, Dr. Bruce Budowle, Texas Forensic Science Commission sponsored training Fort Worth, Texas. 18-20 November 2015.

Mixture Deconvolution Principles, Dr. John Buckleton, Texas Forensic Science Commission sponsored training Fort Worth, Texas. 18-20 November 2015.

Mixture Deconvolution, Dr. Simone Gittelson, Texas Forensic Science Commission sponsored training Fort Worth, Texas. 18-20 November 2015.


Worked Examples and Report Writing, NIST DNA Mixture Interpretation Webcast, Bruce Heidebrecht, 2013.
DNA-09-03 INTERPRETATION FLOWCHARTS

1 Single Source Interpretation

Single Source

- Is there at least 1 complete genotype present?
  - No: Uninterpretable
  - Yes: Is there evidence of allelic drop-out?
    - Yes: Partial Profile
    - No: Full Profile

- RMP stats
  - Heterozygote: $2pq$
  - Homozygote: $p^2 + p(1-p)e$
  - One allele under ST: $2p-p^2$

- RMP stats
  - Heterozygote: $2pq$
  - Homozygote: $p^2 + p(1-p)e$
2 Mixture Interpretation

Diagram:

- Mixture
  - Determine # of contributors
    - Intimate/Indigenous
      - Can you assume K is present?
        - No
        - Environmental
          - Is mixture resolvable?
            - Yes, Mixed Major
            - Yes, Major
            - No
    - Can you assume 2 contributors?
      - Yes
        - Can you resolve a partial or full foreign contributor?
          - Yes
            - CPI inclusion with assumption of 2
          - No
            - CPI inclusion with assumption of 2
      - No
        - Can you resolve a mixed major?
          - Yes
            - CPI inclusion to mixed major w/assumption of 2, follow chart for additional contributors
          - No
            - CPI inclusion

- RMP stats on foreign contributor with assumptions of 2 and K present
- CPI inclusion w/ assumption of 2

- Uninterpretable

- Can you resolve within the mixed major?
  - Yes
    - CPI inclusion to mixed major w/assumption of 2
  - No
    - CPI inclusion

- RMP stats on major and minor w/assumption of 2, (or foreign)

- Significant drop-out observed; data is insufficient for interpretation.

- Data is too complex to interpret.

- Can you assume 2?
  - Yes
    - Is the mixture suitable for CPI?
      - Yes
        - CPI inclusion
      - No
        - Uninterpretable: Are there 4 or more contributors?
          - Yes
            - CPI inclusion
          - No
            - Uninterpretable: Are there 4 or more contributors?
DNA-09-04 STATISTICAL SIGNIFICANCE ESTIMATION

1 Scope

Once an association has been identified and an individual included as a possible source of evidentiary material, the significance of that association is estimated (match probability) to allow investigators, the legal sector, and ultimately a jury of lay persons to place the appropriate emphasis on the conclusion. Although every locus analyzed is evaluated, some loci may provide no information with regard to a particular comparison. This protocol addresses the different methods of calculating significance, when each is to be applied, and the population data from which they are calculated.

Only one statistical calculation model will be reported per DNA profile unless recommended by the laboratory or by court order.

For statistical recalculation requested by the customer with frequencies calculated utilizing the Texas/FBI STR Population database referenced in the DNA SOP.

2 Related Chapters

Manual Autosomal STR Interpretation Guidelines
Texas/FBI STR Population Database
NIST STR Population Database
Report Writing Guidelines

3 Practices

3.1 General Information

A. Evidentiary Stains

The DNA profile obtained from each evidentiary stain shall be classified as uninterpretable, single source, or mixture. Mixtures can also be deconvoluted to obtain major, minor, mixed major and foreign profiles. If the profile fits the criteria for a single source, a major component of a mixture, a minor component of a mixture, or a single foreign contributor to a mixture, the analyst should apply single source significance calculations. Calculations for indistinguishable mixtures will be performed using mixture statistics. A single source and inclusion statistic may both be reported for the same item based on the needs of the case. Uninterpretable profiles are not suitable for comparisons; therefore, no statistics will be calculated for these types of profiles.

B. Minimum and Null Allele Frequencies

Minimum allele frequencies are calculated using 5/2N where N is the number of individuals in the population database. This follows the NRC II recommendation for STR analysis; null allele frequencies are set to 0. (Evett et al. 1996)

C. Off-ladder Alleles

Off-ladder alleles that have been confirmed by re-injection (in the case of STR analysis) or appearance in more than one sample will be used to determine an association and estimate the significance of that association. The allele frequency will be the calculated minimum allele frequency for the locus/population group.
D. Tri-allelic Loci

Tri-allelic loci that have been confirmed by re-amplification, re-injection (in the case of STR analysis) and/or appearance in more than one sample will be used to determine an association. However, the tri-allelic locus will be omitted from the statistical significance calculations for single-source samples.

E. Software

1. The latest available and installable version of the FBI's Popstats software or other validated software will be configured to use the formulae below to calculate significance estimates. The Forensic-Single Sample data input option (Federal Bureau of Investigation. 1997a) will be used for single source significance calculations; the Forensic Mixture data input option and the Mixture Formula (Federal Bureau of Investigation. 1997a, 1997b) will be used for mixed source significance calculations.

2. For CPI, the probability of inclusion will be calculated and the inverse probability of inclusion will be reported.

3. The RMP Tool for Population Frequency Calculations workbook (LAB-DNA-24) will be used to calculate single source or deduced single source samples containing loci with possible allelic drop-out for legacy amplification kit data. This allows the use of the $2p - p^2$ equation and the ability to include multiple genotype combinations to a locus, which is not possible in the FBI's Popstats program.

F. Population Databases

1. Population groups

   a) The significance estimate calculations use empirically determined allele frequencies for each of the represented population groups. Statistics will be routinely calculated and reported for the Caucasian, African American, and Hispanic population groups.

   b) STR allelic frequency data is also available for the Asian populations for use at the analyst’s discretion.

2. STR allele frequencies


   b) Only data for the loci of the associated PCR kit will be utilized.

3.2 Estimation of Significance

A. Only loci considered conclusive for interpretations will be used for statistical calculation.

B. DNA typing results used for statistical analysis must be derived from evidentiary items and not known samples.

C. Although amelogenin is used for declaration of an association, it is not used in calculation of match significance.

D. The random match probability and the combined probability of inclusion will not be combined to create a composite statistic.
E. In situations where multiple options are available, such as CPI and deduced profile RMP, the choice of statistic must be made based on the most informative result.

F. Intimate/Indigenous samples do not require a reported statistic for the donor of the sample for manual interpretations.

3.3 Single Source/Major component Profile Significance Calculation using Random Match Probability (RMP)

A. The single source significance calculation is applicable if the evidentiary profile has been determined to be a single source profile or a component of a mixture profile, which includes a major, minor or single foreign contributor. A major component profile may be suitable for statistical analysis even in the presence of uninterpretable minor contributor results.


1. For each locus the genotypic frequency will be determined as follows:
   a) For homozygotes above stochastic threshold, \( f = p^2 + p(1-p) \theta \), where \( \theta = 0.01 \)
   b) For heterozygotes, \( f = 2pq \)
   c) For a single allele below stochastic threshold, \( f = 2p-p^2 \)
   d) When restricted options are made, the summation of the frequencies for each genotype will be used as the frequency for that locus, (i.e. \( f = 2pq + 2pq \))

2. For all loci used to identify the association, \( F = (f_1 \times f_2 \times f_3 \times f_4 \times \ldots \times f_k) \), where \( k \) is the number of loci.

3. The inverse probability = \( \frac{1}{F} \)

4. For partial profiles, the loci used in the statistic will be identified and reported. Alternatively, the number of loci used for statistical estimation may be reported instead of listing out the loci as long as the loci utilized for statistical estimation are documented in the case record.

3.4 Mixture Profile Significance Calculation using Combined Probability of Inclusion (CPI)

A. The mixture profile significance calculation will be applied if the evidentiary profile has been determined to be a mixture, except for profiles that are able to be resolved into major, minor, or foreign components.

B. Only loci with all alleles above the stochastic threshold and/or no evidence of contributor drop-out may be used to calculate CPI unless assumptions were made and documented during mixture interpretation (i.e. number of contributors).

C. Statistics will be provided when a reference sample cannot be excluded as a possible contributor to an evidentiary profile.

1. Loci will be selected for use in statistics when there is evidence that full contributions from all donors in the mixture are visualized. These loci will be documented in the case record.
2. For each locus, \( f = (p_1 + p_2 + p_3 + p_4 + \ldots + p_k)^2 \) where \( f \) is the probability of inclusion, \( p \) is the estimated frequency of the allele detected for each allele 1 through \( k \), and \( k \) is the number of alleles detected at the locus.

3. For all loci used to identify the association, \( F = (f_1 \times f_2 \times f_3 \times f_4 \times \ldots \times f_k) \), where \( F \) is the combined probability of inclusion and \( k \) is the number of loci.

4. The inverse probability \( = 1/F \).

D. If all loci are not used for statistical calculations, the specific loci used for the statistical calculations will be identified and reported. Alternatively, the number of loci used for statistical estimation may be reported instead of listing out the loci as long as the loci utilized for statistical estimation are documented in the case record.

E. The approximate population of the world will be included in the report.

F. This calculation may be performed using PopStats or other validated software.

4 Records

Calculations shall be documented in the case record, including any re-calculation performed during the technical review process, prior to the release of the report.

RMP Tool for Population Frequency Calculations (LAB-DNA-24)

5 Literature References and Supporting Documentation


DNA-09-05  TEXAS/FBI STR POPULATION DATABASE

1 Scope

These data files are only used for calculating match significance when a recalculation is performed at the request of a customer due to the error in the FBI database files. This would only include legacy kits which include Profiler Plus/Cofiler, Identifiler, and Identifiler Plus.


The format is unedited and reflects the requirements of the approved software. Within each section (Texas, FBI, and Asian), data files are listed alphabetically by locus and then by population group.

2 Software

A. The latest available and installable version of the FBI's Popstats software or other validated software will be configured to use the allele frequencies below to calculate significance estimates.

B. The Forensic-Single Sample data input option (Federal Bureau of Investigation. 1997a) will be used for single source significance calculations.

C. The Forensic Mixture data input option and the Mixture Formula (Federal Bureau of Investigation. 1997a, 1997b) will be used for mixed source significance calculations.

3 Population Databases

Population groups

A. The significance estimate calculations use empirically determined allele frequencies for each of the represented population groups. Statistics will be routinely calculated and reported for the Caucasian, African American, and Hispanic population groups.

B. STR allelic frequency data is also available for the Asian populations for use at the analyst's discretion.

4 Texas STR Data

4.1 General Information

A. The DPS data files for the loci D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 for the Black, Caucasian, and Hispanic populations were created using data collected at the Texas Department of Public Safety CODIS Convicted Offender DNA typing laboratory.

B. Data for 311 African American, 553 Caucasian, and 358 Hispanic individuals, which were collected as part of the convicted offender typing operation, were analyzed by DPS and reviewed and statistically evaluated by Dr. Bruce Budowle, FBI, for suitability for forensic testing. Additional documentation is available on request.

C. The database was approved for use on casework by the Texas DPS DNA Advisory Board and the Crime Laboratory Director on September 2, 1998.
## 1.2 D13S317

### D13S317.BLK

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**Totals**: 622  
Minimum allele frequency = **.00804**  
Null allele frequency = **.00000**

:REM D13S317 allele frequencies for Blacks from the TX DPS database.

### D13S317.CAU

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Null allele frequency = **.00000**

:REM D13S317 allele frequencies for Caucasians from the TX DPS database.
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**Totals** 716 1.00001

Minimum allele frequency = .00698
Null allele frequency = .00000

:REM D13S317 allele frequencies for Hispanics from the TX DPS database.

### D18S51

#### D18S51.BLK

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**Totals** 622 1.00001

Minimum allele frequency = .00804
Null allele frequency = .00000

:REM D18S51 allele frequencies for Blacks from the TX DPS database.
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Minimum allele frequency = 0.00452
Null allele frequency = 0.00000

:REM D18S51 allele frequencies for Caucasians from the TX DPS database.

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D21S11.BLK

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**Totals** | **622** | **1.00001**

Minimum allele frequency = .00698
Null allele frequency = .00000

:REM D18S51 allele frequencies for Hispanics from the TX DPS database.

:REM D21S11 allele frequencies for Blacks from the TX DPS database.
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Totals: 1106, 1.00001

Minimum allele frequency = 0.00452
Null allele frequency = 0.00000

:REM D21S11 allele frequencies for Caucasians from the TX DPS database.

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Minimum allele frequency = **0.00698**

Null allele frequency = **0.00000**

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### 1.5 D3S1358

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Minimum allele frequency = **0.00804**

Null allele frequency = **0.00000**

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:REM D21S11 allele frequencies for Hispanics from the TX DPS database.

:REM D3S1358 allele frequencies for Blacks from the TX DPS database.
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Null allele frequency = 0.00000

:REM D3S1358 allele frequencies for Caucasians from the TX DPS database.

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Null allele frequency = 0.00000

:REM D3S1358 allele frequencies for Hispanics from the TX DPS database.
## 1.6 D5S818

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Totals 622 1.00001

Minimum allele frequency = .00804
Null allele frequency = .00000

:REM D5S818 allele frequencies for Blacks from the TX DPS database.

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Totals 1106 1.0000

Minimum allele frequency = .00452
Null allele frequency = .00000

:REM D5S818 allele frequencies for Caucasians from the TX DPS database.

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**Totals**: 622

Minimum allele frequency = .00804

Null allele frequency = .00000

:REM D7S820 allele frequencies for Blacks from the TX DPS database.

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**Totals**: 716

Minimum allele frequency = .00698

Null allele frequency = .00000

:REM D5S818 allele frequencies for Hispanics from the TX DPS database.
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Totals 716 1.0000
Minimum allele frequency = .00698
Null allele frequency = .00000

:REM D7S820 allele frequencies for Hispanics from the TX DPS database.

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Totals 622 1.0000
Minimum allele frequency = .00804
Null allele frequency = .00000

:REM D8S1179 allele frequencies for Blacks from the TX DPS database.
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**Totals:** 1106  1.0000  
Minimum allele frequency = 0.00452  
Null allele frequency = 0.00000

:REM D8S1179 allele frequencies for Caucasians from the TX DPS database.

## D8S1179.HIS

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**Totals:** 716  0.99999  
Minimum allele frequency = 0.00698  
Null allele frequency = 0.00000

:REM D8S1179 allele frequencies for Hispanics from the TX DPS database.
### 1.9 FGA

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Minimum allele frequency = 0.00804
Null allele frequency = 0.0000

:REM FGA allele frequencies for Blacks from the TX DPS database.

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Totals: 1106 0.99998
Minimum allele frequency = 0.0452
Null allele frequency = 0.00000

:REM FGA allele frequencies for Caucasians from the TX DPS database.
Totals 716 1.00001
Minimum allele frequency = 0.00698
Null allele frequency = 0.0000

:REM FGA allele frequencies for Hispanics from the TX DPS database.

1.10 vWA

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Totals 622 1.000
Minimum allele frequency = 0.00804
Null allele frequency = 0.0000

:REM vWA allele frequencies for Blacks from the TX DPS database.

vWA.CAU

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Null allele frequency = 0.0000

:REM vWA allele frequencies for Caucasians from the TX DPS database.
vWA.HIS

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Totals 716 1.0000

Minimum allele frequency = 0.00698
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:REM vWA allele frequencies for Hispanics from the TX DPS database.

5 FBI STR Data

5.1 General Information


5.2 CSF1PO

CSF1PO.BLK

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Totals 420 1.0000

Minimum allele frequency = 0.0119

Min Allele Frequency calculated as 5/2N where N = 210

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.
### CSF1PO.CAU

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Totals | 404 | 1.0002 |
Minimum allele frequency = 0.0124

Min Allele Frequency calculated as 5/2N where N = 202

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.

### CSF1PO.HIS

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Totals | 418 | 1.0001 |
Minimum allele frequency = 0.0120

Min Allele Frequency calculated as 5/2N where N = 209

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.
### 5.3 D16S539

**D16S539.BLK**

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**Totals** 418 1.0001

Minimum allele frequency = 0.0120

Min Allele Frequency calculated as 5/2N where N = 209

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.

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**D16S539.CAU**

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**Totals** 402 1.0000

Minimum allele frequency = 0.0124

Min Allele Frequency calculated as 5/2N where N = 201

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.

---

**D16S539.HIS**

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Effective Date: 5/4/2020

Issued by: System Quality Manager

*Printed copy is uncontrolled. Refer to electronic copy for current version.*
## 5.4 TH01

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Minimum allele frequency = 0.0119

Min Allele Frequency calculated as 5/2N where N = 210

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.

### TH01.CAU

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Minimum allele frequency = 0.0124

Min Allele Frequency calculated as 5/2N where N = 202

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.
## TPOX

### TPOX.BLK

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**Totals** | 418 | 0.9999 |

Minimum allele frequency = 0.0120

Min Allele Frequency calculated as 5/2N where N = 209

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.

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**Totals** | 418 | 0.9999 |

Minimum allele frequency = 0.0120

Min Allele Frequency calculated as 5/2N where N = 209

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**Totals**: 418  
Minimum allele frequency = 0.0120

Min Allele Frequency calculated as 5/2N where N = 209

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.

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**TPOX.HIS**

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**Totals**: 334  
Minimum allele frequency = 0.0150

Min Allele Frequency calculated as 5/2N where N = 167

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.

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**D2S1338**

**D2S1338.BLK**

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**Totals**: 334  
Minimum allele frequency = 0.0150

Min Allele Frequency calculated as 5/2N where N = 167

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.
### D2S1338.CAU

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Minimum allele frequency = 0.0164

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.

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Minimum allele frequency = 0.0176

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.
### 5.7 D19S433

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Totals: 334, Minimum allele frequency = 0.0150

Min Allele Frequency calculated as 5/2N where N = 167

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.

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Totals: 304, Minimum allele frequency = 0.0164

Min Allele Frequency calculated as 5/2N where N = 152

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**Totals** 284 1.0000

Minimum allele frequency = 0.0176

Min Allele Frequency calculated as 5/2N where N = 142

Data provided in April 2015 by the FBI Laboratory, DNA Support Unit in Quantico, VA. File compiled by Kevin M. Ellis, FBI CODIS Unit.

6 **ASIAN STR Data**

6.1 **General Information**


6.2 **D13S317**

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**Totals** 344 1.000

Minimum allele frequency = .01453
Null allele frequency = .00000


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**Totals** 100 1.000

Minimum allele frequency = .05000
Null allele frequency = .00000

6.3 D18S51

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| Minimum allele frequency = | 0.0500 |
| Null allele frequency = | 0.0000 |


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Minimum allele frequency = 0.0500
Null allele frequency = 0.0000

### 6.4 D21S11

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### 6.5 D3S1358

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Null allele frequency = .00000


### 6.5 D3S1358

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Null allele frequency = .00000


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Minimum allele frequency = .05000
Null allele frequency = .00000

Printed copy is uncontrolled. Refer to electronic copy for current version.
Minimum allele frequency = 0.0500
Null allele frequency = 0.0000


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Totals  344  1.0000
Minimum allele frequency = 0.0145
Null allele frequency = 0.0000


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Null allele frequency = 0.0000

## 6.7 D7S820

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Minimum allele frequency = 0.0500
Null allele frequency = 0.0000


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Minimum allele frequency = 0.0145
Null allele frequency = 0.0000

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**Totals** 100 1.0000

Minimum allele frequency = 0.05000
Null allele frequency = 0.0000


### D8S1179

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**Totals** 100 1.0000

Minimum allele frequency = 0.05000
Null allele frequency = 0.0000

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**Totals** | **344** | **1.0000**

*Minimum allele frequency = 0.01453*

*Null allele frequency = 0.00000*


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*Minimum allele frequency = 0.05000*

*Null allele frequency = 0.00000*

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**FGA.CHI**

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Totals: 100 1.0000

Minimum allele frequency = .05000
Null allele frequency = .00000


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6.10 vWA

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vWA.VET

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Totals 100 1.0000

Minimum allele frequency = 0.05000
Null allele frequency = 0.00000


7 Literature References and Supporting Documentation


DNA-09-06 NIST STR POPULATION DATABASE

1 Scope
These data files are used for calculating match significance. Any statistical calculations as a result of a new request or reinterpretation request (including those for legacy amplification kit data) will use the population data listed below taken from the NIST 1036 database published in Steffen C.R., Coble, M.D., Gettings, K.B., Vallone, P.M. (2017) Corrigendum to ‘U.S. Population Data for 29 Autosomal STR Loci’ [Forensic Sci. Int. Genet. 7(2013) e82-e83] Forensic Sci. Int. Genet. 31: e36-e40. The format is unedited and reflects the requirements of the approved software.

2 Related Chapters
Manual Autosomal STR Statistical Significance Estimation
STRmix Autosomal STR Interpretation Guidelines

3 Software
A. For manual DNA profile interpretation, the latest available and installable version of the FBI’s Popstats software or other validated software will be configured to use the allele frequencies below to calculate significance estimates.
   1. The Forensic-Single Sample data input option (Federal Bureau of Investigation. 1997a) will be used for single source significance calculations.
   2. The Forensic Mixture data input option and the Mixture Formula (Federal Bureau of Investigation. 1997a, 1997b) will be used for mixed source significance calculations.
B. For probabilistic DNA profile interpretation, the latest validated version of STRmix software will be configured to use the allele frequencies below to calculate significance estimates in the form of likelihood ratios.

4 Population Databases
Population groups
A. The significance estimate calculations use empirically determined allele frequencies for each of the represented population groups. Statistics will be routinely calculated and reported for the Caucasian, African American, and Hispanic population groups.
B. STR allelic frequency data is also available for the Asian populations for use at the analyst’s discretion.
C. Statistics will be routinely calculated using the Caucasian, African-American, Hispanic, and Asian population groups if using the STRmix software, and the lowest total likelihood ratio between the population groups reported.

5 NIST STR Data
5.1 Allele Frequency Values for Unrelated African American U.S. Population Samples
Allele frequency values for unrelated African American U.S. population samples at 29 autosomal STR loci in commercial STR multiplex kits. The observed heterozygosity values (Hobs), Hardy-Weinberg equilibrium p-values from exact test (HWE), probability of identity values (PI), and probability of paternity exclusion values (PPE) are listed below for each marker in Table 1. HWE p-values <0.05 are highlighted in yellow.
Results for Population: African American Sample Size = 342 for CSF1PO, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D1S1656, D21S11, D22S1045, D2S1338, D2S441, D3S1358, D5S88, D6S1043, D7S820, D8S179, F13A01, F13B, FESFPS, FGA, LPL, Penta C, Penta D, Penta E, SE33, TH01, and vWA.

Sample Size = 341 for TPOX

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$H_{obs}$: observed heterozygosity

$HWE$: Hardy-Weinberg equilibrium p-value from exact test

$P_I$: probability of identity

$PPE$: probability of paternity exclusion
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\( \text{HWE} \): Hardy-Weinberg equilibrium p-value from exact test

\( \text{P}_{\text{I}} \): probability of identity

\( \text{PPE} \): probability of paternity exclusion
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H_\text{obs}: observed heterozygosity
HWE: Hardy-Weinberg equilibrium p-value from exact test
P_1: probability of identity
PPE: probability of paternity exclusion

### 5.2 Allele Frequency Values for Unrelated Caucasian U.S. Population Samples

Allele frequency values for unrelated Caucasian U.S. population samples (n=361) at 29 autosomal STR loci in commercial STR multiplex kits. The observed heterozygosity values (H_\text{obs}), Hardy-Weinberg equilibrium p-values from exact test (HWE), probability of identity values (PI), and probability of paternity exclusion values (PPE) are listed below each marker in Table 2. HWE p-values <0.05 are highlighted in yellow.

Results for Population: Cauc (Sample Size = 361)

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H_{obs}: observed heterozygosity

HWE: Hardy-Weinberg equilibrium p-value from exact test

P_I: probability of identity

PPE: probability of paternity exclusion
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$H_{obs}$: observed heterozygosity

HWE: Hardy-Weinberg equilibrium p-value from exact test

$P_I$: probability of identity

PPE: probability of paternity exclusion
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Table 2. Caucasian Population Samples (continued)

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Hobs: observed heterozygosity
HWE: Hardy-Weinberg equilibrium p-value from exact test
PI: probability of identity
PPE: probability of paternity exclusion

5.3 Allele Frequency Values for Unrelated Hispanic U.S. Population Samples

Allele frequency values for unrelated Hispanic U.S. population samples at 29 autosomal STR loci in commercial STR multiplex kits. The observed heterozygosity values (Hobs), Hardy-Weinberg equilibrium p-values from exact test (HWE), probability of identity values (PI), and probability of paternity exclusion values (PPE) are listed below for each marker in Table 3. HWE p-values <0.05 are highlighted in yellow.

Results for Population: Hispanic, Sample Size = 236 for CSF1PO, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D1S1656, D21S11, D22S1045, D2S1338, D2S441, D3S1358, D5S88, D6S1043, D7S820, D8S179, F13A01, F13B, FESFPS, FGA, LPL, Penta C, Penta E, SE33, TH01, TPOX and vWA.

Sample Size = 235 for Penta D

See Table 3
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$H_{obs}$: observed heterozygosity

HWE: Hardy-Weinberg equilibrium p-value from exact test

$P_I$: probability of identity

PPE: probability of paternity exclusion
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\(H_{\text{obs}}\): observed heterozygosity

HWE: Hardy-Weinberg equilibrium p-value from exact test

\(P_i\): probability of identity

PPE: probability of paternity exclusion
Table 3. Hispanic Population Samples

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Hobs: observed heterozygosity
HWE: Hardy-Weinberg equilibrium p-value from exact test
PI: probability of identity
PPE: probability of paternity exclusion

5.4 Allele Frequency Values for Unrelated Asian U.S. Population Samples

Allele frequency values for unrelated Asian U.S. population samples (n=97) at 29 autosomal STR loci in commercial STR multiplex kits. The observed heterozygosity values (Hobs), Hardy-Weinberg equilibrium p-values from exact test (HWE), probability of identity values (PI), and probability of paternity exclusion values (PPE) are listed below each marker in Table 4. HWE p-values <0.05 are highlighted in yellow.

Results for Population: Asian (Sample Size = 97)

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$H_{obs}$: observed heterozygosity

HWE: Hardy-Weinberg equilibrium p-value from exact test

$P_I$: probability of identity

PPE: probability of paternity exclusion
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$H_{\text{obs}}$: observed heterozygosity

HWE: Hardy-Weinberg equilibrium p-value from exact test

$P_I$: probability of identity

PPE: probability of paternity exclusion
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Table 4. Asian Population Samples (continued)

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<th>LPL</th>
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<th>Penta_D</th>
<th>Penta_E</th>
<th>SE33</th>
<th>TH01</th>
<th>TPOX</th>
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<td>(H_{\text{obs}})</td>
<td>0.8247</td>
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</table>

\(H_{\text{obs}}\): observed heterozygosity

HWE: Hardy-Weinberg equilibrium p-value from exact test

\(P_i\): probability of identity

PPE: probability of paternity exclusion

6 Literature References and Supporting Documentation


DNA-09-07 REPORT WRITING GUIDELINES

1 Scope

The laboratory report must communicate both the analytical results and the conclusions of the analyst, conveying the essence of the expert testimony in court. The notes and other documentation must support the conclusions of the analyst. Decisions may be made by police officers, attorneys and the courts based on the report alone without analyst clarification, so the report should be able to stand alone.

The report must contain the information required in the Laboratory Reports, Letters, and Certificates chapter of the Crime Laboratory Service Manual and the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories. LIMS reports are compliant with these requirements for proper format.

Typical casework reporting should follow the recommended reporting statements, as appropriate. It is recognized that not every situation can be represented by these statements and that it may be necessary to modify the statements to accurately reflect the results.

Bolded and bracketed portions of the reporting statements indicate situational/case specific wording.

