



TABLE OF CONTENTS

Table of Contents	1
Revision History.....	3
01 Overview	4
CO-01-01 Overview.....	4
CO-01-02 Standard Abbreviations List.....	6
CO-01-03 Glossary of Terms	8
02 Quality Assurance	11
CO-02-01 Personnel.....	11
CO-02-02 Quality Assurance Standards (QAS) Audits	21
CO-02-03 Facilities.....	24
CO-02-04 Emergency Evacuation Plan.....	26
CO-02-05 Validation	28
CO-02-06 Equipment.....	32
CO-02-07 CODIS Records	43
CO-02-08 Critical Reagents	44
CO-02-09 Sample Control	46
CO-02-10 Analytical Controls	49
03 Sample Handling.....	53
CO-03-01 Sample Collection and Handling.....	53
CO-03-02 Sample Destruction	62
CO-03-03 Blood Sample Preparation and Storage	66
CO-03-04 Buccal Swab Preparation and Storage.....	71
CO-03-05 Buccal Lysis Set-up	73
04 Analytical Procedures	78
CO-04-01 EZ1 Advanced XL Extraction.....	78
CO-04-02 QIA Symphony SP Extraction	82
CO-04-03 Investigator 24plex GO! Amplification.....	86
CO-04-04 GlobalFiler Express Amplification	98
CO-04-05 Yfiler Plus Amplification	113
CO-04-06 Capillary Electrophoresis	121
CO-04-07 Data Interpretation Guidelines	129
CO-04-08 Response to Quality Issues	136
CO-04-09 Analytical Review.....	138



CO-04-10	CODIS Upload	142
05	Databasing Practices	143
CO-05-01	CODIS Software and Database	143
CO-05-02	Profile Removal/Expunction Procedures	149
CO-05-03	Searches.....	151
CO-05-04	Partial Matches and Familial Searches	154
CO-05-05	Match Dispositioning.....	159
CO-05-06	Match Verification	161
CO-05-07	Database Monitoring.....	167
06	Reagents.....	169
CO-06-01	Commercial Reagents	169
07	Forms	170
	Directory of Forms	170



REVISION HISTORY

Effective Date	Brief Description of Change(s)
7/30/2020	Original Issue Previous revision history for individual chapters included in archived documents
10/01/2020	Revised: CO-01-02, CO-02-05, CO-02-06, CO-02-08, CO-02-10, CO-04-05, CO-04-06, CO-04-07



01 OVERVIEW

CO-01-01 OVERVIEW

1 Program Description

The State Combined DNA Index System (CODIS) Program is responsible for recording DNA data and establishing and maintaining a computerized database that serves as the central depository in the state for DNA records.

1.1 Purpose

- A. The principal purpose of this system is to assist federal, state, and local criminal justice or law enforcement agencies in the investigation and prosecution of crimes in which biological evidence is recovered.
- B. Other purposes of the CODIS system may include:
 1. Assisting in the recovery or identification of human remains from a disaster or for humanitarian purposes,
 2. Assisting in the identification of living or deceased missing persons, or
 3. Establishing a population database, assisting in identification research and protocol development, or assisting in DNA laboratory quality control. This can only be performed if personal identifying information is removed.

1.2 CODIS Entry

- A. DNA profiles can be entered into the State database in two ways:
 1. Biological samples can be typed by the State CODIS lab
 2. DNA profiles can be uploaded from Local CODIS labs.
- B. Pursuant to Texas Statutes, the Texas Department of Public Safety (DPS) is charged with the task of administering the Combined DNA Index System (CODIS) in the state of Texas. To ensure the quality of the database, it is the policy of the DPS that all data entered into CODIS are generated in a manner consistent with the *FBI Quality Assurance Standards for DNA Databasing Laboratories* and the *NDIS Operational Procedures*.
- C. To comply with Texas Government Code Title 4 Subtitle B Chapter 411 Subchapter G Section §411.142, the State CODIS Program was created effective September 1, 1995. The DNA database must be compatible with the National DNA Index System (NDIS) used by the FBI to the extent required by the FBI to permit the useful exchange and storage of DNA records or information derived from those records.
- D. The State DNA database, consisting of several authorized categories of DNA records, is housed and maintained by the CODIS databasing laboratory in Austin. The State database accepts DNA profile submissions from local NDIS Participating Laboratories in Texas. To be approved, a local laboratory must register with the state program, agree to adhere to specific minimum standards, and submit to regular audits by the state program.



1.3 Approval of Local CODIS Laboratories

- A. A DNA Laboratory in the State of Texas that is maintained by a criminal justice agency may apply to become a CODIS User Laboratory (Local Laboratory) by completing the CODIS User Laboratory Application form (LAB-CO-40) and by providing the requested information. The detailed rules and responsibilities for CODIS User Laboratories are contained in the Texas Administrative Code Title 37 Part 1 Chapter 28 Subchapter G CODIS User Laboratories.
- B. Acceptance as a CODIS User Laboratory, by the FBI, as detailed in the NDIS Operational Procedures, is also required prior to a laboratory connecting to the CJIS-WAN and uploading data to CODIS.
- C. The approval of CODIS Users for a CODIS User Laboratory requires the submission of an Analyst Authorization Form (LAB-CO-41) in addition to the documentation required by NDIS.

1.4 Oversight of DNA Testing Laboratories

- A. The Texas Administrative Code Title 37 Part 1 Chapter 28 Subchapter G requires the Director of the Texas Department of Public Safety to govern the regulation of CODIS user laboratories located in this State.
- B. DPS is required by State and Federal law to maintain compatibility with NDIS which includes following NDIS Operational Procedures prescribed by the FBI and compliance with the *FBI Quality Assurance Standards for Forensic DNA Testing Laboratories and/or DNA Databasing Laboratories*.

2 CODIS Standard Operating Procedures

- A. The CODIS Standard Operating Procedures (SOP) defines policies and procedures for the receipt, processing, analysis and upload of databasing samples.
- B. The DPS CODIS Laboratory does not currently outsource.

3 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.

4 Training Program

- A. The General Laboratory Training Manual addresses the basic understanding of agency and crime laboratory purpose, policy, operation, administrative procedures, ethics, safety, and quality assurance training relevant to the laboratory system.
- B. The CODIS Training Manual contains supplementary materials, reference materials, and resources adequate to ensure the competence of CODIS Technicians and CODIS Analysts in sample handling and DNA analysis to the extent of their participation.

5 Safety Manual

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



CO-01-02 STANDARD ABBREVIATIONS LIST

1 Scope

This is the list of approved abbreviations for use in CODIS documentation. If abbreviations are used that are not contained within this list, the *Glossary of Terms* chapter of this manual, 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.

2 Abbreviations

24plex GO! – Investigator 24plex GO!	DEG – Degraded
AB – Amplification Blank	Dest Plt Pos – Destination Plate Position
AD – Allelic Drop-out	DNAO – DNA Order
ADD DOC – Additional Documentation	DO – Drop Out
Admin – Administrative/Administrative Review	EP – Extra Peaks
AFIS – Automated Fingerprint Identification System	EPP – Electrophoresis Plate Prep
Amel – Amelogenin	EPU – Excessive Pull-up
Amp – Amplification	FS – Familial Search
AmpNeg – Amplification Negative	GFE – GlobalFiler Express
AWB – Analyst Workbench	ILA – Inter/Intra Locus Allele
bp – Base Pair	IP – Imbalanced Peaks
BTR – Blood Tube Rack	IS - Inhibited Sample
CE – Capillary Electrophoresis	judgmt – judgment
CF – Control Failure	LBV – Low Blood Volume
CMF – Common Message Format	LPH – Low Peak Height
CO – Convicted Offender	LS – Low Signal
CODIS – Combined DNA Index System	LTS – Long-term Storage
COS – Conditions of Supervision	MA – Minus A
DB – Dye Blob	MAV – Match Already Verified



MF – Match Folder	RFU – Relative Fluorescent Unit
MID – Match Identification	Rpts – Repeats
MMA – Master Mix Addition	RSMPL – Resampling
MP – Multiple Punches	RXN – Reaction
Multi – Multiple	RXT (or RE) – Re-extract
MV – Match Verification	sec – second
NL – Null Locus	SI – Sequencer Issue
NP – No Punch	SP – Spike
NS – No Signal	ss – Single Sample
NSA – Non-Specific Amplification	SSF – Size Standard Fail
NT – No Template	STR – Short Tandem Repeat
OL – Off Ladder	SWB – Swab (buccal) Storage
OMR - Outside Marker Range	TA – Triple Allele
OS – Off Scale	TDCJ – Texas Department of Criminal Justice
PBS – Permanent Buccal/Blood Storage	TJJD – Texas Juvenile Justice Department
Plt Pos – Plate Position	YP – Yfiler Plus
POS – Positive Control	
PQV – Process Quality Value	
Pt – Point	
PU – Pull-up	
QO – Qualifying Offense	
RA – Re-amplification	
RB – Reagent Blank	



CO-01-03 GLOSSARY OF TERMS

1 Scope

This document defines terms used in the CODIS Manual.

2 Definitions

Adventitious Match: an **association of an evidence DNA profile to the profile of a person who is not the true donor of that profile**; this situation may arise when the DNA profile contains information from a limited number of loci (e.g., from a damaged DNA sample) that are insufficient.

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

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).

Contamination: 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; several reproducible true peaks attributable to exogenous DNA 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.

Control Failure: Used to identify when both positive controls failed to exhibit appropriate results and/or the negative control(s) fail (e.g., due to no addition of the control or alleles present in the control).

Degraded: A term used to describe the exhibited DNA profile. DNA profile can manifest as a low level sample with dropout of the larger loci. Usually has a "ski-slope" appearance to the profile.

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.

Elevated Baseline: Higher than typical fluorescent background noise.

Extra Peaks: A broad term used to describe additional peaks in the profile. Does not solely necessitate contamination.

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

Inhibited Sample: A term used to describe the exhibited DNA profile. The profile may appear to have a ski-slope pattern, inconsistent interlocus peak height patterns or inconsistent dropout of alleles or loci as a result of an inhibitor present in the amplification or too much template DNA.

Inter Locus Allele: An allelic peak that falls between loci.

Intra Locus Allele: An allelic peak that falls into a different locus.

Low Signal: A term used to describe a profile that has low peak height values, may be within the stochastic region, approaching analytical threshold and/or contains drop out.



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.

Microvariants: Alleles that contain incomplete repeating units.

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.

No Signal: The statement given when no alleles are detected at or above analytical threshold.

Non-Specific Amplification: Amplification of nucleic acid sequences other than the target sequence which results from primers hybridizing to sequences other than the target sequence and then serving as a substrate for primer extension.

Null Allele/Locus: 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 or complete absence of alleles.

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

Off Scale Data: Data that exceeds or is approaching the saturation point of the CCD camera of the instrument in the raw data.

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.

Process Quality Value: Used to indicate the quality of data at the sample and marker levels for genotyping within the genetic analysis software.

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

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

Sequencer Issue: A broad term used to describe issues that have arisen due to the capillary instrument. (e.g., bad injection, migration issue, capillary issue, poor resolution.)

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



Size Standard Fail: A broad term used to describe an issue linked to the internal size standard not allowing proper sizing of the DNA profile obtained or no sizing at all.

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, microhaplotypes or mitochondrial DNA.

Triple Allelic Pattern: Three allelic peaks observed at one or more loci 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.



02 QUALITY ASSURANCE

CO-02-01 PERSONNEL

1 Scope

This document provides information related to CODIS personnel. It includes responsibilities for the Technical Leader, State CODIS Administrator, and local CODIS Administrator, as well as contingency plans, job descriptions, procedure for scientific literature review, and general information concerning training, analyst approval, and proficiency testing

The DPS will employ personnel who meet the requirements outlined in the *FBI Quality Assurance Standards (QAS) for DNA Databasing Laboratories*. Only personnel approved by the FBI will have access to the CODIS Software.

2 Related Documents

CLS Manual: Part III – Personnel

National DNA Index System (NDIS) Operational Procedures Manual (external)

3 CODIS Program Manager / State CODIS Administrator

The CODIS Program Manager is responsible for oversight of the CODIS Laboratory operations as well as the point of contact between NDIS and the State of Texas as outlined in the NDIS Operational Procedures.

A. State CODIS Administrator Qualifications

1. Must be a current employee of the laboratory and must be a current or previously qualified database or casework analyst with documented mixture interpretation training.
2. Shall meet the educational and experience qualifications specified in the FBI QAS for DNA Databasing Laboratories.
3. Shall have successfully completed the QAS Auditor training course within one year of assuming the State CODIS Administrator role or position. Prior training in this course is acceptable if that training was over the current QAS Standards at the time of assuming the State CODIS Administrator role or position.
4. Shall participate in the FBI sponsored CODIS software training within six months of appointment if individual has not previously attended such training.
5. Shall obtain and maintain the FBI security clearance required to become a CODIS user and the annual training on DNA Records Acceptable at NDIS.

B. State CODIS Administrator Responsibilities

1. Oversees the full operation of the Texas DPS CODIS Laboratory and CODIS System
2. Administers the laboratory's CODIS network
3. Acts as the central point of contact with the NDIS Custodian and NDIS participating laboratories within the State of Texas
4. Ensures that other participating laboratories in the State comply with the terms and conditions of the NDIS Participation Memorandum of Understanding



5. A State CODIS Administrator or his/her alternate shall attend the regularly scheduled annual CODIS Conference and the regularly scheduled State Administrator Meetings sponsored by the FBI or seek an excused absence from the NDIS Custodian if neither the State CODIS Administrator nor the alternate State CODIS Administrator can attend.
 6. Has the authority over all NDIS Participating Laboratories in the State of Texas to terminate a CODIS user's or laboratory's participation in CODIS until the reliability and security of the computer data can be assured if an issue with the data is identified
 7. Schedules and documents the CODIS required annual training of database analysts
 8. Ensures that the security of data stored in the CODIS database is in accordance with State and/or Federal law and NDIS Operational Procedures
 9. Ensures that the quality of data stored in the CODIS database is in accordance with State and/or Federal law and NDIS Operational Procedures
 10. Ensures that matches are dispositioned in accordance with NDIS Operational Procedures
 11. Compilation and monthly reporting of Investigations Aided and Hit statistics to NDIS
 12. Appoints the Local CODIS Administrator and alternate Local CODIS Administrator for the CODIS Laboratory
 13. Performs other duties as listed in the NDIS Operational Procedures
- C. Contingency Plan
1. In the extended absence of the State CODIS Administrator, the Local CODIS Administrator for the CODIS Databasing Laboratory or a designated acting CODIS Administrator will perform CODIS administrator duties as necessary.
 2. If the positions of both the State CODIS Administrator and the Local CODIS Administrator are unoccupied at the same time, the State will refrain from uploading new DNA data to NDIS during the vacancy.

4 Local CODIS Administrator / Alternate State CODIS Administrator

The Local CODIS Administrator is appointed by the CODIS Program Manager. The Local CODIS Administrator shall act as the point of contact between State CODIS Administrator and the other NDIS participating labs in Texas.

- A. Local CODIS Administrator / Alternate State CODIS Administrator Qualifications
1. Must be a current employee of the laboratory and must be a current or previously qualified database or casework analyst with documented mixture interpretation training.
 2. Shall meet the educational and experience qualifications specified in the FBI QAS for DNA Databasing Laboratories.
 3. Shall have successfully completed the QAS Auditor training course within one year of assuming the State CODIS Administrator role or position. Prior training in this



course is acceptable if that training was over the current QAS Standards at the time of assuming the State CODIS Administrator role or position.

4. Shall participate in the FBI sponsored CODIS software training within six months of appointment if not previously attended such training.
5. Shall obtain and maintain the FBI security clearance required to become a CODIS user and the annual training on DNA Records Acceptable at NDIS.

B. Local CODIS Administrator Responsibilities

1. Acts as Alternate State CODIS Administrator upon the unavailability of the current State CODIS Administrator
2. Acts as the central point of contact with the Texas DPS CODIS Laboratory and NDIS participating laboratories within the State of Texas
3. Assists in administering the laboratory's CODIS network
4. A Local CODIS Administrator or his/her Alternate shall attend the regularly scheduled annual CODIS Conference sponsored by the FBI or seek an excused absence from the NDIS Custodian if neither the Local CODIS Administrator nor the Alternate Local CODIS Administrator can attend.
5. Ensures that the security of data stored in the CODIS database is in accordance with State and/or Federal law and NDIS Operational Procedures
6. Ensures that the quality of data stored in the CODIS database is in accordance with state and/or federal law and NDIS Operational Procedures
7. Ensures that matches are dispositioned in accordance with NDIS Operational Procedures
8. Completion of Annual Audit certification
9. Has the authority to terminate a CODIS Forensic Scientist's or the CODIS laboratory's participation in CODIS until the reliability and security of the computer data can be assured if an issue with the data is identified
10. Performs other duties as listed in the NDIS Operational Procedures

C. Contingency Plan

1. In the extended absence of the Local CODIS Administrator, the State CODIS Administrator or the designated Alternate Local CODIS Administrator will perform Local CODIS Administrator duties as necessary. The Alternate Local CODIS Administrator is appointed by the CODIS Program Manager and must meet the requirements of a Local CODIS Administrator as outlined in the FBI QAS for DNA Databasing Laboratories.
2. If the positions of both the State CODIS Administrator and the Local/Alternate CODIS Administrator are unoccupied at the same time, the State will refrain from uploading new DNA data to NDIS during the vacancy.

5 Technical Leader

A. Technical Leader Qualifications

1. Must be a full time employee of the laboratory system who reports to the Quality Manager and/or CODIS Program Manager



2. Must be a current or previously qualified database or casework analyst with a minimum of three years human-DNA experience as a qualified analyst on database or forensic samples
3. Shall meet the educational and experience qualifications specified in the FBI QAS for DNA Databasing Laboratories.
4. Shall have successfully completed the FBI's DNA Auditor training course within one year of assuming the Technical Leader role or position. Prior training in this course is acceptable if that training was over the current QAS Standards at the time of assuming the Technical Leader role or position.

B. Technical Leader Responsibilities

1. The 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.
2. 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.
3. Laboratory organizational charts reflect the position of the Technical Leader in the management structure.
4. 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:
 - a) *Oversee the technical operations of the laboratory.*
 - i. *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.*
 - b) *Act as advisor with respect to technical issues. Resolve technical problems or issues between primary analyst and reviewer that may be identified in a technical review.*
 - c) *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.*
 - d) *Evaluate and document approval of all validations and methods used by the laboratory.*
 - e) *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, and General Laboratory Training Manual. This review will be documented on the Annual Controlled Document Review form (LAB-508). The CODIS SOP and training manual review will be performed by the CODIS Advisory Board and documented on the Annual Controlled Document Review form (LAB-508).*
 - f) *Direct an annual review of sample processing records. This review is defined to address the representative sample and the time period of processing records to review and is approved prior to the annual review.*



- g) *Review and approve the training, quality assurance and proficiency testing programs in the laboratory. Review all proficiency test results reported as uninterpretable for compliance with laboratory guidelines.*
 - h) *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 at the time of discovery.*
 - i) *Document approval of corrective actions prior to implementation of corrective actions. Implement and document quality action plans.*
 - j) *Ensure that personnel are adequately trained and qualified for assigned duties, to include continuing education opportunities.*
 - k) *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.*
 - l) *Assess a DNA analyst's previous training and ensure it is adequate and documented. If a modified training program is determined to be appropriate, document such modification.*
 - m) *Make recommendations for analyst/technician approval to perform independent analysis.*
 - n) *Review internal and external DNA QAS Audit documents and, if applicable, approve corrective action(s) to ensure that findings, if any, were appropriately addressed; document such review.*
 - o) *Approve program for the annual review of scientific literature that documents the analysts' ongoing reading of scientific literature.*
 - p) *Document approval of the technical specifications of the outsourcing agreement with a vendor laboratory before it is awarded.*
 - q) *Complete the FBI sponsored auditor training within one year of appointment, if such training has not been previously obtained.*
 - r) *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.*
 - s) *Document approval of analysts' review of validation studies and archived procedures for legacy amplification kits.*
5. Newly appointed Technical Leaders shall also be responsible for documented review of the following:
- a) *Validation studies and methodologies currently used by the laboratory*
 - b) *Validation studies and archived procedures for legacy amplification kits that were used by the laboratory*
 - c) *Training records of currently qualified technicians*
 - d) *Educational qualifications and training records of currently qualified analysts and technical reviewers*



6 Contingency Plan for the loss of the Technical Leader

- A. In the event that the Technical Leader is absent for an extended period of time and unable to fulfill the Technical Leader duties, or if the position is vacated, a former Technical Leader who is qualified under the current DNA QAS Databasing Audit document or another current Technical Leader within the system will be immediately appointed by the Laboratory Director (at the recommendation of the CODIS Program Manager) as acting Technical Leader until a permanent replacement is appointed.
- B. If another Technical Leader is not available, a CODIS analyst who is qualified to be a Technical Leader under the current *FBI QAS for Forensic DNA Testing Laboratories and/or DNA Databasing Laboratories* will be appointed as acting Technical Leader until a permanent replacement is appointed.
- C. The laboratory must notify the NDIS Custodian regarding the loss of the Technical Leader and identify the acting Technical Leader. Appendix B in the current QAS Audit document is used for notification.
- D. In the event that the position cannot be filled, the CODIS laboratory will submit a contingency plan for FBI approval. New database analyses will be suspended until the contingency plan is approved by the FBI.