2 Biological Evidence Screening

2.1 Requirements

A. List of items submitted including sub-items for Forensic Biology analysis.
B. Report the results for all items examined.
C. Indicate in report all items not examined related to the request.
D. Indicate in report if trace evidence was collected.
E. Indicate disposition of evidentiary items and collected samples.

2.2 Blood Examinations

A. No visible blood, no presumptive tests performed
   “No visible bloodstains were [observed/detected] [on item].”
   “No stains having the appearance of blood were observed [on item].”
   “No blood was observed [on item].”
   “Stains having the appearance of blood were not observed [on item].”
   “Blood was not observed [on item].”
B. Negative presumptive test(s)
   “No blood was detected [on item].”
   “A presumptive test for the presence of blood was negative [on item].”
   “No detectable bloodstains were found [on item].”
   “Blood was not detected [on item].”
   “Presumptive testing for the presence of blood was negative.”
C. Positive presumptive test(s)
   “A presumptive test indicates the presence of blood [on item].”
   “Apparent blood was detected [on item].”
   “A presumptive test for the presence of blood was positive [on item].”
   “Presumptive testing for the presence of blood was positive.”

D. Uninterpretable presumptive test(s)
   “No interpretable results were obtained; therefore, the presence of presumptive blood cannot be determined.”

E. Positive Hematrace test
   “Apparent human blood was detected [on item].”

F. Negative Hematrace test
   “No human blood was detected [on item].”
   “Human blood was not detected [on item].”

G. Uninterpretable Hematrace test
   “No interpretable results were obtained; therefore, the presence of apparent human blood cannot be determined.”

2.3 Semen Examinations
A. Negative alternate light source
   “No stains having the appearance of semen were observed [on item].”
   “Stains having the appearance of semen were not observed [on item].”

B. Negative AP screening
   “There were no indications of semen [on item].”
   “A presumptive test for the presence of semen was negative [on item].”
   “No acid phosphatase, a nonspecific constituent of semen, was detected [on item].”
   “Acid phosphatase, a nonspecific constituent of semen, was not detected [on item].”
   “Presumptive testing for the presence of semen was negative.”

C. Positive AP, no further testing
   “Presumptive testing for the presence of semen was positive.”
   “Apparent semen was detected [on item].”
   “Semen was indicated but not confirmed [on item].”
   “Acid phosphatase, a nonspecific constituent of semen, was detected [on item].”

D. Negative AP and negative sperm (orifice swabs and/or corresponding slides)
   “No semen was detected [on the orifice swab specimen].”
   “Semen was not detected [on the orifice swab specimen].”
E. Positive AP, negative sperm, negative p30
   “No semen was detected [on item].”
   “Semen was not detected [on item].”
   “A presumptive test for semen was positive [on item]. However, spermatozoa (semen specific constituents) were not detected. Therefore, we cannot confirm the presence of semen.”
   “Semen was indicated [on item]; however, no spermatozoa were detected to confirm the presence of semen.”

F. Negative or positive AP, positive sperm
   “Spermatozoa were detected [on item], confirming the presence of semen.”
   “Spermatozoa, semen specific constituents, were detected [on item].”
   “Semen was detected [on item].”

G. Positive AP, negative sperm, positive p30
   “Presumptive tests for semen were positive [on item]. However, spermatozoa (semen specific constituents) were not detected. Therefore, we cannot confirm the presence of semen.”
   “Acid phosphatase and p30 (nonspecific constituents of semen) were detected [on item]. However, spermatozoa (semen specific constituents) were not detected. Therefore, we cannot confirm the presence of semen.”
   “Semen was indicated [on item]; however, no spermatozoa were detected to confirm the presence of semen.”

2.4 Non-blood, Non-semen Sample Collection
   “Samples were collected for possible DNA analysis.”
   “This item was collected for comparison.”
   “This item was collected to be used as a reference.”

2.5 Significant Location and/or Amount of Stain
   A. In some circumstances it may be useful to provide narrative observations of location and/or amount of stain. Various statements of observation may be combined with screening test results as appropriate.
   B. Examples:
      “Item 5 (suspect’s shirt) contained small stains of apparent blood on the lower front of the shirt. Two stains were selected for further testing.”
      “A minute stain having the appearance of blood and positive to a presumptive test for the presence of blood was present on the right cuff of the shirt.”
2.6 **Species (Human) Origin Determination by Quantifiler/Quantifiler Duo**

The Quantifiler/Quantifiler Duo kits are designed to aid in the determination of the presence and amount of amplifiable human (and higher primate) DNA.

A. **Negative Quantifiler/Quantifiler Duo**
   - “No human DNA was detected on [item].”
   - “Human DNA was not detected on [item].”
   - **[Presumptive statement]** “However, this stain did not respond to human origin testing.”

B. **Positive Quantifiler/Quantifiler Duo**
   - “Human DNA was detected on [item].”

2.7 **Human Male DNA Determination by Quantifiler Duo**

The Quantifiler Duo kit is designed to aid in the determination of the presence and amount of amplifiable human (and higher primate) and male DNA.

A. **Negative Quantifiler Duo Male Quantification**
   - “No human male DNA was detected on [item].”
   - “Human male DNA was not detected on [item].”

B. **Positive Quantifiler Duo Male Quantification**
   - “Human male DNA was detected on [item].”

2.8 **Trace Collection**

Evidence is processed for hair/fiber collection and no examination is performed.

- “Items 1-4 have been processed for the collection of trace evidence. No further analysis was conducted at this time.”
- “Trace evidence was collected from items 1-4. The collected trace evidence has been packaged with the evidence. No further analysis was conducted at this time.”
- “The presence of probative DNA evidence may not require that the preserved trace evidence be examined.”

3 **Male Screening**

3.1 **Requirements**

A. List of items submitted including sub-items for Forensic Biology analysis.
B. Include a description of DNA methodology (i.e. the quantification kit).
C. Report the results for all items tested.
D. Indicate in report all items not examined related to the request.
E. Indicate disposition of evidentiary items and collected samples.

3.2 **Results**

Use the following reporting statements to report male screening results:

A. A “N/A” quantification value was obtained for the presence of male DNA:
   - “No male DNA was detected on this item utilizing Plexor HY.”
B. The sample has a male quant value \( \geq 0.01 \text{ng/\( \mu \text{L} \)} \) or \( \geq 0.001 \text{ng/\( \mu \text{L} \)} \) with a low autosomal/male DNA ratio (less than approximately 200) OR the sample has Check Melts indicated by the quantitation software, there is a melt curve within the window that crosses the threshold (even if it is shifted) and the male quantitation value is \( \geq 0.01 \text{ng/\( \mu \text{L} \)} \) or \( \geq 0.001 \text{ng/\( \mu \text{L} \)} \) with a low autosomal/male DNA ratio (less than approximately 200):

"Male DNA was detected on this item utilizing [insert kit name]."

C. The sample has a male quant value \( < 0.01 \text{ng/\( \mu \text{L} \)} \), but \( \geq 0.001 \text{ng/\( \mu \text{L} \)} \), and a high autosomal/male DNA ratio (greater than approximately 200) OR the sample has Check Melts indicated by the quantitation software, there is a melt curve within the window that crosses the threshold (even if shifted) and the male quantitation value is \( < 0.01 \text{ng/\( \mu \text{L} \)} \), but \( \geq 0.001 \text{ng/\( \mu \text{L} \)} \) with a high autosomal/male DNA ratio (greater than approximately 200):

"Male DNA was detected on this item utilizing [insert kit name]. Due to the presence of high amounts of total DNA relative to the amount of male DNA present, this sample will not be processed for autosomal STR analysis."

D. The sample has a male quantitation value \( < .001 \text{ng/\( \mu \text{L} \)} \), OR the sample has Check Melts indicated by the quantitation software, there is a melt curve within the window (regardless of the presence of other curves outside the window), the threshold may or may not be crossed, and the male quantitation result is \( < .001 \text{ng/\( \mu \text{L} \)} \):

"The detection of male DNA on this item is inconclusive utilizing [insert kit name] due to the low male quantification value."

OR

"Due to the low male quantification value utilizing [insert kit name], the presence of male DNA is inconclusive."

E. The sample has Check Melts indicated by the quantitation software, there is no melt curve in the window, and the male quantitation result is any value:

"No male DNA was detected on this item utilizing [insert kit name]."

F. The sample has Check Melts indicated by the quantitation software, most of the melt curve is outside of the window, the threshold is not crossed, and the male quantitation result is \( < .001 \text{ng/\( \mu \text{L} \)} \):

"The detection of male DNA on this item was inconclusive utilizing [insert kit name] due to potential inhibition and/or non-specific amplification."

4 Manual Autosomal DNA Analysis

4.1 Reporting Requirements

A. List items analyzed for DNA

B. Description of DNA methodology and technology
   1. DNA extraction (if not previously reported)
   2. Differential extractions (if performed and not previously reported)
   3. Analyzed by STR Analysis

C. List loci analyzed (this may be accomplished through use of an appendix).
D. A statement must be present in the report to address all DNA extracts.
   1. If a stain/collection is re-extracted to resolve contamination or a quality incident, the report must indicate that multiple extractions of the same stain/collection were performed due to a quality event. Alternatively, multiple reports may be issued to indicate additional extractions. Comparisons will only be made to profiles where contamination or the quality incident has been resolved.
   2. If a stain/collection is re-extracted in an attempt to gain more information, the report must indicate that multiple extractions of the same stain/collection were performed in an effort to obtain additional information. Alternately, multiple reports may be issued to indicate additional extractions.

E. For cases received and reported using the current LIMS, when DNA comparisons are made the interpretation/conclusions will be reported in the “Evidence Description, Results of Analysis and Interpretation” section of the report. For cases originally received and reported using DRAGNet legacy LIMS, refer to the Legacy Amplification Kit Data Reinterpretation chapter.

F. Statistical Significance Estimate
   1. The statistical estimate may be expressed as either numbers truncated to the last significant digit as generated by the validated statistical software or words for the Caucasian, African American, and Hispanic population groups.
   2. Statistical significance estimate reporting
      A significance statement for each inclusion must be in the report, however intimate and indigenous samples do not require a reported statistic for the donor of the sample.

G. For cases screened using Male Screening with Plexor HY, if no sperm search is performed during DNA analysis, the following statement must be added in the Investigative Leads section of the DNA report:
   “Please note that no testing to confirm the presence of semen was conducted on items [list items here]. If testing to confirm the presence of semen is needed, please contact this laboratory for instructions.”

H. Disposition of evidentiary items on which DNA analysis was performed will be included in the report. This includes evidentiary items depleted during analysis.

4.2 Exclusion
   “[At the loci determined to be suitable for comparison], [Person A] is excluded as the contributor of the [partial] DNA profile from [Item 1, stain 1].”
   OR
   “The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture of at least _ contributors. [At the loci determined to be suitable for comparison], [Person A] is excluded as a possible contributor to this DNA profile.”
   OR
   “The [partial] DNA profile obtained from [Item 1, stain 1] is consistent with a mixture. Assuming two donors to the mixture, [Person A] is excluded as a possible contributor to this DNA profile.”
OR

"[At the loci determined to be suitable for comparison], [Person A] is excluded as the contributor of the [partial] [major/minor] DNA profile from [Item 1, stain 1]."

OR

"The [partial] DNA profile obtained from [Item 1, stain 1] is consistent with a mixture. Assuming two donors to the mixture and that [Person B] is a donor, [Person A] is excluded as a possible contributor to the foreign DNA profile."

OR

"The [partial] DNA profile obtained from [Item 1, stain 1] is consistent with a mixture of at least _ individuals. A mixed major could be resolved. Assuming two donors to the mixed major, [Person A] is excluded as a possible contributor to the [mixed major] DNA profile."

4.3 Inclusion - Single Source

"The [partial] DNA profile from [Item 1, stain 1] is consistent with the DNA profile of [Person A]. [The following loci were used for statistical significance estimation: TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820. At these loci,] The probability of selecting an unrelated person at random who could be the contributor of this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics."

Report the following: “The approximate world population is 7.0 billion.”

4.4 Inclusion – Major/Minor Component

"The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture of at least _ contributors."

OR

"The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture from [Person A and Person B] [or Person C and some unknown individual...]."

AND

"A [complete/partial] DNA profile for the major contributor could be resolved. [Person A] cannot be excluded as the contributor of the [major/minor] component in this DNA profile. [The following loci were used for statistical significance estimation: TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820. At these loci,] The probability of selecting an unrelated person at random who could be the contributor of the [major/minor] component in this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics."

OR

"Assuming two donors to the mixture, a [complete/partial] DNA profile for the [major/minor] contributor could be resolved. [Person A] cannot be excluded as the contributor of the major/minor component in this DNA profile. [The following loci were used for statistical significance estimation: TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338,
4.5 Inclusion - Mixture

“The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture of at least _ contributors.”

OR

“The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture from [Person A and Person B] [or Person C and some unknown individual...].”

AND

“[Person A cannot be excluded as a contributor to the profile. The following loci were used for statistical significance estimation: TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820. At these loci,] The probability of selecting an unrelated person at random who could be a contributor to this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics. The approximate world population is 7.0 billion.”

4.6 Inclusion - Indistinguishable Mixture

“The [partial] DNA profile obtained from [Item 1, stain 1] is consistent with a mixture. Assuming two donors to the mixture, [Person A] cannot be excluded as a possible contributor to this DNA profile. [The following loci were used for statistical significance estimation: TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820. At these loci,] The probability of selecting an unrelated person at random who could be a contributor to this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics. The approximate world population is 7.0 billion.”

4.7 Inclusion - Intimate/Indigenous/Differential Samples

“The [partial] DNA profile obtained from [Item 1, stain 1] is consistent with a mixture of at least _ individuals. Assuming [Person A] is a donor to the mixture, a [partial] DNA profile for the foreign contributor[s] could be resolved. [Person B] cannot be excluded as the foreign contributor to this DNA profile. [The following loci were used for statistical significance estimation: TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820. At these loci,] The probability of selecting an unrelated person at random who could be the contributor of this [foreign] DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics.”

Report the following: “The approximate world population is 7.0 billion.”

OR

“The DNA profile obtained from [Item 1, stain 1] is consistent with a mixture. Assuming two donors to the mixture and that [Person A] is a donor, a [partial] DNA profile for the
foreign contributor could be resolved. **[Person B]** cannot be excluded as the foreign contributor to this profile. **[The following loci were used for statistical significance estimation: TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820. At these loci,] The probability of selecting an unrelated person at random who could be the contributor of this **[foreign]** DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics.**”

**Report the following:** “The approximate world population is 7.0 billion.”

### 4.8 Mixed Major

“**The DNA profile obtained from [Item 1, stain 1] is consistent with a mixture of at least _ individuals. A mixed major could be resolved. Assuming two donors to the mixed major, [Person A] cannot be excluded as a possible contributor to this DNA profile.** **[The following loci were used for statistical significance estimation: TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820. At these loci,] The probability of selecting an unrelated person at random who could be a contributor to the mixed major in this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics.**”

**AND**

**Report the following:** “The approximate world population is 7.0 billion.”

**OR**

“**The DNA profile obtained from [Item 1, stain 1] is consistent with a mixture of at least _ individuals. A mixed major could be resolved. Assuming two donors to the mixed major, and that [Person A] is a donor, [Person B] cannot be excluded as a possible contributor to this DNA profile. **[The following loci were used for statistical significance estimation: TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820. At these loci,] The probability of selecting an unrelated person at random who could be a contributor to the mixed major in this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics.**”

**AND**

**Report the following:** “The approximate world population is 7.0 billion.”

### 4.9 Alternate Reference Samples

**A.** When no standard reference samples are available, alternate reference samples yielding a single source profile may be used.

**B.** If a partial profile is obtained, Technical Leader approval is required in order to use the alternate reference sample for comparison purposes.

**C.** When compared to an evidentiary profile, the appropriate prescribed reporting statement will be modified to read:

“**The [partial] DNA profile from [Item 1, stain 1] is consistent with the DNA profile of [alternate reference]. Assuming [Person A] is the source of the [alternate reference], [Person A] cannot be excluded.**”
OR

“[At the loci determined to be suitable for comparison] and assuming [Person A] is the source of the [alternate reference], [Person A] is excluded as a contributor to the [partial] DNA profile from [Item 1, stain 1].”

D. If the alternate reference sample is determined to be unsuitable for comparisons, the following statements may be used:

“The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture and therefore is unsuitable for use as an alternate known.

OR

“Insufficient data was obtained from [Item 1, stain 1] therefore this item is unsuitable for use as an alternate known.”

4.10 No Comparison to Minor Contributor

“Due to the complexity of data present, no comparisons will be made to the minor component.”

“Due to the quantity and/or quality of DNA obtained, no comparisons will be made to the minor component.”

5 STRmix Reporting Statements

5.1 Reporting Requirements

A. List items analyzed for DNA

B. Description of DNA methodology and technology
   1. DNA extraction (if not previously reported)
   2. Differential extractions (if performed and not previously reported)
   3. Analyzed by STR Analysis

C. List loci analyzed (this may be accomplished through use of an appendix).

D. A statement must be present in the report to address all DNA extracts.
   1. If a stain/collection is re-extracted to resolve contamination or a quality incident, the report must indicate that multiple extractions of the same stain/collection were performed due to a quality event. Alternatively, multiple reports may be issued to indicate additional extractions. Comparisons will only be made to profiles where contamination or the quality incident has been resolved.
   2. If a stain/collection is re-extracted in an attempt to gain more information, the report must indicate that multiple extractions of the same stain/collection were performed in an effort to obtain additional information. Alternately, multiple reports may be issued to indicate additional extractions.

E. For cases received and reported using the current LIMS, when DNA comparisons are made the interpretation/conclusions will be reported in the “Evidence Description, Results of Analysis and Interpretation” section of the report. For cases originally received and reported using DRAGNet legacy LIMS, refer to the Legacy Amplification Kit Data Reinterpretation chapter.
F. Statistical Significance Estimate

1. The statistical estimate used in the report is the lowest total LR between the population groups.
   a) If the LR is ≥0.01 but <100, the number (either generated by STRmix or the calculated 1/LR as applicable) is truncated at the decimal and reported without rounding as either numbers or words (e.g. 2.76 is reported as 2 or two).
   b) If the LR is ≥100, the number is reported as either numbers truncated to the last significant figure as generated by STRmix or words (e.g. 6.26E15 is reported as 6,260,000,000,000,000 or 6.26 quadrillion).

2. A significance statement for each inclusion must be in the report, however intimate and indigenous samples do not require a reported statistic for the donor of the sample.

G. For cases screened using Male Screening with Plexor HY, if no sperm search is performed during DNA analysis, the following statement must be added in the Investigative Leads section of the DNA report:

   “Please note that no testing to confirm the presence of semen was conducted on items [list items here]. If testing to confirm the presence of semen is needed, please contact this laboratory for instructions.”

H. Disposition of evidentiary items on which DNA analysis was performed will be included in the report. This includes evidentiary items depleted during analysis.

5.2 Intimate/Indigenous Samples

A. Intimate/Indigenous single source samples consistent with the assumed donor

   Note: STRmix deconvolution and comparison is not required to support this type of interpretation.

   “The [partial] DNA profile from [this] item [01-01-AA] is consistent with the DNA profile from [Person A].”

   OR

   “The DNA profile is interpreted as originating from a single individual. [Person A] is an assumed contributor to this profile.”

B. Intimate/Indigenous female single source sample with peak(s) at DYS391

   Note: STRmix deconvolution and comparison is not required to support this type of interpretation.

   “The [partial] DNA profile from [this] item [01-01-AA] is consistent with the DNA profile from [Person A].”

   OR

   “The DNA profile is interpreted as originating from a single individual. [Person A] is an assumed contributor to this profile.”

   AND

   Report the following: “There is an indication of at least [#] male contributor(s) at the Y-STR locus; however, this data is insufficient for comparisons.”
C. Intimate/Indigenous male single source sample with multiple peaks at DYS391

Note: STRmix deconvolution and comparison is not required to support this type of interpretation.

“The [partial] DNA profile from [this] item [01-01-AA] is consistent with the DNA profile from [Person A].”

OR

“The DNA profile is interpreted as originating from a single individual. [Person A] is an assumed contributor to this profile.”

AND

Report the following: “There is an indication of at least [#] additional male contributor(s) at the Y-STR locus; however, this data is insufficient for comparisons.”

D. Intimate/Indigenous female single source sample with Y at Amelogenin

Note: STRmix deconvolution and comparison is not required to support this type of interpretation.

“The [partial] DNA profile from [this] item [01-01-AA] is consistent with the DNA profile from [Person A].”

OR

“The DNA profile is interpreted as originating from a single individual. [Person A] is an assumed contributor to this profile.”

AND

Report the following: “There is an indication of at least one male contributor at the Amelogenin locus; however, this data is insufficient for comparisons.”

5.3 Likelihood Ratio of Less than 0.01

Note: STRmix deconvolution and comparison is not required to support these types of interpretation. If STRmix deconvolution and comparison is used to support these types of interpretation, the reporting statement must include information indicating a likelihood ratio result was calculated.

A. For single source profiles:

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single individual. [Based on the likelihood ratio result,] [Person A] is excluded as the contributor of this profile (refer to Appendix).”

B. For female single source profiles with peak(s) at DYS391:

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single individual. [Based on the likelihood ratio result,] [Person A] is excluded as the contributor of this profile (refer to Appendix). There is an indication of at least [#] male contributor(s) at the Y-STR locus; however, this data is insufficient for comparisons.”

C. For female single source profiles with a Y at Amelogenin:

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single individual. [Based on the likelihood ratio result,] [Person A] is excluded as the
contributor of this profile (refer to Appendix). There is an indication of at least one male contributor at the Amelogenin locus; however, this data is insufficient for comparisons."

D. For mixture profiles

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as a mixture of [X] individuals [with Person A as an assumed contributor]. Based on the likelihood ratio result, [Person B] is excluded as a contributor to this profile (refer to Appendix).”

5.4 Likelihood Ratio of more than or equal to 0.01 but Less than or Equal to 0.5

A. For single source profiles:

In these situations, Person A is the donor under Hp, while an unknown individual is the donor under Hd. Enter the 1/LR value in the underscore of each statement and the LR value in the # of each statement as applicable.

\[ LR_{Person\ A} = \frac{Pr(E \mid S_{Person\ A})}{Pr(E \mid S_{Unknown\ Individual})} \]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single individual. The probability of [obtaining] this profile if the DNA came from an unrelated, unknown individual is _ [or 1 divided by #] times greater than the probability of [obtaining] this profile if the DNA came from [Person A]. This likelihood ratio indicates support for the proposition that [Person A] is excluded as a possible contributor of this profile. [Internal validation has shown that] a likelihood ratio of this value may also indicate support for exclusion of a true donor from the profile (refer to Appendix).”

B. For female single source profiles with peak(s) at DYS391:

In these situations, Person A is the donor under Hp, while an unknown individual is the donor under Hd. Enter the 1/LR value in the underscore of each statement and the LR value in the # of each statement as applicable.

\[ LR_{Person\ A} = \frac{Pr(E \mid S_{Person\ A})}{Pr(E \mid S_{Unknown\ Individual})} \]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single female individual. The probability of [obtaining] this profile if the DNA came from an unrelated, unknown individual is _ [or 1 divided by #] times greater than the probability of [obtaining] this profile if the DNA came from [Person A]. This likelihood ratio indicates support for the proposition that [Person A] is excluded as a possible contributor to the female profile. [Internal validation has shown that] a likelihood ratio of this value may also indicate support for exclusion of a true donor from the profile (refer to Appendix).”

AND

Report the following: “There is an indication of at least [X] male contributor(s) at the Y-STR locus; however, this data is insufficient for comparisons. “

C. For female single source profiles with a Y peak at Amelogenin:

In these situations, Person A is the donor under Hp, while an unknown individual is the donor under Hd. Enter the 1/LR value in the underscore of each statement and the LR value in the # of each statement as applicable.

\[ LR_{Person\ A} = \frac{Pr(E \mid S_{Person\ A})}{Pr(E \mid S_{Unknown\ Individual})} \]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single female individual. The probability of [obtaining] this profile if the DNA came from
an unrelated, unknown individual is \[ \text{or 1 divided by } \# \] times greater than the probability of [obtaining] this profile if the DNA came from [Person A]. This likelihood ratio indicates support for the proposition that [Person A] is excluded as a possible contributor to the female profile. [Internal validation has shown that] a likelihood ratio of this value may also indicate support for exclusion of a true donor from the profile (refer to Appendix).”

AND

*Report the following:* “There is an indication of least one male contributor at the Amelogenin locus; however, this data is insufficient for comparisons.”

D. For Mixture Profiles:

In these situations, multiple individuals (Person A, Person B, etc.) are donors under Hp, while unknown individuals are donors under Hd of an assigned X number of contributors mixture. Enter the 1/LR value in the underscore of each statement and the LR value in the # of each statement as applicable.

\[
LR_{\text{Person A}} = \frac{Pr(E \mid S_{\text{Person A} + (X-1) \text{ other contributors}})}{Pr(E \mid S_X \text{ Unknown Individuals})}
\]

OR

\[
LR_{\text{Persons A+B}} = \frac{Pr(E \mid S_{\text{Person A} + Person B + (X-2) \text{ other contributors}})}{Pr(E \mid S_X \text{ Unknown Individuals})}
\]

E. “The [partial] DNA profile from [this] item [01-01-AA] is interpreted as a mixture of [X] individuals. The probability of [obtaining] this [mixture] profile if the DNA came from [X] unrelated, unknown individuals is \[ \text{or 1 divided by } \# \] times greater than the probability of [obtaining] this profile if the DNA came from [Person A and Person B… and (X - 1, 2, etc.) unrelated] unknown individuals. This likelihood ratio indicates support for the proposition that [Person A and Person B…] is/are excluded as [a] possible contributor[s] to the profile. [Internal validation has shown that] a likelihood ratio of this value may also indicate support for exclusion of a true donor from the profile (refer to Appendix).”

In these situations, multiple individuals (Person A, Person B, etc.) are donors under Hp, while some individuals are also donors under Hd of an assigned X number of contributors mixture. Enter the 1/LR value in the underscore of each statement and the LR value in the # of each statement as applicable.

\[
LR_{\text{Person B}} = \frac{Pr(E \mid S_{\text{Person A} + Person B + (X-2) \text{ other contributors}})}{Pr(E \mid S_{\text{Person A} + (X-1) \text{ Unknown Individuals}})}
\]

F. “The [partial] DNA profile from [this] item [01-01-AA] is interpreted as a mixture of [X] individuals [with Person A as an assumed contributor]. The probability of [obtaining] this [mixture] profile if the DNA came from [Person A] and [X] unrelated, unknown individuals is \[ \text{or 1 divided by } \# \] times greater than the probability of [obtaining] the profile if the DNA came from [Person A and Person B… and (X – 1, 2, etc.) unrelated] unknown individuals. This likelihood ratio indicates support for the proposition that [Person B…] is/are excluded as [a] possible contributor[s] to the profile. [Internal validation has shown that] a likelihood ratio of this value may also indicate support for exclusion of a true donor from the profile (refer to Appendix).”
5.5 Likelihood Ratio of more than 0.5 but Less than 2

A. For single source profiles:

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single individual. The probability of [obtaining] this profile if the DNA came from [Person A] is nearly equal to the probability of [obtaining] this profile if the DNA came from an unrelated, unknown individual. The likelihood ratio is uninformative (refer to Appendix).”

B. For female single source profiles with peak(s) at DYS391:

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single individual. The probability of [obtaining] this female profile if the DNA came from [Person A] is nearly equal to the probability of [obtaining] this profile if the DNA came from an unrelated, unknown individual. The likelihood ratio is uninformative (refer to Appendix). There is an indication of at least [X] male contributor(s) at the Y-STR locus; however, this data is insufficient for comparisons.”

C. For female single source profiles with a Y at Amelogenin:

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single individual. The probability of [obtaining] this female profile if the DNA came from [Person A] is nearly equal to the probability of [obtaining] this profile if the DNA came from an unrelated, unknown individual. The likelihood ratio is uninformative (refer to Appendix). There is an indication of least one male contributor at the Amelogenin locus; however, this data is insufficient for comparisons.”

D. For mixture profiles:

1. In these situations, multiple individuals (Person A, Person B, etc.) are donors under Hp, while unknown individuals are donors under Hd of an assigned X number of contributors mixture.

\[
LR_{\text{Person A}} = \frac{Pr(E | S_{\text{Person A}} + (X-1) \text{ other contributors})}{Pr(E | S_X \text{ Unknown Individuals})}
\]

OR

\[
LR_{\text{Persons A+B}} = \frac{Pr(E | S_{\text{Person A}} + \text{Person B} + (X-2) \text{ other contributors})}{Pr(E | S_X \text{ Unknown Individuals})}
\]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as a mixture of [X] individuals. The probability of [obtaining] this profile if the DNA came from [Person A and Person B... and (X - 1, 2, etc.) unrelated,] unknown individuals is nearly equal to the probability of [obtaining] this profile if the DNA came from [X] unrelated, unknown individual(s). The likelihood ratio is uninformative (refer to Appendix).

2. In these situations, multiple individuals (Person A, Person B, etc.) are donors under Hp, while some individuals are also donors under Hd of an assigned X number of contributors mixture.

\[
LR_{\text{Person B}} = \frac{Pr(E | S_{\text{Person A}} + \text{Person B} + (X-2) \text{ other contributors})}{Pr(E | S_{\text{Person A}} + (X-1) \text{ Unknown Individuals})}
\]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as a mixture of [X] individuals [with Person A as an assumed contributor]. The probability of [obtaining] this profile if the DNA came from [Person A and Person B and X-2 unrelated,] unknown individual(s) is nearly equal to the probability of [obtaining]
5.6 Likelihood Ratio of More than or Equal to 2 but Less than or equal to 1000 (or 10,000 for Minifiler)

A. For single source profiles:

In these situations, Person A is the donor under Hp, while an unknown individual is the donor under Hd. Enter the LR value in the underscore of each statement.

\[
\text{LR}_{\text{Person A}} = \frac{\Pr(E \mid S_{\text{Person A}})}{\Pr(E \mid S_{\text{Unknown Individual}})}
\]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single individual. The probability of [obtaining] this profile if the DNA came from [Person A] is _ times greater than the probability of [obtaining] this profile if the DNA came from an unrelated, unknown individual. This likelihood ratio indicates support for the proposition that [Person A] is a possible contributor of the profile. [Internal validation has shown that] a likelihood ratio of this value may also indicate support for a non-donor being a contributor to the profile (refer to Appendix).”

B. For female single source profiles with peak(s) at DYS391:

In these situations, Person A is the donor under Hp, while an unknown individual is the donor under Hd. Enter the LR value in the underscore of each statement.

\[
\text{LR}_{\text{Person A}} = \frac{\Pr(E \mid S_{\text{Person A}})}{\Pr(E \mid S_{\text{Unknown Individual}})}
\]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single female individual. The probability of [obtaining] this female profile if the DNA came from [Person A] is _ times greater than the probability of [obtaining] the profile if the DNA came from an unrelated, unknown individual. This likelihood ratio indicates support for the proposition that [Person A] is a possible contributor of the female profile. [Internal validation has shown that] a likelihood ratio of this value may also indicate support for a non-donor being a contributor to the profile (refer to Appendix).”

AND

Report the following: “There is an indication of at least [X] male contributor(s) at the Y-STR locus; however, this data is insufficient for comparisons. “

C. For female single source profiles with a Y peak at Amelogenin:

In these situations, Person A is the donor under Hp, while an unknown individual is the donor under Hd. Enter the LR value in the underscore of each statement.

\[
\text{LR}_{\text{Person A}} = \frac{\Pr(E \mid S_{\text{Person A}})}{\Pr(E \mid S_{\text{Unknown Individual}})}
\]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single female individual. The probability of [obtaining] this female profile if the DNA came from [Person A] is _ times greater than the probability of [obtaining] the profile if the DNA came from an unrelated, unknown individual. This likelihood ratio indicates support for the proposition that [Person A] is a possible contributor of the female profile. [Internal validation has shown that] a likelihood ratio of this value may also indicate support for a non-donor being a contributor to the profile (refer to Appendix).”

AND
Report the following: “There is an indication of at least one male contributor at the Amelogenin locus; however, this data is insufficient for comparisons.”

D. For Mixture Profiles:

1. In these situations, multiple individuals (Person A, Person B, etc.) are donors under Hp, while unknown individuals are donors under Hd of an assigned X number of contributors mixture.

\[ LR_{Person \ A} = \frac{Pr(E \mid S_{Person \ A + (X-1) \ other \ contributors})}{Pr(E \mid S_X \ Unknown \ Individuals)} \]

OR

\[ LR_{Person \ A+B} = \frac{Pr(E \mid S_{Person \ A + Person \ B + (X-2) \ other \ contributors})}{Pr(E \mid S_X \ Unknown \ Individuals)} \]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as a mixture of [X] individuals. The probability of [obtaining] this [mixture] profile if the DNA came from [Person A and Person B… and (X - 1, 2, etc. unrelated,] unknown individual[s] is _ times greater than the probability of [obtaining] the profile if the DNA came from [X] unrelated, unknown individuals. This likelihood ratio indicates support for the proposition that [Person A and Person B…] is/are [a] possible contributor[s] to the profile. [Internal validation has shown that] a likelihood ratio of this value may also indicate support for a non-donor being a contributor to the profile (refer to Appendix).”

2. In these situations, multiple individuals (Person A, Person B, etc.) are donors under Hp, while some individuals are also donors under Hd of an assigned X number of contributors mixture.

\[ LR_{Person \ B} = \frac{Pr(E \mid S_{Person \ A + Person \ B + (X-2) \ other \ contributors})}{Pr(E \mid S_{Person \ A + (X-1) \ Unknown \ Individuals})} \]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as a mixture of [X] individuals [with Person A as an assumed contributor]. The probability of [obtaining] this [mixture] profile if the DNA came from [Person A and Person B… and (X – 1, 2, etc. [an] unrelated,] unknown individual[s] is _ times greater than the probability of [obtaining] the profile if the DNA came from [Person A, , etc.] and [X -1, 2, etc.] unrelated, unknown individual[s]. This likelihood ratio indicates support for the proposition that [Person B…] is/are [a] possible contributor[s] to the profile. [Internal validation has shown that] a likelihood ratio of this value may also indicate support for a non-donor being a contributor to the profile (refer to Appendix).”

5.7 Likelihood ratio of more than 1000 (more than 10,000 for Minifiler)

A. For single source profiles:

In these situations, Person A is the donor under Hp, while an unknown individual is the donor under Hd. Enter the LR value in the underscore of each statement.

\[ LR_{Person \ A} = \frac{Pr(E \mid S_{Person \ A})}{Pr(E \mid S_{Unknown \ Individual})} \]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single individual. The probability of [obtaining] this profile if the DNA came from [Person A] is _ times greater than the probability of [obtaining] the profile if the DNA came from an unrelated, unknown individual. This likelihood ratio indicates support for the proposition that [Person A] is a possible contributor of the profile (refer to Appendix).”
B. For female single source profiles with peak(s) at DYS391:

In these situations, Person A is the donor under Hp, while an unknown individual is the donor under Hd. Enter the LR value in the underscore of each statement.

\[
\text{LR}_{\text{Person A}} = \frac{\text{Pr}(E \mid S_{\text{Person A}})}{\text{Pr}(E \mid S_{\text{Unknown Individual}})}
\]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single female individual. The probability of [obtaining] this female profile if the DNA came from [Person A] is _ times greater than the probability of [obtaining] this profile if the DNA came from an unrelated, unknown individual. This likelihood ratio indicates support for the proposition that [Person A] is a possible contributor of the profile (refer to Appendix).”

AND

Report the following: “There is an indication of at least [X] male contributor(s) at the Y-STR locus; however, this data is insufficient for comparisons. “

C. For female single source profiles with a Y peak at Amelogenin:

In these situations, Person A is the donor under Hp, while an unknown individual is the donor under Hd. Enter the LR value in the underscore of each statement.

\[
\text{LR}_{\text{Person A}} = \frac{\text{Pr}(E \mid S_{\text{Person A}})}{\text{Pr}(E \mid S_{\text{Unknown Individual}})}
\]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single female individual. The probability of [obtaining] this female profile if the DNA came from [Person A] is _ times greater than the probability of [obtaining] this profile if the DNA came from an unrelated, unknown individual. This likelihood ratio indicates support for the proposition that [Person A] is a possible contributor of the profile (refer to Appendix).”

AND

Report the following: “There is an indication of least one male contributor at the Amelogenin locus; however, this data is insufficient for comparisons.”

D. For Mixture Profiles:

1. In these situations, multiple individuals (Person A, Person B, etc.) are donors under Hp, while unknown individuals are donors under Hd of an assigned X number of contributors mixture.

\[
\text{LR}_{\text{Person A}} = \frac{\text{Pr}(E \mid S_{\text{Person A + (X-1) other contributors}})}{\text{Pr}(E \mid S_{X \text{ Unknown Individuals}})}
\]

\[
\text{OR}
\]

\[
\text{LR}_{\text{Persons A+B}} = \frac{\text{Pr}(E \mid S_{\text{Person A + Person B + (X-2) other contributors}})}{\text{Pr}(E \mid S_{X \text{ Unknown Individuals}})}
\]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as a mixture of [X] individuals. The probability of [obtaining] this [mixture] profile if the DNA came from [Person A and Person B… and (X - 1, 2, etc.) unrelated,] unknown individual(s) is _ times greater than the probability of [obtaining] this profile if the DNA came from [X] unrelated, unknown individuals. This likelihood ratio indicates support for the proposition that [Person A and Person B…etc] is/are [a] possible contributor[s] to the profile (refer to Appendix).”
2. In these situations, multiple individuals (Person A, Person B, etc.) are donors under Hp, while some individuals are also donors under Hd of an assigned X number of contributors mixture.

\[ LR_{Person \ B} = \frac{Pr(E | S_{Person \ A + Person \ B + (X-2) \ other \ contributors})}{Pr(E | S_{Person \ A + (X-1) \ Unknown \ Individuals})} \]

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as a mixture of [X] individuals [with Person A as an assumed contributor]. The probability of [obtaining] this [mixture] profile if the DNA came from [Person A and Person B... and (X – 1, 2, etc.) unrelated], unknown individuals is _ times greater than the probability of [obtaining] the profile if the DNA came from [Person A, , etc.] and [X -1, 2, etc.] unrelated, unknown individual[s]. This likelihood ratio indicates support for the proposition that [Person B …] is/are [a] possible contributor[s] to the profile (refer to Appendix).”

5.8 Alternate Reference Samples for STRmix comparisons

A. When no standard reference samples are available, alternate reference samples yielding a single source profile may be used.

B. If a partial profile is obtained, Technical Leader approval is required in order to use the alternate reference sample for comparison purposes.

C. When compared to an evidentiary profile, the appropriate prescribed reporting statement will be modified to read:

“Assuming [Person A] is the source of the [alternate reference], obtaining this…”

D. If the alternate reference sample is determined to be unsuitable for comparisons, the following statements may be used:

“The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture and therefore is unsuitable for use as an alternate known.

OR

“Insufficient data was obtained from [Item 1, stain 1] therefore this item is unsuitable for use as an alternate known.”

5.9 Consideration of Additional Hypotheses

The following statement may be added to the Investigative Leads section of the report. In particular, if the case includes mixtures and the analyst wishes to communicate that additional, uncalculated LRs may be relevant to the case (e.g. a co-contributor LR or an LR with one or more assumed donors), it is an option to include this statement.

“If additional hypothesis testing is required, please contact this laboratory.”

6 Manual Autosomal DNA Interpretation/ STRmix/ Reporting Statements

6.1 DNA Profiles and CODIS

A. Profile Suitable for CODIS

“A [partial] DNA profile obtained from [Item] [foreign to Person A] has been entered into the Combined DNA Index System (CODIS). All profiles are entered into CODIS in accordance with state and national regulations, where regular searches will be performed.
Notification will be issued if there is a hit in the database or if the profiles are removed from CODIS at any time in the future.

OR

“The [partial] DNA profile obtained from [Item] [foreign to Person A] has been entered into the Combined DNA Index System (CODIS) and will be searched against the local, state, and/or national databases.”

B. Profile Not Suitable for CODIS

“The [partial] DNA profile obtained from [Item] was determined to be unsuitable for entry into the Combined DNA Index System (CODIS).”

6.2 Uninterpretable Results

“No DNA profiles were obtained from [Item 1, stain 1].”

“No interpretable DNA profiles were obtained from [Item 1, stain 1].”

“Insufficient data is present for interpretation.”

6.3 Complexity of Results

“Due to the potential number of contributors, no interpretations will be made using the DNA profile obtained from [Item 1, stain 1].”

“Due to the complexity of this profile, no conclusions can be drawn as to whether [Person A] could be a contributor to the DNA profile from [Item 1, stain 1].”

“Due to the potential relatedness of the individuals, no conclusions can be drawn as to whether [Person A] could be a contributor to the DNA profile from [Item 1, stain 1].”

6.4 No comparison lack of known

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single [male/female] individual. If DNA comparisons are required, please submit a known blood or saliva sample from [any potential contributors].”

OR

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as a mixture of [X] individuals. If DNA comparisons are required, please submit a known blood or saliva sample from [any potential contributors].”

6.5 Re-extraction of samples due to contamination (resolved or unresolved)

“No results can be reported for this item due to a quality event. [This item was/will be re-extracted.]”

OR

“Due to a quality event, this item was extracted twice.”

6.6 Re-extraction of samples to obtain more information

“In an effort to obtain additional information, this item was extracted twice.”

OR

“[Regular reporting statement indicating results of extraction]. This item [was/will be] re-extracted in an effort to obtain additional information.”
7 Y-STR DNA Analysis

7.1 Requirements

A. List items analyzed for Y-STR analysis.

B. Description of DNA methodology and technology
   1. DNA extraction (if not previously reported)
   2. Differential extractions (if performed and not previously reported)
   3. Analyzed by Y-STR Analysis

C. List loci analyzed (this may be accomplished through use of an appendix).
   1. A statement must be present in the report to address all DNA extracts. If a
      stain/collection is re-extracted to resolve contamination or a quality incident, the
      report must indicate that multiple extractions of the same stain/collection were
      performed due to a quality event. Alternatively, multiple reports may be issued to
      indicate additional extractions. Comparisons will only be made to profiles where
      contamination or the quality incident has been resolved.
   2. If a stain/collection is re-extracted in an attempt to gain more information, the
      report must indicate that multiple extractions of the same stain/collection were
      performed in an effort to obtain additional information. Alternately, multiple reports
      may be issued to indicate additional extractions.

D. For cases received and reported using the current LIMS, when DNA comparisons are
   made the interpretation/conclusions will be reported in the “Evidence Description, Results
   of Analysis and Interpretation” section of the report. For cases originally received and
   reported using DRAGNet legacy LIMS, refer to the Legacy Amplification Kit Data
   Reinterpretation chapter.

E. Statistical Interpretation
   1. The statistical interpretation will be reported for the evidentiary profile frequency
      estimation using the counting method as generated by the Y-Chromosome
      Haplotype Reference Database (YHRD) online search tool (http://yhrd.org). The
      profile count will be reported as a statement regarding observations in the
      database and expressed as numbers truncated to the last significant digit.
   2. Statistical interpretation reporting options
      a) A statistical interpretation statement for single source and major components
         inclusions must be in the report.
      b) Statistical interpretation statements will not be reported for mixtures where the
         major and minor contributors cannot be distinguished, or for the minor component
         in a distinguishable mixture.
      c) Intimate and indigenous samples do not require a reported statistic for the donor
         of the sample

F. Disposition of evidentiary items on which Y-STR analysis was performed will be included
   in the report. This includes evidentiary items depleted during analysis.
7.2 Single Source Exclusion

“The [partial] profile from [Item 1, stain 1] is not consistent with the profile of [Person A]. [Person A] is excluded as a contributor to this profile. In addition, all paternally-related male relatives of [Person A] are excluded as contributors to this profile.”

OR

“[Person A] is excluded as a contributor to this [partial] profile. In addition, all paternally-related male relatives of [Person A] [are/may be] excluded as contributors to this profile.”

7.3 Exclusion Major Component of a Mixture

“The [partial] Y-STR profile from [Item 1, stain 1] is interpreted as a mixture of at least _ contributors”. [Person A] is excluded as a contributor to the major component of this profile. In addition, all paternally-related male relatives of [Person A] are excluded as contributors to major component of this profile.”

7.4 Single Source Inclusion

“The [partial] Y-STR profile from [Item 1, stain 1] is consistent with the Y-STR profile of [Person A].

[Person A cannot be excluded as a contributor of the male DNA profile at the following loci: DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385 a/b, DYS456, and Y GATA H4 (if reporting Y23) OR DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385 a/b, DYS393, DYS391, DYS439, DYS635, DYS392, YGATA H4, DYS437, DYS438, and DYS448 (if reporting Yfiler). At these loci,...] The selected profile is found in _ of _____ total individuals within the database. In addition, any paternally-related male relatives of [Person A] [cannot/may not] be excluded as the contributor of this male DNA profile.”

7.5 Inclusion Major Component of a Mixture

“The [partial] Y-STR profile from [Item 1, stain 1] is interpreted as a mixture of at least _ contributors. [Person A] cannot be a contributor of the major component in the male DNA profile at the loci: DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385 a/b, DYS456, and Y GATA H4 (if reporting Y23) OR DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385 a/b, DYS393, DYS391, DYS439, DYS635, DYS392, YGATA H4, DYS437, DYS438, and DYS448 (if reporting Yfiler). At these loci,...] The selected profile is found in _ of _____ total individuals within the database. In addition, any paternally-related male relatives of [Person A] [cannot/may not] be excluded as the contributor of the major component of this male DNA profile.”

7.6 No Comparison Indistinguishable Mixture

“The [partial] Y-STR profile from [Item 1, stain 1] is interpreted as an indistinguishable mixture of at least ____ individuals. No conclusions will be drawn as to whether [Person B, Person C, etc.] could be a contributor to the Y-STR profile from [Item 1, stain 1].”
7.7 No comparison lack of knowns

“The [partial] Y-STR profile from [this] item [01-01-AA] is interpreted as originating from a single individual. If Y-STR comparisons are required, please submit a known blood or saliva sample from any potential male contributors.”

OR

“The [partial] Y-STR profile from [this] item [01-01-AA] is interpreted as a mixture of at least [X] individuals. If Y-STR comparisons are required, please submit a known blood or saliva sample from any potential male contributors.”

7.8 Minor Component of a Mixture

“Due to the low level of data present above our analysis threshold, no comparisons will be made to the minor component.”

“Because statistical calculations will not be conducted, no inclusions regarding the minor component will be made.”

7.9 Alternate Reference Samples

A. When no standard reference samples are available, alternate reference samples may be used as long as they exhibit a single source Y-STR profile.

B. If a partial profile is obtained, Technical Leader approval is required in order to use the alternate reference sample for comparison purposes.

C. When compared to an evidentiary profile, the appropriate prescribed reporting statement will be modified to read:

“The [partial] Y-STR profile from [Item 1, stain 1] is consistent with the Y-STR profile of [alternate reference]. Assuming [Person A] is the source of the [alternate reference], [Person A] cannot be excluded. In addition, any paternally-related male relatives of [Person A] [cannot/may not] be excluded as a contributor to this male DNA profile.”

OR

“The [partial] Y-STR profile from [Item 1, stain 1] is not consistent with the Y-STR profile of [alternate reference]. Assuming [Person A] is the source of the [alternate reference], [Person A] is exclude. In addition, all paternally-related male relatives of [Person A] [are/may be] excluded as contributors to this male DNA profile.”

D. If the alternate reference sample is determined to be unsuitable for comparisons, the following statements may be used:

“The [partial] Y-STR profile from [Item 1, stain 1] is consistent with a mixture and therefore is unsuitable for use as an alternate known.

OR

“Insufficient data was obtained from [Item 1, stain 1] therefore this item is unsuitable for use as an alternate known.”

7.10 Profile Suitable for CODIS

“The Y-STR profile from [Item] was entered into the Combined DNA Index System (CODIS). All profiles are entered into CODIS in accordance with state and national regulations, where regular searches will be performed. Notification will be issued if there is a hit in the database or if the profiles are removed from CODIS at any time in the future.”
OR

“The [partial] Y-STR profile obtained from [Item] [foreign to Person A] has been entered into the Combined DNA Index System (CODIS) and will be searched against the local, state, and/or national databases.”

7.11 Uninterpretable Results

“No Y-STR profiles were obtained from [Item 1, stain 1].”

“No interpretable Y-STR profiles were obtained from [Item 1, stain 1].”

7.12 Complexity of Results

“Due to the potential number of contributors, no interpretations will be made for the Y-STR profile obtained from [Item 1, stain 1].”

7.13 Re-extraction of samples due to contamination (resolved or unresolved)

“No results can be reported for this item due to a quality event. [This item was/will be re-extracted.]”

OR

“Due to a quality event, this item was extracted twice.”

7.14 Re-extraction of samples to obtain more information

“In an effort to obtain additional information, this item was extracted twice.”

OR

“[Regular reporting statement indicating results of extraction]. This item [was/will be] re-extracted in an effort to obtain additional information.”
DNA-09-08 GUIDELINES FOR COUNTING EXAMINATIONS FOR ACTIVITY REPORTS

1 Scope
Forensic Biology, Male Screening, and DNA examinations are counted and tracked for management activity reports in LIMS according to the following guidelines.

2 Practices
2.1 Forensic Biology Request
A. Items Screened – An item handled, labeled and initialed for documentation, and examined for items of evidentiary value should be counted as one item. Examples of one item include: a bloody shirt, a vaginal smear, a vaginal swab, etcetera.

B. Screening Exams – Testing or exams performed on the items screened. Each test performed counts as one exam. Examples include visual inspection, microscopic examinations, tapelifting (or trace evidence collection of) an item, each presumptive test performed, alternate light source examination, etcetera.

C. Trace Evidence Items Collected – Number of items from which trace evidence is collected.

D. Bloodstains – Count the number of stains or swabs that tested presumptive positive.

E. Semen stains – Count the number of positive presumptive tests, positive sperm exams, and positive p30 exams.

F. Other Stains – Count the number of non-blood/non-semen stains collected. Examples of Other Stains include swabs from the mouth of a bottle, known saliva swabs, etcetera.

G. Human Origin – Count the number of positive human origin exams.

2.2 Male Screening Request
A. Number of Items Screened – An item handled, labeled and initialed for documentation, and examined for items of evidentiary value should be counted as one item. Examples of one item include: a vaginal swab, an anal swab, etcetera.

B. Number of Male Screening Exams – Testing or exams performed on the items during male screening process. Count 1 for each sample lysis performed and 1 for quantification performed. Do not count controls.

C. Number of Tubes Prepared – Count the number of tubes prepared for male screening testing.

2.3 DNA Request
A. Blood – Count the blood stains that were analyzed for DNA.

B. Tissue – Count the tissue samples (organs, flesh, etcetera) that were analyzed for DNA.

C. Hair – Count the number of hairs and shafts that were analyzed for DNA.

D. Semen – Count the number of semen stains that were analyzed for DNA.

E. Skeletal – Count the number of bone samples that were analyzed for DNA.

F. Teeth – Count the number of teeth samples that were analyzed for DNA.
G. **Other** – Count other evidentiary materials that were analyzed for DNA that do not fit any other DNA category.

H. **Trace DNA** – Count the number of trace DNA samples analyzed for DNA.

I. **STR Exams** – This number includes exams used to determine DNA type using STR analysis. Count: 1 for each sample extracted; 1 for each quantification; 1 for each amplification; and 1 for each injection. Do not count injection setup, controls, or allelic ladders.

J. **DNA Report Tracking** – On any given case, there may be an entry for all, none, or multiple categories. Do not return and edit this section if a subsequent report changes the way the questions were originally answered. Answers apply to this report as written.

   1. Excluded – Enter the number of suspects excluded using the DNA profiles discussed in this report.
   2. Included – Enter the number of suspects that were included using the DNA profiles discussed in this report.
   3. Uninformative – Enter the number of suspects with uninformative comparison results discussed in this report.
   4. No suspects this analysis – Check the box if no sample from the suspect was run in this report.
   5. Informative Results obtained – Check the box if informative results were obtained for this report.
   6. Negative Y Screen – Check the box if Y quantification results were negative for this report.
   7. Inclusions – Enter the total number of comparisons for all people (including suspects) reported as inclusion for the request. Enter 0 if there were none.
   8. Exclusions – Enter the total number of comparisons for all people (including suspects) reported as exclusion for the request. Enter 0 if there were none.
   9. Uninformative – Enter the total number of comparisons for all people (including suspects) reported as uninformative for the request. Enter 0 if there were none.

K. **CODIS Profile Tracking** – Check the CODIS Yes box if profiles were entered into CODIS that for this report. Select the appropriate profile level using the drop down. Check the CODIS No box if no profiles were entered into CODIS for this report.
DNA-09-09 CODIS

1 Scope

The policies regarding the entry, searching, and notification to the customer of DNA profiles in CODIS are included in this guideline.

The original core set of 13 CODIS autosomal STR loci were selected in November 1997 and are required by the Federal Bureau of Investigation (FBI) for upload of DNA profiles to the U.S. national DNA database. In March of 2015, the FBI published the expansion of the original core 13 loci to a new CODIS core containing 20 loci with an implementation date of January 2017 for U.S. Laboratories.

Policies regarding Y-STR profiles are also included in this guideline.

2 Related Documents

Laboratory Records (CLS Manual)
Expunction and Destruction of Laboratory Records and Information (CLS Manual)
Autosomal STR Interpretation Guidelines
Y-STR Interpretation Guidelines
STRmix Autosomal STR Interpretation
CODIS Entry Worksheet (LAB-DNA-15)
CODIS Y-STR Entry Worksheet (LAB-DNA-21)
CODIS Y23 Y-STR Data Worksheet (LAB-DNA-23)

3 Policy

3.1 Legal Basis

A. The DNA Identification Act of 1994 as amended included provisions establishing the FBI’s Combined DNA Index System, a national DNA database program.

B. Texas CODIS law allows for the inclusion in the state database of authorized samples legally obtained in the investigation of a crime. The law further allows for the inclusion of voluntarily submitted samples. The inclusion of samples in a Suspect/Legal Index is authorized by an administrative decision.

1. The laws governing CODIS are found in Government Code Chapter 411, subchapter G (beginning at 411.141).

2. The rules governing CODIS are found in the Texas Administrative Code Title 37, Part 1, Chapter 28.

3.2 Database Operation

A. The State CODIS Manager has the authority to terminate a local laboratory's participation in CODIS in the event of a problem until the reliability of the data can be assured.

B. The records in the CODIS database are confidential and not subject to open records disclosure under Texas Government Code Chapter 552. A record includes both the profile and the identity of the individual whose profile is in the database. Neither the profile nor the identity of the individual whose profile is in the database will be released except to a criminal justice agency for law enforcement identification purposes, for a judicial
proceeding, if otherwise admissible under law, or for criminal defense purposes to a defendant if related to the case in which the defendant is charged.

C. The CODIS server and workstations will be located in the laboratory which is a secure environment. Additional security features may exist but will vary between laboratories. The CODIS Administrator will ensure only authorized individuals have access and the hardware will be password protected at all times.

D. Each laboratory must have at least one DNA staff member assigned or appointed as the casework CODIS Administrator and a designated alternate. The alternate must meet all of the qualifications of the CODIS Administrator and handle the same responsibilities at the request of or in the absence of the Administrator.

E. A laboratory shall not upload DNA profiles to NDIS in the event that the casework CODIS Administrator position is unoccupied.

3.3 Case Evaluation

A. All cases containing biological evidence will be evaluated for possible entry into CODIS.

1. If the victim reports having a consensual partner within 96 hours prior to the time of evidence collection, the laboratory must document a request for a consensual partner elimination sample to make the evidentiary profile eligible for CODIS entry. If DNA analysis has already been performed on the evidentiary sample prior to this request and the DNA profile matches a profile from a suspect known, the profile is considered eligible for CODIS entry without this documented request.