7 Contingency plan for less than two full-time employees who are qualified DNA analysts

- A. If the Technical Leader position is vacant and the laboratory has less than two full-time employees who are qualified CODIS analysts, in addition to the plan for loss of a Technical Leader outlined in section 6, another analyst currently qualified in the methods used by the laboratory and authorized for independent work will be appointed by the Laboratory Director (at the recommendation of the CODIS Program Manager) to perform/review databasing analysis onsite at the laboratory for at least 1 day a week until the laboratory has at least two full-time employees who are qualified CODIS analysts that are authorized for independent work.
- B. If the Technical Leader position is not vacant and the laboratory has less than two full-time employees who are qualified CODIS 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 (at the recommendation of the CODIS Program Manager) to perform technical review for all analysis completed during this time (including mentored/supervised work).
- C. If another qualified analyst that is authorized for independent work is not available for assignment, the laboratory will suspend new database analysis until the laboratory has at least two full-time employees who are qualified CODIS analysts that are authorized for independent work. The CODIS laboratory will submit a contingency plan for FBI approval. Appendix B from the most recent QAS Audit document is used for notification.
- D. Mentored/supervised work 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.
- E. Mentored/supervised work 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 analysis.



- F. 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, approve their qualifications prior to independent work analysis, and document such review.
- G. 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 Job Descriptions

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

9 Training

- A. Training will be conducted according to the CODIS Training Manual.
 - 1. The CODIS training manual references the competency requirements and addresses the qualifying examinations for personnel approval.
 - 2. Training beyond what is required in the training manuals will be performed at the discretion of the Technical Leader and/or supervisor.
- B. The Technical Leader, State/Local CODIS Administrator, and each qualified CODIS Forensic Scientist 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.

10 Review of Scientific Literature

- A. Personnel assigned to the CODIS section that perform analysis on database samples are responsible for the ongoing reading of scientific literature and documentation of such.
- B. The CODIS 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.
- C. Documentation of completion of the readings will be maintained through QualTrax.
- D. The 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 CODIS.
- E. 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.
- F. Members of the CODIS section may meet to discuss the reading of scientific literature during the year as directed by the Technical Leader and/or Section Supervisor.



11 Analyst/Technician Approval

- A. 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 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 and 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 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 Section for inclusion with the analyst/technician's credential file.
 4. For analysts/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 databasing applications.
- B. Analysts/technicians previously qualified by DPS through completion of a specific unit of training 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.
1. The Technical Leader will review the circumstances and previous work of the analyst/technician to determine the extent of retraining necessary, and a documented retraining plan will be developed.
 2. Once the Technical Leader has approved the retraining plan, the most current version of the CODIS 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.
 3. 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.



12 Proficiency Testing

- A. Each CODIS analyst and technical reviewer is required to successfully complete a proficiency test in DNA analysis performed to the full extent in which they participate in database analysis 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 (Investigator 24plex GO!, GlobalFiler Express, Yfiler Plus) at least once per calendar year.
 3. Based on qualification, a test must be completed using at least one method in each methodology (extraction, 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 test interval is based on the date the results are submitted to the external test provider.
- C. The CODIS laboratory employs two automated methods for extraction. Each CODIS analyst shall use at least one of these automated methods for performing their proficiency; however, this does not preclude the possibility that both methods may be administered on a single proficiency. The CODIS Laboratory does not employ a manual extraction method.
- D. 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.
- E. The CODIS Laboratory can employ a team approach for conducting DNA analysis; therefore, CODIS analysts responsible for pre-amplification steps shall participate in a team proficiency to the full extent in which they participate in DNA analysis at least once per year. However, each analyst performing interpretation and reporting must be assigned their own test to complete the interpretation and reporting portions.
- F. Employees are considered qualified analysts in the relevant category of testing once the Laboratory Director has authorized them to conduct work in that category.
- G. Newly qualified CODIS analysts shall be added to the examiner assessment schedule within eight months of the date of qualification by the Laboratory Director.
- H. Typing of all CODIS core loci shall be attempted for each technology performed.
- I. 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 and in accordance with CLS policies. This test shall serve as proof of continued competency after a leave of absence.
- J. Proficiency tests will be evaluated by the System Quality Assurance Section. The grading criteria used for DNA proficiency tests include an evaluation of the correctness of all reported genotypes and/or phenotypes. A satisfactory grade is attained for a proficiency test when there are no analytical errors for the DNA profile-typing data.
- K. 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.



-
- L. 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.

13 Records

DNA Technical Leader Checklist (LAB-DNA-48)



CO-02-02 QUALITY ASSURANCE STANDARDS (QAS) AUDITS

1 Scope

In order to participate in NDIS, DPS is required to comply with the FBI DNA audit document (FBI Quality Assurance Standards Audit for DNA Databasing Laboratories). 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 DNA Databasing 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 a 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 (as applicable); 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 CODIS Technical Leader and CODIS Program Manager as appropriate throughout the audit to apprise them of audit progress including any potential findings.

3.3 Post-Internal Audit Activities

- A. Once the audit is received, the CODIS Technical Leader will review the QAS audit document for accuracy and completeness. The document may be returned to the audit team as necessary to ensure that it has been completed.
- B. The CODIS Technical Leader and the State CODIS Administrator shall review the QAS audit document and provide responses to any findings to System Quality Assurance within 30 calendar days of the laboratory’s receipt of the final audit document.
- C. Findings may be contested by providing documentation to System Quality Assurance.
- D. The laboratory will be notified by System Quality Assurance once the audit has been closed.
- E. After the audit has been closed, the Technical Leader and State 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.
- F. The finalized internal QAS audit document record consists of the audit document, responses to any findings, and Technical Leader and State CODIS Administrator initials and date. This record will be forwarded to System Quality Assurance. Completed audit document records will be stored electronically.
- G. 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.
- B. If corrections are required, the CODIS Technical Leader will provide System Quality Assurance with a list of corrections. System Quality Assurance will forward the request for corrections to the auditors.
- C. Once the final (corrected) QAS audit is received, the CODIS Technical Leader and State CODIS Administrator will prepare responses to any findings. The responses should include supporting documentation to show how the issue was remediated.
- D. 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 Quality Assurance 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.
- E. 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 CODIS laboratory. The CODIS Program Manager should be informed when the audit has been submitted to NDIS for review.

- F. If an acknowledgement of receipt of audit is provided by NDIS, this acknowledgement must be retained as part of the final audit record.
- G. 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.
- H. After the audit has been submitted to NDIS, the CODIS Technical Leader and State 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.
- I. The finalized external QAS audit record consists of the audit document, CODIS Technical Leader and State 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. Completed audit document records will be stored electronically.

4 Literature References and Supporting Documentation

FBI. Quality Assurance Standards for DNA Databasing Laboratories. www.fbi.gov (current version)

FBI. National DNA Index System (NDIS) Operational Procedures Manual (current version)



CO-02-03 FACILITIES

1 Scope

The laboratory is designed to maintain the integrity of databasing samples throughout processing by minimizing contamination and controlling unauthorized access to the operational laboratory and sample areas.

Security, limited authorized access, and system-wide facilities requirements are described in the Crime Laboratory Service Manual.

2 DNA Work Areas

The laboratory is oriented as a one way workflow system mirroring DNA processing steps. Sample receiving, DNA extraction, PCR setup, and capillary electrophoresis occur in separate rooms. Amplified DNA product areas are physically separate from all other areas of the laboratory. Doors to these rooms are maintained in a closed position.

2.1 Sample Receiving Room

CODIS DNA collection kits are opened in the sample receiving room. Samples are stored in this room until they are put into process.

2.2 Blood and Buccal Sample Preparation Room

Blood samples are spotted onto archive cards and buccal swabs are lysed in the sample preparation room.

2.3 Extraction Room

DNA extraction is performed in the DNA extraction room at separate times or in separate spaces from amplification.

2.4 PCR Set-up Room

Amplification set-up steps are performed in the PCR set-up room. A laminar flow hood is used when preparing the amplification master mix and when adding template and/or controls. PCR plates are sealed and centrifuged before proceeding to the thermal cycler (PCR) room.

2.5 Amplified DNA Product Areas

The generation of amplified DNA product are performed in the thermal cycler (PCR) room. Once amplified, the samples will be transferred to the capillary electrophoresis room. No amplified samples will leave the amplified DNA product areas unless appropriately packaged. Equipment, reagents, and supplies in the amplified product area are dedicated to that area and will not be removed unless properly decontaminated.

3 Good Laboratory Practices

3.1 Prevention and Decontamination

- A. Clean work surfaces thoroughly after use with appropriate cleaning solutions such as 10% bleach solutions/wipes, commercial DNA decontaminates, or ethanol. Be mindful of areas and/or equipment where bleach is not recommended for use.
- B. Dedicated laboratory coats are available for each room. Laboratory coats worn during PCR setup may not leave the PCR setup room. Special care should be taken to store and launder pre and post amplification laboratory coats separately.



- C. Wear disposable gloves during testing and sample handling. Change gloves frequently or when they become contaminated. Discard gloves when leaving work areas.
- D. Clean scissors, forceps, and single-hole punchers with ethanol between each sample.
- E. Aliquot reagents whenever possible to minimize contaminating stock reagents.
- F. Use aerosol-resistant pipette tips when handling pre-amplified DNA. Sterile pipette tips may be used for amplified DNA. Change pipette tips between samples.
- G. Quarterly laboratory cleanings will be initiated by the Technical Leader.

3.2 Cleaning glassware

- A. Wear gloves when cleaning
- B. Thoroughly rinse glassware with distilled or deionized water after each use and invert to air-dry.



CO-02-04 EMERGENCY EVACUATION PLAN

1 Scope

Any emergency which requires evacuation of the laboratory will call for the activation of this plan.

Chapters 5 and 22 of the DPS General Manual contain additional information regarding the safe evacuation of DPS occupied facilities. These chapters should be reviewed by each employee.

2 Policy

2.1 Definitions

A more detailed list and/or description of these terms are available in the DPS General Manual Chapter 22.

- A. "All Clear" – An oral announcement by the Building Evacuation Coordinator that it is safe to return to the building.
- B. Alternate (alt) – An individual designated to perform the duties of the Evacuation Coordinator if that individual is not present during the emergency.
- C. Assembly Site (Rally Point) – A point away from the building where employees should assemble and wait until the emergency is resolved.
- D. Building Evacuation Coordinator – The person responsible for:
 - 1. Ensuring all building occupants understand the emergency evacuation plan
 - 2. Maintaining a current list of Evacuation Coordinators
 - 3. Facilitating and assisting in the safe exit of any employee or visitor
 - 4. Assigning an Evacuation Buddy to any employee needing assistance
 - 5. Ensuring the evacuation plan is implemented correctly
 - 6. Communicating between building occupants and the command center during an evacuation
- E. Evacuation Buddy – An employee who will aid those who need assistance to exit the building.
- F. Primary Evacuation Route – The quickest and safest accessible route from an employee's location to the assembly site at the time evacuation is necessary.
- G. Secondary Evacuation Route – The next quickest and safest route to a designed assembly site if the primary route is unsafe for use.

2.2 Evacuation Coordinators

Emergency Information Sheets listing current Evacuation Coordinators, Evacuation Buddies, and Alternates are updated and distributed as necessary.

2.3 Evacuation Notification

During an emergency, employees will be notified to evacuate by an audible alarm. At this point, employees should implement the emergency evacuation plan.



2.4 Evacuation Instructions

- A. When evacuating the building, all office and hallway doors should be closed (but not locked) when possible to help suppress an actual fire.
- B. It is not necessary to card out of the building during an emergency situation.
- C. Evacuation diagrams have been placed in multiple places throughout the building. They are marked with evacuation routes.
- D. Upon evacuation notification, all laboratory employees will immediately proceed to their nearest accessible evacuation route and proceed to their assembly (rally) point. Evacuation Buddies will assist those in need prior to exiting the building. Take only those personal belongings within reach and do not return to the building until the all clear sign is given.
- E. Prior to leaving the building, the Building Evacuation Coordinator (or their designee) will verify that the building has been evacuated.
- F. After evacuation has been completed, the Building Evacuation Coordinator (or their designee) will account for each employee with use of the Building Evacuation Log.

3 Records

Building Evacuation Log (LAB-SAF-08)

4 Literature References and Supporting Documentation

General Manual, Chapters 5 and 22.



CO-02-05 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 CODIS.

2 Related Documents

CLS Manual: Laboratory Equipment

CLS Manual: Validations and Performance Verifications

3 Practices

3.1 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. Verification using an appropriate and available certified reference material
 - 4. Database-type samples
 - 5. Accuracy (repeatability and reproducibility) studies
 - 6. Contamination assessment (evaluation of controls)
 - 7. 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 an 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 (evaluation of controls)
 - 7. Database-type samples



- D. For robotic workstations, the studies performed will depend upon the procedure being automated. Initial system validation of a robotic workstation 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 (evaluation of controls)
 5. Changes to the robot programming after validation which may affect DNA databasing results will require additional validations.

3.2 Software Validation

- A. New software or new modules of existing software associated with significant 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. Include determination of which studies will and will not be conducted,
 2. Specify the type of software being evaluated (equipment, analysis/interpretation, or statistical),
 3. Specify if the analytical process is impacted or not, and
 4. Include software tools developed by the laboratory.
- B. If any studies are determined to be not applicable, the summary must acknowledge and address the reason why this determination was made.
- C. Validation of a 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.
- D. The Technical Leader is responsible for reviewing and approving software validations and testing prior to implementation. The Technical Leader may engage in or direct such activities.

3.3 Required Studies for Software

- A. Validation of new software or modifications of existing software that are associated with significant equipment must include the following studies and associated summaries:
1. Functionality testing
 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. Functionality testing
 2. Reliability testing
 3. Precision and accuracy studies (as applicable)



4. Sensitivity studies (as applicable)
5. Specificity studies (as applicable)

3.4 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 validations,
 - a) *An initial (system) validation has already been performed,*
 - 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 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 CODIS SOP (e.g. changes to thermal cycling parameters, CE run voltage and time, etcetera).*
 7. CODIS documents have been reviewed to determine if they are applicable to the validated method/instrument. Any proposed deviation to current CODIS SOP 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.
- C. Technical review of validation studies must be completed by personnel that are qualified in the general discipline under which the validation falls.
- 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.



3.5 NIST Traceability

- A. The laboratory must check its DNA database procedures, at a minimum from amplification to characterization, either annually or whenever substantial changes are made to a procedure against an appropriate and available NIST standard reference material (SRM) or certified reference material (CRM) traceable to a NIST standard.
- B. The laboratory must generate typing results for each technology (e.g., STRs, Y-STRs, mtDNA) performed in the laboratory.
- C. Substantial changes include a change in test kit, platform, software, or methodology.
- D. The Technical Leader shall document approval of the results.
- E. Documentation of the traceability of a CRM will be maintained on-site.

4 Records

Validation Plan Form (LAB-407)

Validation Form (LAB-408a)

5 Literature References and Supporting Documentation

Scientific Working Group on DNA Analysis Methods. *Validation Guidelines for DNA Analysis Methods*. Approved at the Scientific Working Group on DNA Analysis Methods. www.swgdam.org (most recent version).

FBI. Quality Assurance Standards for DNA Databasing Laboratories. www.fbi.gov (most recent version).



CO-02-06 EQUIPMENT

1 Scope

Only validated, properly maintained, calibrated, and/or performance verified equipment will be used in the analysis of CODIS samples.

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 an instrument or piece of equipment, a performance check or verification should be performed immediately.

Records of maintenance, service, performance checks, and calibration must be maintained and be readily available for inspection. **The LAB-405 is used in conjunction with STaCS 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

- Robotic Workstations
- Thermal Cycler
- Thermal Cycler temperature-verification system
- Mechanical Pipettes
- Capillary Electrophoresis (CE) Instrument
- NIST-traceable thermometer
- Dry Incubators/Thermomixers

3.2 Robotic Workstations

- A. Robotic workstations perform a variety of functions in the CODIS laboratory. Most robotic workstations are liquid handling systems while others only perform single functions such as punching dried DNA samples on FTA paper.
- B. Refer to the Validation chapter for specific validation requirements for newly purchased robotic workstations.
- C. Planned Maintenance
 1. Robotic workstations require annual planned maintenance by a qualified service engineer to assess the functionality of the robotic instrument, Plate Filler excluded.
 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 Quality Control for Robotic Workstations (LAB-CO-25) and documented on LAB-408b.*



- c) *The robotic workstation is considered to have acceptable performance if the performance verification ends in successful downstream results for the process being tested.*

D. Quality Control for the Plate Filler:

1. A performance check is required annually.
2. This will be accomplished by replacing the tube dispensing cassette annually and the factory calibration report for the tube dispensing cassette will serve as documentation for the performance check.

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 repairs on LAB-405 and the performance verification on the LAB-408b.

F. Preventive Measures

Robotic workstations should be cleaned as needed following the manufacturer's guidelines. LAB-405 and LAB-408b 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 Procedures
 1. A performance check must be performed annually.
 2. A performance check of the thermal cyclers will consist of temperature verification, temperature non-uniformity, and running system diagnostic tests.
 - a) *Follow manufacturer's instructions for performing these tests and document the results on the Thermal Cycler Quality Control Record (LAB-CO-51 or LAB-CO-52) and the LAB-405.*
 - b) *For the Veriti Thermal Cyclers, the results generated from the instrument may be attached to the Thermal Cycler Quality Control Record.*
 3. The thermal cycler uses established parameters to determine if temperature verification, temperature non-uniformity, and running of system 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 of a qualified service technician may repair the equipment. Any repair must be documented.
 - 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 and recorded on LAB-408b.*
2. Record repairs on LAB-405.

F. Preventive Measures

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

G. Precautions

The heater block and heated cover can be at or in excess of 100°C. Care should be taken to be sure that the block temperature, as indicated on the front panel display, is at or below 25°C before opening the heated cover.

3.4 Thermal Cyclers Temperature-Verification System

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



F. Preventive Measures

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

3.5 Mechanical Pipette

- A. Some pipettes are dedicated to a specific function, such as those used in the post PCR amplification process.
- B. External calibration by an approved supplier is required annually.
- C. 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 a LAB-408b.
- D. Newly purchased pipettes must undergo performance verification using one of the quality control procedure options prior to being placed into service.
 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 μ L.*
 - 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 μ L, it is recommended that the liquid be dispensed into a microcentrifuge tube containing at least 50 μ L of water to ensure accurate delivery. For example, if using a p10 pipette set to 5 μ L, 5 μ L of water is dispensed into a tube containing at least 50 μ L 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 μ L, 5 μ L 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 μ L, the amount of liquid remaining in the microcentrifuge tube should be approximately 45 μ L.*
 - 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 an amplification 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. CE plate results in expected allele calls from the ladder, positive, and negative control).*



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

E. 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 the 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 repairs on LAB-405 and performance verification on the LAB-408b.

F. Preventive Measures

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-408b are not required.

G. Precautions

Care must be taken to ensure that pipettes are not contaminated with biohazardous materials. Following the possible contamination of a pipette with biohazardous materials, the pipette should be wiped clean with a soft cloth and 10% bleach followed by a rinse with deionized water or ethanol.