2. Suspect known reference samples submitted to the laboratory prior to the reporting of the screening results of the associated evidence shall be processed for entry into CODIS, regardless if DNA analysis will be performed on any of the evidentiary samples unless it has been verified through SDIS that the suspect is already in CODIS.

3. Suspect known reference samples submitted without associated evidence or after negative screening and/or DNA results have been reported will not be processed for entry into CODIS.

4. A suspect profile will be entered into CODIS if the sample was:
   a) obtained legally
   b) originally collected for the investigation of a crime
   c) a biological sample from a suspect in a criminal investigation
   d) not typed solely for the purpose of eliminating a non-suspect (e.g., consensual sex partner)

5. All suspect profiles remain the property and responsibility of the submitting local laboratory.

6. For autosomal profiles, the set of core loci currently required for participation in NDIS is as follows: TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D12S391, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820.

7. For autosomal profiles, the original core loci required for participation in NDIS included only: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, and CSF1PO.
8. For Y-STR profiles, there are no set core loci required for entry into CODIS. Y-STR profiles are only stored and searched at LDIS unless they are associated with autosomal profile data.

B. Any DNA profiles from sexual assault kits that are determined to be eligible for CODIS entry will be entered into the database and searched no more than 30 days after administrative review is complete.

3.4 Profile Evaluation

A. For evaluation of data suitable for CODIS entry see the Manual Autosomal STR Interpretation Guidelines; Y-STR Interpretations Guidelines; and STRmix Autosomal STR Interpretation Guidelines chapters.

B. Specimen identification will include the case number and item number/unique identifier. The item description may also be included.

C. Suspect profiles will be identified in the database by the case number and item number/unique identifier and will not include the name of the individual.

D. For outsource cases the specimen identification will include either the LIMS case number or the vendor laboratory case number and item number/unique identifier. The item description may also be included; however, suspect profiles will not include the name of the individual.

E. Prior to any search at SDIS/NDIS, each profile entered into CODIS will be reviewed by a second analyst to confirm the correct DNA types, correct specimen category (including a review of inverse MRE/MME as applicable), and specimen eligibility. This review will be reflected on the CODIS Data Worksheet (LAB-DNA-15, LAB-DNA-21, or LAB-DNA-23) or in the case record.

F. For autosomal profiles, the inverse Match Rarity Estimate (MRE) or Moderate Match Estimate (MME) shall be evaluated on any partial or mixture evidentiary profile prior to upload. This can be accomplished by using the Match Estimation Tool located in Popstats or through STR data entry.

1. Match estimation results will be included in the case record either by adding a printout of the match estimation or recording the results of the match estimation on the CODIS Entry Worksheet (LAB-DNA-15) or in the case record.

2. To evaluate MRE using the Match Estimation Tool located in Popstats:

   a) Open the Match Estimation Tool in Popstats.
   b) Enter the profile using only the original 13 core loci.
   c) Determine if the combined inverse MRE is greater than or equal to 1.0E+7. If the number is greater, the profile is marked for upload to NDIS, and no further calculation is needed.
   d) If the combined inverse MRE is greater than or equal to 1.0E+5 and less than 1.0E+7, the profile is marked for upload to SDIS in either the SDIS Forensic Partial index or the SDIS Forensic Mixture index, and no further calculation is needed.
   e) If the combined inverse MRE is less than 1.0E+5, recalculate the profile in the Match Estimation Tool. This time enter the original 13 core loci, D2S1338 and D19S433. If upon recalculation the combined inverse MRE is at least 1.0E+5, the
profile is marked for upload to SDIS in either the SDIS Forensic Partial index or the SDIS Forensic Mixture index.

3. To evaluate the MME using STR data entry:
   a) Open STR data entry
   b) Enter the entire profile into STR data entry. Include a specimen id, specimen index, etcetera as you would enter a profile for upload.
   c) Once save has been clicked, an MME value will appear for the profile. (Note: the MME value will not appear unless the profile is assigned to a mixture or partial evidentiary index.)
   d) Determine if the inverse MME is greater than or equal to 1.0E+7. If the number is greater, the profile is marked for upload to NDIS, and no further calculation is needed.
   e) If the inverse MME is greater than or equal to 1.0E+5 and less than 1.0E+7, the profile is marked for upload to SDIS in either the SDIS Forensic Partial index or the SDIS Forensic Mixture index, and no further calculation is needed.
   f) If the inverse MME is less than 1.0E+5, recalculate the profile in the Match Estimation Tool. Click the Popstats button to automatically import the profile from STR data entry into the Match Estimation Tool in Popstats. If upon recalculation the inverse MRE is at least 1.0E+5, the profile is marked for upload to SDIS in either the SDIS Forensic Partial index or the SDIS Forensic Mixture index.

4. Profiles not meeting match estimation thresholds may be stored at the LDIS level based on local policy. Consult the local CODIS Administrator for resolution. These profiles may also be considered for the Forensic Targeted category.

5. Forensic Targeted category:
   a) Based on case circumstances it could be beneficial to place a profile in the Forensic Targeted category over the SDIS Forensic Mixture or SDIS Forensic Partial categories. This can be evaluated on a case by case basis with the approval of the local CODIS Administrator.
   b) It may also be beneficial to reevaluate profiles that have been searched at SDIS or LDIS that have not had any positive associations (hits) of value to see if they can be placed in the Forensic Targeted category. This can be evaluated on a case by case basis with the approval of the local CODIS Administrator.
   c) Completeness for forensic targeted category for both SDIS and NDIS is 8 original core loci with an MRE value of 1 in 10 million.

6. To evaluate a Forensic Partial specimen for entry into the Forensic Targeted specimen category:
   a) Check the profile to see if any loci should be set to partial. If the locus is marked partial, it will be searched at moderate stringency. Loci not set as partial will be evaluated at high stringency. The MRE will be calculated in the STR data entry following the rules above.
   b) To evaluate the MRE using the Match Estimation Tool enter the profile using only the original 13 core loci and set loci to partial as appropriate. Loci not set to partial should be evaluated at high stringency by changing the stringency in the grid.
c) It is important to note that a match could be missed if partial loci needing to be searched at moderate stringency are not identified.

7. To evaluate a Forensic Mixture specimen for entry into the Forensic Targeted specimen category:
   a) Check the profile to see if any loci can be updated to have a required allele. Required alleles will increase the rarity of the locus and boost the MRE value, but only when the locus is set to be searched at moderate stringency.
   b) It is critical for loci with a required allele to either be marked partial or have more than 2 alleles for the required allele to have an effect in this search. Since loci with more than two alleles will be evaluated at moderate stringency, evaluate the profile to see if any loci can be updated to have less than three alleles. Loci with two alleles or less will be evaluated at high stringency and boosts the MRE value.
   c) Evaluate loci having two alleles or less to determine if these loci should be flagged as partial.
   d) The MRE will be calculated in the STR data entry following the rules above.
   e) To evaluate the MRE using the Match Estimation Tool enter the profile using only the original 13 core loci. Set loci to partial and add any required allele designations as appropriate. To calculate any loci at high stringency change the stringency in the grid.

G. For autosomal profiles, the required allele designation (+) may be used once per locus to indicate obligate perpetrator alleles.

H. If a profile of interest can be reliably and completely deconvoluted from a mixture profile, ONLY that portion of the mixture will be entered into LDIS/SDIS/NDIS.
   1. In order to pull a profile from a mixture, alleles must be evaluated at each locus.
      a) Visual examinations, peak height ratios, STRmix genotype combination tables may be used in this evaluation.
      b) Profiles of assumed contributors (exempting suspect knowns) may be used in this evaluation.
   2. It is not acceptable to use the profile from one unknown contributor to deduce the profile of a second unknown contributor in a mixture profile if this information is obtained from two different samples.
   3. For Y-STR profiles, the only portion of a mixture profile that may be entered into CODIS is the major component of a mixture profile.

3.5 Determination of Specimen Category

A. LDIS (Local DNA Index System)
   1. Required number of loci for entry of autosomal profiles into LDIS is determined by local policy.
   2. Specimen categories existing in LDIS are determined by local policy. These categories are not uploaded and are searched only at LDIS.
   3. DNA Y-STR profiles are entered into a local Y-STR index at LDIS.
      a) Required number of loci for entry into the LDIS Y-STR index is a minimum of 10 from any Y-STR loci.
b) Unresolved mixture profiles from Y-STR data are not entered into CODIS

B. SDIS (State DNA Index System)

4. Required number of loci for entry of autosomal profiles into SDIS is a minimum of 7 from the original core and D2S1338 and D19S433 (not including Amelogenin).

5. Specimen categories existing in SDIS include SDIS Forensic Partial, SDIS Forensic Mixture, and Suspect Known. Autosomal profile data in these categories is uploaded to SDIS and searched at both LDIS and SDIS.

   a) SDIS Forensic Partial – this category contains single source profiles or fully deduced profiles originating from mixtures. The profiles have either locus or allelic information missing at any of the core loci and meet an MME or MRE value of 1.00E+5. There are no more than three alleles at any one locus in the profile.

   b) SDIS Forensic Mixture – this category contains profiles with DNA from more than one source. The profiles shall not have more than 4 alleles at any one locus. The profiles meet an MME or MRE value of 1.00E+5.

   c) Suspect Known – this category contains profiles developed from a suspect reference sample where locus or allelic information is missing at any of the following loci: TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D12S391, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820. This category also contains partial or complete profiles developed as alternate knowns for suspects.

6. Y-STR data that is associated with an autosomal profile is uploaded to but not searched at SDIS.

C. NDIS (National DNA Index System)

7. For autosomal profiles, NDIS requires that all core loci be attempted and will accept specimens with data for 8 of the original 13 core loci (not including Amelogenin) for searching. Amelogenin is accepted at NDIS although not searched.

8. Specimen categories existing in NDIS include Forensic Unknown, Forensic Partial, Forensic Mixture, Forensic Targeted, and Legal. Autosomal profile data in these categories is uploaded to SDIS and NDIS and searched at both SDIS and NDIS.

   a) Forensic Unknown – this category contains single source profiles or fully deduced profiles originating from mixtures having all core loci and shall not have more than 3 alleles at any one locus in the profile.

   b) Forensic Partial – this category contains single source profiles or fully deduced profiles originating from mixtures with 8 or more original core loci. The profiles have either locus or allelic information missing at any core loci and meet an MME or MRE value of 1.00E+7. There are no more than 3 alleles at any one locus in the profile. For example, profiles with required/obligate allele(s), locus indicator of partial (p), or profile indicator of partial “yes” must be entered into this index.

   c) Forensic Mixture – this category contains profiles with DNA from more than one source with 8 or more original core loci. The profiles shall not have more than 4 alleles at any one locus. The profiles meet an MME or MRE value of 1.00E+7.

   d) Forensic Targeted- this category is intended for Forensic Partial and Forensic Mixture specimens that do not meet a 1 in 10 million MME value. This category will only be searched against full single source profiles (Forensic Unknown, Convicted Offender, Arrestee, Detainee, and Legal). This category should be
considered a specimen category of last resort for NDIS and specimens that meet MME should remain in the Forensic Partial or Forensic Mixture specimen category over placing them in the Forensic Targeted specimen category. Specimens in this category must have 8 or more original core loci with an MRE (stringency by locus) value of 1 in 10 million. The Forensic Targeted index is only searched “stringency by locus” at NDIS. Loci marked partial and loci with more than 2 alleles are searched at Moderate and all other loci are searched at High Stringency. Loci with the required allele designation do not get automatically searched at Moderate.

e) Legal – this category includes eligible profiles from suspect reference samples with complete information at all 20 core loci. If suspect profiles do not meet the requirement for this index, they will be entered into the Suspect Known index.

9. Y-STR data that is associated with an autosomal profile is not uploaded to or searched at NDIS at this time.

4 Personnel

4.1 Casework CODIS Administrator(s)

A. A casework CODIS Administrator shall be a current or previously qualified casework DNA analyst and shall be appointed by the DNA Section Supervisor/Manager.

B. A newly appointed casework CODIS Administrator shall complete the Computer Based Training (CBT) modules associated with the most recent version of CODIS software within six months of assuming casework CODIS Administrator duties (unless previously completed).

C. A newly appointed casework CODIS Administrator shall successfully complete FBI sponsored auditor training within one year of assuming administrator duties (unless previously attended).

D. The casework CODIS Administrator shall be responsible for the following:

   1. Administration of the laboratory’s local CODIS network.
   2. Scheduling and documenting CODIS computer training of casework analysts.
   3. Assuring that the security and quality of data stored in CODIS is in accordance with state and/or federal law and NDIS operational procedures.
   4. Making a good faith effort to ensure matches are dispositioned within 30 business days of the receipt of the request for follow-up.
   5. Designating an alternate CODIS administrator with the approval of the DNA Section Supervisor/Manager.
   6. Terminating an analyst’s or laboratory’s participation in CODIS until the reliability and security of the computer data can be assured in the event an issue with the data is identified.

4.2 CODIS User(s)

Authorization to become a CODIS user comes following application by the casework CODIS Administrator on behalf of the DNA analyst to the FBI.

A. The following materials will be submitted by the casework CODIS Administrator to the State CODIS Administrator on or about when the forensic scientist begins DNA training:
2. Two completed fingerprint (10 Print) cards from the employee (FBI form).
3. Questionnaire for Non-Sensitive Positions (FBI form).
4. Sensitive Information Nondisclosure Agreement (FBI form)
5. As required: Proof of Citizenship Documentation (Naturalization, Birth Abroad, etcetera)
6. The CODIS User Information form will be completed by the State CODIS Administrator.

B. Once the forensic scientist is approved by the FBI, he/she will be added as a new user by the casework CODIS Administrator.
   1. A user name should be chosen according to the following format: the first initial of the first name followed by the last name not to exceed a total of eight characters. This user name must match the user name submitted on the application.
   2. The Administrator designates a temporary password assigned to the User. The new user will be prompted to change this password at first login.
   3. The User is assigned to a group (administrative or user).
   4. The new user must be added and assigned CODIS rights.
   5. The start date entered must match the date on the LAB-CO-41.

C. In the event of a change of information for an approved CODIS user (such as a name change), the casework CODIS Administrator may submit a revised Analyst Authorization Form (LAB-CO-41) to the State CODIS Administrator, who will forward the request to the NDIS Custodian.

D. If the user separates from service or is deemed ineligible, his/her access must be removed.
   1. The request for removal of the user must be submitted to the State CODIS Administrator within 20 working days of separation.
   2. The revised Analyst Authorization Form (LAB-CO-41) must be submitted to the State CODIS Administrator with the stop date indicated.
   3. The corresponding stop date will be entered next to the relevant user and the change(s) will be saved.
   4. Any data associated with this user uploaded after the stop date will be rejected.

5 Practices
5.1 Casework CODIS Administrator Duties
A. Weekly
   1. Review and/or enter eligible profiles (if generated) and perform a search against the staff profiles accordingly.
   2. Check CODIS Message Center.
   3. Disposition matches if needed.
   4. Perform an incremental upload if new profiles entered.
5. CODIS data is backed up automatically on an external drive on a weekly basis. On a routine basis, but no less than once per month, backup media shall be stored in a secure physical location other than the NDIS participating laboratory in a locked container.

6. Review reconciliation reports.

B. Monthly

1. Submit hit counts to the State CODIS Program manager. The submission of the previous month’s counts is requested by the 5\textsuperscript{th} of the current month.

2. Check for Windows updates on server and workstation(s) and install as necessary.

C. Quarterly – perform a full upload.

D. Annually

1. Archive messages (may be done more frequently).

2. Ensure each CODIS user (including CODIS Administrators) successfully completes the NDIS Annual Review.

3. Complete the Laboratory Audit Certification Form (FBI form) available in the NDIS Operation Procedures and submit the form to the State CODIS Administrator.

4. Complete the Annual Update of Points of Contacts form (FBI form) available in the NDIS Operational Procedures and submit the form to the State CODIS Administrator.

5. Attend the National CODIS Conference

   a) The CODIS Administrator or Alternate CODIS Administrator shall attend the National Conference.

   b) Pursuant to NDIS Procedures, if neither Administrator is available to attend, a request for an excused absence must be made to the NDIS Custodian in writing, on agency letterhead and signed by the Administrator’s Laboratory System Director.

5.2 Evaluation of Matches

A. Evaluate matches by examining the stringency of the match at each locus and the information present in both profiles.

B. Review the case record for the associated profile.

1. If there is a request for reinterpretation, any profile with a CODIS entry must be reviewed to determine if the profile is still considered interpretable.

   a) If the profile is no longer considered interpretable, it must be removed from CODIS.

   b) Any associated matches will not be deleted from CODIS.

   c) For any previous matches that have already been communicated to the customer, the laboratory must inform the agency where the profile originated that the profile no longer has a CODIS entry and is no longer considered interpretable. The laboratory should also inform any non-DPS CODIS Administrator involved in the match of the change in profile status.

      i. These notifications may be done via email/fax.
ii. Suggested Wording: “Due to changes in profile interpretation policies, the DNA profile generated from [item #] is no longer eligible for CODIS entry and has been deleted from the CODIS database.”

d) For any current matches, the laboratory will set the disposition to No Match and no further action is necessary.

2. If the profile was generated by a non-DPS laboratory and entered on behalf of another agency, it may be necessary to contact the non-DPS laboratory to verify if the interpretation methods have changed in a way that would make the profile with the CODIS entry uninterpretable.

C. If, following evaluation, the profile appears to be No Match, set the disposition as such. No further action is necessary.

D. If, following evaluation, the profile appears to be a match, query the submitting agency as necessary to determine the status of the case using LIMS to determine if the case is solved or unsolved and set the disposition as appropriate.

5.3 CODIS Communications

See the Outsourcing to a Contract Laboratory chapter for outsourced CODIS profile communications

A. CODIS Entry

1. The requesting official must be informed via LIMS if a profile was entered into CODIS.

2. This can be accomplished by either including the CODIS entry information in the DNA report or in a supplemental letter containing only the CODIS entry information.


B. Deletion of a Profile from CODIS (for Legal Index specimens see Section 6.3)

1. Deletion of a profile is at the discretion of the CODIS Administrator.

2. The requesting official must be notified when any profile related to the respective case is removed from the CODIS database.

   a) This can be accomplished through use of LIMS or personal communication.

   b) Documentation of the communication must be retained in the case record.

C. One-Time Searches

1. Performing a one-time search is at the discretion of the casework CODIS Administrator.

2. If a one-time search is conducted, the requesting official must be notified of both the search and any results.

   a) If the result of the search is a positive association of value, parties associated with both profiles must be notified in writing of the result, specific case information, and contact information for investigating officials. The notification will be in the form of a letter in LIMS if the case is a DPS case.

   b) If the result is not a positive association of value, a Hit Notification email/fax must be sent to the requesting official.
c) If no match is obtained, an email/fax must be sent to the requesting official.

D. A letter must be distributed to the investigative official informing them of positive associations (hits) of value. This letter must not include the names of any suspects. In order to ensure that suspect names are not included, it is necessary to check both the body of the letter and the header.

1. Forensic Hits
   a) Forensic hits are matches between a forensic unknown or forensic mixture profile in an unsolved case and a forensic unknown or forensic mixture profile from another solved OR unsolved case.
   b) A letter will be generated in LIMS for each case involved with a forensic hit. The letter will include specific case information along with contact information for investigating officials, if from different agencies, involved in any of the cases.
   c) Suggested Wording for autosomal profile hits
      “The purpose of this communication is to inform you of a possible investigative lead.
      During a search of the CODIS database, an association occurred between the DNA profiles generated from [Item #] from the [submitting agency name’s] case [agency case number] and [Item #] from your case referenced above. This association may require additional investigation. Please contact [other agency contact name] at [phone and/or email] for more information concerning this investigative lead. If the Texas Department of Public Safety Crime Laboratory Service can be of any further assistance, please contact this laboratory.”
   d) Suggested Wording for Y-STR profile hits
      “This letter is to inform you of a Local level case-to-case hit in the CODIS (Combined DNA Index System) database. The Y-STR DNA profile from [Item #] for your case referenced above hit with an evidentiary profile from [Item #][submitting agency][agency case number]. This hit should be considered an investigative lead. If Y-STR DNA comparisons are needed for your case, please submit a known blood sample or buccal swab from the suspect. If the Texas Department of Public Safety Crime Laboratory Service can be of any further assistance, please contact this laboratory.”

2. Case to Offender (Offender Hits), Arrestee (Arrestee Hits), or Detainee (Detainee Hits)
   a) These hits are matches between a known individual’s DNA profile in the respective index and the DNA profile from a forensic unknown or forensic mixture profile in an unsolved forensic case.
   b) For interstate hits: A CODIS DNA Match Data Request form (FBI form) available from the NDIS Procedures may be completed and forwarded to the appropriate CODIS Offender Laboratory. A copy of the laboratory report and contact information for the case investigating official may be included in the documentation provided to the CODIS Database Laboratory.
   c) For intrastate hits: A CODIS DNA Match Data Request form (LAB-CO-29) is required. The form shall be sent via email in Excel format. A copy of the laboratory report and contact information for the case investigating official is not required to be forwarded with the LAB-CO-29.
3. Case to Suspect Known (hits to Suspect Known Index or Legal Index)
   
   a) Matches between suspect profiles and previously unlinked forensic profiles provide an investigative lead. The suspect profile, if appropriate, may be verified with a newly collected suspect sample.
   
   b) Follow-up of any match between a suspect profile and a forensic profile is the responsibility of the local laboratories that submitted the matching profiles.
   
   c) A letter will be generated in LIMS for each case involved with an unsolved case to suspect known hit or legal index hit. The letter will include contact information for investigating officials if from different agencies, involved in the cases. Personal Identifiable Information of the suspect known sample will not be released.
   
   d) In the event of a hit of a suspect known profile or Legal index profile to a solved case, Personal Identifiable Information may be released to the other CODIS participating laboratory in order to verify the name associated with the matching profile.
   
   e) Suggested Wording for autosomal profile hits
   
   “The purpose of this communication is to inform you of a possible investigative lead related to a known reference sample. During a search of the CODIS database, an association occurred between the DNA profiles generated from [item #] from the [submitting agency name’s] case [agency case number] and [item #] of your case referenced above. This association may require additional investigation. Please contact [other agency contact name] at [phone and/or email] for more information concerning this investigative lead. Please note that if DNA comparisons are required, a known reference sample will be necessary. If the Texas Department of Public Safety Crime Laboratory Service can be of any further assistance, please contact this laboratory.”
   
   f) Suggested Wording for Y-STR profile hits
   
   “This letter is to inform you of a Local level hit between an evidentiary profile and a suspect profile in the CODIS (Combined DNA Index System) database. The Y-STR DNA profile from [item #] for your case reference above hit with a suspect profile from [item #] [submitting agency] [agency case number]. This hit should be considered an investigative lead. It is not a statement of identity. It should be noted that all paternally-related male relatives cannot be excluded as a contributor to this male profile.”
If Y-STR DNA comparisons are needed for your case, please submit a known blood sample or buccal swab from the suspect.”
If the Texas Department of Public Safety Crime Laboratory Service can be of any further assistance, please contact this laboratory.”

g) Hits from unsolved cases to the Suspect Known Index may be dispositioned as offender hit or forensic hit. It is recommended that the lab with the forensic sample determine which disposition to use. Regardless, the disposition from both labs must match.

h) Hits from unsolved cases to the Legal Index will be dispositioned as a legal hit.

i) Hits from a solved case to either the Suspect Known Index or Legal Index may be dispositioned as conviction match or investigative information. It is recommended that the lab with the forensic sample determine which disposition to use. The disposition of conviction match is preferred; however, ultimately the disposition from both labs must match.

4. If a positive association of value occurs and it is determined that one of the profiles is no longer CODIS eligible, the association shall still be communicated but the ineligible profile will be deleted.

E. For all other positive associations not considered positive associations of value, a Hit Notification email/fax must be sent to the requesting official. This notification must not include the names of any suspects. In order to ensure that suspect names are not included, it is necessary to check both the body of the notification and the header.

1. A copy of the notification email/fax must be retained in the case record.

2. Suggested Wording

“This is a Hit Notification [email, fax] to inform you of a positive association between the specimen (item xx) listed in your case {agency #, lab #} to {a convicted offender sample, suspect sample, another forensic sample}. Because {your case is solved, the specimen was determined to be CODIS ineligible}, it has been determined that the hit does not provide an investigative lead. Therefore, no action is necessary on your part and no further action will be taken by the Department.
If the Texas Department of Public Safety Crime Laboratory Service can be of any further assistance, please contact this laboratory.”

3. Examples of positive associations (not considered positive associations of value) which require hit notification include:

a) Investigative Information

Investigative information dispositions may occur in a variety of circumstances: when a match does not provide probative information and/or does not fit the other disposition categories, such as

i. when a subsequent match between the same specimen numbers occurs
ii. when two forensic unknowns match and each case is previously solved

b) Benchwork Match

When forensic profiles linked externally to CODIS are also matched by CODIS

4. If a positive association occurs and it is determined that one of the profiles is no longer CODIS eligible, the submitting agency shall still be notified of the association via email/fax but the ineligible profile will be deleted.
F. Conviction Matches (CM)

Conviction matches occur when a case is solved. The suspect reference sample has been analyzed by the laboratory or the case has been adjudicated with or without DNA analysis (assuming there are no discrepancies with the name associated). No communication to the customer is required.

G. No Match

If the profile evaluation leads the administrator to determine that there is an exclusion, the disposition is “no match.” No communication to the customer is required.

H. Moderate Stringency Hits with Forensic Mixtures

If a moderate stringency hit occurs at SDIS between a known individual and a Forensic Mixture specimen or a Forensic specimen derived from a mixture and yields information which may be valuable to the investigator, the casework laboratory should consult with the State CODIS Administrator (or designee) to determine if the match provides an investigative lead prior to any communication with the agency.

I. Partial Match

See Texas DPS CODIS Standard Operating Procedure concerning partial matches indicating a potential relative.

6 Expunction Procedure for Legal Index Samples

6.1 Background Information on Expunction

A. The DPS recognizes the need to remove from CODIS DNA profiles of individuals who have obtained a court-ordered expunction. The following procedures are designed to ensure the rights of individuals are protected by allowing for the destruction of any specimen, and the removal of any DNA profile, which does not meet the Texas statutory requirements for inclusion in CODIS as provided in Texas Government Code. § 411.1471(e).

B. Federal law requires that the laboratory, as a participant in NDIS, expunge the DNA records of persons whose qualifying convictions have been overturned.

1. An amendment to the Federal DNA Identification Act in 2001 requires that the laboratory “shall promptly expunge from that index the DNA analysis (DNA profile) of a person included in the index by that state if the responsible agency or official of that state receives, for each conviction of the person of an offense on the basis of which that analysis (profile) was or could have been included in the index, a certified copy of a final court order establishing that such conviction has been overturned.”

2. A court order is not considered “final” for these purposes if time remains for an appeal or application for discretionary review with respect to the order.


C. Amendments made by the DNA Fingerprint Act of 2005 (P. L. 109-162) require expungements of DNA data of arrestees, indicted persons or similar legal specimens in the event the charge is dismissed or results in an acquittal or no charge was filed within the applicable time period.
D. The laboratory is required to expunge from NDIS the DNA analysis of a person included in NDIS by that State if “the person has not been convicted of an offense on the basis of which that analysis was or could have been included in the index, and the responsible agency or official of that State receives, for each charge against the person on the basis of which the analysis was or could have been included in the index, a certified copy of a final court order establishing that such charge has been dismissed or has resulted in an acquittal or that no charge was filed within the applicable time period.” See 42 U.S.C. § 14132(d)(2)(A)(ii).

6.2 Administrative Removal

A. The DPS Laboratory Director, local CODIS Administrator, or designee, can order removal of a DNA profile from the database if it is determined to be ineligible for entry into CODIS (see 5.3 B for deletion instructions).

B. An individual wishing to petition the DPS for removal of his/her DNA profile from CODIS must provide such a request in writing to the DPS.