3.6 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. 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.*
 - b) *An amplification positive, an amplification negative, and a ladder must be run and analyzed under normal conditions. Results of the controls from the first plate run after annual planned maintenance, service, repair, installation of a new array, or following a spectral calibration may be used to satisfy this requirement.*
 - c) *The CE instrument is considered to have acceptable performance if all peaks in the allelic ladder and amplification positive are called correctly and if no peaks attributable to DNA are present in the amplification negative.*



2. The documentation of the completion of the quality control procedure and the analyzed data must be recorded using the Quality Control for CE Instrument Form (LAB-CO-22). LAB-408b 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, amplification positive and amplification negative appropriate to the dye set used must be run and analyzed with the new spectral under normal conditions. Results of the controls from the first plate run after annual planned maintenance, service, repair, installation of a new array, or following a spectral calibration may be used to satisfy this requirement.*
 - d) *The analyzed data from this run must be documented using the Quality Control for CE Instrument Form (LAB-CO-22) and LAB-408b must be completed.*
 - e) *The CE instrument is considered to have acceptable performance if all peaks in the allelic ladder and amplification positive are called correctly and if no peaks attributable to DNA are present in the amplification negative.*
- 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 below 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. After service engineer repair:
 - a) *An amplification positive, an amplification negative, and a ladder must be run and analyzed under normal conditions. Results of the controls from the first plate run after annual planned maintenance, service, repair, installation of a new array, or following a spectral calibration may be used to satisfy this requirement.*
 - b) *The CE instrument is considered to have acceptable performance if all peaks in the allelic ladder and amplification positive are called correctly and if no peaks attributable to DNA are present in the amplification negative.*
 - c) *Documentation for performance verification after repair includes completion of Quality Control for CE Instrument (LAB-CO-22) and LAB-408b.*
 3. Record repairs on LAB-405 and performance verification on the LAB-408b.



G. Preventive Measures

1. Preventive measures must be completed weekly on the 3130xl genetic analyzers. Instruments not in use for more than a week must have preventive measures completed prior to use. Preventive measures include, but are not limited to:
 - a) *Water Trap Flush*
 - b) *Water Wash Wizard*
 - c) *Water/Buffer change*
 - d) *Computer restart*
 - e) *Array Port Flush, if needed*
2. Preventive measures must be completed biweekly on the 3500xl genetic analyzers. Instruments not in use for more than two weeks must have preventive measures completed prior to use. Preventive measures include, but are not limited to:
 - a) *Water Trap Flush*
 - b) *Water Wash Wizard*
 - c) *Water/Buffer change*
 - d) *Computer restart*
 - e) *Array Port Flush, if needed*
3. When preventive measures are completed, they are recorded in STaCS. LAB-405 and LAB-408b are not required.

3.7 NIST-Traceable Thermometers

- A. A NIST traceable thermometer is used to performance check critical equipment and also to verify the accuracy of thermometers listed in section 4.4.
- 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-408b.
- D. Newly purchased thermometers require a performance verification using the quality control procedure prior to placing the thermometer into service. Complete LAB-408b
- E. Quality Control Procedure
 1. Set a wet/dry incubator or thermomixer 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 $\pm 2^{\circ}\text{C}$ of the set point.

F. Preventive Measures

There are no recommended preventive measures for NIST traceable thermometers.



3.8 Dry Incubators/Thermomixers

- A. 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 the option 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-408b.
- 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/Thermomixer Quality Control Record (LAB-CO-53) 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-408b 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 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 repair on LAB-405 and performance verification on LAB-408b.
- F. Preventive Measures
 1. Incubators will be cleaned as needed.
 2. When preventive measures are performed, they should be recorded on the Dry Bath/Thermomixer Quality Control Record (LAB-CO-53). Completion of LAB-405 and LAB-408b is not required.
- G. Any adjustment of internal thermometers will be recorded on the Dry Bath/Thermomixer Quality Control Record (LAB-CO-53).



4 Non-Significant Equipment

4.1 Centrifuges

- A. Centrifuges are not to be operated if they have not been installed properly, have been partly dismantled, or the rotor is not installed securely on the rotor shaft.
- B. Centrifuge housing, rotor chamber, and rotor accessories should be cleaned as necessary.
- C. There is no scheduled maintenance for centrifuges.

4.2 Refrigerator/Freezer

- A. Refrigerators/freezers used in the laboratory should be capable of maintaining the optimum temperature range required for storing reagents and samples.
- B. Quality Control Procedure
 - 1. Required ranges are indicated on the Temperature Verification Log (LAB-CO-44).
 - a) *For storage of reagents, set points on refrigerator and freezer equipment will be adjusted to maintain temperatures within the required ranges designated as:*
 - i. *Refrigerators – between 2 and 10 oC*
 - ii. *Freezers – between -30 and -10 oC*
 - b) *For storage of samples, set points on refrigerator and freezer equipment will be adjusted to maintain temperatures within the ranges designated as:*
 - i. *Refrigerators – between 1 and 15 oC*
 - ii. *Freezers – at or below 0 oC*
 - 2. Each unit will be equipped with a thermometer that is within current certification of NIST Calibration.
- C. Refrigerators/freezers are observed during working hours and regular access to ascertain that they are functioning within the required ranges.
- D. Weekly records of temperature readings are maintained using the Temperature Verification Log (LAB-CO-44). Temperature readings are documented using the min/max recordings.
- E. If the min/max readout for a unit is found to be outside of the acceptable range, document the current temperature reading in the comments section. If the current temperature is within the required range, no further action is needed.
- F. If the current temperature in a unit is found to be outside of the acceptable range (e.g., by observation or alarm):
 - 1. Verify:
 - a) *That the temperature set-point is at the proper setting and adjust if necessary*
 - b) *That sensors are not blocked and re-organize materials if overcrowded*
 - c) *If there has been a high frequency of recent access in and out of the unit or if a large quantity of material has been recently added to the unit*
 - 2. Re-check between 1 and 24 hours later. If temperature is within the required range, no further action is needed.



3. If the temperature is still out of range, notify Technical Leader and/or Section Manager.
 - a) *Determine whether contents require transfer to another unit. Comment in the Temperature Verification Log (LAB-CO-44).*
 - b) *Any corrective maintenance performed must be documented identifying the individual/company who performed the maintenance, date performed, and the nature of the maintenance. This documentation will be filed with weekly reading logs.*
 - c) *If the condition may have affected sample or reagent integrity or if there are recurring problems, then a Quality Incident will be initiated.*

4.3 Biohazard Safety Cabinets (PCR Setup)

- A. These are hoods or cabinets that do not vent to the outside and have HEPA filters.
- B. Quality Control Procedure
 1. The hood must be re-certified at least annually, or at the operator's discretion, by an external vendor.
 2. All repair, maintenance, and certification/calibration documents will be retained.
- C. Maintenance Procedure
 1. There is no scheduled maintenance for biohazard safety cabinets.
 2. Decontamination using a bleach solution should be done before or after use or if a spill occurs that may contribute to contamination.
 3. Decontamination records are maintained using the Biohazard Safety Cabinet Cleaning Log (LAB-CO-45).

4.4 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 for use with dry baths or thermomixers during DNA extraction.
- B. NIST-traceable electronic thermometers with a certification are used in the CODIS laboratory. Documentation of their certification will be maintained.
- C. A NIST-traceable thermometer certified for two years that is not used for conducting performance checks does not require the annual performance checks and may be used until the certification expires.

4.5 Water Purification System

- A. The water purification system ensures water purity of greater than 15 megohm-cm at all times. See the manufacturer's guidelines for a description of the individual system components.
- B. Quality Control Procedure
 1. Scheduled maintenance of the system consists of yearly replacement of the activated carbon prefilter capsule, the deionization (DI) module, the UV sterilizer, and the final filter capsule. The Ultrafilter is replaced every two years.



2. Yearly scheduled maintenance is documented using the Solution 2000 Filter Replacement Log (LAB-CO-46).

C. Maintenance Procedure

All unscheduled maintenance should be performed by a qualified service technician.

5 Records

Equipment Log (LAB-405)

Performance Verification (LAB-408b)

Equipment Out of Service (LAB-410)

Quality Control for CE Instrument (LAB-CO-22)

Quality Control for Robotic Workstations (LAB-CO-25)

Temperature Verification Log (LAB-CO-44)

Biohazard Safety Cabinet Cleaning Log (LAB-CO-45)

Solution 2000 Filter Replacement Log (LAB-CO-46)

9700 Thermal Cycler Quality Control Record (LAB-CO-51)

Veriti Thermal Cycler Quality Control Record (LAB-CO-52)

Dry Bath/Thermomixer Quality Control Record (LAB-CO-53)

6 Literature Reference and Supporting Documentation

Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started and Maintenance Guide (current version).

Applied Biosystems 3500/3500xl Genetic Analyzer User Guide (current version).

Applied Biosystems Veriti Thermalcycler User Guide (current version).

EZ1 Advanced XL User Manual (current version).

GeneAmp PCR System 9700 User's Manual, Applied Biosystems (current version).

Tecan Application Manual HID EVOLution (current version).

Tecan Freedom Evo Operating Manual (current version).

Multidrop DW Manual (current version).

BSD 600 Duet Manual (current version).

QIASymphony User Manual (current version).



CO-02-07 CODIS RECORDS

1 Scope

These policies are established as minimum requirements for documentation and record keeping in the CODIS laboratory. CODIS records are retained according to the Texas Department of Public Safety Records Retention Schedule. Destruction of any records not listed in the approved schedule requires special authorization from the Texas State Library and Archives Commission (TSLAC). Quality control measures are put into practice to ensure that information in STaCS is being entered into the system accurately.

2 Submission Records

Submission records begin when the sample is entered into STaCS at data entry (login date). Submission records may include personally identifiable information, collection date, current status, and storage information. Submission records are maintained in STaCS.

3 Technical Records

- A. Technical records are maintained in STaCS. Analysis Forms (LAB-CO-05) and supporting plate information (if applicable) are documented and maintained in hard copy format. Raw analytical data is archived in electronic format.
- B. Technical records for Profile Runs begin when samples are designated in STaCS as In Process after AFIS Verification and end after Analysis Review.
- C. Technical records for QA/QC Runs begin when samples are initiated in the "QA/QC Runs" module in STaCS and end after Analysis Review.
- D. Technical records for Hit Confirmation Runs begin when samples are initiated in the "Hit Confirmation" module in STaCS and end after Analysis Review.

4 Match Verification Records

- A. Match verification records are identified by the unique CODIS number associated with the match.
- B. Administrative records are maintained in the match verification folder or in STaCS.
- C. Hit Confirmation technical records are maintained in STaCS. Raw analytical data is archived in electronic format.

5 Familial Search Records

- A. Familial Search records are identified by the date the familial search is initiated (e.g. FS-MMDDYY).
- B. The Familial Search Document Checksheet (LAB-CO-23) lists the specific documents required in the batch and maintained in hard copy format.
- C. Technical records are maintained in STaCS. Raw analytical data is archived in electronic format.



CO-02-08 CRITICAL REAGENTS

1 Scope

In order to maintain the quality of work performed, 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 database samples. This testing ensures that reagents are reliable and are suitable for use in the laboratory and that there is no unnecessary loss of sample.

2 Related Chapters/Documents

Analytical Controls

Data Interpretation Guidelines

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 stated guidelines, it may not be utilized in database typing.
 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 database typing.
- C. Expired reagents with acceptable quality control test results may be used for training purposes only. Such reagents must clearly be marked "Expired" or "training".

3.2 List of Critical Reagents

The following are considered critical reagents:

- Amplification test kits
- Allelic Ladder (individually purchased)

3.3 Quality Control

- A. Each new amplification test kit lot is subjected to an internal quality control test prior to use. At a minimum, a positive control and an amplification negative control are amplified with the amplification test kit. All components of the amplification test kit are used to demonstrate the efficacy of the kit.
- B. Individually purchased ladder is subjected to an internal quality control test prior to use. At a minimum, a positive and negative control will be run with the allelic ladder.

4 Interpretation

- A. The positive amplification control indicates the activity of the critical reagent. Acceptable performance is indicated if a complete profile with the expected alleles, as listed in the *Analytical Controls* chapter, is obtained.



- B. The negative amplification control indicates the purity of the critical reagent. Acceptable performance is indicated if no contamination, as defined in the *Analytical Controls* chapter, is apparent.
- C. The allelic ladder must be analyzed. Acceptable performance is indicated if all of the appropriate alleles are detected.
- D. Results must conform to the *Data Interpretation Guidelines* chapter.

5 Records

- A. A record shall be maintained for the quality control of all critical reagents.
- B. The record shall consist of the following worksheet(s), as relevant to the reagent being tested, along with the supporting documentation necessary to demonstrate the reagent met the relevant guidelines as listed above:
 1. Amplification Kit Reagent Quality Control (LAB-CO-21)
 2. Inventory, lot records, and QA/QC records are maintained in STaCS.



CO-02-09 SAMPLE CONTROL

1 Scope

The integrity, preservation, and security of databasing samples submitted to the Texas DPS CODIS Laboratory are of the utmost importance. This document includes policies and procedures for the identification, inventory control, storage, security, and retention of databasing samples.

2 Related Documents

CLS Manual: Evidence and Database Sample Integrity

3 Sample Integrity

3.1 Sample Identification

- A. Acceptable CODIS samples are assigned a unique CODIS number (barcode) upon receipt from the submitting agency.
- B. The CODIS number is affixed to the sample and the DNA Database Card.

3.2 Sample Inventory Control

- A. Sample information, storage location, and current status are tracked and documented through STaCS.
- B. Sample submission information is entered into STaCS at Data Entry. Records include, but are not limited to, CODIS number, receipt date (login date), personally identifiable information (PII), collection date, contributor type (specimen category), and storage location. This information may be retrieved in the Submission module or Submission History Report.
- C. Submission Audit History reports list changes to sample submission information post Data Entry along with current status information.
- D. Sample History reports contain processing records which include analytical procedures performed, date performed, analyst who performed task, plate information (which is uniquely identified based on the analytical procedure performed), well position, equipment used, reagents used, sample storage location, CODIS DNA profile, and current status.

3.3 Sample Storage

- A. The laboratory takes measures to protect samples under its control from deterioration, loss, damage, cross-transfer, or contamination.
- B. Databasing samples are located in the Sample Receiving Room, File Room, and walk-in freezers.
 1. Collection kits, blood tubes, and buccal swabs waiting to be processed are located in the Sample Receiving Room.
 - a) *Blood tubes shall be refrigerated upon receipt.*
 - b) *Blood tubes are stored in numerical order in labeled blood tube racks. The rack identifier (BTR#-###) and rack position are documented in STaCS.*
 - c) *Buccal swabs shall be stored at room temperature. The swab envelope must be securely sealed.*
 - d) *Buccal swabs are stored in numerical order in labeled storage boxes. The storage box identifier (rack identifier - SWB#-###) is documented in STaCS.*



2. FTA blood archive samples, long-term storage (LTS) plates, and buccal archive samples are stored in the File Room.
 - a) *Swabs, LTS plates, and archive cards are securely sealed in envelopes.*
 - b) *FTA and buccal samples are arranged in numerical order.*
 - c) *LTS plates are arranged by batch number.*
 - d) *Samples are stored at room temperature.*
3. Samples prior to CODIS number 309000 (including non-FTA blood archive cards and a few FTA blood archive cards and buccal archive swabs) are stored in the walk-in freezers.
 - a) *Archive cards and buccal swabs are securely sealed in envelopes.*
 - b) *Samples are arranged in numerical order.*
 - c) *Samples are stored at or below freezing (°C).*
4. DNA sample extracts and buccal daughter plates are not considered evidence, but will be retained frozen by the laboratory whenever possible.
5. Amplification products and buccal lysis plates are considered work product and not considered evidence. These items should be properly discarded after sample status verification has been completed.
6. When samples are stored under refrigerated or frozen conditions, the storage location conditions are logged on the Temperature Verification Log (LAB-CO-44).

3.4 Sample Handling

- A. Databasing samples are rejected upon receipt if the kit seal on the exterior box/envelope is missing or damaged.
- B. During processing, samples must be kept in a manner that protects them from loss, deterioration, and contamination. This includes but is not limited to:
 1. Ensuring FTA blood archive cards are thoroughly dried before inserting into archive envelopes.
 2. Ensuring samples are properly sealed. This includes envelopes, plates, and tubes.
 3. Ensuring samples are stored within appropriate temperature ranges.
 4. Whenever possible, handling samples one at a time to prevent cross-contamination.
- C. Analysts should make a diligent effort to complete examinations within a reasonable amount of time and shall properly seal and store samples when testing is complete.

3.5 Sample Security

- A. The laboratory is designed to maintain the integrity of databasing samples throughout processing by controlling unauthorized access to the operational laboratory and sample storage areas.
- B. Security, limited authorized access, and system-wide facilities requirements are described in the CLS Manual.
- C. Only authorized personnel are allowed in sample storage areas.



3.6 Sample Retention

- A. All acceptable database samples will have a portion retained indefinitely or until consumed.
- B. The archived sample will be used for hit confirmations, familial searches, quality assurance purposes, validations, and for future testing when new technologies are available, unless prohibited by law.
 1. A sample will only be consumed to obtain a complete and searchable profile as per NDIS Procedures. The Technical Leader will be notified prior to sample depletion for approval. Documentation of sample depletion will be maintained in STaCS.
 2. An attempt will be made to recollect the sample if the subject is still under supervision.

4 Records

Submission and processing records are maintained in STaCS.

5 Literature References and Supporting Documentation

STaCS Manual



CO-02-10 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, amplification negative controls, and amplification positive controls.

2 Related Documents

CLS Manual: Laboratory Equipment

CLS Manual: Standards, Reference Materials/Collections, Databases, and Controls

3 Analytical Controls

3.1 Extraction Controls

A. Reagent Blank (Negative in STaCS)

1. Reagent blanks will be used appropriately when using direct amplification kits (i.e. Blood samples will be amplified with a combined reagent blank and amplification negative control).
2. A reagent blank will be prepared and concurrently extracted 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 DNA. It will be handled in such a way that it will detect contamination from extraction to final analysis.
3. A reagent blank will be amplified using the same primers, the same thermal cycler model, and the most sensitive volume conditions as the samples.
4. If samples require amplification using a second primer set, the associated reagent blank must be amplified with this set. If the reagent blank has been depleted or does not have sufficient volume remaining, then the set of samples must be re-extracted with a new reagent blank.
5. The reagent blank shall be typed utilizing the same instrument model and most sensitive injection and volume conditions of the extraction set.
6. If the reagent blank fails due to a sequencer problem, it may be re-injected or the plate will be set up again.
7. The reagent blank should not exhibit a DNA profile above the analytical threshold. Multiple labeled allelic peaks will result in the failure of the reagent blank.
 - a) *Analysts should determine if labeled peaks are true alleles. Peaks such as known amplification artifacts are not cause for rejection but should be documented.*
 - b) *It is not unusual to encounter low levels of contamination due to carry-over/cross-talk. Carry-over/cross-talk is not cause for rejection but must be documented either in STaCS or on the Analysis Form.*
 - c) *Analysts should use their best effort and judgment to determine the source of the contaminant and document their observations on the Analysis Form and/or in STaCS. If evidence of a possible DNA profile is present, refer to Response to Quality Issues chapter in the CODIS SOP.*



- d) *The analyst may consult with the technical leader or section supervisor for guidance.*

3.2 Amplification Controls

A. Amplification Negative Control (Ampblank in STaCS)

1. The amplification negative control contains all amplification reagents without the addition of template DNA.
2. The amplification negative control detects contamination during the amplification procedure.
3. The amplification negative control is amplified concurrently using the same thermal cycler model and is typed with the same primer set as the database samples.
4. If the amplification negative control fails due to a sequencer problem, it may be re-injected or the plate will be set up again.
5. The amplification negative control should not exhibit a DNA profile above the analytical threshold. Multiple labeled allelic peaks will result in the failure of the amplification negative control.
 - a) *Analysts should determine if labeled peaks are from true alleles. Peaks such as known amplification artifacts are not cause for rejection but should be documented.*
 - b) *It is not unusual to encounter low levels of contamination due to carry-over/cross-talk. Carry-over/cross-talk is not cause for rejection but must be documented either in STaCS or on the Analysis Form.*
 - c) *Analysts should use their best effort and judgment to determine the source of the contaminant and document their observations on the Analysis Form. If evidence of DNA is present, refer to Response to Quality Issues chapter in the CODIS SOP.*

B. Amplification Positive Control

1. The positive control tests for proper amplification of samples and ensures the genotyping software is working properly.
2. Off-scale data is acceptable as long as it exhibits the appropriate allele calls.
3. The positive control is amplified concurrently using the same thermal cycler model and is typed with the same primer set as the database samples.
4. At least one positive control in the amplification set must exhibit a complete profile. If all positive controls fail in the amplification set, the corresponding samples must be re-amplified.
5. If the positive control fails due to a sequencer problem, it may be re-injected using the same initial injection time or set up again.
6. The positive control must exhibit the following appropriate DNA profile:



Investigator 24plex GO!