C. If needed, DPS Legal Counsel may be contacted to provide a recommended course of action to assure that DPS and the casework laboratory are in compliance with applicable statutes.

6.3 Court Ordered Expunction

A. In the event of acquittal or the qualifying offense of an individual whose DNA profile is included in CODIS is overturned or dismissed by the courts; or the adjudication of a juvenile record is sealed, the DNA profile may be expunged from CODIS.

B. The DPS Casework Laboratory must be notified, in writing, of the need to expunge. This may be accomplished by:

1. The District Attorney prosecuting the case stating that the qualifying offense has been removed. A certified copy of the court order must be provided to the DPS Casework Laboratory.

2. An individual whose conviction has been overturned, or legal counsel thereof, may request the DNA profile be expunged. Such a request must be in writing. A certified copy of the court order overturning the qualifying offense must accompany the request.

6.4 Expunction Operations

A. Upon order from the DPS Laboratory Director, or designee, court order, or receipt of a proper request from the collecting agency or District Attorney’s Office as described above, the Local CODIS Administrator will ensure the expunction or administrative removal of all information related to the DNA profile in question.

B. This will include the removal, if applicable, of all DNA profile information from all levels of the CODIS DNA database.

C. See the Expunction and Destruction of Laboratory Records and Information chapter of the CLS manual for additional information regarding the expunction of case records.
6.5 Notification and Records

A. Notification of removal or expunction will be in writing from the Local CODIS Administrator, or designee to the requestor through a communication generated in LIMS.

B. The DPS Casework Laboratory may retain documents relating to the administrative removal but shall destroy any records relating to court ordered expunction of a sample. (see 5.3 B for deletion instructions)

C. In the event that an interstate candidate match(es) have been generated to the Legal Index profile, the DPS Casework Laboratory expunging the DNA record will notify any other laboratory(ies) involved in the candidate match that the expunction is being performed. This notification may be done via email/fax.

D. Suggested Wording

“The DNA profile generated from [item #] has been deleted from the CODIS database.”

7 Records

A. Documentation of each CODIS entry will be recorded in the appropriate case record.

B. Documentation of profile deletions and positive associations will be maintained in the appropriate case record.

8 Literature References and Supporting Documentation


DNA-09-10 Y-STR INTERPRETATION GUIDELINES

1 Scope

These guidelines are in place to ensure that conclusions are scientifically supported by the analytical data with appropriate standards and controls. Interpretations are made as objectively and consistently as possible from analyst to analyst. Interpretation guidelines are based upon validation studies, literature, instrumentation and casework experience. Not every situation can nor should be covered by a rule; consultation with the Technical Leader and further documentation is required for scenarios not addressed in this chapter.

Y chromosome markers represent “lineage markers.” They are passed down through the paternal line without changing (except for mutational events).

2 Related Chapters

Glossary
Analytical Controls
Guidelines for Technical Review
Capillary Electrophoresis
Report Writing Guidelines

3 Preliminary Evaluation of Data

The first step in data evaluation is to determine whether the results are of sufficient quality for interpretation purposes including identifying the presence of artifacts that could affect interpretations. The following criteria have been determined by evaluating data generated by the laboratory.

3.1 DNA Analysis Software

A. GeneMapper IDX must be used for all final data analysis and printouts.
B. The DNA analysis software will be set to the default settings and/or validated in-house methods for all kit dependent values. The analysis range may vary for each run.
C. Process Quality Values (PQVs) will not be used to replace analyst data interpretation. Any flags or color designations provided in the software will not be used during analysis of sample data.
D. The DNA analysis software includes a profile comparison tool which must be used to aid in comparing profiles within a project to each other as well as comparing lab reference profiles to all samples within a project.

3.2 Thresholds

A. All data shall be analyzed at an analytical threshold established by each laboratory through validation.
B. Data from the DYS385 locus should be evaluated using an instrument-specific stochastic threshold established by each laboratory through validation.
C. The maximum analytical threshold for evaluating Y-STR profiles is the saturation point of the CCD camera of the instrument in the raw data.
D. If the data exceeds the maximum analytical threshold at any locus, the off scale locus must be called uninterpretable or the sample reanalyzed using any of the following methods:
   1. Inject the sample for less time; as supported in validations for the instrument being used.
   2. Dilute the amplified product in dH₂O or TE⁻⁴ buffer and add the diluted amplified product to the formamide/size standard mixture.
   3. Re-amplify the sample with less template DNA.

E. If the maximum threshold is exceeded for the size standard, the analyst may use the data as long as any associated pull-up does not interfere with data interpretation.

F. To increase peak height, the sample may be reanalyzed using one of the following methods:
   1. Inject the sample for more time; as supported in validations for the instrument being used.
   2. Re-amplify the sample with more or less template DNA.

3.3 Internal Size Standard Evaluation
A. Internal size standards must be run with every sample.
B. The LIZ size standard is used in conjunction with Yfiler, and an analysis range of 75 to 400 bp is required for proper sizing.
C. The WEN size standard is used in conjunction with Y23, and an analysis range of 60-500 bp is required for proper sizing.

3.4 Allelic Ladder Evaluation
A. When interpreting Y-STR results, haplotypes are assigned to sample alleles by comparing their sizes to those obtained for the known alleles in the allelic ladders.
B. At a minimum, one acceptable Y-STR ladder must be present within each run for each respective kit.
C. Each ladder used for analysis must have the appropriate number of properly labeled alleles present for each locus when analyzed.

3.5 Analytical Controls
Analytical controls must be evaluated according to the Analytical Controls chapter.

3.6 Spectral
A. The Yfiler kit uses a five dye system which includes 6-FAM, VIC, NED, PET, and LIZ which is used as the internal lane size standard.
B. The PowerPlex Y23 kit also uses a five dye system which includes: fluorescein, JOE, TMR, CXR, and WEN which is used as the internal lane size standard.
C. Spectral files vary between instruments, virtual filter sets, and run conditions on a single instrument. However, the spectral overlap between the dyes is reproducible under constant run conditions.
4 Data Analysis

4.1 Allele Identification

An allele is defined as any peak that meets or exceeds established analytical threshold values, has Gaussian morphology, and is not an artifact. Allelic data may be present below analytical threshold.

4.2 Artifacts

A. General Guidelines

1. Generally, artifacts are easily characterized and do not pose a problem in identifying true allele peaks.

2. Artifacts or anomalies (including elevated baseline) may be detected on the electropherogram. Profiles which contain artifacts that occur within the analysis range and do not interfere with interpretation may be used providing the artifacts are identified and labeled. Analysts are encouraged to consult with the technical leader if the identity of an artifact is in question.

3. It is important to try to identify and document the cause for the appearance of the artifact.

4. Kit-associated artifacts (e.g. dye artifacts) may be documented and loci may be used.

B. Spikes

If a spike occurs and/or is labeled by the DNA analysis software the profile may be used or the sample may be re-injected.

C. Stutter

1. Stutter is a biological artifact that is known to occur during PCR due to slippage of DNA strands. Stutter occurs most commonly at the N-4 position, but is also known to occur at the N+4 position and rarely at N–2 and N+2.

2. Stutter peaks are evaluated by the ratio of the stutter peak height to the height of the appropriate allele.

3. When determining the most appropriate designation for a peak that is only minimally above the expected stutter ratio, the overall profile must be carefully evaluated, taking into account number and ratio of donors. Reamplification of the sample may help clarify whether a peak is stutter or allelic.

4. An analyst may designate a peak as stutter outside of existing thresholds in single source or mixture samples by labeling the peak to that effect.

D. Non-Template Addition (Minus A)

1. Minus A may occur when too much amplification product is generated due to incomplete adenylation during PCR. If this occurs, the analyst may re-analyze the sample using any of the following methods:

   a) **Re-inject for a shorter period of time as supported in validations for the instrument being used,**

   b) **Dilute the amplified product in dH2O or TE-4 buffer and add the diluted amplified product to the formamide/size standard mixture and reinject, or**
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DNA-09-10  Y-STR Interpretation Guidelines  (4.3)

Effective Date: 5/4/2020
Issued by: System Quality Manager

Printed copy is uncontrolled. Refer to electronic copy for current version.

4.3 Off-ladder Alleles

A. The number designation for alleles containing an incomplete repeat unit, falling within the range spanned by the ladder alleles, should include the number of complete repeats, and separated by a decimal point, the number of base pairs in the incomplete repeat.

B. If an allele falls outside the smallest or largest allele at a locus, it will be designated as greater than or less than the appropriate ladder allele. No calculations are required for these alleles.

C. Off-ladder alleles must be verified by re-injection of the sample or by confirmation in multiple samples.

D. Off-ladder alleles will be used for comparisons and for frequency estimation with the counting method.

E. Calculate off-ladder alleles using the following formulas and document in the case record.

\[ L_y = \text{the nearest ladder allele y} \]
\[ S_{OL} = \text{sample OL allele} \]
\[ C = \text{The relative peak shift of the OL allele from the nearest ladder allele} \]
\[ C = \left| S_{OL} - L_y \right| \]

For ladder alleles \((L_y)\), it is acceptable to use an average basepair size from all ladders in the run or to use the basepair size from a single ladder.

4.4 Tri-allelic Loci

A. Tri-allelic patterns must be verified by re-injection and/or re-amplification of the sample or by confirmation in multiple samples.

B. Tri-allelic loci will be used for comparisons and for frequency estimation with the counting method.

c) Re-amplify the sample with less template DNA.
4.5 Null alleles and Duplications
   A. A null allele presents as the failure of a locus to amplify and is often the result of a primer binding site mutation.
   B. A duplication presents as the presence of more than one allele at a locus in a single source profile and is often the result of mutation on the Y-chromosome.
   C. Null alleles and duplications have been observed during Y-STR analysis. The potential for the presence of null alleles and duplications should be considered during evaluation of data for interpretation.
   D. Duplications at loci will be used for comparisons and for frequency estimation with the counting method.

4.6 Composite Data: Single Amplification Kit
   A. Composite profiles (i.e., generated by combining typing results from multiple amplifications from the same kit and/or multiple injections of the same amplicon) will not be used in Y-STR interpretation.
   B. If a sample is subjected to additional amplifications or injected at different injection times, the profile with the most informative data will be used for interpretation.

4.7 Combined Data: Multiple Amplification Kits
   A. Yfiler and Y23 results from the same sample extract may be combined. For loci that are shared between the kits, the locus with the most information from either kit will be used to create the combined profile. Combined profiles must use the kit specific thresholds for the respective loci.
   B. When results are discordant between Yfiler and Y23, the Technical Leader must be consulted.

5 Analysis of Results
5.1 Characterization
   A. The evidentiary profile is characterized prior to comparison to known samples. Characterizations will be recorded in the case record.
      1. If no peaks are present at or above the analytical threshold, the profile is interpreted as no result.
      2. When peaks are present at or above the analytical threshold, an attempt should be made to characterize the profile. Possible characterizations are single source or mixture (major/minor or indistinguishable) and partial or full.
         a) When considering data, it may be impossible to characterize a profile. This type of profile would be considered uninterpretable.
         b) Allelic data may be present below analytical threshold. This data may be used for characterization of a profile. The number of sub-threshold peaks, baseline noise and amplification artifacts should be taken into consideration.
   B. Typical single source indicators include:
      1. No more than one allele per locus (Exception: no more than two alleles at DYS385).
      2. Sample origin
C. Typical mixture indicators include:
   1. More than one allele at one or more loci (Exception: more than two alleles at DYS385).
   2. Sample origin
   3. Peaks in stutter position that exceed the expected stutter ratio
   4. Significantly imbalanced alleles

D. Determine if the profile is partial or full:
   1. Single source profiles are considered partial if there is allelic dropout at the DYS385 locus or if there is complete locus dropout at any of the single copy loci.
   2. Mixture profiles are considered partial if there is complete locus dropout.

5.2 Single Source Evidentiary Profile Interpretation and Comparison

A. Interpretation
   Determine if there is enough data present to call a single source profile.
   1. A profile can be designated as single source with the observation of one or more complete haplotypes.
   2. Determine if allelic drop-out is reasonably suspected at any locus
      a) If both alleles are present in the stochastic region for the DYS385 locus, this locus is considered to have a complete haplotype.
      b) If only one allele is present for the DYS385 locus and this allele is under stochastic threshold, then this locus may be exhibiting drop-out.

B. Comparison to Reference Samples
   1. Comparisons will be made between the evidentiary profile and known profiles of possible contributors. The conclusions will be recorded in the case record.
   2. Known reference sample profiles developed by an external, accredited laboratory can be used with the approval of the Technical Leader.

5.3 Mixture Evidentiary Profile Interpretation and Comparison

A. Interpretation
   1. Mixture Deconvolution
      a) Major components of mixed profiles should be resolved whenever possible.
      b) Considerations for mixture deconvolution include:
         i. Evaluation of the profiles moving from the smaller loci to the larger loci.
         ii. Base line quality (e.g. low noise, no pull-up, spikes etc.)
         iii. Profile quality (e.g. degradation, low level, inhibition, etc.)
         iv. Mixture ratio consistency across the profile.
   2. Assign the minimum number of contributors and document in the case record.
      a) Evaluating the number of unambiguous alleles per locus and peak height ratios will aid in the determination of the minimum number of potential contributors. All
alleles at or above the analytical threshold may be used in this assessment. As an example, if at most 3 alleles are detected in a locus other than DYS385, then the DNA typing results are consistent with having arisen from at least three individuals.

b) Allelic data may be present below analytical threshold and may be used to determine number of contributors.

c) It should not be assumed that the number of alleles present is always a definitive method for determining the minimum number of potential contributors, especially in low level template samples.

d) The possibility of degradation, drop out, additive effects, peak height ratio, baseline noise, and increased stutter should be considered when determining number of contributors.

e) To determine number of contributors for complex mixtures, find the locus other than DYS385 with the maximum number of alleles. This number is indicative of the number of contributors.

f) For the DYS385 locus, count the number of alleles present and round this number to the nearest even number and divide by 2. With this number, try to pair the alleles at the DYS385 locus.

g) Using the information from all loci, assess the entire profile to ensure the number of contributors makes sense across the profile.

3. Determine if drop out is probable by evaluating the entire profile.

a) Allele drop out can still occur at the DYS385 locus with alleles that minimally exceed the stochastic threshold due to additive and stochastic effects.

b) Loci on either side of a locus and/or in a different dye channel can suggest that there could be potential contributor drop out.

c) Allele sharing is a more likely assumption for loci with high RFU values and a close ratio of contributors as they are unlikely to be experiencing drop out.

4. Major component (distinguishable mixture) resolution

a) Comparisons will be made and statistical frequencies will be generated for a Y-STR haplotype when the major profile can be determined. If the smaller peak is less than 40% of the larger peak, the major and minor contributors can usually be distinguished.

b) It is possible that multiple donor profiles to a mixture will degrade at different rates. This could result in a mixture that exhibits a major/minor pattern at some loci (typically the lowest molecular weight loci) but relatively equal intensities at other loci (typically the highest molecular weight loci). Caution should be exercised when identifying a major component to such a mixture. If a stochastic threshold is used, alleles in in the stochastic region for DYS385 can be designated as major contributor alleles as long as careful evaluation of the entire profile has been performed.

c) Documentation of the peak height ratios at each appropriate locus is required to support a major component determination.

d) Loci where a major cannot be resolved will be deemed uninterpretable for statistical calculation.

e) No comparisons will be made and no statistical frequencies will be generated for the minor component in the profile.
5. Indistinguishable mixtures
   a) **When the criteria for resolving a major component is not met, the profile is considered an indistinguishable mixture.**
   b) **No comparisons will be made and no statistical frequencies will be generated for a profile if a major component cannot be resolved.**

B. Comparison

1. Inclusions and exclusions are determined by evaluation of the entire DNA profile produced by the loci tested.
2. It is acceptable for comparisons to be made when one or more of the loci yield uninterpretable results. A comparison statement will be based only on loci that yield conclusive results.
3. Reference profiles will be compared to deduced major components.
4. If the laboratory has determined a stochastic threshold, the analyst may use the data in the stochastic region of DYS385 to determine if sufficient information exists to exclude the reference profile through application of this threshold.
5. If a locus was determined to be suitable for interpretation (i.e. a component of a distinguishable mixture was resolved or all alleles were determined to be present) and subsequently during comparison with reference, a true haplotype was eliminated and/or allelic drop out is indicated, the profile will be deemed uninterpretable and no comparisons will be reported.
   a) **If complete allelic drop out is observed, additional testing of the sample (extraction and/or amplification) may be attempted to determine if further information may be obtained for comparisons with Technical Leader approval.**
   b) **If the comparison was to a sample previously entered into CODIS, consult with the Technical Leader.**
6. No comparisons will be made to indistinguishable mixtures or minor components of distinguishable mixtures.

C. Possible Conclusions

1. **Exclusion/Excluded** – The alleles obtained for the reference haplotype are not included in the alleles obtained for the evidentiary Y-STR profile. The reference male is excluded as a possible contributor of the Y-STR DNA profile obtained from the evidentiary sample.
2. **Inclusion/Included/Not Excluded** – The alleles obtained for the reference haplotype are included in the alleles obtained for the single source or major component of the evidentiary Y-STR profile. The reference male cannot be excluded as a possible contributor of the Y-STR DNA profile obtained from the evidentiary sample.
3. **Uninterpretable** – the DNA data cannot be interpreted and may not be able to be fully characterized; therefore, the data is not suitable for comparisons. This could be the result of poor or limited data quality, data that is too complex, or data that fails to meet laboratory quality requirements.
4. **No Result** – No DNA profile was obtained.
6 Profiles for CODIS Entry
   A. Regular upload of Y-STR profiles to CODIS is optional and based on local policy.
   B. The analyst may use the data in the stochastic region for DYS385 to develop profiles for CODIS entry.
   C. Any new or additional data obtained for CODIS eligible profiles through reamplification or reanalysis with a different amplification kit should be uploaded to CODIS.
   D. If a profile has been determined to be unsuitable for comparison there will be no attempt to develop a profile for CODIS.

7 Records
   A. All reference and evidentiary profiles will be recorded as haplotypes.
   B. All conclusions will be documented in the case record.

8 Literature References and Supporting Documentation
   Applied Biosystems. DNA Analysis Software User’s Manual. (current version)
DNA-09-11 Y-STR STATISTICAL INTERPRETATION

1 Scope

Y-STR loci are located on the nonrecombining part of the Y-chromosome and, therefore, should be considered linked as a single locus. Statistical calculations for a random match probability cannot involve the product rule because of the linkage. A Y-STR database must consist of haplotype frequencies rather than allele frequencies. The basis for haplotype frequency estimation is the counting method. The autosomal statistical results will not be combined with haplotype frequency estimation.

While Y chromosome DNA exclusions can aid forensic investigations, a match between a suspect and the evidence only means that the individual in question could have contributed the forensic stain, as could any male from the same paternal lineage.

2 Related Chapters

Report Writing Guidelines
Y-STR Interpretation Guidelines

3 Practices

3.1 Counting Method for Frequency Estimation

A. The basis for the haplotype frequency estimation is the counting method. According to the Scientific Working Group on DNA Analysis Methods, reporting a haplotype count without a confidence interval is acceptable as a factual statement regarding observations in the database.

B. Our laboratory system will use the Y-chromosome Haplotype Reference Database (YHRD), and will report the number of times a haplotype has been seen overall in the United States database.

1. Caucasian, African American, Hispanic, Asian, and Native American databases are also available.
2. Only loci considered conclusive for interpretations will be used for frequency estimation.
3. The search will be performed using the database associated with the amplification kit used. Multiple database searches using different amplification kit databases will not be undertaken at this time.

C. The YHRD can be located on the internet at the following address: http://yhrd.org This database was designed for use in estimating Y-STR haplotype population frequencies for forensic casework purposes.

3.2 Instructions for Using YHRD

A. In the “Search the Database” tab, analysts may choose to manually enter the haplotype to search or enter multiple haplotypes using a spreadsheet or GeneMapper IDX export table.

B. To use manual entry perform the following:

1. Click on the “manually enter the haplotype/haplotypes to search for” button
2. Select the correct kit by clicking the “Yfiler” or “PowerPlex Y23” button.
3. Enter the alleles for the haplotype including any duplications under the appropriate loci.
   a) \textit{Tab may be used to move from locus to locus}
   b) \textit{For loci with duplications, allele entries are separated by a comma}
   c) \textit{For the DYS385 locus, two alleles must be entered or the search will give an error. If there is only a single allele at this locus, enter that number twice, separated by commas.}

4. Click the “search” button.

5. The screen that pops up gives the worldwide count; click the “add feature to this report” button below the entered haplotype and select “National Database (with Subpopulations, 2014 SWGDAM-compliant)”. 

6. There should now be 6 results under “National Database (with Subpopulations)” in the “Observed” header. The United States (Overall) is the number that is reported.

7. Print the YHRD report or save it to pdf for inclusion in the case record.

C. To use a spreadsheet or GeneMapper IDX export table perform the following:

1. Open or create project in GeneMapper IDX.

2. Check samples and remove any artifacts and/or samples needing reinjection. Only whole projects can be exported.

3. Export the project using the “Export Combined Table” selection in the File menu

4. Name the file and select the location where it will be saved. Save the file as a text tab delimited file.

5. Open the YHRD website.

6. Optional step: in the “Tools” menu, select “Data File Validator”. This step will check the file to make sure it is in concordance with Y-STR nomenclature.
   a) \textit{In the “Choose your file” field, browse to where the export file was saved and select it.}
   b) \textit{Under the “select the type of validation” field, choose “search”}
   c) \textit{Click the “Go” button.}
   d) \textit{If the export file passes validation, proceed with search instructions. If not, follow the advice given at each unsuccessful check in order to resolve any issues prior to searching.}

7. In the “Search the Database” tab, click the “Search using your Excel-, OpenOffice- or CSV-spreadsheet OR your Applied Biosystems GeneMapper ID/ID-X or ABI PRISM Genotyper export-file” button.

8. In the pop-up window, navigate to the saved export file and select it.

9. Select the sample or samples (can search multiple samples at one time) that are intended for searching.

10. Select the appropriate kit for each sample.

11. Click the “search” button.
12. The screen that pops up gives the worldwide count; click the “add feature to this report” button below the entered haplotype and select “National Database (with Subpopulations, 2014 SWGDAM –compliant)”.

13. There should now be 6 results under “National Database (with Subpopulations)” in the “Observed” header. The United States (Overall) is the number that is reported.

14. Print the YHRD report or save it to pdf for inclusion in the case record.

4 Interpretation

A. On single source samples and on mixtures where a major contributor can be deduced, the laboratory will report the haplotype count, without a confidence interval, as a factual statement regarding observations in the database.

B. Statistical significance will not be reported for indistinguishable mixtures.

5 Records

The YHRD report including the Y-STR haplotype and the count summation stating the number of times the haplotype is seen in each of the relevant databases will be included in the case record.

6 Literature References and Supporting Documentation


DNA-09-12 STRMIX AUTOSOMAL STR INTERPRETATION GUIDELINES

1 Scope
These guidelines are established for performing STRmix analysis of Identifiler Plus, Investigator 24plex QS, and Minifiler data after electrophoresis of samples on a 3130 or 3500 (Investigator 24plex QS only) series genetic analyzer. STRmix is a software program that applies a fully continuous probabilistic approach to DNA profile interpretation. It standardizes both inter- and intra-laboratory analysis of profiles by using Markov-Chain-Monte-Carlo (MCMC) and Metropolis-Hastings algorithms along with variance models of the results derived from validation data. STRmix can be used to analyze samples with or without reference samples and under varying hypotheses to calculate Likelihood Ratios to aid in answering questions commonly asked in criminal legal proceedings.

2 Related Chapters
- Glossary of Terms
- Analytical Controls
- Guidelines for DNA Technical Review
- Capillary Electrophoresis
- Autosomal STR Data Evaluation and Profile Characterization Guidelines
- Report Writing Guidelines
- CODIS

3 Data Preparation

A. Artifact Editing
1. For Identifiler Plus and Minifiler kits, all artifacts, with the exception of n-4 stutter on evidentiary profiles must be removed from the electropherogram prior to exporting for entry into STRmix.
2. For Investigator 24Plex QS kit all artifacts, with the exception of n±1 repeat stutter on evidentiary profiles, must be removed from the electropherogram prior to exporting for entry into STRmix.
3. Editing is accomplished by right clicking on the artifact peak in GeneMapper ID-X and selecting the ‘Custom Artifact Label’ option.
   a) The type of artifact must be entered into the label field and user initials may also be entered for documentation purposes.
   b) If user specific log-ons are used for GeneMapper ID-X, initials need not be entered, but if only a global log-on is available for users, user initials must follow the artifact description for documentation purposes.

B. Stutter Filters
1. STRmix software requires that stutter filters be turned off when evaluating questioned samples and that stutter filters are turned on for reference samples.
2. It is required that separate analysis methods are created in GeneMapper ID-X software for both evidence and reference samples.
3. It is also recommended that evidence samples be manually evaluated with stutter filters turned off so that the analysts will be evaluating the same data as the software. If manual evaluation is completed on evidence samples with the stutter filter on, the electropherogram with the stutter filter off must be maintained in the case record.

C. Exporting Files from GeneMapper ID-X

1. Exporting Reference Files for STRmix
   a) Create or use a table setting that contains at least the following information in the recorded order:
      
      Sample Name/File, Marker, Allele (1-10), Allele Size (1-10)
   
   b) Display the Genotypes Table in GeneMapperID-X, export the table and save a text document (.txt) in a desired location.
   
   c) When no standard reference samples are available, alternate reference samples yielding a single source profile may be used. Only loci with full/complete allelic representation shall be used for comparison. Loci without full/complete allelic representation shall be omitted from the profile prior to comparison in STRmix. This can be accomplished by either editing the electropherogram or the table.
   
   d) Known reference data from the 3500 series genetic analyzer amplified in Identifier Plus and Investigator 24plex QS may be used for comparisons.
   
   e) Known reference data from a Minifiler amplification or combined data from Investigator 24plex QS, Identifier Plus and/or Minifiler amplification kits to generate a complete known profile may be used for comparisons as long as sizing information is not considered by the software.
   
   f) Known reference data from Identifier, Minifiler, and Investigator 24plex QS data run on a 3130 model genetic analyzer may be used for comparisons.
   
   g) Known reference sample profile developed by an external accredited laboratory can be used with the approval of the Technical Leader.
   
   h) Tri-allelic peak patterns will result in the locus needing to be omitted in the STRmix software. This can be accomplished by either editing the electropherogram or the table.

2. Exporting Evidence Files for STRmix
   a) Edit the electropherogram to remove any artifacts (e.g. pull-up, n+2 and n-2 stutter, spikes, etcetera.) and re-label a verified OL allele to its appropriate allele value (e.g. 10.1). Leave off stutter filters and do not remove the n+1 repeat and n-1 repeat stutter peaks from the electropherogram.
      
      i. OL alleles within marker range need to be relabeled with the correct allele value.
      
      ii. Presence of an OL allele outside a marker range will result in the locus needing to be omitted from the STRmix software (see section 4.2.B.4). This can be accomplished by either editing the electropherogram or the table.
      
      iii. Tri-allelic peak patterns will also result in the locus needing to be omitted in the STRmix software (see section 4.2.B.4). For mixture profiles consult the Technical Leader.
b) Edited or deleted alleles/artifacts need to be displayed on the epg for technical review purposes.
   i. Turn on the labels in GeneMapper ID-X software and document reason(s) for edit(s) in the software.
   ii. When editing artifacts, label as Custom Artifact in the software; when editing true alleles (i.e. OL) use the Edit Allele function. This will ensure proper exporting of the genotypes.

c) Create or use a table setting that contains the following information in the recorded order:
   i. Sample Name/File,
   ii. Marker,
   iii. Allele (1-10),
   iv. Allele Size (1-10),
   v. Allele Height (1-10)

d) Display the Genotypes Table in GeneMapper ID-X, export the table and save a text document (.txt) in a desired location.

4 Interpretation of Results
4.1 Single Source Evidentiary Profile Interpretation and Comparison

A. Interpretation

Determination if the profile is interpretable or not is made through application (and documentation of such application) of the appropriate stochastic threshold.