Amel	TH01	D3S1358	vWA	D21S11	TPOX	DYS391	D1S1656
X,Y	6,9.3	15,17	17,17	29,30	8,9	10	14,17
D12S391	SE33	D10S1248	D22S1045	D19S433	D8S1179	D2S1338	D2S441
18,24	23.2,26.2	12,15	16,18	13,14	12,13	23,23	11,12
D18S51	FGA	D16S539	CSF1PO	D13S317	D5S818	D7S820	
15,18	24,26	11,11	10,11	11,11	11,13	11,11	

GlobalFiler Express

D3S1358	vWA	D16S539	CSF1PO	TPOX	Yindel	Amel	D8S1179
15,16	14,16	9,10	11,12	8, 8	2	X,Y	12,13
D21S11	D18S51	DYS391	D2S441	D19S433	TH01	FGA	D22S1045
28,31	12,15	11	14,15	14,15	7,9.3	24,26	11,16
D5S818	D13S317	D7S820	SE33	D10S1248	D1S1656	D12S391	D2S1338
11, 11	11, 11	7,12	17,25.2	12,15	13,16	18,19	20,23

Yfiler Plus

DYS576	DYS389I	DYS635	DYS389II	DYS627	DYS460	DYS458	DYS19
19	13	24	29	21	11	17	15
YGATAH4	DYS448	DYS391	DYS456	DYS390	DYS438	DYS392	DYS518
13	19	11	15	24	12	13	37
DYS570	DYS437	DYS385a/b	DYS449	DYS393	DYS439	DYS481	DYF387S1
17	15	11,14	30	13	12	22	35,37
DYS533							
13							

7. The positive control is considered acceptable as long as the profile contains the appropriate allele calls.
8. If the positive control is non-concordant, notify the Technical Leader or Section Supervisor for assistance in resolution.

4 Investigator 24plex GO! Quality Sensors

- A. The Investigator 24plex GO! primer set includes two internal PCR quality controls which help identify the amplification efficacy.
- B. Interpreting Quality Sensor results:

Allele Peaks	QS1	QS2	Interpretation
Present	Present	Present	Successful Profile
Absent	Present	Present	No DNA
Absent	Absent	Absent	Failed PCR
Ski-Slope Profile	Present	Dropdown	Inhibitors Present
Ski-Slope Profile	Present	Present	Degraded DNA



- C. A dropdown of the QS2 signal below 20% of the QS1 signal indicates inhibition of the PCR reaction.
- D. Failed quality sensors are not cause for rejection provided the profile meets data interpretation guidelines.



03 SAMPLE HANDLING

CO-03-01 SAMPLE COLLECTION AND HANDLING

1 Scope

The policy provides direction to CODIS personnel on how to properly accession, evaluate for acceptance, and store database samples for inclusion into CODIS.

2 Related Documents:

CLS Manual: CODIS DNA Procedural Guidelines

3 Safety

Wear personal protective clothing while processing kits, including gloves and a lab coat.

4 Sample Collection

- A. Database samples are, by law, obtained for the purpose of identification in order to provide investigative leads. As such, CODIS specimens are considered reference samples.
- B. No database sample shall be analyzed for use as substantive evidence in the prosecution of an offense, as a substitute for a legally obtained casework known reference sample, or for any other purpose other than identification, unless otherwise permitted by State or Federal law.

4.2 Mandatory Collection

DNA database samples are collected in accordance with applicable State laws. All samples shall be collected following the *CODIS DNA Procedural Guidelines* chapter of the CLS Manual. Only with the approval of the CODIS Program Manager and/or the CODIS Interagency Liaison, will a sample be accepted without meeting all of the requirements listed in the *CODIS DNA Procedural Guidelines*.

4.3 Voluntary Submission

Under Texas Government Code Title 4 Subtitle B Chapter 411 Subchapter G §411.149 – Voluntary DNA Record, an individual may at any time voluntarily provide or cause to be provided to a criminal justice agency a sample to be forwarded to the Texas Department of Public Safety for the purpose of creating a DNA record.

5 DNA Collection Kit Shipments

- A. The DNA Database blood and buccal swab collection kits are obtained through the DPS CODIS lab.
- B. Orders for blood and/or buccal swab collection kits may be requested using the CODIS Collection Kit Order Form (LAB-CO-08) or email.
 1. Give request documentation to CODIS Interagency Liaison or designee for order verification. Once approved, the CODIS Interagency Liaison will create a list of kit orders needing to be processed.
 2. Generate a mailing label.
 3. Prepare a packing slip letter for the shipment. Be sure to use the correct packing slip.



4. Pack the requested items and the packing slip into a box for shipment and seal. Affix label and place box(es) for mail pickup.
5. Initial and date the kit order list and return to the CODIS Interagency Liaison.
6. The CODIS Interagency Liaison or designee will enter the request and shipment data into a tracking file.

6 Sample Receiving and Storage

6.1 Fingerprints

Inked fingerprints on DNA Database Cards submitted with CODIS samples are verified by the AFIS section. See the **AFIS Verification** section for policies and procedures regarding the verification process.

6.2 Blood

A. General Information

1. When convicted offender samples are received, the laboratory technicians process the blood tubes and DNA Database Cards.
 - a) *The blood tubes are cataloged until analysis can be conducted.*
 - b) *The DNA Database Cards are forwarded to the AFIS section for verification unless rejected. **Rejects shall have the rejection reason, technician's initials, and date documented in the upper left corner of the DNA Database Card.***
2. The CODIS Interagency Liaison has the ability to override rejections in situations where discrepancies with identification information (e.g. SID number, date of birth, submission of fingerprints) supplied by the submitting agency can be reconciled.

B. Creating Storage Unit label (STaCS Supervisor only)

1. Log in at the appropriate workstation. Double click on STaCs icon.
2. On the Group Bar under the Main tab, select the "Storage Subsystem" module. Select "Manage Storage Units" button.
3. Enter Storage Type (BTR# - ###) and the appropriate number of labels. Click "Print", then "Close".

C. Printing CODIS Barcode Labels

1. Log in at the appropriate workstation. Double click on the STaCS icon.
2. On the Group Bar under the Main tab, select the "Submission Bar Code" module. Enter the quantity of unique barcodes (CODIS numbers) needed and the quantity of copies of that unique barcode. Click "Create", then "Close".

D. Processing Blood Kits

1. Process kits one at a time.
2. Check seal for integrity. If unsealed or seal is broken, reject the sample and place tube into rejects tray. File the DNA Database Card with the CODIS Interagency Liaison.



3. Break the seal and remove the contents of a kit.
 - a) *Check the name and/or SID # on the tube to ensure that it matches the DNA Database Card.*
 - b) *If tube is wrong type (must be purple top), less than 0.5 cm, unlabeled, or did not match the DNA Database Card, reject the sample. File the DNA Database Card with the CODIS Interagency Liaison.*
 - c) ***If the blood volume is between 0.5 - 2.0 cm, label the card with "Low Blood Volume" on the upper left hand corner.***
4. If blood contaminated items are present within the kit, dispose of in an appropriate biohazard container. If there are bloodstains on the DNA Database Card, cover the area with clear tape while avoiding the fingerprints. Dispose of other kit components in regular trash.
5. **Check the kit for fingerprints**, either printed on the DNA Database Card or on a ten-print card or other CODIS Interagency Liaison approved document intended for use for fingerprint identification. If there are no fingerprints included in the kit, reject the sample. File the DNA Database Card with the CODIS Interagency Liaison.
6. If the DNA Database Card is incomplete, AFIS can still verify the card if the name and DOB are included (even if the SID # is not included).
 - a) ***Do not reject samples due to lack of SID numbers.***
 - b) *If the SID # is not listed on the data card but is listed on the blood tube, transfer the SID # to the datacard and initial and date.*
7. If information is insufficient, the CODIS Interagency Liaison will contact the agency for required information and document changes on the DNA Database Card. If several samples from the same agency are lacking the SID #'s, contact the agency and request that the SID #'s for the future samples be included on the DNA Database Card.
8. If collection was correct, place the matching CODIS number (barcode label) on both the blood tube and DNA Database Card. Place one Storage Unit barcode label (BTR# - ###) on an empty blood tube rack and the second label on the back of the first DNA Database Card of the set. Place the tube in the next sequential slot on the labeled blood tube rack.
9. Court orders and **any** paperwork submitted with the kit must be labeled with the CODIS number. In addition, the top left-hand corner of the DNA Database Card should be stamped indicating additional paperwork was received with the kit.
 - a) *The court orders and paperwork will be scanned and stored electronically by the CODIS number.*
 - b) *Original paper copies will be shredded after verification that the scanned documentation is readable and of acceptable quality.*
10. When finished, verify that there is a DNA Database Card for every sample and that no cards or samples are missing. Store all blood tubes in refrigerator.



6.3 Buccal

A. General Information

1. When buccal swab database samples are received, the laboratory technician will process the swabs and DNA Database Cards.
 - a) *The swabs are cataloged until analysis can be conducted.*
 - b) *The DNA Database Cards are forwarded to the AFIS section for verification unless rejected. **Rejects shall have the rejection reason, technician's initials, and date documented in the upper left corner of the DNA Database Card.***
2. The CODIS Interagency Liaison has the ability to override rejections in situations where discrepancies with identification information (e.g. SID number, date of birth, submission of fingerprints) supplied by the submitting agency can be reconciled.

B. Creating Storage Unit label (**STaCS Supervisor ONLY**)

1. Log in the appropriate workstation. Double click on STaCs icon.
2. On the Group Bar under the Main tab, select the "Storage Subsystem" module. Select "Manage Storage Units" button. Enter Storage Type (SWB# - ####) and appropriate number of labels. Click "Print", then "Close".

C. Printing CODIS Barcode labels

1. Log in the appropriate workstation. Double click on the STaCS icon.
2. On the Group Bar under the Main tab, select the "Submission Bar Code" module. Enter the quantity of unique barcodes needed (CODIS numbers needed) and the quantity of copies of that unique barcode. Click "Create", then "Close".

D. Processing Buccal Kits

1. Process kits one at a time.
2. Check seal for integrity. If unsealed or seal is broken, reject the sample. Store the rejected swab envelope in the "Rejected Buccal Samples" box. File DNA Database Card with the CODIS Interagency Liaison.
3. Break the seal and remove the contents of a kit.
 - a) *Check seal of inside envelope. If the swab envelope is unsealed, reject the sample.*
 - b) *Check the name, SID #, and/or date of birth on the swab envelope to ensure that it matches the DNA Database Card.*
 - c) *If the swab envelope does not match the DNA Database Card, or is blank, reject the sample. If the swab envelope is blank, discard into the appropriate biohazard container. File DNA Database Card with the CODIS Interagency Liaison.*
4. **Check the kit for fingerprints**, either printed on the DNA Database Card or on a ten-print card or other CODIS Interagency Liaison approved document intended for use for fingerprint identification.
 - a) *If fingerprints are included in the form of a separate document and if the agency listed on the DNA Database Card or fingerprint documentation is an arresting agency, stamp the top left of the card with 'HB 1399'. Paperclip fingerprint documentation to the database card as securely as possible to prevent the fingerprint card from getting lost.*



- b) If there are no fingerprints included in the kit, reject the sample. File the DNA Database Card with the CODIS Interagency Liaison.*
5. **Check to see if there are two swab samples.** If not, reject the sample. If the DNA Database Card is inside the swab envelope, open the swab envelope to remove the card. Seal the envelope with appropriate lab tape.
6. If the collection was completed correctly, place the matching CODIS number (barcode label) on both the DNA Database Card and the swab envelope. Place one Storage Unit barcode label on an empty buccal box and the second on the back of the first DNA Database Card of the set.
7. Place swab envelope into a labeled storage box (SWB# - ###).
 - a) Place the labeled swab envelopes in sequential order in the labeled buccal box.*
 - b) Store all samples in the mobile filing system in the kit opening room keeping the storage boxes consecutive.*
 - c) When finished, verify that there is a DNA Database Card for every sample and that no cards or samples are missing.*
8. Court orders and **any** paperwork submitted with the kit must be labeled with the CODIS number. In addition, the top left-hand corner of the DNA Database Card should be stamped indicating additional paperwork was received with the kit.
 - a) The court orders and paperwork will be scanned and stored electronically by the CODIS number.*
 - b) Original paper copies will be shredded after verification that the scanned documentation is readable and of acceptable quality.*

7 Recording DNA Database Card Information

7.1 Data Entry

1. Log in at the appropriate workstation. Double click the STaCS icon. On the Group Bar, under the Main tab, click on the “Data Entry” module.
2. Scan the CODIS barcode on the first card to be entered. Scan the Storage Unit barcode. For Buccal samples, select appropriate Sample Nature before scanning Storage Unit barcode.
3. Enter the subject’s information from the DNA Database Card. You cannot save the record unless the yellow highlighted fields (Last Name, SID #, Gender, Agency, Sample Nature, and Contributor Type) are populated. **Note: Alternate names for Community Supervision and Correction Departments (CSCDs) are often used on DNA Database Cards.**
4. If the SID number is invalid, enter “00000000” for the SID number.
5. If the DNA Database Card is marked with “Low Blood Volume,” mark the sample “Yes” under “Problem Kit” with “Low Blood Volume” as the reason. If the DNA Database Card is marked with ‘HB 1399’, select “HB 1399” under “Qualifying Reason”.



6. Check to ensure the Data Entry record and DNA Database Card match. Click “Save” first, then “Next” to go to the next sample to be entered. For Blood samples, this also brings up the next available Rack Position. **Note: Gender, Race, Sample Nature, and Contributor Type fields will automatically carry over to the next record. Make any corrections needed for the following sample.**

7.2 Rejects

1. Log in at the appropriate workstation. Double click on the STaCS icon. On the Group Bar, under the Main tab, click the “Data Entry” module.
2. Click the “Reject” button. Look up the subject’s SID # (or name and DOB) in CCH and use the information from CCH to populate the appropriate fields. If the subject is not in CCH, enter the information from the DNA Database Card. Under the Redraw/Reject field, select the appropriate reason for rejection from the drop-down. The record cannot be saved unless the yellow highlighted fields (last name, SID, Sample Nature, Gender, Agency, and Contributor Type) are populated.
3. Verify all information is correct before clicking Save, since errors cannot be changed once the record is saved. Click “Save”. Once the submission is saved, STaCS performs the Duplicate Check as described in the AFIS Verification Worksheet section with the only exception that it is not possible to list the sample as a Potential Duplicate. Duplicates made through this module will set the Submission Status to “Duplicate” and the Redraw Status to “Rejected” for the rejected sample; the sample will not be placed on Redraw Management.

7.3 Redraws

Texas Administrative Code Title 37 Part 1 Chapter 28 Subchapter H Rule § 28.126 requires the laboratory to notify the submitting agency if a sample is rejected.

1. Log in at the appropriate workstation. Double click on the STaCS icon. On the Group Bar under the Main tab, click on the “Redraw Management” module.
2. Select the sample that needs a redraw letter issued to the collector of the sample.
3. Verify the agency, contact, and method information is correct. If needed, add a new contact by clicking on the box with the ellipsis.
4. Click on the “Issue Request” button. Once the letter opens, print the letter if using the letter for a fax or email.
5. Fax letter to the collector/collecting agency. If the fax fails to send, delete the request by highlighting the request and clicking “Delete Request”. Change the method to a method other than Fax and click “Issue Request” once the notification is successfully made to the agency.

8 AFIS Verification

- A. AFIS Verification consists of an AFIS Send and an AFIS Verification module.
- B. Once the DNA Database Cards have been entered into STaCS and are sent out to AFIS, the submissions are placed onto the AFIS Verification worklist. After verification, the submissions are sent for lab processing.
- C. All submissions received into the CODIS Laboratory must be sent out for AFIS verification prior to processing. Submissions are not processed until they are AFIS verified.



8.2 AFIS Send Worklist

- A. Once a submission is entered into the STaCS system and saved via the Data Entry module, it is placed on the AFIS Send worklist.
- B. If a submission is placed on the Redraw Management worklist, it will not be placed on the AFIS Send worklist. However, if the submission is placed on the Problem Kit worklist, it is also placed on the AFIS Send worklist.
- C. To generate the AFIS Send File
 1. In the Group Bar under Main tab, click “AFIS Send” module to display the AFIS Send worklist.
 2. Select only the entries that you want to forward to AFIS.
 3. Click “Create” to generate and save the AFIS Send file. The file is located in **M:\AFIS File** folder. The file is named **INTAKE.DBF**. Click “Close”.
 4. Transfer the file to a flash drive. The database file is uploaded manually to the AFIS scanning computers or appropriate network location.
 5. All accepted DNA Database Cards from kit opening go to AFIS for verification.

8.3 AFIS Verification Worklist

1. To update submission records after AFIS verification, log in the appropriate workstation. Double click on the STaCS icon. On the Group Bar, under the Main tab, click “AFIS Verification” module to display the AFIS Verification worklist.
2. Select the submission entries from the list and click “Add” to transfer them to the AFIS Verification Personal List.
3. Click “Edit” to begin verifying the list of submissions.
4. **Check each card individually.** At this time, any changes made by AFIS should be corrected in the computer and **each SID number should be checked. Include suffixes and hyphens** if they are included on the AFIS worklist or CCH printout.
5. The **FBI Number** needs to be entered if a CCH print-out is stapled to the DNA Database card. Otherwise, this field can be left blank.
6. If the **Qualifying Reason** field is blank but the card has “HB 1399” stamped on the card, change this field to “HB 1399”
7. **Check the Problem Kit field** to see if the submission was put on the Problem Kit worklist. Change the “Yes” to “No” in the dropdown if the reason was corrected through verification. If the submission is marked “Low Blood Volume,” file the DNA Database Card with the CODIS Interagency Liaison.
8. If AFIS rejects the submission, send the submission to either the Redraw Management worklist or Problem Kit worklist by changing either requirement field as applicable from “No” to “Yes” and supplying a reason.
 - a) *For cards rejected for quality of prints by AFIS, choose “Yes” under “Redraw Required” and under “Reason”, choose “AFIS Rejected Due to Quality of Fingerprints” and then click “Save”. File DNA Database Card with CODIS Interagency Liaison.*



- b) For cards that are marked “No Record”, under “Problem Kit”, choose “Yes” and “Could not confirm identity”. Periodically, these cards will be checked for SID assignments. The SID #s for new offenders may take several months before they are entered into the DPS system.
 - c) For cards marked “AFIS Reject”, under “Problem Kit”, choose “Yes” and “AFIS Reject”. File DNA Database Card with CODIS Interagency Liaison.
 - d) For cards marked “No Match”, under “Problem Kit”, choose “Yes” and “No Match”. Do not select “No Match” under “Redraw”. File DNA Database Card with CODIS Interagency Liaison.
9. For buccal DNA Database Cards marked **Registered Sex Offender**, or **Convicted** change the “Contributor Type” from **Arrestee** to **Convicted Offender** from the drop down selection. In addition, the following agencies also require the “Contributor Type” changed from **Arrestee** to **Convicted Offender: TDCJ Parole, TJJD, DPO, RPD-DNA, ELIM, ELIM for TDCJ and DPO Parole**.
10. After all information is verified and updated, click “Save”. Once the submission is saved, STaCS performs the Duplicate Check, marks the submission as Print Verified, removes the submission from the AFIS Verification Personal list, and places the submission (CODIS number) onto the Plate Create worklist or Blood Spotting worklist.

Note: The submission (CODIS Number) is not placed on the Plate Creation worklist or Blood Spotting worklist if it is on either the Redraw Management or Problem Kit worklists or is marked as a Confirmed Duplicate.

8.4 Duplicates

- A. A duplicate check is initiated by the database once the "Save" button is clicked while in the AFIS Verification module. By default, submissions marked as “Confirmed Duplicates” are not processed in the lab.
- B. STaCS performs a duplicate check based on the SID or a combination of Last Name and Date of Birth. One of the values on the submissions being checked as duplicates must match. Therefore a duplicate check match may be as a result of one of the following occurrences:
 - 1. The SID matches between two or more submissions,
 - 2. The SIDs do not match but the Last Names and the Birth Dates are a match, or
 - 3. Both conditions.
- C. If one or more matches are found, the “Duplicate Check” form is displayed with all potential duplicates. Compare the displayed potential duplicates (submission matches), under the bold face original record, to the search conditions on the left hand side of the window.
- D. If it is determined the record is **NOT** a duplicate, click the "Close" button while in the duplicate check module. The module will close and the record will be saved as an original submission. Do NOT click “Save” again or the duplicate check module will reopen. Click “Next” to move to the next record.
- E. If the record is a duplicate, select the original submission or master duplicate from the duplicate list. Check the "Submission Match" box, and click “Save”. The Current Status will change from “AFIS Pending” to “Duplicate”.



- F. If it cannot be determined whether the record is a duplicate, click on "Potential Submission Match" and click "Save". The record will be placed on the Potential Duplicates Worklist where it will later be verified by the CODIS Interagency Liaison. Database Cards from potential duplicates are given to the CODIS Interagency Liaison.

9 Storage

Store cards sequentially by CODIS number in the mobile filing system.

10 Literature References and Supporting Documentation

Texas Administrative Code Title 37 Part 1 Chapter 28 Subchapter H Rule §28.123 – Eligible Individual

Texas Administrative Code Title 37 Part 1 Chapter 28 Subchapter H Rule §28.125 – Collection of Sample

Texas Administrative Code Title 37 Part 1 Chapter 28 Subchapter H Rule § 28.126 – Processing of Sample

Texas Government Code Title 4 Subtitle B Chapter 411 Subchapter G



CO-03-02 SAMPLE DESTRUCTION

1 Scope

The CODIS laboratory receives samples from many different sources. Space limitations require that only a portion of that sample be retained indefinitely either on an approved blood archive card or as an unused buccal swab portion. These procedures provide direction to CODIS personnel on how to properly destroy the unused portions of the sample collected once successful DNA typing analysis has been confirmed.

This does not include duplicate offender samples, which do not need to be retained, or mistakenly collected samples, which are destroyed when it is determined they are no longer needed or by specific court order.

2 Practice

2.1 Sample Expunction or Administrative Removal

- A. Texas Government Code Title 4 Subtitle B Chapter 411 Subchapter G Section §411.151 EXPUNCTION OR REMOVAL OF DNA RECORDS specifies that CODIS records shall be expunged if the DPS is notified in writing that the record has been court ordered to be expunged from the database. The person making the request must provide the DPS with a certified copy of the court order that expunges the record.
- B. There are times when a sample may have been inadvertently collected from an individual that is not required by statute to provide a sample. When this occurs, the agency having the sample taken must provide a formal request on agency letterhead specifying that the sample should be removed. Before removing the sample, a check of the offender's criminal history should be conducted to verify that there are no qualifying offenses. If an individual is determined to have a qualifying offense, notify the agency that has requested the sample destruction by giving them the offense information and that the sample cannot be destroyed.
- C. There may be other reasons determined by the CODIS Administrator(s) or CODIS Interagency Liaison for administrative removal of a sample such as (but not limited to) concern for the integrity of the sample or the data associated with that sample. The agency will be notified when a sample is administratively removed from CODIS.
- D. Perform the following procedure to administratively remove or expunge records.
 1. Open the STaCS database.
 2. On the Group Bar, go to **Main > Submission Removal**. Click **New**.
 3. On the New Submission Removal screen, accept the current date or select an alternate **Date of Request**.
 4. Choose an option from the **Removal Type** drop down list based on the type of removal desired. If needed, type a comment in the comment field. If the administrative removal or expunction was initiated by an outside agency, choose that agency in the drop down menu.
 5. Click **Search**. A Duplicate Check form will appear. Use this form to search for the sample you wish to remove using one of the fields present (such as CODIS number, etc.). Click **Search**. All matches that reflect the search condition(s) entered are displayed in the **Submission Matches** list.



6. Select a matching entry and select the **Submission Match** check box. Click **Save**. If the selected submission is a duplicate, a message is displayed to confirm if you want to remove all the related submissions (legal expunction) or just the selected submission (administrative removal). The duplicates to be included in the removal are displayed in the **Associated Submission** section of the form.
 7. Verify that the sample info (name, DOB, SID) matches the info on the destruction request/expunction order.
 8. For **Administrative Removal**, click **No** to remove only the selected submission.
 9. For **Legal Expunction**, click **Yes** to remove all related submissions. Once the removal is completed, STaCS removes all pertinent information from each duplicate submission included in the removal.
 10. Click **Save**. The selected submission is displayed on the **New Submission Form**. Any related submission CODIS numbers are also displayed if selected for removal.
 11. Follow the necessary items on the checklist click the completed steps. For **Legal Expunction**, check the boxes marked **Clear Personal Data** and **Include Associated Submissions**.
 12. Click **Yes** to complete the removal. Once completed, the selected submission(s) is removed from any worklist on which it is currently located. The **Removal Status** changes to **Confirmed**.
- E. For expunctions perform the following steps:
1. Retrieve the sample DNA Database Card(s).
 - a) *Verify that the CODIS number matches the one that was removed.*
 - b) *Verify that the identifying information from the DNA Database Card, the STaCS database, and the destruction request letter all match and are from the same individual.*
 2. Get a blank DNA Database Card for each of the sample(s) being removed.
 - a) *Print out a barcode label with the sample's CODIS number and affix to the blank DNA Database Card(s).*
 - b) *On the blank card under "Name:" write "Expunged" and the date the sample was expunged.*
 3. If the expunction also includes the sample, destroy the archive sample and liquid sample (if applicable).
 - a) *File the new blank DNA Database Card(s) in the proper place and destroy (shred) the sample DNA Database Card(s).*
 - b) *Notify the proper agency (see below) of the removal.*
 4. For removals initiated by an agency, write a letter to the appropriate authority stating that the sample and its corresponding data has been destroyed and or expunged.
 - a) *Fax the letter to the requesting authority.*
 - b) *Staple the signed letter and the fax verification printout to the removal request and file in the folder maintained by the CODIS Administrator or Liaison.*



5. If the sample was a court-ordered expunction, write a letter to the appropriate authority.
 - a) *The letter will state that the sample and its corresponding data have been destroyed and or expunged.*
 - b) *Following notification of the proper authority, the expunction order will be destroyed.*

Note: The following two steps are only to be performed by the CODIS Program Manager or their designee.

6. If the sample has been uploaded, open the CODIS software.
 - a) *Search for the specimen number.*
 - b) *Delete the specimen.*
7. If the specimen has been transferred to NDIS, additional steps will need to be taken as described in the NDIS Procedures Manual, including:
 - a) *Sending an upload to NDIS*
 - b) *Notifying the NDIS custodian to have the upload processed*
 - c) *Documenting verification the upload was processed and specimen was deleted*

2.2 Aborting a Submission Removal

STaCS Database allows you to abort an initiated submission removal. However, confirmed removals cannot be aborted.

1. On the Group Bar, go to **Main > Submission Removal**.
2. Click Edit.
3. Add a reason for aborting the removal in the Abort Reason Field.
4. Click Save.
5. Click Yes to confirm that you want to abort the submission removal.
6. Click Close.

2.3 Lysis Plate/PCR Plate Disposal

- A. Periodically, lysis plates and PCR plates need to be destroyed in order to make room in the refrigerators/freezers.
- B. It is extremely important to ensure that all the samples on the plate have either been completed and CODIS confirmed or abandoned. This can be accomplished by checking the plate status in STaCS.
- C. Once this has been completed, the plates can be removed and destroyed properly.

2.4 Blood Tube Disposal

- A. Periodically, blood tubes need to be destroyed in order to make room in refrigerator storage. The following procedure will only be performed on blood samples that have a complete DNA profile uploaded into CODIS and an archive sample preserved.
- B. Samples successfully processed through STaCS and uploaded into the CODIS database will automatically be placed in the "Sample Disposal" module in STaCS immediately following the Administrative Review.



C. For Samples on the Sample Disposal list

1. Open STaCS.
2. Under the Main tab, select the “Sample Disposal” module.
3. Locate the blood tubes corresponding to the CODIS numbers in the Sample Disposal list.
4. Scan the tubes (up to 72 at a time) in order and ensure a check was put in the box as each one is scanned.
5. When scanning is complete, check the List Count number and verify that the number decreases by the number of tubes scanned once Saved. Click “Save”, then “Yes” to dispose of the selected samples. The tubes are then removed from the list as the module refreshes after the Save.
6. Open Storage Subsystem module. Click “View Contents”, select the appropriate Long Term Storage Location and note the Item Count number.
7. Click “Store Items”, and scan the barcode of the Long Term Storage Location, then scan the tubes to be destroyed.
8. Open “View Contents”, select the appropriate Long Term Storage Location, and ensure the Item Count was updated to reflect the addition of the scanned tubes.
9. Double bag a biohazard container with two biohazard bags. Empty the racks of blood tubes into the bin. Tie the bags with a ziptie and place the bag in the appropriate location for biohazard disposal.

D. For Samples not on Sample Disposal list

1. Tubes that shouldn't be destroyed will produce an error when scanned in the Sample Disposal module. To clear this error, scan the tube again or click “OK” then set the tube aside.
2. The appropriate tubes will then be put into a Duplicate rack. Under the main tab, select “Storage Subsystem” module and scan the appropriate Duplicate Storage Rack barcode, then scan the tubes by placing them in the order scanned into the rack.

E. Cleaning Blood Tube Racks

1. Remove barcodes from blood tube storage racks.
2. Thoroughly clean blood tube storage racks with bleach solution.



CO-03-03 BLOOD SAMPLE PREPARATION AND STORAGE

1 Scope

These procedures cover the process to properly prepare blood samples for blood spotting / archiving using the Tecan robot.

2 Safety

- A. This process involves handling opened blood tubes and therefore requires the operator to observe the utmost of caution. Suitable personal protective equipment shall be used by technicians and analysts.
- B. Any physical contamination with blood tube contents (either by injection or ingestion) or other exposures will be reported immediately to the appropriate personnel.
- C. Physical safety features built into the Tecan robot and heat sealer should never be compromised for the purpose of expedience.
- D. Some robotic workstations utilize large moving arms with sharp tips which could easily pierce the skin. While some safeguards exist to protect the operator, injury may still occur if not properly operated. Decks should not be accessed while an arm is in motion.

3 Equipment and Material

- Tecan Freedom EVO 150 robot with Tecan EVOware Standard software
- Archive cards
- Electrically conductive (black) 1 mL tips

4 Instructions

4.1 Selecting Samples for Blood Spotting

- A. Typical Processing
 1. Open the "Blood Spotting" module in STaCS.
 2. Either select "Auto Select" or manually select 84 samples.
 3. Click "Print Selection". Two sets of barcode labels will be printed.
 4. Print a copy of the "Blood Spotting Sample Selection". This sheet will be used to pull the appropriate blood tubes.
- B. Low Blood Volume (LBV) Processing
 1. Open the "Blood Spotting Manual" module in STaCS to select LBV samples.
 2. Click "Print" to print a copy of the "Blood Spotting Manual" list.
 3. Refer to "Blood Spotting Manual" list to pull the appropriate blood tubes.
 4. Open STaCs "Print Bar Code" found in "Utilities" tab to print two sets of barcodes corresponding to each blood tube.



4.2 Preparation of Archive Cards

- A. Place first set of barcode labels on archive cards.
 1. Put on gloves and place the archive cards on a sterile work surface.
 2. Barcode the archive cards where indicated, labeling vertically from right to left and in sequential order. (See diagram on next page.)
 3. Place the archive cards in a large envelope.
- B. Place the second set of barcode labels on the envelopes
 1. Keep the labeled envelopes in sequential order.
 2. File in appropriate archive box.

Number refers to the order in which samples are spotted onto the archive card.

TEXAS DEPARTMENT OF PUBLIC SAFETY CODIS PROGRAM 6 BAR CODE LABEL HERE	TEXAS DEPARTMENT OF PUBLIC SAFETY CODIS PROGRAM 5 BAR CODE LABEL HERE	TEXAS DEPARTMENT OF PUBLIC SAFETY CODIS PROGRAM 4 BAR CODE LABEL HERE	TEXAS DEPARTMENT OF PUBLIC SAFETY CODIS PROGRAM 3 BAR CODE LABEL HERE	TEXAS DEPARTMENT OF PUBLIC SAFETY CODIS PROGRAM 2 BAR CODE LABEL HERE	TEXAS DEPARTMENT OF PUBLIC SAFETY CODIS PROGRAM 1 BAR CODE LABEL HERE
TEXAS DEPARTMENT OF PUBLIC SAFETY CODIS PROGRAM 12 BAR CODE LABEL HERE	TEXAS DEPARTMENT OF PUBLIC SAFETY CODIS PROGRAM 11 BAR CODE LABEL HERE	TEXAS DEPARTMENT OF PUBLIC SAFETY CODIS PROGRAM 10 BAR CODE LABEL HERE	TEXAS DEPARTMENT OF PUBLIC SAFETY CODIS PROGRAM 9 BAR CODE LABEL HERE	TEXAS DEPARTMENT OF PUBLIC SAFETY CODIS PROGRAM 8 BAR CODE LABEL HERE	TEXAS DEPARTMENT OF PUBLIC SAFETY CODIS PROGRAM 7 BAR CODE LABEL HERE

4.3 Setting up Blood Tubes on Tecan Racks

1. Refer to the “Blood Spotting Sample Selection”/“LBV Sample” sheet to set up the blood tube racks.
2. Pull blood tubes from appropriate blood tube rack (BTR) and place into the appropriate order and position on the Tecan rack. If the batch will not be run the same day, replace the racks in the storage refrigerators.
3. If they will be run that day, proceed to the extraction room.

4.4 Blood Tube Opening

1. Place Tecan rack in the safety hood.
2. Shake or invert tubes appropriately ensuring the contents are well mixed.
 - a) *Additional mixing may be required to ensure a uniform appearance (there shouldn't be blood/serum partitioning within the tube).*
 - b) *For low blood volume samples, shake tubes individually to a homogenous mixture without inverting and without allowing bubble formation.*



3. Check “Blood Spotting Sample Selection”/“LBV Sample” to make sure that the blood tube is in that batch and in the correct numerical position.
4. Position tube over biohazard waste bin.
5. Gently pull off the top with an absorbent wipe and dispose the cap.
6. Place blood tubes consecutively back into the Tecan rack.
7. Make sure blood samples are devoid of significant surface bubbles.
8. It may be necessary to use one of the small sterile wooden sticks to remove large bubbles. Do not use the same stick on more than one tube.

4.5 Tecan Preparation

1. Position all components on the Tecan deck.
2. If needed, place a disposable plastic liner in the tip disposal bin and put the bin in its template under the tip-removal slot.
3. Place black conductive 1 mL disposable tips in the rack position.
4. Arrange the archive cards in the card racks in the proper order for spotting.
5. Position blood tubes on the robot deck.
 - a) *Orient barcodes in the appropriate position so the barcode reader can scan the barcodes.*
 - b) *Check the sample order with the “Blood Spotting Sample Selection”/“LBV Sample” sheet.*

4.6 Blood Spotting

A. Typical Processing

1. Open the “Blood Spotting” module in STaCS.
2. Ensure the Tecan is on and scan the robot.
3. Select “Bed Scan”.
4. STaCS will launch the EVOware Standard software.
5. Select “Run Direct” from the “Run” drop-down menu.
6. The Tecan will begin scanning the blood tubes. If an error occurs follow prompts. **Note: Do not select “retry bedscan”. Select “enter” instead.**
7. After script completes successfully, close “Runtime Controlled” window
8. Exit EVOware Standard software.
9. Select “Yes” when prompted to move arms to home position.
10. If needed, check “Blood Spotting Sample Selection” sheet against the sample selected in STaCS to verify the appropriate samples were selected.
11. Click “Process”. STaCS will launch EVOware Standard software.
12. Select “Run Direct” from the “Run” drop-down menu.
13. Verify appropriate values are selected. Select “OK”.



14. For each set of racks:
 - a) *Follow prompts so the Tecan can scan the blood tubes and archive cards.*
 - b) *The program will transfer two 25 μ L spots and one 75 μ L spot to each archive card.*
 - c) *Remove the spotted archive cards and replace with the next set. Place the archive cards on shelves to dry.*
 - d) *Replace empty tip racks and check biohazard waste container as needed.*
 - e) *Repeat until the Tecan completes the run.*
 15. Exit the "Runtime Controlled" window.
 16. Exit EVOware Standard software.
 17. Select "Yes" when prompted to move arms to home position.
 18. If needed, check "Blood Spotting Sample Selection" sheet against the sample selected in STaCS to verify the appropriate samples were selected.
 19. In the "Blood Spotting" module in STaCS, select "Save".
 20. Click "Close" to exit the "Blood Spotting" module.
 21. Remove the blood tubes from the Tecan deck for re-racking into appropriate carriers for transport.
 22. Re-cap blood tubes.
 23. Replenish tips and refill water jug if needed.
 24. Wipe down workspace using bleach.
 25. When finished for the day, log off the computer and turn off the Tecan.
- B. Low Blood Volume Processing
1. Turn on the Tecan and launch the EVOware Standard software.
 2. Select "Run an Existing Script". Click on "Spotting_Archives" followed by clicking "Run" when prompted.
 3. Verify appropriate transfer volumes and number of racks. Continue by selecting "OK".
 4. For each set of racks:
 - a) *Initiate the Tecan check of blood tube barcodes on the set of racks against the corresponding barcodes on archive cards and correct any errors in mismatch as prompted. DO NOT CONTINUE ON TO SPOTTING YET.*
 - b) *Gently shake the first set of four blood tubes one more time to ensure that any nucleated cells that have settled are in a homogenous mixture again. Follow the prompts and continue on to spotting.*
 - c) *While the Tecan is spotting the first set of four samples, continue shaking the second set of four in sequence. Pause the spotting script between every set of four if necessary to complete shaking of all tubes.*
 - d) *Remove the spotted archive cards and replace with the next set. Place the archive cards on shelves to dry.*
 - e) *Repeat until all LBV tubes on all racks have been spotted onto corresponding archive cards.*



5. When the script is completed, exit and unload drivers in EVOware Standard software. Turn the Tecan off and log off the computer.
6. Re-cap all blood tubes.
7. Replenish tips and refill water jug if needed.
8. Wipe down workspace using bleach.
9. To continue on to the "Punch" module, all LBV samples must to be moved from "Blood Spotting Manual" list to the "Plate Create" list in STaCs by the analyst.
 - a) *Open "Blood Spotting Manual" module in STaCs.*
 - b) *Scan archive card/envelope barcodes for LBV samples to be selected for transfer.*
 - c) *Click "SAVE" to complete the transfer of LBV samples to "Plate Create".*

4.7 Storage

1. Return all tubes/racks in the kit receiving room to the refrigerators in the appropriate storage location.
2. Once the archive cards are dry, they are ready to be stuffed into envelopes.
 - a) *Wipe the counter appropriately.*
 - b) *Spread labeled envelopes and pull the corresponding archive card sheet.*
 - c) *Individually separate each archive card and place on top of the correct envelope.*
 - d) *Check once again that the archive card and envelope have the same barcode. Slide card into envelope.*
3. When finished, wipe the counter and the shelves with bleach solution.
4. Place the envelopes in the "To be punched" or "LBV" box in the PCR set-up room.
5. After punching is complete, seal envelopes using the heat sealer.
6. Place the sealed envelopes in sequential order into the appropriate archive box.

5 Records

- A. Processing information is recorded in STaCS.
- B. Archive cards will be retained indefinitely.

6 Literature References and Supporting Documentation

Tecan. Freedom EVO Operating Manual.

Tecan. Freedom EVOware Standard Getting Started Guide.