B. Comparison to Reference Samples

1. If the profile is determined to be interpretable, visual comparison of the profile to any known DNA profiles may be performed.

2. If the result of the comparison is exclusion, then further analysis in STRmix is not required to support this conclusion.

3. With the exception of single source profiles originating from intimate/indigenous samples consistent with the profile of the assumed donor, any single source profile comparison resulting in inclusion will be compared using STRmix in order to generate a likelihood ratio.

   Single source profiles originating from intimate/indigenous samples that are consistent with the profile of the assumed donor are not required to be compared using STRmix and do not require a likelihood ratio.

4. When a reference sample from a person of interest is available and has been analyzed, a comparison to a STRmix deconvolution can be performed.

5. After obtaining the STRmix results, a manual comparison must be done that includes review of the electropherogram to ensure that likelihood ratios and conclusions are intuitively supported.

   a) If intuitive support is not indicated, the analyst should consult with the Technical Leader to troubleshoot any issues and determine possible solutions.
4.2 Mixture Evidentiary Profile Interpretation and Comparison

A. Interpretation

1. A number of contributors must be assigned for each deconvolution run in the software and documented in the case record.

2. The following are considered when assigning number of contributors:
   a) Evaluating the number of unambiguous alleles per locus. All alleles at or above the analytical threshold may be used in this assessment. As an example, if at most five alleles are detected per locus, then the DNA typing results are consistent with having arisen from at least three individuals.
   b) It should not be assumed that the number of alleles present is always a definitive method for determining the number of potential contributors, especially in low level template samples. As an example, some 3 or 4 person mixtures may not have more than four alleles present at any given locus.
   c) The possibility of degradation, drop out, additive effects, peak height ratio, baseline noise, and increased stutter should be considered when determining number of contributors.
   d) Assess the entire profile to ensure that number of contributors makes sense across the profile.
   e) Data may be present below analytical threshold and may be used to determine the number of contributors; however, contributors thought to make no unique or substantial addition to the profile above the analytical threshold should not be considered.
   f) In general, overestimation of number of contributors may increase adventitious support for the inclusion of a non-donor and underestimation of number of contributors may increase adventitious support for the exclusion of a true donor.

3. If it is not readily apparent how the number of contributors is assigned, designations should be included for at least one locus to show the reason why that number of contributors was assigned.

4. Mixtures assigned to be of 5 or more individuals are too complex for interpretation, due in part to, hardware limitations that render the STRmix software unable to deconvolve these complicated profiles.

5. If the number of contributors is too difficult to confidently assign, multiple deconvolutions of the profile under different number of contributors may be performed to ensure the most informative and accurate conclusion is reported.
   a) A review of the run diagnostics and possible genotype combinations may aid in assigning the number of contributors.
   b) If after multiple considerations or due to the complexity of the profile, the number of contributors cannot be confidently assigned, the profile will be determined to be uninterpretable.
   c) If multiple contributor assignments are being considered, comparisons and LR calculations will not be performed initially. LR calculations will only be performed for considerations that resulted in acceptable deconvolutions. All tested deconvolutions will be retained in the case record.
B. Comparison to Reference Samples

1. When a reference sample from a person of interest is available and has been analyzed, a comparison to a STRmix deconvolution can be performed.

2. Assumed contributors (contributors in both the Hp and Hd) should not be limited to intimate samples.

3. Hypotheses with multiple contributors which require the assumption of the presence of an individual on an indigenous or environmental sample must first test that individual as the only donor to Hp against unknown donors in Hd.
   a) This LR is not required to be reported.
   b) Only after this individual comparison has resulted in a LR value greater than 1000 for Identifier Plus/24plex samples or 10,000 for Minifiler samples, may that individual be an assumed donor under Hp and Hd.
   c) In general, it is not necessary to test an individual as the only donor to Hp against unknown donors in Hd for an intimate sample.

4. The case scenario may warrant multiple hypotheses to be tested based on plausible assumed contributors.
   a) Multiple deconvolutions may be conducted prior to comparisons (i.e. A profile may be run with an assignment of two contributors or three contributors).
   b) Justification for each additional deconvolution must be documented.
   c) Analysts may calculate various likelihood ratios under different hypotheses.

5. Hypotheses which require multiple known donors to be considered in the Hp must first test each known donor against the profile individually. Only after an individual comparison has resulted in a LR value greater than 1000 for Identifier Plus/24plex samples or 10,000 for Minifiler samples, may that individual be entered into a multiple donor Hp.

6. After obtaining the STRmix results, a visual comparison must be done that includes review of the electropherogram to ensure that results including, but not limited to, mixture proportions, likelihood ratios, and conclusions are intuitively supported.
   a) If intuitive support is not indicated, the analyst should consult with the Technical Leader to troubleshoot any issues and determine possible solutions.

4.3 STRmix Software

A. Start STRmix by double-clicking the STRmix icon on the desktop or select STRmix from Start > STRmix. The STRmix main menu will display (Figure 1).

![Figure 1](image.png)
B. Each of the functions within STRmix can be accessed from the Startup screen. To exit the software click the Exit button, the top right-hand X button has been disabled.

C. Single Sample Analysis

1. Select Start Mixture Analysis to open the Configure Analysis window (Figure 2). This initiates a three-step process for the interpretation of single source and mixed DNA profiles.

![Figure 2](image)

2. Fill in the Case Number. In the Sample ID field, add a sample name or unique identifier, any case notes (optional), and the number of contributors for the analysis. Select the appropriate DNA kit for the analysis.

3. All analyses require the validated number of 500,000 MCMC accepts and 100,000 burn-in accepts and should not be changed.
   a) If longer analyses are required to better resolve complex mixtures, the Technical Leader may approve additional burn-in of 1,000,000 and MCMC accepts of 5,000,000.
   b) Careful evaluation of the STRmix diagnostic parameters should be completed to ensure a proper deconvolution was performed.

4. The ignore loci in Run Settings may be changed as needed. If, through manual review of the profile, it is determined that the software did not model drop out correctly, consider additional MCMC accepts prior to ignoring the locus in Run Settings.

5. The degradation max setting shall not be changed.

6. STRmix results folders are prefixed with the information entered into the Case Number field, followed by sample ID, then the date and time in the format yyyy-mm-dd-hh-mm-ss.

7. Select confirm.

8. In the Add Profile Data screen select Add EPG to open Add EPG window (Figure 3) or drag and drop.
9. Select Find Text file, Find STRmix file or Create File as appropriate to enter an evidence input file. Navigate to the appropriate file and select the sample to be analyzed. Select Add EPG to add the input file and proceed or Cancel to return to the Add EPG screen.

10. The software allows for multiple EPGs of multiple amplifications of the same DNA extract, amplification concentration and injection time to be added together and used within its replicate function. This technique is considered separate and free from the restrictions placed on composite profiles as invoked by the *Autosomal STR Data Evaluation and Profile Characterization* chapter. This feature may be used for any samples that display stochastic effects and may benefit from additional data.

   a) *Technical Leader approval is required before re-amplification is performed.*
   
   b) *A maximum of two amplification electropherograms per extract may be used.*
   
   c) *Profiles from this method are ineligible for upload to CODIS.*

11. Set Reference Samples

   a) *In the Set Reference EPGs screen, repeat the steps above for any reference input file(s) as appropriate.*

   b) *Reference input files will automatically be assigned as a known contributor under Hp and denoted by an “X” in the Hp box*

   c) *If any references can reasonably be assumed, they may be added at this step. Should you require a reference sample to be an assumed contributor under Hd, select the reference of interest with the cursor so it is highlighted then press the Change Hd button. Assumed contributor epgs must be entered into the software prior to contributors that will only appear in one of the hypotheses.*

   d) *Comparisons are generally performed after the initial deconvolution of the sample, but may be done in conjunction with the initial deconvolution.*

   e) *If a deconvolution is not done initially and a subsequent deconvolution is required, the analyst must ensure that all STRmix settings, including Model Maker parameters, are set to the appropriate values for the amplification kit at the time of the original amplification.*
12. Select Confirm Settings to proceed to the next step. (Refer to Figure 4.)

![Figure 4](image)

**a)** Select the appropriate population(s) from the drop down menu. If reference samples have been added, population groups for any likelihood ratio calculations can be added at this step by choosing Add Pop for each highlighted population of interest from the drop-down menu.

**b)** Population settings are only required when reference samples have been selected in Step 8.

**c)** If no reference samples are selected, the settings in Step 12 are disabled (as in Figure 4).

**d)** Add population data for Caucasian, African American and Hispanic populations and report the lowest total likelihood ratio between the population groups.

**e)** The Asian population shall also be added with the use of the Investigator 24plex QS kit.

13. Select Start to start the calculation.

14. Once the run is complete, **Run Report** and **Create PDF**. A PDF report cannot be created after the fact without re-running this mixture. Close the Advanced report box, and click **Finish**.

15. Results for this run will be located on the local computer in the STRmix Results folder.

16. Run time is dependent on mixture complexity.

D. **Batch Sample Analysis**

1. Multiple samples may also be added to STRmix to be processed sequentially by selecting Batch Mode from the screen in Figure 1.

2. The process proceeds in the same way as Single Sample Analysis by going through steps 1 through 14.
3. After step 14, when Start is selected, the software does not actually start a deconvolution, but will return you to the screen in Figure 5.

![Figure 5](image)

4. Once at the screen in Figure 5, additional STRmix analyses can be queued by repeating step 2 above.

5. Once all the samples for the batch have been added, selecting Start Batch will commence the deconvolutions. The deconvolutions will proceed in alpha-numeric order.

E. Likelihood Ratio from Previous Analysis

1. When performed after the initial deconvolution, select LR from Previous Analysis from the STRmix main menu (Figure 1).

2. Navigate to the previously analyzed sample folder of choice, highlight the settings.ini file and select Open.

3. The window in Figure 2 will open, but analysis settings will be unavailable for change. Fill in the Case Number, Sample ID, and any case notes (optional). The sample ID box should be altered to contain the initials for the reference sample to be compared (e.g., XXXXXXXX LR Ref XX). Any loci to be ignored can be set in Others Settings. Select confirm.

4. The window in Figure 3 will open. Only the Set Reference EPGs will be available. If any contributors need to be assumed at this point, the deconvolution should be repeated as this is not an available feature when conducting an LR from Previous Analysis. Navigate to the reference input file(s) as appropriate. Files may be added by drag and drop.

5. Reference input files will automatically be assigned as a known contributor under Hp. Select Confirm Settings.

6. The window in Figure 4 will open. Select the appropriate population(s) from the drop down menu and choose Add Pop. Select Start to start the calculation.

7. Once the run is complete, Run Report and Create PDF. A PDF report cannot be created after the fact without re-running this mixture. Close the Advanced report box, and click Finish.

8. Results for this run will be located on the local computer in the STRmix Results folder.
F. Using the Database Search Module for Exclusions

1. The Database Search function can be used, with Technical Leader approval, to potentially decrease the number of individual STRmix analyses required for a case that includes a large number of references.

2. This tool allows the user to search a deconvoluted evidence profile against a database of reference profiles.

3. All database comparisons are calculated by considering the database individual (POI) in an “N” person mixture by:
   \[ Hp = POI + N-1 \text{unknowns} \]
   \[ Hd = N \text{unknowns}. \]

4. The calculated LR is a point estimate using a theta value of 0.

5. Comparisons resulting in a LR of 0 may be reported as exclusionary with no additional STRmix analysis.

6. Comparisons resulting in a non-zero LR will require separate STRmix analysis in which the lowest LR is calculated.

7. The database file must be a .csv type file in the following format:
   Case number, sample ID, locus 1 allele 1, locus 1 allele 2, …, locus N allele 2.

8. Select Search Database from the STRmix main menu. A file chooser window will open.

9. Navigate to the previously analyzed results folder of choice and highlight the settings.ini file. Select Open.

10. Select Choose Database to open a file chooser. Navigate to the reference profile database created above and select Open. The number of profiles within the database appears in the window.

11. Enter 0 for Minimum LR.

12. Choose a population (i.e. allele frequency database) for the search. For this application, the Database Search tool is simply being used to identify comparisons with a resulting LR of 0, which means the profile cannot be explained under Hp. Allele frequencies are not utilized in a calculation when this occurs, and therefore, the specific allele frequency database that is selected at this step will not affect the results.

13. Check the box for Standard to undertake a standard database search.

14. Select Start to start the search. On completion of the search, select Finish to close the window.

15. The results will be saved as a .txt file named DBSearchResults in the STRmix Results folder.
4.4 STRmix Advanced Report

The advanced run report contains information that will allow the analyst to evaluate if an appropriate deconvolution was performed. The following criteria must be reviewed by both the reporting analyst and the technical reviewer to ensure proper results were obtained through the software. Profiles with criteria that fall out of the ranges listed below may be used with discretion. If necessary samples may be re-processed to include re-injection on the CE instrument, re-amplification and/or re-extraction.

A. Case Number and Sample ID
   These should correlate the epg deconvolved within STRmix to the item being reported.

B. Kit Used
   This should be the same amplification kit as the kit used to amplify the sample extract.

C. Number of Contributors
   1. The analyst and technical reviewer should agree on this designation for the profile.
   2. If a consensus between the reporting analyst and the technical reviewer cannot be achieved, the Technical Leader will be consulted.
   3. If a consensus still cannot be reached, the profile is too complex for meaningful interpretation.

D. Propositions Under Hp (H1) and Hd (H2)
   The analyst will ensure that proper reference samples were entered in the software to address the plausible court questions about the origin/composition of the DNA profile.

E. Mixture Proportions
   The analyst and technical reviewer should evaluate whether the contributor specific proportions STRmix calculated are similar to the proportions or ratios they may have determined independent of deconvolution. This evaluation may include some mixture proportion calculations where possible.

F. Contributor Specific degradation
   These values should correlate to the analysts’ determination if no, low, moderate or severe degradation has occurred. As the severity of the degradation increases, the RFU/bp (Relative fluorescent units per base pair) value also increases within the software.

G. Acceptance Rate
   1. This value is calculated by dividing the number of post burn-in MCMC accepts by the total iterations.
   2. A low acceptance rate (for example, 1 in thousands or 1 in millions) may indicate the need for additional MCMC accepts in order for proper deconvolution to occur.
   3. The acceptance rate is calculated by the software and given as a 1 in ____ number.

H. Total Iterations
   1. This value displays how many total post burn-in proposed profiles the software used before reaching the number of required MCMC accepts.
2. This number should be less than 400 million for the default post burn-in accepts of 400,000.

3. If the number is higher, it may indicate additional MCMC accepts are required for a proper deconvolution.

I. Effective Sample Size

1. This value is the number of independent genotypes proposed by the software to obtain the number of MCMC accepts.

2. If this value is lower than the manufacturer recommended number of 100, an additional deconvolution through STRmix with increased MCMC accepts should be considered.

J. Average Log (Likelihood)

1. This value is the average log of the probability of the proposed genotypes.

2. The higher this value the better STRmix has been able to describe the observed data. Low or negative values should be investigated and may be due to:
   a) Low level data that do not allow for large LR’s to be calculated. This result is expected and should not be reason for reanalysis of the profile through the software.
   b) The assigned number of contributors is incorrect.
   c) Allele or stutter information has been removed from the epg.
   d) Artifact information has not been removed from the epg.
   e) Replicate amplifications with large stochastic effects will naturally lower this value.

K. Gelman-Rubin Convergence Diagnostic

1. This diagnostic describes how closely the independent chains of the MCMC process converged on the same genotypes by the end of the deconvolution.

2. A value between 1 and 1.2 is optimal as per the manufacturer recommendation.

3. Large Gelman-Rubin values mean that increased MCMC accepts may be necessary for the chains of the MCMC to converge.

L. Allelic and Stutter Variance

1. This describes how far from the mean of observed allele and stutter values the software had to deviate to explain the observed data.

2. Low-level and stochastic samples are expected to have larger deviations, while robust and simpler profiles should have variances that are closer to the mode.

3. The below table indicates the values calculated during our empirical study for DPS samples.

<table>
<thead>
<tr>
<th></th>
<th>ID+ Mode</th>
<th>24plex Mode (3130 series)</th>
<th>24plex Mode (3500 series)</th>
<th>Minifiler Mode</th>
</tr>
</thead>
<tbody>
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<td>Allelic Variance</td>
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<td>3.942706</td>
<td>4.951</td>
</tr>
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<td>Stutter Variance</td>
<td>7.01</td>
<td>5.878496</td>
<td>6.019582</td>
<td>5.141</td>
</tr>
</tbody>
</table>
M. Verify Input Files

Compare the input file to the electropherograms for both evidence and known profiles to ensure correct genotypes were imported.

4.5 Calculating Likelihood Ratios in STRmix

A. STRmix calculates likelihood ratios using the following equation:

\[ LR_C = \frac{\sum_{j} w_j \Pr(S_j | H_1)}{\sum_{u} w_u \Pr(S_u | H_2)} \]

- \( LR_C \): Likelihood ratio for the continuous model
- \( \Pr \): Probability of the evidence given the hypothesis, i.e. \( \Pr(E|H) \)
- \( w_j \): Probability weight of genotype "\( j \)"
- \( w_u \): Probability weight of unknown genotype "\( u \)"
- \( S_j \): Genotype "\( j \)"
- \( S_u \): Unknown genotype "\( u \)"
- \( H_1 \): Hypothesis 1 or the prosecution hypothesis (\( H_P \))
- \( H_2 \): Hypothesis 2 or the defense hypothesis (\( H_D \))

1. Calculations use the Balding and Nichols formulae:

   - **Homozygote Loci**: \( P(A_iA_i|A_iA_i) = \left[ 2\Theta+(1-\Theta)p_i \right] \left[ 3\Theta+(1-\Theta)p_i \right] / \left[ (1+\Theta)(1+2\Theta) \right] \)
   - **Heterozygote Loci**: \( P(A_iA_j|A_iA_j) = 2\left[ \Theta+(1-\Theta)p_i \right] \left[ \Theta+(1-\Theta)p_j \right] / \left[ (1+\Theta)(1+2\Theta) \right] \)

2. Allele Frequency

   Allele frequencies are updated to the posterior mean frequency using the equation:

   \[ \frac{x_i + \frac{1}{k}}{N_a + 1} \]

   - \( N_a \): Number of alleles sampled for the database
   - \( x_i \): number of observations of allele \( i \) in the database. Can be calculated by \((N_a \times \text{database allele frequency})\) rounded to nearest whole number
   - \( k \): number of allele designations with non-zero observations in the database

B. The lowest, 99% 1-sided lower bound HPD (Highest Posterior Density), total likelihood ratio between the population groups will be reported.

4.6 Likelihood Ratio Considerations

A. All evidence profiles amplified with Investigator 24plex QS with corresponding reference samples will be evaluated with STRmix for statistical calculations, with the exception of known contributors to intimate samples, known contributors to single source indigenous samples, and exclusions made to single source samples.

1. Identifiler Plus and Minifiler profiles may be interpreted using either manual autosomal STR interpretation or STRmix.

B. After obtaining the STRmix results, a visual comparison must be done that includes review of the electropherogram to ensure that results including, but not limited to, mixture proportions, likelihood ratios, and conclusions are intuitively supported.
C. STRmix runs that are generated unnecessarily (e.g. administrative mistakes, wrong profile importation or conditioned needlessly) are not reported. They are not retained in the case record unless the mistake is detected during technical case review.

D. STRmix runs reflecting diagnostic issues that indicate the deconvolution is a poor description of the data (e.g. stutter variance, allele variance or Gelman-Rubin convergence are out of range or HPD LR versus point source LR are inconsistent) are not reported, but must be maintained in the case record. A reason for the rejection of the data must be given along with the initials of the person making the rejection.

E. LRs that are generated to support an inclusion for a co-contributor hypothesis or conditioning of an individual to a mixed DNA profile do not need to be reported, but must be maintained in the case record.

F. When an LR is generated and subsequently a determination is made that the comparison/conclusion is not intuitively supported, this LR is not reported but must be maintained in the case record along with documentation as to why it was not used.

G. If during the technical review there is a disagreement regarding the interpretation of the DNA profiles, the analyst and technical reviewer will follow the appropriate Case Review Resolution procedure outlined in the Crime Laboratory Service Manual. If an agreement is reached the reasons for the change must be documented in the case record.

1. The analyst has the option of reporting all LR calculations (generated prior to or during technical review) or adding a statement to the report to notify the customer of the additional LRs.

2. If the latter option is chosen the relevant portions of the case record must be released following the report within a reasonable timeframe.

4.7 Possible Conclusions for Single Source Evidentiary and Mixture Evidentiary Profiles

A. Exclusion/Excluded – The alleles and/or genotypes obtained for the reference sample are not included in the alleles and/or genotypes obtained for the evidentiary sample. The reference sample is excluded as a possible contributor of the DNA profile obtained from the evidentiary sample.

1. After review of the profile, if the resultant LR is less than $1 \times 10^{-2}$, the conclusion will be reported as an exclusion for all amplification kits.

2. After review of the profile, if the resultant LR is 0.01 to 0.5, the conclusion will be reported as an exclusion for all amplification kits and a cautionary statement regarding the possibility of support for exclusion of a true donor from the profile added.

3. If the LR is exclusionary and an evaluation of the DNA profile determines this conclusion is intuitively supported, the conclusion may not be reported as uninformative or inclusionary.

4. If the LR is exclusionary and an evaluation of the DNA profile determines this conclusion is NOT intuitively supported, the analyst should consult with the Technical Leader to troubleshoot any issues and determine possible solutions.

a) After completion of trouble shooting, if intuitive support is still not indicated, the profile will be reported as uninterpretable.

5. STRmix analysis is not required to support the conclusion of exclusion for single source profiles.
B. **Inclusion/Included/Not Excluded** – The alleles and/or genotypes obtained for the reference sample are included in the alleles and/or genotypes obtained for the evidentiary sample. The reference sample cannot be excluded as a possible contributor of the DNA profile obtained from the evidentiary sample.

1. After review of the profile, if the resultant LR is greater than or equal to 2, the LR gives a degree of support for the conclusion of inclusion.

2. For LR values between 2 and 1000 for Identifiler Plus and 24plex samples and 2 and 10,000 for Minifiler samples, a cautionary statement regarding the possibility of support for inclusion of a non-donor to the profile is added.

3. STRmix analysis is not required to support the conclusion of inclusion for the assumed donor of single source intimate or indigenous samples.

4. If the LR is inclusionary and an evaluation of the DNA profile determines this conclusion is NOT intuitively supported, the analyst should consult with the Technical Leader to trouble shoot any issues and determine possible solutions.
   
   a) After completion of trouble shooting, if intuitive support is still not indicated, the profile may be reported as an exclusion, uninformative, or uninterpretable as applicable with Technical Leader approval.

   b) The profile evaluation and Technical Leader approval must be documented.

C. **Uninformative** – No determination can be made concerning whether or not the alleles obtained for the reference sample are excluded or included for the evidentiary sample.

1. After review of the profile, if the resultant LR is greater than 0.5 but less than 2, the conclusion will be reported as uninformative for all amplification kits.

2. If the LR is uninformative and an evaluation of the DNA profile determines this conclusive is NOT intuitively supported, the analyst should consult with the Technical Leader to trouble shoot any issues and determine possible solutions.

   a) After completion of trouble shooting, if intuitive support is still not indicated, the profile may be reported as an exclusion or uninterpretable as applicable with Technical Leader approval.

   b) The profile evaluation and Technical Leader approval must be documented.

D. **Uninterpretable** – the DNA data cannot be interpreted and may not be able to be fully characterized; therefore, the data is not suitable for comparisons. This could be due to poor or limited data quality, data that is too complex, or data that fails to meet laboratory quality requirements.

E. **No results** – no alleles above analytical threshold at any locus.

4.8 **Profiles for CODIS entry**

A. When attempting to deconvolve a mixture into its component genotypes for CODIS data entry, STRmix may be used to aid in the deconvolution.

B. If a profile has been determined to be unsuitable for comparison there will be no attempt to develop a profile for CODIS.
5 Records
   A. The Advanced report of each deconvolution and comparison will be uploaded into the LIMS system as part of the case record.
   B. Digital results and files created by the STRmix software will be electronically archived.

6 Literature References and Supporting Documentation

   Valid-Software-SYS-DNA-IdentifilerPlus-STRmix-v2.3.07-2016-0314
   Valid-Software-SYS-DNA-Minifiler-STRmix-v2.3.07-2017-0217
   Valid-Software-SYS-DNA-24plex-STRmix-v2.4.06-2017-0713
   Valid-Software-SYS-DNA-IdentifilerPlus-STRmix-v2.4.06-2017-0523
   Valid-Software-SYS-DNA-Minifiler-STRmix-v2.4.06-2017-0713
   Valid-Software-SYS-DNA-24plex-RevisedNIST1036-STRmix-v2.4.06-2017-0815
   Valid-Software-SYS-DNA-IdentifilerPlus-RevisedNIST1036-STRmix-v2.4.06-2017-0815

   STRmix User's Manual v2.4.
   STRmix Operation Manual v2.4


   Estimating the number of contributors to a mixture powerpoint, Texas Forensic Science Commission sponsored training Fort Worth, Texas. 18-20 November 2015.
DNA-09-13 LEGACY AMPLIFICATION KIT DATA REINTERPRETATION

1 Scope
Changes in mixture interpretation guidelines and recent revision of population frequency databases have prompted requests for reinterpretation of DNA data. This policy provides guidance for completion of reinterpretation requests for data developed using legacy amplification kits.

2 Related Chapters
Analytical Controls
Technical Review
Autosomal STR Data Evaluation and Profile Characterization Guidelines
Manual Autosomal STR Interpretation Guidelines
Interpretation Flowcharts
Manual Autosomal STR Statistical Significance Estimation
Texas/FBI STR Population Database
NIST STR Population Database
Report Writing Guidelines
STRmix Autosomal STR Interpretation Guidelines
LIMS Manual: DNA Workflow
LIMS Manual: Amended DNA Statistical Reports

3 Evaluation of Controls for Legacy Amplification Kit Data
A. An attempt must be made to locate all analytical controls including the reagent blank, positive control, and negative control. If an analytical control is unable to be located or reanalyzed, a notation must be made in the case record regarding why the control was not reanalyzed (e.g., not located, data file corrupted).

B. If accessible, analytical controls must be reanalyzed in the analysis software using the same analysis parameters as the samples (e.g., 50 rfu analytical threshold).

1. If the reagent blank and/or negative control is unable to be reanalyzed, any associated samples extracted prior to July 1, 2009 may still be reinterpreted using a validated lower analytical threshold. A statement will be included in the report informing the requestor that the reagent blank and/or negative control was unable to be reanalyzed.

2. If the reagent blank is unable to be reanalyzed, any associated samples extracted after July 1, 2009 will not be reinterpreted and will be reported as uninterpretable due to the lack of accessible analytical controls.

3. If the negative control is unable to be reanalyzed, any associated samples extracted after July 1, 2009 may still be reinterpreted provided an associated reagent blank has been reanalyzed with the evidentiary samples.
4. The reagent blank or negative control must not exhibit contamination.
   a) If contamination is exhibited, the associated samples will not be reinterpreted further and will be reported as uninterpretable.
   b) Contamination can be recognized as observation of one or more called alleles at or above analytical threshold in a blank or negative control; the observation of three or more reproducible, recognizable peaks below analytical threshold in a blank or negative control; the presence of an analyst’s DNA type in a sample, and/or evidence of carry-over between samples.

C. Reagent blanks were not historically required to be amplified using all typing systems available. This situation may exist in samples extracted prior to July 1, 2009. As an example, reagent blanks were routinely amplified using Profiler Plus but were not amplified using Cofiler.

D. For all samples, regardless of when they were extracted, if the positive control is unable to be reanalyzed, the associated samples may still be reinterpreted provided another positive control is reanalyzed with the samples in order to show the genotyping software is functioning correctly.

E. If associated controls from legacy amplification kit data contain artifacts including spikes, pull up, stutter, and minus A that are labeled by the DNA analysis software, the data from the run may be used as long as the artifacts have been labeled by the analyst with their identity. Technical Leader approval is not required; however, analysts are encouraged to consult with the Technical Leader if the identity of an artifact is in question.