CO-03-04 BUCCAL SWAB PREPARATION AND STORAGE

1 Scope

This policy outlines the procedure to properly prepare buccal swabs for lysis.

2 Related Chapters

Buccal Lysis Set-up

3 Safety

Suitable personal protective equipment shall be used by technicians and analysts.

4 Equipment and Materials

- S Block
- Appropriate plate seal
- Tweezers, as needed
- Ethanol, as needed

5 Instructions

5.1 Creating Permanent Storage (PBS) Labels (Program Coordinator/LIMS Specialist only)

This label will only need to be printed when the container in STaCS is full (99,999 samples).

1. Open STaCS and click on “Storage Subsystem” module.
2. Select “Manage Storage Units”.
3. Single click on “STaCS Storage System” to activate the “New Storage Unit” button.
4. Under the “Class” drop-down menu, select “Permanent Buccal Storage”
5. Enter the next available storage unit (PBS# - ####) and select “1” unit.
6. Advance label printer to the next available label to ensure optimal printing. Click “Print”.
7. Select “Close” to exit the “Storage Subsystem” and exit out of STaCS.

5.2 Selection of Samples

1. Open STaCS and select the “Plate Create” module.
2. Select “Buccal Plate” from the “Plate Type” drop-down menu. The other fields will default appropriately.
3. Click “Location” column twice to go to the oldest sample.
4. Select the appropriate number of samples. Avoid the red and green font samples as these are match confirmation and re-work samples respectively.
5. Click “Print Selection”. Print a copy of the “Sample Selection”. This sheet will be used to pull the appropriate buccal swabs.



5.3 Pulling Samples from Storage

1. Using the "Sample Selection" sheet, pull the samples from the appropriate SWB location and place into a new box.
2. Transfer samples from SWB location to PBS location.
 - a) *Open STaCS and select the "Storage Subsystem" module.*
 - b) *Click "View Contents". Select the appropriate PBS Storage Unit and note the item count number.*
 - c) *Scan appropriate PBS unit and scan all swabs.*
 - d) *Click "View Contents" to verify samples were properly transferred by adding the number of buccal swabs scanned to the original item count.*
 - e) *Click "Close" to exit "Storage Subsystem" module.*
3. Place "Sample Selection" sheet in box with swabs.

5.4 Plate Create

1. Open STaCS and select the "Plate Create" module.
2. Select "Buccal Plate" from the "Plate Type" drop-down menu. The other fields will default appropriately.
3. Select "Create Plate".
4. Open the envelope and verify there are two swabs inside. If there isn't or there are more than two swabs, do not process the sample and notify the CODIS Interagency Liaison.
5. Scan envelope and manually eject buccal swab into appropriate well designated in STaCS.
 - a) *Eject second swab tip into envelope and discard stick. Reseal envelope.*
 - b) *The remaining buccal swab will be retained infinitely (or until consumed) for archiving / verification purposes.*
6. When all the swabs have been scanned and plated, click "Save".
7. Place barcode on appropriate deep well plate.
8. Click "Close" to exit the "Plate Create" module.
9. Seal the plate and store at room temperature.
10. File envelopes into appropriate archive box.
11. Proceed to *Buccal Lysis Set-up* chapter.

6 Records

Processing information is recorded in STaCS.



CO-03-05 BUCCAL LYSIS SET-UP

1 Scope

This procedure outlines the process to properly prepare buccal swab plates for direct amplification. Some steps of this process are performed by approved robotic platforms. The multidrop DW plate filler is used to add the lysis solution to the deep well plate. The Tecan 200 is used to aliquot 100 μ L of sample lysate into a 96-well daughter plate.

2 Related Chapters

Data Interpretation Guidelines

3 Safety

- A. Body fluids and extracts may contain infective agents. Use universal precautions during handling. Appropriate personal protective equipment must be worn during reagent preparation and use.
- B. Some robotic workstations utilize large moving arms with sharp tips which could easily pierce the skin. While some safeguards exist to protect the operator, injury may still occur if not properly operated. Decks should not be accessed while an arm is in motion. Physical safety features built into robots should never be compromised for the purpose of expedience.

4 Equipment and Materials

- Centrifuge
- Pipettes, adjustable
- Pipette tips
- Appropriate Seals
- STR GO! Lysis Buffer
- Prep-n-Go Buffer
- Tecan Freedom EVO 200 MCA
- Tecan EVOware Standard software
- Beaker
- TYPE I Water
- Thermomixer
- Heat block
- Plate filler (Multidrop DW or equivalent)
- 96-well daughter plate

5 Standards, Controls, and Calibration

Reagent Blank



6 Procedure

6.1 Buccal Plate Lysis Procedure using STR GO! Lysis Buffer

A. Lysis

1. In STaCS, open the “Lysis” module.
2. Scan the appropriate buccal plate.
3. Select “Get Scenario(s)”.
4. Select correct scenario and click “OK”
5. Scan the appropriate buccal plate and reagents.
6. Click “Process”.

B. Operation of Multidrop DW plate fillers

1. Turn on the instrument.
2. Open the rotor cover and insert the dispensing cassette as described in the user manual.
3. Close the rotor cover.
4. Insert the eight tube end with tubing weight into the reservoir solution to be used.
5. Check that the priming vessel is empty and firmly in place.
6. Press and hold the “Prime/Drop” key until all eight channels are dispensing continuously into the priming vessel and there are no more air bubbles in the tubes.
7. Set the reagent volume to 500 μ L using the thumbwheels.
8. Set the number of columns to be dispensed to 12 using the thumbwheels.
9. Place the appropriate buccal plate on the carrier.
10. Press the “Start” key to begin dispensing.
 - a) *Any number of plates can be dispensed by changing the plate and pressing “Start” as long as there is enough solution in the reservoir.*
 - b) *The instrument primes itself before each plate is dispensed. Check the priming vessel before each plate is filled and empty as necessary.*
11. Verify lysis buffer has been aliquoted to appropriate wells.
12. Seal plate(s) and place in a thermomixer at 95°C shaking at 1000 rpm for 5 minutes.
13. Allow plate(s) to cool to room temperature before proceeding to Daughter Plate Create.
14. Briefly centrifuge the plate to remove any solution from underneath the seal.
15. In the “Record Activity Completion Result” window in STaCS, select the appropriate result and click “OK”.
16. Select “Close” to exit the “Lysis” module.



17. After the last plate is filled, press the “Empty” key to return any unused reagent in the tubes to the reservoir.
18. When finished with the instrument for the day, wash the tubes and dispensing cassette with TYPE I water using the “Prime/Drop” and “Empty” keys. Empty and replace the priming vessel when finished.
19. Store the cassette in the resting position.
20. Turn off the instrument and put the dust cover on.

6.2 Buccal Plate Lysis Procedure using Prep-n-Go Buffer

A. Lysis

1. In STaCS, open the “Lysis” module.
2. Scan the appropriate buccal plate.
3. Select “Get Scenario(s)”.
4. Select correct scenario and click “OK”
5. Scan the appropriate buccal plate and reagents.
6. Click “Process”.

B. Operation of Multidrop DW plate fillers

1. Turn on the instrument.
2. Open the rotor cover and insert the dispensing cassette as described in the user manual.
3. Close the rotor cover.
4. Insert the eight tube end with tubing weight into the reservoir solution to be used.
5. Check that the priming vessel is empty and firmly in place.
6. Press and hold the “Prime/Drop” key until all eight channels are dispensing continuously into the priming vessel and there are no more air bubbles in the tubes.
7. Set the reagent volume to 400 μ L using the thumbwheels.
8. Set the number of columns to be dispensed to 12 using the thumbwheels.
9. Place the appropriate buccal plate on the carrier.
10. Press the “Start” key to begin dispensing.
 - a) *Any number of plates can be dispensed by changing the plate and pressing “Start” as long as there is enough solution in the reservoir.*
 - b) *The instrument primes itself before each plate is dispensed. Check the priming vessel before each plate is filled and empty as necessary.*
11. Verify lysis buffer has been aliquoted to appropriate wells.
12. Seal plate(s) and place in a heat block at 90°C for 20 minutes.
13. Allow plate(s) to cool to room temperature before proceeding to Daughter Plate Create.
14. Briefly centrifuge the plate to remove any solution from underneath the seal.



15. In the “Record Activity Completion Result” window in STaCS, select the appropriate result and click “OK”.
16. Select “Close” to exit the “Lysis” module.
17. After the last plate is filled, press the “Empty” key to return any unused reagent in the tubes to the reservoir.
18. When finished with the instrument for the day, wash the tubes and dispensing cassette with TYPE I water using the “Prime/Drop” and “Empty” keys. Empty and replace the priming vessel when finished.
19. Store the cassette in the resting position.
20. Turn off the instrument and put the dust cover on.

6.3 Daughter Plate Creation

1. In the “Daughter Plate Creation” module in STaCS, select “Create Plate”.
2. The plate options should already be defaulted. If not, select the appropriate “Plate Layout” and “Plate Source”.
3. Select the appropriate lysis plate.
4. Click “Allocate” and then select “Create”.
5. Place barcode on the daughter plate and scan.
6. Click “Close”.
7. Scan the appropriate daughter plate.
8. Select “Get Scenario(s)”.
9. Place the daughter plate and lysis plate on the Tecan deck.
10. Scan the robot, daughter, and lysis plate.
11. Ensure the Tecan is on and select “Process”.
12. STaCS will launch the EVOware Standard software.
13. Select “Run Direct” from the “Run” drop-down menu.
Note: *To avoid the swab head being lifted out of the well in the lysis plate, manipulate the plate as the Tecan lifts the tips from the lysis plate.*
14. When the Tecan has completed the process, click “Cancel”.
15. In the file drop-down menu, select “Exit, unload drivers”.
16. Select “Yes” when prompted to move arms to the home position.
17. Verify lysate has been aliquoted to appropriate wells and note any discrepancies in “Plate Comment” or “Well Comment” in STaCS.
18. In the “Record Activity Completion Result” window in STaCS, select the appropriate result and click “OK”.
19. Select “Close” to exit the “Daughter Plate Create” module.
20. Seal the plate(s) and store at 4°C.
21. Wipe countertop with 70% ethanol.



7 Records

Processing information is recorded in STaCS.

8 Literature Records and Supporting Documentation

Qiagen. Investigator 24plex GO! Handbook.

Applied Biosystems. AmpF ℓ STR GlobalFiler Express PCR Amplification Kit User Guide.

Thermo Electron Corp. Multidrop DW User Manual.

Tecan. Freedom EVO Operating Manual.

Tecan. Freedom EVOware Standard Getting Started Guide.



04 ANALYTICAL PROCEDURES

CO-04-01 EZ1 ADVANCED XL EXTRACTION

1 Scope

Samples may require an extraction separate from the work routinely performed in the CODIS Laboratory. The following extraction procedure is typically used for match verifications, familial searches, or sample reworks.

2 Safety

- A. 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.
- B. Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling.
- C. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

3 Equipment and Materials

- STaCS computer
- Qiagen EZ1 Advanced XL robotic workstation
- Qiagen EZ1 DNA Investigator Kit, including
 - Stock Buffer G2
 - Proteinase K
- Single hole puncher
- Heat block
- Vortex
- Scissors
- Pipettes, adjustable
- Pipette tips
- Tweezers
- Ethanol
- Kimwipes
- Tube rack
- Blank FTA card
- UltraPure distilled water
- Diluted Buffer G2

4 Standards, Controls, and Calibration

- A. At least one reagent blank must be processed for each extraction batch as a negative control.
- B. An extraction batch is defined as a set of samples processed during one robotic run.

5 Procedure



5.1 Plate Create in STaCS

1. Open STaCS and select the "Plate Create" module.
2. In the "Plate Type" drop-down menu, select "EZ1 Plate". "Protocol" should be set to "Not Defined".
3. If needed, a printed list of samples may be generated by highlighting appropriate samples and clicking "Print Selection".
4. Select "Create Plate".
5. Scan barcode on the sample envelope to place that sample onto the newly created plate. Scan each additional sample to be included on the plate. When completed, select "Save". **Note: Sample order cannot change once the plate is created.**
6. Click "Close" to exit the "Plate Create" module.
7. Place printed plate barcode on designated tube rack.

5.2 Sample Preparation

1. As needed, prepare diluted Buffer G2 using a 1:1 ratio of the stock Buffer G2 and UltraPure distilled water.
 - a) *Expiration date is the same as on the Qiagen EZ1 DNA Investigator Kit.*
 - b) *Store at room temperature in the same manner as the kit.*
2. Add 190 μ L of diluted Buffer G2 to the sample tube.
3. Add 10 μ L Proteinase K.
4. For each sample, plus the negative:
 - a) *Scan the barcode (archive card for punches) using Print Barcode from Utilities menu and print two small barcodes. Place on the 2 μ L sample tube and the elution tube.*
 - b) *Place the appropriate amount of sample into a properly labeled screw cap 2.0 mL sample tube.*
5. Vortex briefly and place tube(s) into the 56 °C heat block for 15 minutes.

5.3 Purification using the EZ1 Advanced XL

- A. Set-up/Prepare equipment
 1. Switch on the EZ1 instrument.
 2. Press "Start" to display the "Protocols" menu.
- B. Purification
 1. Select appropriate protocol. (Press 2 for Tip Dance protocol)
 2. Select TE as the elution buffer.
 3. Select the appropriate elution volume.
 4. Open the workstation door.



5. Repeat all of the following steps as necessary for each sample and control to be extracted:
 - a) *Invert reagent cartridges to mix magnetic particles, and then tap to deposit reagents at the bottom of the wells. Check that the magnetic particles are completely re-suspended.*
 - b) *Load reagent cartridges into the cartridge rack, pressing down until they each click in place.*
 - c) *Load opened elution tube(s) into the first row of the tip rack.*
 - d) *Load tip holder(s) containing filter tip(s) into the second row of the tip rack.*
 - e) *Load opened sample tube(s) from incubator into the back row of the tip rack. (Row 3 will remain empty)*
6. Close the workstation door.
7. Press "Start". When the run is completed, the LCD displays "Protocol finished".
8. Remove elution tubes containing purified DNA. Use immediately or store at 2-8 °C.
9. To run again, press "ESC", otherwise press "ESC" twice, and close the door.
10. Follow the onscreen instructions to perform a UV decontamination of the worktable surfaces after use or at the end of the day.
11. Switch the power off.
12. Clean EZ1 instrument as needed. Maintenance instructions are in the EZ1 Advanced XL User Manual.

5.4 EZ1 Processing in STaCS

1. Open STaCS and select "EZ1 Processing" module.
2. Scan the EZ1 robot.
3. Scan the sample tubes.
4. Scan "EZ1 DNA Investigator Kit".
5. Select "Add Set".
6. Click "Process".
7. When run is complete, in the "Record Activity Completion Result Window", select the appropriate result and click "OK".
8. Click "Close" to exit the "EZ1 Processing" module.

5.5 Post Extraction Activities

1. Proceed to amplification
2. Store samples at 2-8°C.

6 Records

Processing information is recorded in STaCS.



7 Literature References and Supporting Documentation

Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. Chapter 2 DNA Extraction Methods.

Qiagen. EZ1 DNA Investigator Handbook.

Qiagen. EZ1 Advanced XL User Manual.



CO-04-02 QIASYMPHONY SP EXTRACTION

1 Scope

Instructions for use of the QIASymphony SP. The following extraction procedure is typically used for match verifications and familial searches.

2 Related Documents

None

3 Safety

- A. Appropriate personal protective equipment must be worn during sample preparation and use. Body fluids may contain infective agents. Use universal precautions during handling.
- B. Use caution when operating robotic equipment. The hood should be closed to avoid moving parts while the instrument is running.
- C. Liquid waste should be disposed of properly.

4 Equipment and Materials

- QIASymphony SP
- QIASymphony DNA Investigator Kit
- Reagent cartridge and holder
- Enzyme rack
- Piercing lid
- 8-rod covers
- Sample prep cartridges
- Disposable filter tips
- Spin baskets
- 1.5 µL microcentrifuge tubes
- S-block or round bottom plate
- Buffer ATL
- Proteinase K
- Carrier RNA
- Biohazard bag
- Heat block
- Pipettors, adjustable, and tips
- Vortex
- Appropriate plate seal
- Hole punch
- Tweezers
- Scissors

5 Standards, Controls, and Calibration

Reagent Blank



6 Procedures

6.1 General

- A. The QIASymphony SP is designed for automated purification of DNA from a variety of sample substrates.
- B. This robot will allow for up to 24 samples and/or reagent blanks to be loaded onto a rack. Racks can be loaded one at a time or up to four racks can be loaded at once. Up to 96 samples and/or reagent blanks can be processed in a defined run.

6.2 Inventory Scan (as needed)

1. Ensure all drawers are closed before turning on the QIASymphony.
2. Wait until the initialization procedure is complete and log on. This will unlock the drawers.
3. Ensure there are enough tips, 8-rod covers, sample prep cartridges and reagent cartridge(s) in the “Reagents and Consumables” drawer to allow for all the samples to be processed.
Note: the magnetic particles must be completely re-suspended. This can be done by vortexing the magnetic particle trough for at least four minutes.
4. Ensure the waste drawer is loaded with an appropriate amount of empty unit boxes, liquid waste bottle, and biohazard bag for tip disposal.
5. When the drawers are closed, select “Inventory Scan” for tip racks, unit boxes and reagents. Buffer bottle and alcohol trough are not scanned.

6.3 Plate Create

1. Open STaCS and select the “Plate Create” module.
2. In the “Plate Type” drop-down menu, select “QIASymphony Plate”. “Protocol” should be defaulted to “Not Defined”.
3. If needed, a printed list of samples may be generated by highlighting the appropriate samples and selecting “Print Selection”.
4. Click “Create Plate.”
5. Scan the barcode on the sample envelope to place that sample on the newly created plate. Scan each additional sample to be included on the plate.
6. Once all the samples have been scanned, click “Save”. Select “Yes” when prompted to place controls after the last DNA well.
7. Place printed barcode on either an S block plate or round bottom plate.
8. Click “Close” to exit the “Plate Create” module.

6.4 QIASymphony Processing

1. Open STaCS and select the “QIASymphony Processing” module.
2. Scan the appropriate S block/round bottom plate, the QIASymphony, and the DNA Investigator kit.
3. Place a spin basket into a 1.5 µL microcentrifuge tube and pipette 680 µL of ATL buffer and 20 µL Proteinase K into the tube.



4. For each sample to be placed on the tube rack:
 - a) *In the "Utilities" tab in STaCS, select "Print Barcode". Make sure the "Small" label is selected.*
 - b) *Scan the sample (type in samples on LTS plates) and print barcode. Place barcode on the microcentrifuge tube.*
 - c) *Cut or punch substrate and place into the appropriate tube. Two blood punches, one LTS punch, and the tip of a buccal swab is optimal; however, more punches or sample may be used as necessary. Close the tube cap.*
5. Place the samples in the 56°C heat block for 15 minutes.
6. On the QIASymphony deck, load an appropriate plate into the elution slot. Using the sample screen, designate the type of plate and scan the plate.
 - a) *For S Block plate, select deep well QIA19585 S-Block96*
 - b) *For round bottom plate, select Micro Plate CO#3795 MTP96RB*
7. Snip off the folding edge of the cap and place the microcentrifuge tube in the rack.
8. Remove the lid and spin basket.
9. Select "Start Process".
10. Minimize the STaCS screen and open the "QIASymphony Management Console".
11. From the "File" drop-down menu select "Login". Use the login you created on the QIASymphony. Ensure the instrument IP address is selected for login.
12. From the "File Format" drop-down menu, select "Work list". Select the appropriate worklist and transfer it to the remote site.
13. Exit the "QIASymphony Management Console".
14. Slide the rack onto the QIASymphony deck.
15. Ensure all barcodes were read during the loading of the rack by checking the sample screen on the QIASymphony. If necessary, re-scan to correctly identify each sample.
16. Verify the correct extraction procedure is correlated with each sample. Select the elution plate loaded earlier and verify the elution volume.
17. Press the "Queue" button.
18. Press the "Run" button to begin the extraction.

Note: *if an error message is received due to lack of reagents, load additional reagent cartridges and repeat inventory scan.*
19. When the run is complete, remove the elution plate from the QIASymphony deck and remove the plate on the elution screen.
20. Open the "QIASymphony Processing" module in STaCS.
21. Scan the appropriate plate to complete the process.
22. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
23. Click "Close" to exit the "QIASymphony Processing" module.
24. At the end of the day (if no run is in process), the reagent cartridge should be removed and capped to prevent evaporation.



7 Post Extraction Activities

1. Proceed to amplification
2. Store samples at 2-8 °C.

8 Records

Processing information is recorded in STaCS.

9 Literature References and Supporting Documentation

Qiagen. QIASymphony SP/AS User Manual.



CO-04-03 INVESTIGATOR 24PLEX GO! AMPLIFICATION

1 Scope

The Investigator 24plex GO! amplification kit is a 22 marker multiplex PCR kit designed specifically for direct amplification from blood or buccal cells.

2 Related Chapters

Buccal Lysis Set-up

EZ1 Advanced XL Extraction

Capillary Electrophoresis

3 Safety

- A. Wear lab coat and gloves when working in the laboratory. Face masks may be used as appropriate.
- B. Some robotic workstations utilize large moving arms with sharp tips which could easily pierce the skin. While some safeguards exist to protect the operator, injury may still occur if not properly operated. Decks should not be accessed while an arm is in motion. Physical safety features built into robots should never be compromised for the purpose of expedience.

4 Equipment and Materials

- STaCS computer
- Thermal cycler
- Centrifuge
- Microcentrifuge tubes , 1.5 mL
- Vortex
- 96-well amplification plate
- Amplification cover
- Appropriate plate seal
- Pipettes, adjustable
- Pipette tips
- Investigator 24plex GO! kit
- BSD 600 (instrument, computer, and appropriate software)
- Tecan Freedom EVO 100 (instrument, computer, and appropriate software)
- Investigator STR GO! Punch Buffer
- STR GO! Lysis Buffer
- TYPE I water

5 Standards, Controls, and Calibration

- A. At least one positive control must be included with each amplification set. However, more may be used if needed.
- B. Reagent blanks are denoted as “Negative” and amplification negatives as “Ampblank” in STaCS.



- C. An amplification negative control must be included with each amplification set. This negative control consists of all amplification reagents without the addition of template DNA.
- D. Blood plates have a combined reagent blank/amplification negative control.
- E. For buccal plates and DNA extracts, the reagent blank must be amplified using the most sensitive template volume.

6 Procedure

6.1 General

- A. The primer set also includes two Quality Sensors which are internal PCR controls that provide information about the efficiency of the PCR reaction and/or determine the presence of PCR inhibitors. Half reaction volumes are used when amplifying buccal samples with this kit. However, full reaction volumes may be used when necessary.

Note: when amplifying blood samples using the full reaction volume, select the 26-cycle program on the thermal cycler.

- B. Investigator 24plex GO! Kits are to be stored in the freezer.
- C. Inhibited blood samples may require a water wash prior to amplification.

6.2 Amplifying FTA or Non-FTA Blood Samples

A. Plate Create for Blood Samples

1. In STaCS, select the "Plate Create" module.
2. Select "FTA-96 Wells" from the drop-down menu. Ensure the "Protocol" and "Sample Source" are "Investigator 24Plex GO!" and "Blood" respectively.
3. Select "Create Plate"
4. Scan archive envelopes to allocate samples onto the plate.
5. Select "Save".
6. Click "Yes" to move controls to the next available well.
7. Place barcode on amplification plate with plate name facing up and fold excess barcode underneath the plate edge.
8. Click "Close" to exit "Create Plate" module.

B. Manual Plate Preparation for FTA or Non-FTA Blood samples:

1. Open the "Plate Preparation" module in STaCS.
2. Scan the appropriate PCR plate.
3. Click "Get Scenario(s)".
4. Select the appropriate scenario.
5. Scan the consumables.
6. **Optional:** To print a plate layout, select "Plate Content" and "Layout View" and then "Print."
7. In the "Plate Preparation" module of STaCS, click "Process".



8. If the "Select Transfer Map Directory" Screen appears, select "c:", double click "C:\\" to expand the folder, and double click the "PickSample" folder. Click "OK". Exit the Excel workbook. This file will be used to create the plate layout for the amplification worksheet.
9. To print the amplification worksheet, go to the Start menu, navigate to "M:\STaCS Production" and open "STaCS pickSample.xls".
10. Press "Ctrl+W" and select the pickSample.csv file from the "C:\PickSample" folder.
11. Enter the number of reactions. To ensure enough PCR reaction mix is prepared, include controls and additional reactions in the calculation.
12. Click "Save as" and save to separate folder or select "Print".
13. Exit and do not save.
14. When performing a water wash, continue to step a). (If not, skip to step 15 – Prepare PCR reaction mix)
 - a) *Aliquot 20 µL TYPE I water to the appropriate wells.*
 - b) *In the "Record Activity Completion Result" window, select the appropriate result and click "OK".*
 - c) *Click "Close" to exit the "Plate Preparation" module.*
 - d) *Punch samples following section – Punching FTA or non-FTA Samples Using the BSD 600*
 - e) *Spin down plate to ensure punches are submerged in water*
 - f) *Remove water from wells using a mechanical pipette.*
15. Prepare PCR reaction mix:
 - a) *Vortex the PCR components well and centrifuge briefly prior to use.*
 - b) *Prepare the PCR reaction mix by adding the following volumes per reaction in a microcentrifuge tube or use the values calculated in the PickSample amplification worksheet. To ensure enough PCR reaction mix is prepared, factor additional reactions to the calculation.*

i.	<i>Fast Reaction Mix</i>	<i>7.5 µL</i>
ii.	<i>Primer Mix</i>	<i>12.5 µL</i>
iii.	<i>Investigator STR GO! Punch Buffer</i>	<i>2.0 µL</i>

Note: When amplifying non-FTA samples, do not add punch buffer.
 - c) *Vortex the reaction mix and spin briefly.*
16. Aliquot 22 µL (**20 µL for Non-FTA samples**) of PCR reaction mix into appropriate wells on the 96-well amplification plate.
17. If performing the water wash step, skip to [Amplification](#) section
18. In the "Plate Preparation" module of STaCS, click "Process".
19. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".



20. Click "Close" to exit the "Plate Preparation" module.
 21. Proceed to – Punching FTA or non-FTA Blood Samples Using the BSD 600.
- C. Automated Plate Preparation for FTA or non-FTA Blood Samples:
1. In STaCS, select the "Plate Preparation" module.
 2. Scan the appropriate PCR plate on the designated location on the robot deck.
 3. Click "Get Scenario(s)".
 4. Select the appropriate scenario.
 5. Scan the robot and consumables.
 6. Turn on robot.
 7. Prepare PCR reaction mix as listed above in part B.
 8. Place PCR reaction mix in appropriate location on the robot deck.
 9. In the "Plate Preparation" module in STaCS, click "Process".
 10. STaCS will launch the EVOware software.
 11. Select "Run Direct" from the "Run" drop-down menu.
 12. When the Tecan has completed the process, click "Cancel".
 13. In the "File" drop-down menu, select "Exit, unload drivers".
 14. Select "Yes" when prompted to move arms to home position.
 15. Verify PCR reaction mix was aliquoted to the appropriate wells.
 16. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
 17. Click "Close" to exit "Plate Preparation" module.
 18. Proceed to – Punching FTA or non-FTA Blood Samples Using the BSD 600.
- D. Punching FTA or Non-FTA Blood Samples 600 Using the BSD:
1. Turn on the BSD robot and pressure pump. Ensure enough TYPE I water is in the Humidifier Bottle and adjust flow control screws for appropriate air pressure.
 2. Open STaCS and select the "Punch" module.
 3. Scan the appropriate BSD instrument and PCR plate.
 4. Click "Create Input File".
 5. STaCS will launch the "BSD Duet Menu" software. Type the user name and password.
 6. The BSD will automatically initialize.
 7. Select "Continue" and follow additional prompts.
 8. Select the "STaCS plate" or other appropriate plate layout. Make sure "Samples" and "Cleaning" are selected before continuing.
 9. Scan the PCR plate and place on appropriate location on the plate table, Select "Continue" and follow additional prompts



10. Scan the appropriate archive card and follow prompts for punching and cleaning steps.
 11. Repeat for each additional sample. Respond to query boxes as necessary.
 12. When punching is complete, pull the amplification plate off the plate table and place in the PCR hood.
 13. Verify all punches are present in appropriate wells and note any discrepancies in "Plate Comment" or "Well Comment" in STaCS.
 14. Select "End Run".
 15. Exit the BSD software. Select "Yes" to end run and "No" to print.
 16. Exit the "BSD Main Menu" by selecting "Exit"..
 17. Select "Yes" when prompted that "File Verification has completed, continue saving files?"
 18. For each amplification set, add 1 μ L of positive control to the appropriate well(s) in the amplification plate.
 19. Cover the amplification plate with an appropriate seal being careful not to cover the barcode and centrifuge for 1 minute.
 20. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
 21. Select "Close" to exit "Punch Module"
 22. When finished for the day, turn off pump and robot and clean out the chute mechanism when appropriate.
 23. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.
- E. Amplification
1. Transport the amplification plate into the PCR room.
 2. Open STaCS. Select the "Amplification" module. Place amplification plate on thermal cycler. Scan the thermal cycler and plate. Close the thermal cycler cover.
 3. Select the 26 cycle thermal cycler program and begin method.
 4. In STaCS, click on "Start Process". Click "Close" to exit the "Amplification" module.
 5. The thermal cycler is programmed using the "Max Mode" according to the chart below:



Temperature	Time	Number of cycles
98°C	30sec	3 cycles
64°C	40sec	
72°C	5sec	
96°C	10sec	23 cycles
61°C	40sec	
72°C	5sec	
68°C	2min	-
60°C	2min	-
10°C	∞	-

6. When PCR has completed, open STaCS. Select the “Amplification” module. Scan the appropriate amplification plate in “Available Plates” and click “Complete Process”. Click “Close” to exit the “Amplification” module.
7. Amplification plates should be stored in the freezer if not proceeding directly with capillary electrophoresis.

6.3 Procedure for Buccal Samples

A. Master Mix Addition

1. In STaCS, select the “Master Mix Addition” module.
2. Select “Create Plate”.
3. In the “Plate Layout” drop-down menu, select the appropriate layout.
4. In the “Plate Source” drop-down menu, select the appropriate source.
5. If applicable, select the appropriate amplification kit in the “Amplification Plate Protocol” drop-down menu.
6. In the “Amplification Plate Analytical Process” drop-down menu, select “Investigator 24Plex GO!” (if applicable).
7. Select the appropriate plate from the “Plate Create Worklist” and click “Allocate”. Samples may be allocated from multiple plates when amplifying rework samples.
8. Select “Create”.
9. If applicable, click “Yes” to move controls to next available well.
10. Place barcode on amplification plate with plate name facing up and fold excess underneath the plate edge. Scan plate.
11. Click “Close”.
12. Place amplification plate (PCR) and buccal lysis daughter plate in appropriate location on Tecan deck or for re-work plate proceed to [Manual PCR Preparation for Buccal Samples](#).



13. Scan the appropriate amplification plate.
 14. Select "Get Scenario(s)".
 15. If applicable, select the appropriate scenario and click "OK".
- B. Manual PCR Preparation for Buccal Samples:
1. Scan the appropriate reagents and appropriate daughter and amplification plates.
 2. In the "Master Mix Addition" module in STaCS, click "Process".
 3. If the "Select Transfer Map Directory" Screen appears, select "c:", double click "C:\\" to expand the folder, and double click the "PickSample" folder. Click "OK". Exit the Excel workbook. This file will be used to create the plate layout for the amplification worksheet.
 4. To print the amplification worksheet, go to the Start menu, navigate to "M:\STaCS Production" and open "STaCS pickSample.xls".
 5. Press "Ctrl+W" and select the pickSample.csv file from the "C:\PickSample" folder.
 6. Enter the number of reactions. To ensure enough PCR reaction mix is prepared, include controls and additional reactions in the calculation.
 7. Click "Save as" and save to separate folder or select "Print".
 8. Exit and do not save.
 9. Prepare PCR reaction mix:
 - a) *Vortex the PCR components well and centrifuge briefly prior to use.*
 - b) *Prepare the PCR reaction mix by adding the following volumes in a microcentrifuge tube or use the values calculated in the PickSample amplification worksheet. To ensure enough PCR reaction mix is prepared, factor additional reactions to the calculations.*
 - i. *Fast Reaction Mix 3.75 μ L X # of samples*
 - ii. *Primer Mix 6.25 μ L X # of samples*
 - c) *Vortex the reaction mix and spin briefly.*
 10. Aliquot 10 μ L of PCR reaction mix into appropriate wells on the 96-well amplification plate.
 11. Add 1 μ L of swab lysate to each well containing PCR reaction mix. 0.5-3.0 μ L of swab lysate may also be used.
 12. For each amplification set, add 1 μ L of positive control to the appropriate well(s) in the amplification plate.
 13. Cover the amplification plate with an appropriate seal being careful not to cover the barcode and centrifuge for 1 minute.
 14. In the "Record Activity Completion Result" window in STaCS, select the appropriate result and click "OK".
 15. Click "Close" to exit the "Master Mix Addition" module.
 16. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.



C. Automated PCR Preparation for Buccal Samples:

1. Scan the robot, appropriate reagents, lysis daughter plate and PCR plate.
2. Turn on robot.
3. Prepare PCR reaction mix as listed above in part B.
4. Place PCR reaction mix in the appropriate location on the robot deck.
5. In the "Master Mix Addition" module in STaCS, click "Process"
6. STaCS will launch the EVOware software.
7. Select "Run Direct" from the "Run" drop-down menu. Enter appropriate template volume.
8. When the Tecan has completed the process, click "Cancel".
9. In the "File" drop-down menu, select "Exit, unload drivers".
10. Select "Yes" when prompted to move arms to home position.
11. Verify PCR amplification mix has been aliquoted to the appropriate wells.
12. For each amplification set, add 1 μ L of positive control to the appropriate well(s) in the amplification plate.
13. Cover the amplification plate with an appropriate seal being careful not to cover the barcode and centrifuge for 1 minute.
14. In the "Record Activity Completion Result" window in STaCS, select the appropriate result and click "OK".
15. Click "Close" to exit the "Master Mix Addition" module.
16. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.

D. Amplification

1. Transport the amplification plate into the PCR room.
2. Open STaCS. Select the "Amplification" module. Scan the thermal cycler and amplification plate. Close the thermal cycler cover.
3. Select the 27 cycle thermal cycler program and begin method.
4. In STaCS, click on "Start Process". Click "Close" to exit the "Amplification" module.
5. The thermal cycler is programmed using the "Max Mode" according to the chart below:



Temperature	Time	Number of cycles
98°C	30sec	3 cycles
64°C	40sec	
72°C	5sec	
96°C	10sec	24 cycles
61°C	40sec	
72°C	5sec	
68°C	2min	-
10°C	∞	-

6. When PCR has completed, open STaCS. Select the “Amplification” module. Scan the appropriate amplification plate in “Available Plates” and click “Complete Process”. Click “Close” to exit the “Amplification” module.
7. Amplification plates should be stored in the freezer if not proceeding directly with capillary electrophoresis.

6.4 Procedure for Extracted Samples

A. Master Mix Addition

1. In STaCS, select the “Master Mix Addition” module.
2. Select “Create Plate”.
3. In the “Plate Layout” drop-down menu, select the appropriate layout.
4. In the “Plate Source” drop-down menu, select the appropriate source.
5. If applicable, select the appropriate amplification kit in the “Amplification Plate Process Type” drop-down menu.
6. In the “Amplification Plate Analytical Process” drop-down menu, select “Investigator 24Plex GO!” (if applicable).
7. Select the appropriate plate from the “Plate Create Worklist” and click “Allocate”. Samples may be allocated from multiple plates when amplifying rework samples.
8. Select “Create”.
9. If applicable, click “Yes” to move controls to next available well.
10. Place barcode on amplification plate with plate name facing up and fold excess underneath the plate edge. Scan plate.
11. Click “Close”.
12. Scan the appropriate amplification plate.
13. Select “Get Scenario(s)”.
14. If applicable, select the appropriate scenario and click “OK”.



B. Manual PCR Preparation for Extracted Samples:

1. Scan the appropriate reagents and appropriate extraction and amplification plates.
2. In the "Master Mix Addition" module in STaCS, click "Process".
3. If the "Select Transfer Map Directory" Screen appears, select "c:", double click "C:\\" to expand the folder, and double click the "PickSample" folder. Click "OK". Exit the Excel workbook. This file will be used to create the plate layout for the amplification worksheet.
4. To print the amplification worksheet, go to the Start menu, navigate to "M:\STaCS Production" and open "STaCS pickSample.xls".
5. Press "Ctrl+W" and select the pickSample.csv file from the "C:\PickSample" folder.
6. Enter the number of reactions. To ensure enough PCR reaction mix is prepared, include controls and additional reactions in the calculation.
7. Click "Save as" and save to separate folder or Select "Print".
8. Exit and do not save.
9. Prepare PCR reaction mix:
 - a) *Vortex the PCR components well and centrifuge briefly prior to use.*
 - b) *Prepare the PCR reaction mix by adding the following volumes per reaction in a microcentrifuge tube or use the values calculated in the PickSample amplification worksheet. To ensure enough PCR reaction mix is prepared, factor additional reactions to the calculations.*
 - i. *Fast Reaction Mix 3.75 μ L X # of samples*
 - ii. *Primer Mix 6.25 μ L X # of samples*
 - c) *Vortex reaction mix and spin briefly.*
10. Aliquot 10 μ L of PCR reaction mix into appropriate wells on the 96-well amplification plate.
11. Add 1 μ L of DNA extract to each well containing PCR reaction mix. 0.5-4.0 μ L of DNA extract may also be used.
12. For each amplification set, add 1 μ L of positive control to the appropriate well(s) in the amplification plate.
13. Cover the amplification plate with an appropriate seal being careful not to cover the barcode and centrifuge for 1 minute.
14. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
15. Click "Close" to exit the "Master Mix Addition" module.
16. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.

C. Automated PCR Preparation for Extracted Samples:

1. Scan the robot, appropriate reagents, extraction plate and amplification plate.
2. Turn on robot.
3. Prepare PCR reaction mix as listed above in part B.



4. Place PCR reaction mix in appropriate location on the robot deck.
5. In the "Master Mix Addition" module in STaCS, click "Process"
6. STaCS will launch the EVOware software.
7. Select "Run Direct" from the "Run" drop-down menu.
8. When the Tecan has completed the process, click "Cancel".
9. In the "File" drop-down menu, select "Exit, unload drivers".
10. Select "Yes" when prompted to move arms to home position.
11. Verify PCR amplification mix has been aliquoted to the appropriate wells.
12. For each amplification set, add 1 µL of positive control to the appropriate well(s) in the amplification plate. 0.5 µL may also be used.
13. Cover the amplification plate with an appropriate seal being careful not to cover the barcode and centrifuge for 1 minute.
14. In the "Record Activity Completion Result" window in STaCS, select the appropriate result and click "OK".
15. Click "Close" to exit the "Master Mix Addition" module.
16. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.

D. Amplification

1. Transport the amplification plate into the PCR room.
2. Open STaCS. Select the "Amplification" module. Scan the thermal cycler and amplification plate. Close the thermal cycler cover.
3. Select the 26 cycle thermal cycler program and begin method. 27 cycles may also be used for failed samples.
4. In STaCS, click on "Start Process". Click "Close" to exit the "Amplification" module.
5. The thermal cycler is programmed using the "Max Mode" according to the chart below:

Temperature	Time	Number of cycles
98°C	30sec	3 cycles
64°C	40sec	
72°C	5sec	
96°C	10sec	23-24 cycles
61°C	40sec	
72°C	5sec	
68°C	2min	-
10°C	∞	-



6. When PCR has completed, open STaCS. Select the “Amplification” module. Scan the appropriate amplification plate in “Available Plates” and click “Complete Process”. Click “Close” to exit the “Amplification” module.
7. Amplification plates should be stored in the freezer if not proceeding directly with capillary electrophoresis.

11 Interpretations

Refer to the *Data Interpretation Guidelines* chapter

12 Records

Processing information is recorded in STaCS.