F. Positive controls for data typed with legacy amplification kits must exhibit the following appropriate DNA profile. If the appropriate DNA profile is not exhibited (such as a partial profile is obtained, a mixture profile is obtained, or incorrect genotype is obtained), notify the Technical Leader for resolution.

**Profiler Plus**

<table>
<thead>
<tr>
<th>D3S1358</th>
<th>vWA</th>
<th>FGA</th>
<th>Amel</th>
<th>D8S1179</th>
<th>D21S11</th>
<th>D18S51</th>
<th>D5S818</th>
<th>D13S317</th>
<th>D7S820</th>
</tr>
</thead>
<tbody>
<tr>
<td>14,15</td>
<td>17,18</td>
<td>23,24</td>
<td>X,X</td>
<td>13,13</td>
<td>30,30</td>
<td>15,19</td>
<td>11,11</td>
<td>11,11</td>
<td>10,11</td>
</tr>
</tbody>
</table>

**Cofiler**

<table>
<thead>
<tr>
<th>D3S1358</th>
<th>D16S539</th>
<th>Amel</th>
<th>TH01</th>
<th>TPOX</th>
<th>CSF1PO</th>
<th>D7S820</th>
</tr>
</thead>
<tbody>
<tr>
<td>14,15</td>
<td>11,12</td>
<td>X,X</td>
<td>8,9.3</td>
<td>8,8</td>
<td>10,12</td>
<td>10,11</td>
</tr>
</tbody>
</table>

**Identifiler/Identifiler Plus**

<table>
<thead>
<tr>
<th>D8S1179</th>
<th>D21S11</th>
<th>D7S820</th>
<th>CSF1PO</th>
<th>D3S1358</th>
<th>TH01</th>
<th>D13S317</th>
<th>D16S539</th>
<th>D2S1338</th>
</tr>
</thead>
<tbody>
<tr>
<td>13,13</td>
<td>30,30</td>
<td>10,11</td>
<td>10,12</td>
<td>14,15</td>
<td>8,9.3</td>
<td>11,11</td>
<td>11,12</td>
<td>19,23</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D19S433</th>
<th>vWA</th>
<th>TPOX</th>
<th>D18S51</th>
<th>Amel</th>
<th>D5S818</th>
<th>FGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>14,15</td>
<td>17,18</td>
<td>8,8</td>
<td>15,19</td>
<td>X,X</td>
<td>11,11</td>
<td>23,24</td>
</tr>
</tbody>
</table>
4 Preliminary Evaluation of Data

A. Determine the correct analytical threshold for use.
   1. The analytical threshold for the Profiler Plus, Cofiler, and Identifiler kits is 50 rfu.
   2. The analytical threshold for the Identifiler Plus kit run on 3130 series instruments is 50 rfu.
   3. The analytical threshold for the Identifiler Plus kit run on 3500 series instruments is the validated threshold established by local validation.

B. Determine the correct size standard to use.
   1. Data from the Profiler/Cofiler kit requires ROX 400 size standard to be selected.
   2. Data from the Identifiler or Identifiler Plus kit requires LIZ 500 size standard to be selected.

C. If re-analyzing legacy kit data previously run on a 310 genetic analyzer, an appropriate matrix file must be applied to the data during data analysis in the DNA analysis software or the software will be unable to analyze the sample.

D. Use the DNA analysis software to reanalyze the data and apply the new analytical threshold.

E. Evidentiary samples, reference sample, alternate reference samples, and corresponding reagent blanks will be reanalyzed using the new analytical threshold.

F. Process Quality Values (PQVs) will not be used to replace individual data analysis completed by the analyst. Any flags or color designations provided in the software will not be used during analysis of sample data.

G. The maximum analytical RFU for evaluating STR profiles is the saturation point of the CCD camera of the instrument in the raw data, except at the Amelogenin locus. If the data exceeds the maximum analytical RFU at any locus except for Amelogenin, the off scale locus must be called inconclusive.

H. If the maximum RFU is exceeded for the size standard, the analyst may use the data as long as any associated pull-up does not interfere with data interpretation.

I. Evaluate the allelic ladder to determine that it contains the appropriate number of properly labeled alleles present for each locus when analyzed. At a minimum, one acceptable allelic ladder must be present within each run for each respective kit.

5 Data Analysis

A. Determine the correct stochastic threshold for use.
   1. The stochastic threshold for the Profiler Plus kit is 165 rfu for 5 second injections and 200 rfu for 10 second injections.
   2. The stochastic threshold for the Cofiler kit is 140 rfu for 5 second injections and 195 for 10 second injections.
   3. The stochastic threshold for the Identifiler and Identifiler Plus kit is the validated threshold established by local validation.
B. Refer to the *Autosomal STR Data Evaluation and Profile Characterization Guidelines* chapter for allele identification, artifacts, off-ladder alleles, tri-allelic loci, and composite data from a single amplification kit.

C. Legacy amplification kit data exhibiting artifacts may be used without first taking steps to minimize or eliminate artifacts. Caution should be used in reinterpreting these kinds of samples.
   1. The reanalysis process has the potential to cause numerous artifacts to appear in the data that were not present originally.
   2. If the presence of these artifacts has caused the data to become too complex for meaningful interpretation, the analyst may choose to not reinterpret the data if Technical Leader approval is obtained.

D. Data results from the same sample extract developed using kits that share loci may be combined.
   1. For loci that are shared between the kits, the locus with the most information from either kit will be used to create the combined profile. Combined profiles must use the kit specific stochastic threshold for the respective loci.
   2. When results are discordant between the kits, the Technical Leader must be consulted.

6 Analysis of Reinterpretation Results

6.1 Profiler Plus, Cofiler, Identifiler, and Identifiler Plus with Manual Autosomal STR interpretation

A. Refer to the *Autosomal STR Data Evaluation and Profile Characterization Guidelines* chapter for information on characterization of profiles.

B. The Profiler Plus, Cofiler, and Identifiler legacy data amplification kits must be reinterpreted using manual autosomal STR interpretation. Refer to the *Manual Autosomal STR Interpretation Guidelines* chapter for information on single source evidentiary profile interpretation and comparison, mixture evidentiary profile interpretation and comparison for these samples.

C. Please note that balanced peak heights for heterozygous alleles are typically ≥ 70% for Identifiler/Identifiler Plus and ≥ 75% for Profiler Plus/Cofiler.

D. Peak height ratios that can be utilized to eliminate possible genotype combinations in mixtures samples are usually ≥ 50% in Identifiler/Identifiler Plus and ≥ 40% for Profiler Plus/Cofiler. Caution should be taken when doing this in the stochastic region, as peak height ratios are known to fluctuate more at that level.

6.2 Identifiler Plus with STRmix

A. The only legacy data amplification kit for which probabilistic genotyping was validated is Identifiler Plus. Identifiler Plus data may be reinterpreted using either manual autosomal STR interpretation or STRmix. For Identifiler Plus, refer to the *STRmix Autosomal STR Interpretation Guidelines* chapter if probabilistic genotyping is to be used for reinterpretation.

B. For 3130 series genetic analyzers, all Identifiler Plus data shall be analyzed at an analytical threshold of 50 rfu.
C. All artifacts with the exception of n-4 stutter on evidentiary profiles must be removed from the electropherograms prior to exporting for entry into STRmix.

7 Profiles for CODIS Entry in Reinterpretation Cases
A. The analyst may use the data in the stochastic region to develop profiles for CODIS entry if at least one locus used for CODIS entry is above stochastic threshold and suitable for statistics.
B. Any new or additional data obtained for CODIS eligible profiles through reamplification or reanalysis (including reinterpretation of legacy amplification kit data) will be uploaded to CODIS.
C. If reinterpretation of data was performed, CODIS profiles must be reviewed and updated based on the reinterpretation results. This may involve removing profiles from the database if the profile was determined to be unsuitable for comparison or if a different deduced profile was selected through reinterpretation.

8 Statistical Significance Estimation for Legacy Amplification Data Cases
A. For samples reinterpreted using manual autosomal STR interpretation, refer to the Statistical Significance Estimation chapter for information regarding statistical significance estimation using RMP and CPI.
   1. The Texas/FBI STR population database referenced in the DNA SOP will be used for calculating match significance when a recalculation is performed at the request of a customer due to the error in the FBI database files.
   2. Any statistical calculations as a result of a reinterpretation request unrelated to the error in the FBI database files will use the NIST STR population database referenced in the DNA SOP.
B. Samples reinterpreted using probabilistic genotyping with STRmix will report statistical significance estimation using a likelihood ratio.
C. A significance estimate for each inclusion must be in the report, however intimate and indigenous samples do not require a reported statistic for the donor of the sample.

9 Reporting Reinterpretation Cases
9.1 Reporting Requirements
A. Follow instructions in section 4.4 of the DNA Workflow chapter of the LIMS manual to add information regarding requests for reinterpretation of DNA results; a statement explaining the reason for the reinterpretation must be added to the report.
B. If the request is a recalculation of statistics due to the error in the FBI database files, follow the instructions in the Amended DNA Statistical Reports chapter of the LIMS manual.
C. List items analyzed for DNA
D. Description of DNA methodology and technology
   1. DNA extraction (if not previously reported)
   2. Differential extractions (if performed and not previously reported)
   3. Analyzed by STR Analysis
E. List loci analyzed
F. A statement must be present in the report to address all DNA extracts.
   1. If a stain/collection is re-extracted to resolve contamination or a quality incident, the report must indicate that multiple extractions of the same stain/collection were performed due to a quality event. Alternatively, multiple reports may be issued to indicate additional extractions. Comparisons will only be made to profiles where contamination or the quality incident has been resolved.
   2. If a stain/collection is re-extracted in an attempt to gain more information, the report must indicate that multiple extractions of the same stain/collection were performed in an effort to obtain additional information. Alternately, multiple reports may be issued to indicate additional extractions.

G. For cases originally received and reported using DRAGNet legacy LIMS, the analyst may:
   1. Not relate evidence to the request if the parent container in LIMS cannot be easily identified.
   2. If evidence is not related to the request, submission information must be communicated to the customer by typing the date and method of submission for the items reinterpreted in another section of the report or by making reference to the original report if this report contains the submission information.
   3. If evidence is not related to the request, the statement “The following are the Results of Analysis and Interpretation” must be included to designate any DNA interpretation/conclusions as such.

H. The statistical estimate may be expressed as either numbers truncated to the last significant digit as generated by the validated statistical software or words for the Caucasian, African American, and Hispanic population groups for RMP and CPI statistics.

I. For likelihood ratios generated in STRmix, the stratified statistical estimate may be expressed as either numbers or words. This statistic is calculated using the Caucasian, African American, Hispanic, and Asian population groups.

J. Disposition of evidentiary items on which DNA analysis was performed will be included in the report. This includes evidentiary items depleted during analysis.

K. Legacy data from the Identifiler Plus kit can also be reinterpreted with probabilistic genotyping using STRmix. If the data has been reinterpreted with STRmix, use the reporting statements found in the Report Writing Guidelines chapter, section 5: STRmix Reporting Statements.

9.2 Exclusion

“[At the loci determined to be suitable for comparison], [Person A] is excluded as the contributor of the [partial] DNA profile from [Item 1, stain 1].”

OR

“[At the loci determined to be suitable for comparison], [Person A] is excluded as a possible contributor to this DNA profile.”

OR

“The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture of at least _ contributors. [At the loci determined to be suitable for comparison], [Person A] is excluded as a possible contributor to this DNA profile.”

OR

“The [partial] DNA profile obtained from [Item 1, stain 1] is consistent with a mixture. Assuming two donors to the mixture, [Person A] is excluded as a possible contributor to this DNA profile.”

Printed copy is uncontrolled. Refer to electronic copy for current version.
OR

“[At the loci determined to be suitable for comparison], [Person A] is excluded as the contributor of the [partial] [major/minor] DNA profile from [Item 1, stain 1].”

OR

“The [partial] DNA profile obtained from [Item 1, stain 1] is consistent with a mixture. Assuming two donors to the mixture and that [Person B] is a donor, [Person A] is excluded as a possible contributor to the foreign DNA profile.”

OR

“The [partial] DNA profile obtained from [Item 1, stain 1] is consistent with a mixture of at least _ individuals. A mixed major could be resolved. Assuming two donors to the mixed major, [Person A] is excluded as a possible contributor to the [mixed major] DNA profile.”

9.3 Inclusion - Single Source

Statements of Inclusion

“The [partial] DNA profile from [Item 1, stain 1] is consistent with the DNA profile of [Person A]. [The following loci were used for statistical significance estimation: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 (if using Profiler Plus) D3S1358, D16S539, TH01, TPOX, CSF1PO, D7S820 (if using Cofiler) OR D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA (if using Identifiler or Identifiler Plus). At these loci.] The probability of selecting an unrelated person at random who could be the contributor of this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics.”

Report the following: “The approximate world population is 7.0 billion.”

9.4 Inclusion – Major/Minor Component

Reporting Statement

“The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture of at least _ contributors.”

OR

“The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture from [Person A and Person B] [or Person C and some unknown individual...].”

AND

“A [complete/partial] DNA profile for the major contributor could be resolved. [Person A] cannot be excluded as the contributor of the [major/minor] component in this DNA profile. [The following loci were used for statistical significance estimation: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 (if using Profiler Plus) D3S1358, D16S539, TH01, TPOX, CSF1PO, D7S820 (if using Cofiler) OR D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA (if using Identifiler or Identifiler Plus). At these loci.] The probability of selecting an unrelated person at random who could be the contributor of the [major/minor] component in this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics.”
“Assuming two donors to the mixture, a [complete/partial] DNA profile for the [major/minor] contributor could be resolved. [Person A] cannot be excluded as the contributor of the major/minor component in this DNA profile. [The following loci were used for statistical significance estimation: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 (if using Profiler Plus) D3S1358, D16S539, TH01, TPOX, CSF1PO, D7S820 (if using Cofiler) OR D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA (if using Identifiler or Identifiler Plus). At these loci,] The probability of selecting an unrelated person at random who could be the contributor of the [major/minor] component in this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics.”

Report the following: “The approximate world population is 7.0 billion.”

9.5 Inclusion - Mixture

Reporting Statement

“The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture of at least _ contributors.”

OR

“The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture from [Person A and Person B] [or Person C and some unknown individual…].”

AND

[Person A cannot be excluded as a contributor to the profile. The following loci were used for statistical significance estimation: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 (if using Profiler Plus) D3S1358, D16S539, TH01, TPOX, CSF1PO, D7S820 (if using Cofiler) OR D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA (if using Identifiler or Identifiler Plus). At these loci,] The probability of selecting an unrelated person at random who could be a contributor to this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics. The approximate world population is 7.0 billion.”

9.6 Inclusion - Indistinguishable Mixture

“The [partial] DNA profile obtained from [Item 1, stain 1] is consistent with a mixture. Assuming two donors to the mixture, [Person A] cannot be excluded as a possible contributor to this DNA profile. [The following loci were used for statistical significance estimation: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 (if using Profiler Plus) D3S1358, D16S539, TH01, TPOX, CSF1PO, D7S820 (if using Cofiler) OR D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA (if using Identifiler or Identifiler Plus). At these loci,] The probability of selecting an unrelated person at random who could be a contributor to this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics.”
9.7 Inclusion - Intimate/Indigenous/Differential Samples

“The [partial] DNA profile obtained from [Item 1, stain 1] is consistent with a mixture of at least _ individuals. Assuming [Person A] is a donor to the mixture, a [partial] DNA profile for the foreign contributor[s] could be resolved. [Person B] cannot be excluded as the foreign contributor to this DNA profile. [The following loci were used for statistical significance estimation: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 (if using Profiler Plus) D3S1358, D16S539, TH01, TPOX, CSF1PO, D7S820 (if using Cofiler) OR D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA (if using Identifiler or Identifiler Plus). At these loci,] The probability of selecting an unrelated person at random who could be the contributor of this [foreign] DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics.”

Report the following: “The approximate world population is 7.0 billion.”

OR

“The DNA profile obtained from [Item 1, stain 1] is consistent with a mixture. Assuming two donors to the mixture and that [Person A] is a donor, a [partial] DNA profile for the foreign contributor could be resolved. [Person B] cannot be excluded as the foreign contributor to this profile. [The following loci were used for statistical significance estimation: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 (if using Profiler Plus) D3S1358, D16S539, TH01, TPOX, CSF1PO, D7S820 (if using Cofiler) OR D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA (if using Identifiler or Identifiler Plus). At these loci,] The probability of selecting an unrelated person at random who could be the contributor of this [foreign] DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics.”

Report the following: “The approximate world population is 7.0 billion.”

9.8 Mixed Major

“The DNA profile obtained from [Item 1, stain 1] is consistent with a mixture of at least _ individuals. A mixed major could be resolved. Assuming two donors to the mixed major, [Person A] cannot be excluded as a possible contributor to this DNA profile. [The following loci were used for statistical significance estimation: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 (if using Profiler Plus) D3S1358, D16S539, TH01, TPOX, CSF1PO, D7S820 (if using Cofiler) OR D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA (if using Identifiler or Identifiler Plus). At these loci,] The probability of selecting an unrelated person at random who could be a contributor to the mixed major in this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics.”

AND

Report the following: “The approximate world population is 7.0 billion.”

OR
"The DNA profile obtained from [Item 1, stain 1] is consistent with a mixture of at least _ individuals. A mixed major could be resolved. Assuming two donors to the mixed major, and that [Person A] is a donor, [Person B] cannot be excluded as a possible contributor to this DNA profile. [The following loci were used for statistical significance estimation: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 (if using Profiler Plus) D3S1358, D16S539, TH01, TPOX, CSF1PO, D7S820 (if using Cofiler) OR D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA (if using Identifiler or Identifiler Plus). At these loci,] The probability of selecting an unrelated person at random who could be a contributor to the mixed major in this DNA profile is approximately 1 in _ for Caucasians, 1 in _ for African Americans, and 1 in _ for Hispanics.”

AND

Report the following: “The approximate world population is 7.0 billion.”

9.9 Alternate Reference Samples

A. When no standard reference samples are available, alternate reference samples yielding a single source profile may be used. If a partial profile is obtained, Technical Leader approval is required in order to use the alternate reference sample for comparison purposes. When compared to an evidentiary profile, the appropriate prescribed reporting statement will be modified to read:

“The [partial] DNA profile from [Item 1, stain 1] is consistent with the DNA profile of [alternate reference]. Assuming [Person A] is the source of the [alternate reference], [Person A] cannot be excluded.”

OR

“[At the loci determined to be suitable for comparison] and assuming [Person A] is the source of the [alternate reference], [Person A] is excluded as a contributor to the [partial] DNA profile from [Item 1, stain 1].”

B. If the alternate reference sample is determined to be unsuitable for comparisons, the following statements may be used:

“The [partial] DNA profile from [Item 1, stain 1] is consistent with a mixture and therefore is unsuitable for use as an alternate known.

OR

“Insufficient data was obtained from [Item 1, stain 1] therefore this item is unsuitable for use as an alternate known.”

9.10 DNA Profiles and CODIS

A. Profile Suitable for CODIS

“A [partial] DNA profile obtained from [Item] [foreign to Person A] has been entered into the Combined DNA Index System (CODIS). All profiles are entered into CODIS in accordance with state and national regulations, where regular searches will be performed. Notification will be issued if there is a hit in the database or if the profiles are removed from CODIS at any time in the future.”

OR
“The [partial] DNA profile obtained from [Item] [foreign to Person A] has been entered into the Combined DNA Index System (CODIS) and will be searched against the local, state, and/or national databases.”

B. Profile Not Suitable for CODIS

“The [partial] DNA profile obtained from [Item] was determined to be unsuitable for entry into the Combined DNA Index System (CODIS).”

9.11 Uninterpretable Results

“No DNA profiles were obtained from [Item 1, stain 1].”

“No interpretable DNA profiles were obtained from [Item 1, stain 1].”

“Insufficient data is present for comparison.”

“Due to the quantity and/or quality of DNA obtained, no comparisons can be made to the DNA profile obtained from [Item 1, stain 1].”

“Due to the quantity and/or quality of DNA obtained, no comparisons can be made to the DNA profile obtained from the additional contributor(s) to [Item 1, stain 1].”

9.12 Complexity of Results

“Due to the potential number of contributors, no comparisons will be made to the DNA profile obtained from [Item 1, stain 1].”

“Due to the complexity of this profile, no conclusions can be drawn as to whether [Person A] could be a contributor to the DNA profile from [Item 1, stain 1].”

“Due to the potential relatedness of the individuals, no conclusions can be drawn as to whether [Person A] could be a contributor to the DNA profile from [Item 1, stain 1].”

9.13 No comparison lack of known

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as originating from a single [male/female] individual. If DNA comparisons are required, please submit a known blood or saliva sample from [any potential contributors].”

OR

“The [partial] DNA profile from [this] item [01-01-AA] is interpreted as a mixture of [X] individuals. If DNA comparisons are required, please submit a known blood or saliva sample from [any potential contributors].”

9.14 Re-extraction of samples due to contamination (resolved or unresolved)

“No results can be reported for this item due to a quality event. [This item was/will be re-extracted.]”

OR

“Due to a quality event, this item was extracted twice.”

9.15 Re-extraction of samples to obtain more information

“In an effort to obtain additional information, this item was extracted twice.”

OR

“[Regular reporting statement indicating results of extraction]. This item [was/will be] re-extracted in an effort to obtain additional information.”
10 Reinterpretation Requests

A. Requests for reinterpretation are generally made by the customer and should be in writing.
B. If a subpoena is received for a case worked with legacy amplification kits, the case must be evaluated to determine if it would benefit from reinterpretation. If so, the prosecuting attorney must be advised regarding the recommendation and potential for reinterpretation.
C. Additionally, if known samples are received for cases that were originally worked with legacy amplification kits, the evidentiary data must be reinterpreted using the most current methods prior to comparison to the known sample and a new report issued with the results.

11 Records

A. All reference and evidentiary profiles may be recorded as phenotypes.
B. All conclusions, characterizations, and classifications (intimate, indigenous, environmental) will be documented in the case record.

12 Literature References and Supporting Documentation

Applied Biosystems. AmpF/STR COfiler PCR amplification kit user bulletin (current version).
Applied Biosystems. AmpF/STR Identifier amplification kit users guide (current version).
SWGDAM. SWGDAM Clarification on the Reinterpretation of Data Typed with Legacy Amplification Test kits. www.swgdam.org (current version).
10  REAGENT PREPARATION

DNA-10-01  AP TEST REAGENT

1  Scope
Acid phosphatase (AP) test reagent undergoes a color change in the presence of acid
phosphatase, which is found in highest concentration in semen. Instructions for use and
interpretation are in the Presumptive Semen Test – Acid Phosphatase chapter. AP test reagent
may be purchased. Two options for preparation of the reagent appear below and may be selected
based on the availability of Aerosol 22. Larger amounts of reagent may be prepared as needed by
proportionally increasing the components.

2  Related Documents
CLS Manual: Laboratory Equipment

3  Specification
Acid phosphatase (AP)

4  Safety
A. The reagents, α-naphthyl acid phosphate, o-dianisidine, sodium acetate and Aerosol 22,
are irritants. Avoid contact and inhalation.
B. The reagent, o-dianisidine, is a suspected carcinogen. Avoid contact and inhalation; use
powder only in a chemical fume hood when possible.
C. Glacial acetic acid causes burns and is extremely destructive to tissues of the upper
respiratory tract, eyes, and skin. Use only in a chemical fume hood.
D. Appropriate personal protective equipment must be used during preparation and use.
Clothing may protect unbroken skin; broken skin must be covered.

5  Equipment and Materials
• Mortar and pestle
• Stir plate
• Darkened container for storage
• Funnel and filter paper
• dIH₂O
• α-naphthyl acid phosphate calcium or disodium salt
• Aerosol 22 (detergent)
• o-dianisidine, tetrazotized (Fast Blue B salt)
• Acetic acid (glacial)
• Sodium acetate, trihydrate
• Sodium chloride
• SERI AP Spot Test PMR
6 Procedure

6.1 Solution containing Aerosol 22:

1. Add 1 L dIH₂O into a beaker. Pour ~10 mL of this water into a small beaker.
2. Place 2 g α-naphthyl acid phosphate in the mortar. Thoroughly grind the powder with the 1 mL Aerosol 22 and the water from the small beaker. Set aside.
3. On a stir plate, add one at a time to the large beaker of water 4 g o-dianisidine, 5 mL acetic acid, 20 g sodium acetate, and 210 g sodium chloride.
4. When completely dissolved, add the α-naphthyl acid phosphate solution from step 2.
5. Continue stirring for about 30 minutes. Let stand at room temperature for about 1 hour.
6. Filter through filter paper and store in a darkened container at 2-8°C.

6.2 Solution without Aerosol 22:

A. Prepare Solution A as follows:
1. Place 0.2 g o-dianisidine and 4 g sodium acetate into an appropriate container.
2. Add 20 ml diH₂O and 2 ml acetic acid to the container.
3. Stir the solution to mix thoroughly until all of the o-dianisidine and sodium acetate dissolve.

B. Prepare Solution B as follows:
1. Place 0.16 g α-naphthyl acid phosphate into an appropriate container.
2. Add 2 ml diH₂O to the container.
3. Stir the solution to mix thoroughly until all of the α-naphthyl acid phosphate has dissolved.

C. Prepare the Final Solution as follows:
1. Place 20 ml of Solution A and 2 ml of Solution B into an appropriate container.
2. Add 178 ml diH₂O to the container for a final volume of 200 ml.
3. Stir the solution to mix thoroughly.
4. Aliquot into suitable containers for long term storage in the freezer.

6.3 SERI AP Spot Test:

1. Place 0.26 g SERI AP Spot Test PMR into an appropriate container.
2. Add 10 ml di H₂O to the container.
3. Mix thoroughly.

7 Testing, Storage, Expiration, and Disposal

A. Solution containing Aerosol 22: The solution will initially be dark purple but will change to the characteristic clear amber after standing and filtration. Store in a darkened container. AP test reagent can be stored at 2-8°C for one year. The precipitate that forms after several days does not affect sensitivity.
B. Solution without Aerosol 22: Store aliquots frozen for one year. Any unused portion of the aliquot will be disposed of at the end of the day on which it was thawed.

C. SERI AP Spot Test: May be made fresh daily and any unused portion disposed of at the end of the day on which it was made. Alternately, the solution may be prepared and stored refrigerated for up to 4 weeks.

D. For all solutions:
   1. Minimum labeling includes specification above, initials, and date prepared.
   2. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal. Discard any used filter paper in trash.

8 Records

A. Lot numbers of chemicals used to prepare the reagent, date of preparation expiration date, and preparer’s initials are recorded in the Acid Phosphatase Reagent Preparation Form (LAB-DNA-28) and placed in appropriate reagent logs.

B. If SERI AP Spot reagent is prepared fresh daily, the lot numbers of chemicals used for preparation may be recorded in the case record.

9 Literature References and Supporting Documentation


DNA-10-02  CHELEX 5% W/V

1 Scope
Chelex is a chelating resin that has a high affinity for polyvalent metal ions. The Chelex resin is composed of styrene divinylbenzene copolymers containing paired iminodiacetate ions, which act as chelating groups. The Chelex solutions are used in DNA extraction procedures.

2 Related Documents
CLS Manual: Laboratory Equipment

3 Specification
5% Chelex

4 Safety
A. Explosive when mixed with oxidizing substances; do not mix with oxidizing substances.
B. Avoid contact with skin and eyes.
C. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

5 Equipment and Materials
- Stir plate and bar
- Sterile beaker or bottle
- Chelex 100 Resin (100-200 mesh, sodium form, biotech grade)
- Sterile dIH₂O

6 Procedure
1. Weigh out 5 g Chelex 100 Resin into a sterile bottle or beaker with a stirring bar.
2. Add 100 mL sterile dIH₂O.
3. Check pH as necessary - pH should be alkaline.

7 Testing, Storage, Expiration, and Disposal
A. Quality Control Procedure
   1. Perform extraction, amplify and type according to the relevant DNA SOP and evaluate results.
   2. Reagents will be approved for use if a full DNA profile consistent with previously typed DNA profile is obtained. This approval is indicated by the analyst.
B. Store tightly closed and refrigerated.
C. Minimum labeling includes specification above, initials, and date prepared.
D. Chelex 5% can be stored at 2-8°C one year. May be made up in smaller quantities on the day of use.
E. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.
8 Records

Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, preparer's initials and QC paperwork are recorded on Chelex Reagent Preparation Form (LAB-DNA-41) and maintained in the appropriate reagent logs.
DNA-10-03 DIGEST BUFFER (SPERM WASH SOLUTION) pH 7.5

1 Scope
Digest buffer (10mM Tris-HCl, 10mM EDTA, 50mM NaCl, 2% Sodium Dodecyl Sulfate) is used in organic DNA extraction procedures in combination with other reagents. The chemicals in digest buffer are identical to the sperm wash solution used in washing sperm cells during differential extraction procedures.

2 Related Chapters/Documents
Sodium Chloride 5M
CLS Manual: Laboratory Equipment

3 Specification
Digest buffer or Sperm wash

4 Safety
A. Tris-HCl and EDTA solutions are irritants.
B. 20% Sodium Dodecyl Sulfate is toxic and an irritant.
C. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin during use; broken skin must be covered.

5 Equipment and Materials
- Sterile glassware
- 1 M Tris-HCl, pH 7.5, ultra pure grade 1 mL
- 0.5 M EDTA, pH 8.0, ultra pure grade 2 mL
- 5 M NaCl, ultra pure grade 1 mL
- 20% SDS (sodium dodecyl sulfate), ultra pure grade 10 mL
- Sterile dH2O 86 mL

6 Procedure
1. Mix together the following: 1 mL Tris-HCl, 2 mL EDTA, 1 mL NaCl, 10 mL 20% Sodium Dodecyl Sulfate, and 86 mL sterile dH2O.
2. As necessary, aliquot into sterile tubes for long term storage and to prevent possible contamination.

7 Testing, Storage, Expiration, and Disposal
A. Quality Control Procedure
1. Perform extraction, amplify and type according to the relevant DNA SOP and evaluate results.
2. Reagents will be approved for use if a full DNA profile consistent with previously typed DNA profile is obtained. Mixtures are acceptable if due to separation of differential extractions. Approval is indicated by analyst.
B. Store tightly closed at room temperature. Minimum labeling includes specification above, initials, and date prepared.
C. Digest buffer or sperm wash can be stored for one year.
D. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.

8 Records

Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, preparer’s initials, and QC paperwork are recorded on Digest Buffer Reagent Preparation Form (LAB-DNA-35) and maintained in the appropriate reagent logs.
DNA-10-04  DTT – 0.39M / 1M

1  Scope

DTT (dithiothreitol) is a reducing agent used in DNA extraction buffers to allow lysis of cells with thiol-rich membrane proteins, such as spermatozoa. Instructions for use and interpretation are in the test procedures for extraction.

2  Related Documents

CLS Manual: Laboratory Equipment

3  Specification

DTT, 0.39M

DTT, 1M

4  Safety

A. Dithiothreitol is an irritant. It is incompatible with bases, oxidizing and other reducing agents, and alkali metals and may decompose on exposure to moisture.

B. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin during use; broken skin must be covered.

5  Equipment and Materials

- Microcentrifuge tubes
- Sterile glassware
- Dithiothreitol (DTT), molecular biology grade
- Sterile dIH₂O

6  Procedure

6.1 0.39M DTT

1. Dissolve 601.2 mg DTT in 10 mL sterile dIH₂O.
2. As necessary, aliquot into sterile tubes for long term storage and to prevent possible contamination.
3. Do not autoclave.

6.2 1M DTT

1. Dissolve 1.54 g DTT in 10 mL sterile dIH₂O.
2. As necessary, aliquot into sterile microcentrifuge tubes for long term storage and to prevent possible contamination.
3. Do not autoclave.

7  Testing, Storage, Expiration, and Disposal

A. Quality Control Procedure

1. Perform extraction, amplify and type according to the relevant DNA SOP and evaluate results.
2. Reagents will be approved for use if a full DNA profile consistent with previously typed DNA profile. Mixtures are acceptable if due to separation in differential extractions. Approval is indicated by the analyst.

B. Store in tightly closed microcentrifuge tubes and place tubes in a storage container in the freezer. Label storage container with specification, initials, and date prepared. Label tubes to link them to storage container.

C. Store aliquots frozen for up to two years. Thaw tubes as needed.

D. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.

8 Records
Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, preparer’s initials and QC paperwork are recorded on DTT Reagent Preparation Form (LAB-DNA-40) and maintained in the appropriate reagent logs.

9 Literature References and Supporting Documentation
DNA-10-05 LEUCOMALACHITE GREEN (LMG) SOLUTION

1 Scope
Leucomalachite green (LMG) solution is used for presumptive blood identification, because it oxidizes to blue-green malachite green in the presence of heme and hydrogen peroxide.

2 Related Documents
CLS Manual: Laboratory Equipment

3 Specification
Leucomalachite green (LMG)

4 Safety
A. Leucomalachite green is an irritant; avoid contact or inhalation.
B. Glacial acetic acid is corrosive, combustible, and causes severe burns.
C. Avoid contact with or inhalation of powdered zinc.
D. Solid reagents must be handled in a chemical fume hood.
E. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin during use; broken skin must be covered.

5 Equipment and Materials
- Hot plate
- Fume hood
- Leucomalachite green 1 g
- dIH₂O 150 mL
- Glacial acetic acid 100 mL
- Powdered zinc 5 g

6 Procedure
1. Add 1.0 g leucomalachite green in 150 mL dIH₂O.
2. Slowly add 100 mL glacial acetic acid, swirling to mix.
3. Using low heat, warm solution with 5 g powdered zinc until colorless. It may be necessary to add powdered zinc periodically throughout reagent storage to retain a colorless solution.

7 Testing, Storage, Expiration, and Disposal
A. Minimum labeling includes specification above, initials, and date prepared.
B. LMG solution can be stored at 2-8°C for one year.
C. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.
D. Powdered zinc in contact with limited amounts of water liberates hydrogen, an extremely flammable gas. Store dry, and keep residues thoroughly wet until disposal.

1. Either carefully dry zinc residue in a chemical fume hood and dispose of in a container to keep dry or dispose in a container to keep very wet.
2. Do not dispose of damp residue in the trash where generated heat could cause a fire.

8 Records
Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, and preparer’s initials are recorded in the LMG Reagent Preparation Form (LAB-DNA-31) and placed in appropriate reagent logs.

9 Literature References and Supporting Documentation
DNA-10-06 NUCLEAR FAST RED (NFR) SOLUTION

1 Scope
Nuclear fast red solution is used with picroindigocarmine in the staining of microscopic slides for spermatozoa examination. This reagent may be purchased.

2 Related Documents
CLS Manual: Laboratory Equipment

3 Specification
Nuclear Fast Red solution (NFR)

4 Safety
A. Aluminum sulfate and nuclear fast red are irritants and may be harmful by inhalation, ingestion, or skin absorption.
B. Solid reagents should be handled in a chemical fume hood when possible.
C. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

5 Equipment and Materials
- Hot plate
- Stir plate
- Funnel and filter paper
- Aluminum sulfate $\text{Al}_2(\text{SO}_4)_3$ 5 g
- Nuclear fast red 0.1 g
- dH$_2$O 100 mL

6 Procedure
1. Dissolve 5 g aluminum sulfate in 100 mL hot dH$_2$O.
2. Add 0.1 g nuclear fast red and stir to dissolve.
3. Allow to cool. Filter.

7 Testing, Storage, Expiration, and Disposal
A. Quality Control Procedure
1. Prepare a slide using material (such as a mock differential swab) known to contain both sperm cells and epithelial cells.
2. Stain the slide and view the results at a minimum of 400x.
3. Reagents will be approved for use if at least one sperm head and at least one epithelial cell are observed. The cells must stain the appropriate color. This approval is indicated by the analyst.
4. The quality control procedure is not required if the laboratory is using a purchased reagent.
B. Minimum labeling includes specification above, initials, and date prepared.
C. NFR can be stored at 2-8°C or at room temperature for one year.
D. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.

8 Records

Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, and preparer's initials are recorded in the Nuclear Fast Red Reagent Preparation Form (LAB-DNA-29) and placed in appropriate reagent logs.

9 Literature References and Supporting Documentation

DNA-10-07  PHENOLPHTHALIN (PHT) SOLUTION

1  Scope
Phenolphthalein (PHT) solution is used for presumptive blood identification because it oxidizes to pink phenolphthalein in the presence of heme and hydrogen peroxide. This reagent may be purchased.

2  Related Documents
CLS Manual: Laboratory Equipment

3  Specification
Phenolphthalein (PHT)

4  Safety
A. Phenolphthalein is an irritant. Phenolphthalein may be carcinogenic and may cause reproductive disorders.
B. Potassium hydroxide is corrosive.
C. Ethanol is highly flammable.
D. Avoid contact with or inhalation of powdered zinc.
E. Solid reagents must be handled in a chemical fume hood.
F. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin during use; broken skin must be covered.

5  Equipment and Materials
- Fume hood
- Dark bottle for storage
- Phenolphthalein 2 g
- Potassium hydroxide 20 g
- dIH₂O 100 mL
- Powdered zinc 20 g
- Ethanol 4/5 total volume of desired working solution

6  Procedure
1. Dissolve 2 g phenolphthalein and 20 g potassium hydroxide in 100 mL dIH₂O.
2. Reflux with 20 g powdered zinc until solution is colorless (2-3 hours).
3. Decant solution into dark bottle with a small amount of zinc. This is the PHT stock solution.
4. Prepare PHT working solution by mixing one part PHT stock solution with four parts ethanol.

7  Testing, Storage, Expiration, and Disposal
A. Minimum labeling includes specification above, initials, and date prepared. PHT stock and working solutions can be stored at 2-8°C for one year.

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Printed copy is uncontrolled. Refer to electronic copy for current version.
B. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.

C. Powdered zinc in contact with limited amounts of water liberates hydrogen, an extremely flammable gas. Store dry, and keep residues thoroughly wet until disposal.
   1. Either carefully dry zinc residue in a chemical fume hood and dispose of in a container to keep dry or dispose in a container to keep very wet.
   2. Do not dispose of damp residue in the trash where generated heat could cause a fire.

8 Records
Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, and preparer’s initials are recorded in the PHT Reagent Preparation Form (LAB-DNA-30) and placed in appropriate reagent logs.

9 Literature References and Supporting Documentation
DNA-10-08 PICROINDIGOCARMINE (PIC) SOLUTION

1 Scope
Picroindigocarmine solution is used with nuclear fast red in the staining of microscopic slides for spermatozoa examination. This reagent may be purchased.

2 Related Documents
CLS Manual: Laboratory Equipment

3 Specification
Picroindigocarmine (PIC)

4 Safety
A. Picric acid is flammable, toxic, and explosive when dry; keep container tightly closed and ensure sufficient water coverage. Avoid contact with metals; forms very sensitive explosive metallic compounds. Contact of picric acid with concrete floors may form the friction-sensitive calcium salt.
B. Indigocarmine is an irritant and may be harmful by inhalation, ingestion, or skin absorption.
C. Reagents must be handled in a chemical fume hood.
D. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

5 Equipment and Materials
- Stir plate
- Funnel and filter paper
- Ceramic spatula
- Indigocarmine
- Picric acid
- dIH₂O

OR
- Saturated picric acid solution (~1.3%)

6 Procedure
1. Use 300 mL purchased saturated picric acid solution or prepare saturated picric acid solution by letting 4 g picric acid stand overnight in 300 mL dIH₂O. Use a ceramic spatula to measure picric acid!
2. Dissolve 1 g indigocarmine in 300 mL saturated picric acid solution.
3. Filter. Thoroughly rinse picric acid solution from filter paper after use and prior to disposal.

7 Testing, Storage, Expiration, and Disposal
A. Quality Control Procedure
1. Prepare a slide using material (such as a mock differential swab) known to contain both sperm cells and epithelial cells.
2. Stain the slide and view the results at a minimum of 400x.

3. Reagents will be approved for use if at least one sperm head and at least one epithelial cell are observed. The cells must stain the appropriate color. This approval is indicated by the analyst.

4. The quality control procedure is not required if the laboratory is using a purchased reagent.

B. Minimum labeling includes specification above, initials, and date prepared.

C. PIC can be stored at 2-8°C or at room temperature for one year.

D. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.

8 Records

Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, and preparer's initials are recorded in the PIC Reagent Preparation Form (LAB-DNA-42) and placed in appropriate reagent logs.

9 Literature References and Supporting Documentation

DNA-10-09 PROTEINASE K SOLUTION – 10 mg/mL

1 Scope
Proteinases are protein and peptide splitting enzymes. Proteinase K (10 mg/mL) in combination with other reagents is used in DNA extraction procedures. The source of Proteinase K is from *Tritirachium album*. This reagent may be purchased.

2 Related Documents
CLS Manual: Laboratory Equipment

3 Specification
Proteinase K (Pro K or PK)

4 Safety
A. Proteinase K and solutions of Proteinase K can be irritating to mucous membranes. Avoid inhalation and skin contact.
B. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin during use; broken skin must be covered.

5 Equipment and Materials
- Microcentrifuge tubes
- Sterile glassware
- Proteinase K, molecular biology grade
- Sterile dIH₂O
- 20 mg/mL Proteinase K concentrated liquid

6 Procedure
6.1 Preparing from powder:
1. Dissolve 100 mg Proteinase K in 10 mL sterile dIH₂O.
2. As necessary, aliquot into sterile tubes for long term storage and to prevent possible contamination.

6.2 Preparing from liquid:
1. Dilute 5 mL of 20 mg/mL Proteinase K concentrated liquid stock with 5 mL sterile dIH₂O.
2. As necessary, aliquot into sterile tubes for long term storage and to prevent possible contamination.

7 Testing, Storage, Expiration, and Disposal
A. Quality Control Procedure
1. Perform extraction, amplify and type according to the relevant DNA SOP and evaluate results.
2. Reagents will be approved for use if a full DNA profile consistent with previously typed profile is obtained. This approval is indicated by the analyst.
B. Solution prepared from powder:
   1. Store in tightly closed microcentrifuge tubes and place tubes in a storage container
      in the freezer.
   2. Store aliquots frozen for up to two years.
   3. Thaw tubes as needed for appropriate number of extractions.

C. Solution prepared from liquid:
   Store in tightly closed microcentrifuge tubes following manufacturer storage
   recommendations for the 20 mg/mL Proteinase K concentrated liquid stock.

D. For both methods of preparation:
   1. Label storage container with specification, initials, and date prepared. Label tubes
      to link them to storage container.
   2. Properly dispose in marked waste container. Refer to Safety Data Sheet for
      additional information on proper disposal.

8 Records
Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration,
preparer’s initials, and QC paperwork are recorded on ProK Reagent Preparation Form (LAB-
DNA-38) and maintained in the appropriate reagent logs.
DNA-10-10  SARCOSYL SOLUTION – 20% w/v

1 Scope
Sarcosyl, N-lauroylsarcosine (C_{15}H_{28}NNaO_{3}), solution is used in differential DNA extraction procedures in combination with other reagents. The solution is used as a detergent and foaming agent. This reagent may be purchased.

2 Specification
20% Sarcosyl

3 Safety
A. May be harmful if inhaled, ingested, or absorbed through the skin. Avoid contact and inhalation.
B. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin during use; broken skin must be covered.

4 Equipment and Materials
- Analytical filter unit, 0.45 µm, 150 mL
- Vacuum pump or apparatus
- Sterile microcentrifuge tubes
- N-lauroylsarcosine, sodium salt, molecular biology grade 20 g
- Sterile dH_{2}O 100 mL

5 Procedure
1. Add 20 g N-lauroylsarcosine in 100 mL sterile dH_{2}O.
2. Mix thoroughly until powder is in solution.
3. Sterilize by passage through a 0.45 µm analytical filter. Filtration may be facilitated by use of a vacuum apparatus.
4. As necessary, aliquot into sterile microcentrifuge tubes for long term storage and to prevent possible contamination.

6 Testing, Storage, Expiration, and Disposal
A. Quality Control Procedure
1. Perform extraction, amplify and type according to the relevant DNA SOP and evaluate results.
2. Reagents will be approved for use if a full DNA profile consistent with previously typed profile is obtained. Mixtures are acceptable if due to separation in differential extractions. This approval is indicated by the analyst.
3. The quality control procedure is not required if the laboratory is using a purchased reagent.
B. Store tightly closed at room temperature. Minimum labeling includes specification above, initials, and date prepared.
C. 20% Sarcosyl can be stored for one year.
D. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.

7 Records

Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, preparer’s initials, and QC paperwork are recorded on Sarcosyl Reagent Preparation Form (LAB-DNA-39) and maintained in the appropriate reagent logs.

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DNA-10-11  SODIUM CHLORIDE – 5M

1 Scope
Sodium chloride is a salt that is used in making Digest Buffer and TNE solution for differential extractions. This reagent may be purchased.

2 Specification
NaCl, 5M

3 Safety
A. Sodium chloride is an irritant.
B. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Glassware
- Stir plate
- Sodium chloride (NaCl) 292.2 g
- Sterile dH₂O 1 L

5 Procedure
1. Dissolve 292.2 g NaCl in 800 mL dH₂O.
2. Adjust the final volume to 1.0 L with dH₂O.
3. Autoclave as necessary.

6 Testing, Storage, Expiration, and Disposal
A. Store tightly closed. Minimum labeling includes specification above, initials, and date prepared.
B. NaCl can be stored at room temperature for five years. Follow expiration date on purchased reagent.
C. Discard in regular sink, flush with copious amounts of water.

7 Records
Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, and preparer’s initials are recorded on 5M Sodium Chloride Reagent Preparation Form (LAB-DNA-51) and maintained in the appropriate reagent logs.
DNA-10-12 SODIUM HYDROXIDE – 5N

1 Scope
Sodium hydroxide is used to adjust pH of solutions. This reagent may be purchased.

2 Specification
NaOH, 5N

3 Safety
A. Sodium hydroxide is corrosive, causes burns, and is exothermic in water. It is incompatible with strong acids, strong oxidizing agents, and organic materials. Keep container tightly closed and never add water to sodium hydroxide.

B. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

4 Equipment and Materials
- Stir plate
- Sodium hydroxide, pellets (NaOH) 100 g
- Sterile dH₂O 400 mL

5 Procedure
1. Dissolve 100 g NaOH in 400 mL sterile dH₂O.
2. Stir to dissolve. Allow to cool.
3. Adjust the final volume to 500 mL with sterile dH₂O.
4. Mix thoroughly.

6 Testing, Storage, Expiration, and Disposal
A. Store tightly closed. Minimum labeling includes specification above, initials, and date prepared.

B. NaOH, 5N, can be stored at room temperature for five years.

C. To discard, flush in sink with copious amounts of water.

7 Records
Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, and preparer’s initials are recorded on 5N Sodium Hydroxide Reagent Preparation Form (LAB-DNA-52) and maintained in the appropriate reagent logs.
DNA-10-13  STAIN EXTRACTION BUFFER

1  Scope
Stain extraction buffer (10mM Tris, 100 mM NaCl, 10 mM EDTA, 2% Sodium Dodecyl Sulfate) is used to lyse cells and digest proteinaceous materials during the isolation of nucleic acids. This buffer may contain 39 mM DTT when complete.

2  Related Documents
CLS Manual: Laboratory Equipment

3  Specification
Stain Extraction Buffer (SEB)

4  Safety
A. Tris base is an irritant.
B. Dithiothreitol is an irritant; is incompatible with bases, oxidizing and reducing agents, and alkali metals; and may decompose on exposure to moisture.
C. Sodium dodecyl sulfate (Sodium Dodecyl Sulfate) is toxic, is fetotoxic, is an irritant, and is incompatible with strong oxidizing agents.
D. Hydrochloric acid is corrosive, is toxic by inhalation, causes burns, and reacts violently in water. It is incompatible with bases, amines, alkali metals, copper, and aluminum. Never add water to hydrochloric acid. Use concentrated hydrochloric acid only in a chemical fume hood.
E. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin during use; broken skin must be covered.

5  Equipment and Materials
- Stir plate
- pH paper
- Sterile glassware and stir bar
- Tris base (Tris (hydroxymethyl) aminomethane)  1.21 g
- Sodium chloride (NaCl)  5.84 g
- Sterile dIH₂O  ~500 mL
- EDTA solution, 0.5M  20 mL
- 20% w/v Sodium Dodecyl Sulfate  100 mL
- Hydrochloric acid, concentrated (HCl) to pH
- Dithiothreitol (DTT), molecular biology grade  6.02 g

6  Procedure
1. Dissolve 1.21 g Tris base, 5.84 g NaCl, and 6.02 g DTT (if adding) in 500 mL sterile dIH₂O.
2. Add 20 mL 0.5 M EDTA solution and 100 mL 20% w/v Sodium Dodecyl Sulfate.
3. Adjust the pH to 8.0 (± 0.2) with HCl.
4. Bring to a final volume of 1 L with sterile dIH₂O.
5. If DTT is not already incorporated into the reagent, add 6.02 g DTT prior to use.

6. Solution with DTT must be aliquoted in single-use volumes into tubes and stored frozen.

7. Do not autoclave.

7 Testing, Storage, Expiration, and Disposal

A. Quality Control Procedure

1. Perform extraction, amplify and type according to the relevant DNA SOP and evaluate results.

2. Reagents will be approved for use if a full DNA profile consistent with previously typed profile is obtained. This approval is indicated by the analyst.

B. Store tightly closed. Minimum labeling includes specification above, initials, and date prepared.

C. Stain Extraction Buffer without DTT can be stored at room temperature for one year.

D. Stain Extraction Buffer with DTT can be stored frozen for two years.

E. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.

8 Records

Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, preparer’s initials, and QC paperwork are recorded on SEB Reagent Preparation Form (LAB-DNA-37) and maintained in the appropriate reagent logs.
DNA-10-14 TE-4 BUFFER

1 Scope
TE-4 Buffer (10 mM/0.1 mM, pH 8.0) contains a common buffering agent for nucleic acid analysis as well as EDTA to chelate DNases. This reagent may be purchased.

2 Related Documents
CLS Manual: Laboratory Equipment

3 Specification
TE-4, TE

4 Safety
A. Tris-HCl and EDTA solutions are irritants.
B. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin during use; broken skin must be covered.

5 Equipment and Materials
- Sterile glassware
- Tris-HCl, 1 M, pH 8.0 10 mL
- EDTA solution, 0.5M 200 µL
- Sterile dH2O 990 mL

6 Procedure
1. Add 10 mL 1 M Tris-HCl and 200 µL 0.5 M EDTA to 990 mL sterile dH2O.
2. Mix thoroughly.
3. As necessary, aliquot into sterile tubes for long term storage and to prevent possible contamination.
4. Autoclave if not using sterile dH2O and sterile glassware.

7 Testing, Storage, Expiration, and Disposal
A. Quality Control Procedure
1. Perform extraction, amplify and type according to the relevant DNA SOP and evaluate results.
2. Reagents will be approved for use if a full DNA profile consistent with previously typed profile is obtained. This approval is indicated by the analyst.
3. The quality control procedure is not required if the laboratory is using a purchased reagent.
B. Store tightly closed. Minimum labeling includes specification above, initials, and date prepared.
C. TE-4 can be stored at room temperature or 2-8°C for one year.
D. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.

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8  Records

Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, preparer's initials, and QC paperwork are recorded on the TE Reagent Preparation Form (LAB-DNA-34) and maintained in the appropriate reagent logs.
DNA-10-15 TETRAMETHYL BENZIDINE (TMB) SOLUTION

1 Scope
Tetramethylbenzidine (TMB) solution is used for presumptive blood identification because it oxidizes to a blue-green form in the presence of heme and hydrogen peroxide.

2 Related Documents
CLS Manual: Laboratory Equipment

3 Specification
Tetramethylbenzidine (TMB)

4 Safety
A. Tetramethylbenzidine is a mutagen and an irritant; avoid contact with metals.
B. Glacial acetic acid is corrosive, combustible, and causes severe burns.
C. Ethanol is highly flammable.
D. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin during use; broken skin must be covered.

5 Equipment and Materials
- 3, 3', 5, 5' – tetramethylbenzidine 20 mg
- Ethanol 10 mL
- Glacial acetic acid 5 drops

6 Procedure
1. Dissolve 20 mg tetramethylbenzidine in 10 mL ethanol.
2. Add 5 drops glacial acetic acid and swirl to mix.

7 Testing, Storage, Expiration, and Disposal
A. Minimum labeling includes specification above, initials, and date prepared.
B. TMB solution can be stored at 2-8°C for one year.
C. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.

8 Records
Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, and preparer’s initials are recorded in the TMB Reagent Preparation Form (LAB-DNA-33) and placed in appropriate reagent logs.

9 Literature References and Supporting Documentation
DNA-10-16  TNE SOLUTION, pH 8.0

1  **Scope**

TNE (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 8.0) solution is used in differential DNA extraction procedures in combination with other reagents.

2  **Related Chapters/Documents**

Sodium Chloride 5M

Sodium Hydroxide 5N

CLS Manual: Laboratory Equipment

3  **Specification**

TNE

4  **Safety**

A. Tris base may be an irritant to skin, eyes, and mucous membranes.

B. NaCl is an irritant to eyes, respiratory system and skin.

C. EDTA is an irritant.

D. Appropriate personal protective equipment must be worn during preparation and use. Clothing may protect unbroken skin during use; broken skin must be covered.

5  **Equipment and Materials**

- Sterile glassware
- pH paper
- Tris-HCl, 1 M, pH 8.0 10 mL
- NaCl, 5 M 20 mL
- EDTA, 0.5 M 2 mL
- NaOH or HCl to adjust pH
- Sterile dH₂O to 1 L volume

6  **Procedure**

1. Add 10 mL 1 M Tris (pH 8.0), 20 mL 5 M NaCl, and 2 mL 0.5 M (pH 8.0) EDTA to 848 mL sterile dH₂O.

2. Titrate to pH 8.0 using either NaOH or HCl.

3. Bring to final volume of 1 L with sterile dH₂O

4. Autoclave solution if sterile water and glassware not used.

5. As necessary, aliquot into sterile tubes for long term storage and to prevent possible contamination.

7  **Testing, Storage, Expiration, and Disposal**

A. Quality Control Procedure

1. Perform extraction, amplify and type according to the relevant DNA SOP and evaluate results.
2. Reagents will be approved for use if a full DNA profile consistent with previously obtained profile is obtained. Mixtures are acceptable if due to separation in differential extraction. This approval is indicated by the analyst.

B. Store tightly closed. Minimum labeling includes specification above, initials, and date prepared.

C. TNE Solution can be stored at room temperature or at 2-8°C for one year.

D. Properly dispose in marked waste container. Refer to Safety Data Sheet for additional information on proper disposal.

8 Records

Lot numbers of chemicals used to prepare the reagent, date of preparation, date of expiration, preparer’s initials, and QC paperwork are recorded on TNE Reagent Preparation Form (LAB-DNA-36) and maintained in the appropriate reagent logs.
DNA-10-17 COMMERCIAL REAGENTS

1 Scope

The following policy concerns the use of commercial reagents in the laboratory.

2 Practice

A. Reagents that do not have expiration dates provided by the manufacturer shall be assigned an expiration date five years from the date when the reagent is opened.
   1. This expiration date may be extended at one year intervals thereafter provided that the reagent is performance checked and the reagents are being stored according to the manufacturer's guidelines.
   2. A letter from the manufacturer may also be used to extend the expiration date.

B. If the manufacturer has provided an expiration or re-assay date for the reagent, this date shall be used as the expiration date of the reagent. The reagent will not be used in casework after the date of expiration.

C. The expiration date for dry chemicals will be indefinite.

D. Commercial reagents do not require performance checks prior to use in casework unless they are listed as critical reagents, presumptive blood tests, or presumptive semen tests.
### 11 FORMS

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