13 Literature References and Supporting Documentation

Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. Chapter 4 PCR Amplification: Capabilities and Cautions

Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. Chapter 5 Short Tandem Repeat (STR) Loci and Kits

Qiagen. Developmental Validation of the Investigator 24plex GO! Kit. June 2015.
<https://www.qiagen.com/us/resources/>

Kraemer, Melanie, et al. “Developmental validation of QIAGEN Investigator 24plex QS Kit and Investigator 24plex GO! Kit: Two 6-dye multiplex assays for the extended CODIS core loci.” *Forensic Science International: Genetics*. 2017. 29:9-20

Qiagen. Investigator 24plex GO! Handbook.

Tecan. Freedom EVO Operating Manual.

Tecan. Freedom EVOware Standard Getting Started Guide.

BSD 600-Duet Semi-Automated Dried Sample Punch Instrument Operator Guidelines.



CO-04-04 GLOBALFILER EXPRESS AMPLIFICATION

1 Scope

The GlobalFiler Express PCR amplification kit is a 23 marker multiplex PCR kit designed specifically for direct amplification from blood or buccal cells. This procedure may be performed manually or by using an approved robotic platform.

2 Related Chapters

Buccal Lysis Set-up

EZ1 Advanced XL Extraction

Capillary Electrophoresis

3 Safety

- A. Wear lab coat and gloves when working in the laboratory. Face masks may be used as appropriate.
- B. Some robotic workstations utilize large moving arms with sharp tips which could easily pierce the skin. While some safeguards exist to protect the operator, injury may still occur if not properly operated. Decks should not be accessed while an arm is in motion. Physical safety features built into robots should never be compromised for the purpose of expedience.

4 Equipment and Materials

- STaCS computer
- Thermocycler
- Centrifuge
- Microcentrifuge tubes, 1.5 mL
- Vortex
- 96-well amplification plate
- Appropriate plate seal
- Pipettes, adjustable
- Pipette tips
- GlobalFiler Express PCR Amplification Kit
- BSD 600 (instrument, computer, and appropriate software)
- Tecan Freedom EVO 100 (instrument, computer, and appropriate software)
- TE Buffer
- Prep-n-Go Buffer
- TYPE I water

5 Standards, Controls, and Calibration

- A. At least one positive control must be included with each amplification set. However, more may be used if needed.
- B. An amplification negative control must be included with each amplification set. This negative control consists of all amplification reagents without the addition of template DNA.



- C. Reagent blanks are denoted as “Negative” and amplification negatives as “Amplank” in STaCS.
- D. Blood plates have a combined reagent blank/amplification negative control.
- E. For buccal plates and extracted plates, the reagent blank must be amplified using the most sensitive template volume.

6 Procedures

6.1 General

- A. Inhibited blood samples may require a water wash prior to amplification.
- B. GlobalFiler Express kits should be stored in the freezer upon receipt.
- C. After initial use, kits should be refrigerated for no more than 6 months or until the expiration date on the kit (whichever comes first). **Do not refreeze kits after thawing.**

6.2 Adding Master Mix Additive to Master Mix

1. In STaCS, click on the “Receiving” tab.
2. Open the “Chemical Preparation” module.
3. In the “Material/Chemical/Reagent” drop-down menu, select “GlobalFiler Master Mix + Additive.”
4. Scan the Master Mix and Master Mix Additive.
5. Vortex the Master Mix and Master Mix Additive.
6. Briefly spin tubes in a microcentrifuge.
7. For 1,000 reaction kits, add 390 μ L Master Mix Additive to the Master Mix tube.
8. For 200 reaction kits, add 80 μ L Master Mix Additive to the Master Mix tube.
9. Gently mix by pipetting or inverting the tube until appropriately mixed.
10. Spin Master Mix tube briefly in a microcentrifuge.
11. In the “Chemical Preparation” module, select the “SMALL” label from the “Label Type” drop-down menu.
12. Click “Save” and place barcode on Master Mix bottle/tube.
13. Close “Chemical Preparation” module.
14. Master Mix is good for up to 6 months or until the expiration date on the kit (whichever comes first).
15. Consume Master Mix Additive in STaCS and discard tube.

6.3 Amplifying FTA Blood Samples

- A. Plate Create
 1. In STaCS, select the “Plate Create” module.
 2. Select “FTA-96 Wells” from the “Plate Type” drop-down menu. Ensure the “Protocol” and “Sample Nature” are “GlobalFiler Express” and “Blood” respectively.
 3. Select “Create Plate”.
 4. Scan sample barcodes to allocate samples onto the plate.



5. Select "Save".
 6. Click "Yes" to move controls to the next available well.
 7. Place barcode on PCR plate with plate name facing up and fold excess barcode underneath the plate edge.
 8. Click "Close" to exit "Create Plate" module.
- B. Automated Plate Preparation for FTA Samples
1. In STaCs, select the "Plate Preparation" module.
 2. Scan the appropriate PCR plate on the designated location on the robot deck.
 3. Click "Get Scenario(s)".
 4. Select the appropriate scenario.
 5. Scan the robot and consumables.
 6. Turn on robot.
 7. Prepare PCR reaction mix:
 - a) *Vortex the PCR components and centrifuge briefly prior to use.*
 - b) *Prepare the PCR reaction mix by adding the following volumes in a microcentrifuge tube. To ensure enough PCR reaction mix is prepared, factor additional reactions to the calculation.*
 - i. *Master Mix* 6 μ L X # of reactions
 - ii. *Primer Set* 6 μ L X # of reactions
 - iii. *TE⁻⁴ Buffer* 3 μ L X # of reactions
 - c) *Vortex the reaction mix and spin briefly.*
 8. Place PCR reaction mix in appropriate location on the robot deck.
 9. In the "Plate Preparation" module in STaCS, click "Process".
 10. STaCS will launch the EVOware software.
 11. Select "Run Direct" from the "Run" drop-down menu.
 12. When the Tecan has completed the process, click "Cancel".
 13. In the "File" drop-down menu, select "Exit, unload drivers".
 14. Select "Yes" when prompted to move arms to home position.
 15. Verify PCR reaction mix was aliquoted to the appropriate wells.
 16. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
 17. Click "Close" to exit "Plate Preparation" module.
- C. Manual Plate Preparation for FTA samples
1. Open the "Plate Preparation" module in STaCS.
 2. Scan the appropriate PCR plate.
 3. Click "Get Scenario(s)".



4. Select the appropriate scenario.
 5. Scan the consumables.
 6. **Optional:** To print a plate layout, select “Plate Content” and “Layout View” and then “Print.”
 7. In the “Plate Preparation” module of STaCS, click “Process”.
 8. Exit the Excel workbook. This file is used to create the plate layout for the amplification worksheet.
 9. To print the amplification worksheet, go to the Start menu, navigate to “M:\STaCS Production” and open the appropriate pickSample.xls file.
 10. Press “Ctrl+W” and select the pickSample.csv file from the “C:\PickSample” folder.
 11. Enter the number of reactions. To ensure enough PCR reaction mix is prepared, include controls and additional reactions in the calculation.
 12. Select “Print”.
 13. Exit and do not save.
 14. Prepare PCR reaction mix as listed in [Section 6.3; Part B](#)
 15. Aliquot 15 µL of PCR reaction mix into appropriate wells on the 96-well amplification plate.
 16. In the “Record Activity Completion Result” window, select the appropriate result and click “OK”.
 17. Click “Close” to exit the “Plate Preparation” module.
- D. Punching FTA Samples Using the BSD 600:
1. Turn on the BSD robot and pressure pump. Ensure enough TYPE I water is in the Humidifier Bottle and adjust flow control screws for appropriate air pressure.
 2. Open STaCS and select the “Punch” module.
 3. Scan the appropriate BSD instrument and PCR plate.
 4. Click “Create Input File”.
 5. STaCS will launch the “BSD Duet Menu” software. Type the appropriate user name and password.
 6. The BSD will automatically initialize.
 7. Select “Continue” and follow additional prompts.
 8. Select the “STaCS plate” or other appropriate plate layout. Make sure “Samples” and “Cleaning” are selected before continuing.
 9. Scan the PCR plate and place on the appropriate location on the plate table. Select “Continue” and follow additional prompts.
 10. Scan appropriate archive card and follow prompts for punching and cleaning steps.
 11. Repeat for each additional sample.
 12. Respond to query boxes as necessary.



13. When punching is complete, pull the amplification plate off the plate table and place in the PCR hood.
14. Verify all punches are present in appropriate wells and note any discrepancies in "Plate Comment" or "Well Comment" in STaCS.
15. Select "End Run".
16. Exit the BSD software. Select "Yes" to end run and "No" to print.
17. Exit the "BSD Main Menu" by selecting "Exit"..
18. Select "Yes" when prompted that "File Verification has completed, continue saving files?"
19. For each amplification set, add 3 μ L of the positive control to the appropriate wells in the amplification plate.
20. Cover the amplification plate with an appropriate seal being careful not to cover the barcode and centrifuge for 1 minute.
21. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
22. Select "Close" to exit "Punch Module"
23. When finished for the day, turn off pump and robot and clean out the chute mechanism when appropriate.
24. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.

E. Amplification

Proceed to [Section 6.6, Part C](#)

6.4 Amplifying Non-FTA Blood Samples

A. Plate Create

1. In STaCS, select the "Plate Create" module.
2. Select "FTA-96 Wells" from the "Plate Type" drop-down menu. Ensure the "Protocol" and "Sample Nature" are "GlobalFiler Express" and "Blood" respectively.
3. Select "Create Plate".
4. Scan sample barcodes to allocate samples onto the plate.
5. Select "Save".
6. Click "Yes" to move controls to the next available well.
7. Place barcode on PCR plate with plate name facing up and fold excess barcode underneath the plate edge.
8. Click "Close" to exit "Create Plate" module.

B. Plate Preparation for non-FTA samples

1. Open the "Plate Preparation" module in STaCS.
2. Scan the appropriate PCR plate.
3. Click "Get Scenario(s)".



4. Select the appropriate scenario.
 5. Scan the consumables.
 6. **Optional:** To print a plate layout, select “Plate Content” and “Layout View” and then “Print.”
 7. In the “Plate Preparation” module of STaCS, click “Process”.
 8. Exit the Excel workbook. This file creates the plate layout for the amplification worksheet.
 9. To print the amplification worksheet, go to the Start menu, navigate to “M:\STaCS Production” and open the appropriate pickSample.xls file.
 10. Press “Ctrl+W” and select the pickSample.csv file from the “C:\PickSample” folder.
 11. Enter the number of reactions. To ensure enough PCR reaction mix is prepared, include controls and additional reactions in the calculation.
 12. Select “Print”.
 13. Exit and do not save.
 14. Add 3 μ L Prep-n-Go solution to the appropriate wells.
 15. In the “Record Activity Completion Result” window, select the appropriate result.
 16. Click “Close” to exit the “Plate Preparation” module.
- C. Punching non-FTA samples using the BSD 600
1. Turn on the BSD robot and pressure pump. Ensure enough TYPE I water is in the Humidifier Bottle and adjust flow control screws for appropriate air pressure.
 2. Open STaCS and select the “Punch” module.
 3. Scan the appropriate instrument and plate.
 4. Click “Create Input File”.
 5. STaCS will launch the “BSD Duet Menu” software. Type the appropriate user name and password.
 6. The BSD will automatically initialize.
 7. Select “Continue” and follow additional prompts.
 8. Select the “STaCS plate” or other appropriate plate layout. Make sure “Samples” and “Cleaning” are selected before continuing.
 9. Place the amplification plate on the appropriate location on the BSD plate table and scan plate, Select “Continue” and follow additional prompts by clicking “Continue.”
 10. Scan appropriate archive card and follow prompts for punching and cleaning steps.
 11. Repeat for each additional sample.
 12. Respond to query boxes as necessary.
 13. When punching is complete, pull the amplification plate off the BSD plate table and place in the PCR hood.
 14. Select “End Run”.



15. Exit the BSD software. Select “Yes” to end run and “No” to print.
16. Exit the “BSD Main Menu” by selecting “Exit”.
17. Select “Yes” when prompted “File Verification has completed, continue saving files?”
18. Spin down plate to submerge punches in Prep-n-Go buffer.
19. Preparing PCR reaction mix:
 - a) *Vortex the PCR components well and centrifuge briefly prior to use.*
 - b) *Prepare the PCR reaction mix by adding the following volumes in a microcentrifuge tube. To ensure enough PCR reaction mix is prepared, factor additional reactions to the calculation.*
 - i. *Master Mix 6 μ L X # of reactions*
 - ii. *Primer Set 6 μ L X # of reactions*
 - c) *Vortex the reaction mix and spin briefly.*
20. Aliquot 12 μ L of PCR reaction mix into appropriate wells on the 96-well amplification plate.
21. For each amplification set, add 3 μ L of the positive control to the appropriate wells in the amplification plate.
22. Cover the amplification plate with an appropriate seal being careful not to cover the barcode and centrifuge for 1 minute.
23. In the “Record Activity Completion Result” window, select the appropriate result and click “OK”.
24. Select “Close” to exit “Punch Module”.
25. When finished for the day, turn off pump and robot and clean out the chute mechanism when appropriate.
26. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.

D. Amplification

Proceed to [Section 6.6, Part C](#)

6.5 Amplifying FTA Blood Samples Requiring a Water Wash

A. Plate Create

1. In STaCS, select the “Plate Create” module.
2. Select “FTA-96 Wells” from the “Plate Type” drop-down menu. Ensure the “Protocol” and “Sample Nature” are “GlobalFiler Express” and “Blood” respectively.
3. Select “Create Plate”.
4. Scan sample barcode to allocate samples onto the plate.
5. Select “Save”.
6. Click “Yes” to move controls to the next available well.
7. Place barcode on PCR plate with plate name facing up and fold excess barcode underneath the plate edge.
8. Click “Close” to exit “Create Plate” module.



- B. Plate Preparation for FTA samples requiring a water wash
1. In STaCS, select the “Plate Preparation” module.
 2. Scan the appropriate PCR plate.
 3. Click “Get Scenario(s)”.
 4. Select the appropriate scenario.
 5. Scan the consumables.
 6. **Optional:** To print a plate layout, select “Plate Content” and “Layout View” and then “Print.”
 7. In the “Plate Preparation” module of STaCS, click “Process”.
 8. Exit the Excel workbook. This file creates the plate layout for the amplification worksheet.
 9. To print the amplification worksheet, go to the Start menu, navigate to “M:\STaCS Production” and open the appropriate pickSample.xls file.
 10. Press “Ctrl+W” and select the pickSample.csv file from the “C:\PickSample” folder.
 11. Enter the number of reactions. To ensure enough PCR reaction mix is prepared, include controls and additional reactions in the calculation.
 12. Select “Print”.
 13. Exit and do not save.
 14. Aliquot 20 µL TYPE I water to the appropriate wells.
 15. In the “Record Activity Completion Result” window, select the appropriate result and click “OK”.
 16. Click “Close” to exit the “Plate Preparation” module.
- C. Punching water washed FTA samples using the BSD 600
1. Turn on the BSD robot and pressure pump. Ensure enough TYPE I water is in the Humidifier Bottle and adjust flow control screws for appropriate air pressure.
 2. Open STaCS and select the “Punch” module.
 3. Scan the appropriate instrument and plate.
 4. Click “Create Input File”.
 5. STaCS will launch the “BSD Duet Menu” software. Type the appropriate user name and password.
 6. The BSD will automatically initialize.
 7. Select “Continue” and follow additional prompts.
 8. Select the “STaCS plate” or other appropriate plate layout. Make sure “Samples” and “Cleaning” are selected before continuing.
 9. Place the amplification plate on the appropriate location on the BSD plate table and scan plate. Select “Continue” and follow additional prompts by clicking “Continue.”
 10. Scan appropriate archive card and follow prompts for punching and cleaning steps.



11. Repeat for each additional sample.
12. Respond to query boxes as necessary.
13. When punching is complete, pull amplification plate off the BSD plate table and place in a centrifuge. Spin down plate to ensure punches are submerged in water.
14. Select "End Run".
15. Exit the BSD software. Select "Yes" to end run and "No" to print.
16. Exit the "BSD Main Menu" by selecting "Exit"..
17. Select "Yes" when prompted "File Verification has completed, continue saving files?"
18. Remove water from wells using a mechanical pipette.
19. Prepare PCR reaction mix:
 - a) *Vortex the PCR components and centrifuge briefly prior to use.*
 - b) *Prepare the PCR reaction mix by adding the following volumes in a microcentrifuge tube. To ensure enough PCR reaction mix is prepared, factor additional reactions to the calculation.*
 - i. *Master Mix* 6 μ L X # of reactions
 - ii. *Primer Set* 6 μ L X # of reactions
 - iii. *TE⁻⁴ Buffer* 3 μ L X # of reactions
 - c) *Vortex the reaction mix and spin briefly.*
20. Aliquot 15 μ L of PCR reaction mix into appropriate wells on the 96-well amplification plate.
21. For each amplification set, add 3 μ L of the positive control to the appropriate wells in the amplification plate.
22. Cover the amplification plate with an appropriate seal being careful not to cover the barcode and centrifuge for 1 minute.
23. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
24. Select "Close" to exit "Punch Module".
25. When finished for the day, turn off pump and robot and clean out the chute mechanism when appropriate.
26. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.

D. Amplification

Proceed to [Section 6.6, Part C](#)

6.6 Amplifying Buccal Samples

A. Automated Master Mix Addition for Buccal Samples

1. In STaCS, select the "Master Mix Addition" module.
2. Select "Create Plate".
3. In the "Plate Layout" drop-down menu, select the appropriate layout.



4. In the "Plate Source" drop-down menu, select the appropriate source.
5. If applicable, select "GlobalFiler Express" in the "Amplification Plate Process Type" drop-down menu.
6. Select the appropriate plate from the "Plate Create Worklist" and click "Allocate".
7. Select "Create".
8. If applicable, click "Yes" to move controls to next available well.
9. Place barcode on PCR plate with plate name facing up and fold excess underneath the plate edge. Scan plate.
10. Click "Close".
11. Scan the appropriate PCR plate on the designated location on the robot deck.
12. Select "Get Scenario(s)".
13. If applicable, select the appropriate scenario and click "OK".
14. Scan the robot and appropriate reagents. Place the appropriate daughter plate on the robot deck and scan.
15. Turn on robot.
16. Prepare PCR reaction mix:
 - a) *Vortex the PCR components well and centrifuge briefly prior to use.*
 - b) *Prepare the PCR reaction mix by adding the following volumes in a microcentrifuge tube. To ensure enough PCR reaction mix is prepared, factor additional reactions to the calculation.*
 - i. *Master Mix 6 μ L X # of reactions*
 - ii. *Primer Set 6 μ L X # of reactions*
 - c) *Vortex reaction mix and spin briefly.*
17. Place PCR reaction mix in the appropriate location on the robot deck.
18. In the "Master Mix Addition" module in STaCS, click "Process"
19. STaCS will launch the EVOware software.
20. Select "Run Direct" from the "Run" drop-down menu.
21. When the Tecan has completed the process, click "Cancel".
22. In the "File" drop-down menu, select "Exit, unload drivers".
23. Select "Yes" when prompted to move arms to home position.
24. Verify PCR reaction mix was aliquoted to the appropriate wells.
25. For each amplification set, add 3 μ L of the positive control to the appropriate wells in the amplification plate.
26. Cover the amplification plate with an appropriate seal being careful not to cover the barcode and centrifuge for 1 minute.
27. In the "Record Activity Completion Result" window in STaCS, select the appropriate result and click "OK".



28. Click "Close" to exit the "Master Mix Addition" module.
 29. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.
- B. Manual Master Mix Addition for Buccal Samples
1. In STaCS, select the "Master Mix Addition" module.
 2. Select "Create Plate".
 3. In the "Plate Layout" drop-down menu, select the appropriate layout.
 4. In the "Plate Source" drop-down menu, select the appropriate source.
 5. If applicable, select "GlobalFiler Express" in the "Amplification Plate Process Type" drop-down menu.
 6. Select the appropriate plate from the "Plate Create Worklist" and click "Allocate". You may allocate samples from multiple plates when amplifying rework samples.
 7. Select "Create".
 8. Click "Yes" to move controls to next available well.
 9. Place barcode on PCR plate with plate name facing up and fold excess underneath the plate edge. Scan plate.
 10. Click "Close".
 11. Scan the appropriate PCR plate.
 12. Select "Get Scenario(s)".
 13. If applicable, select the appropriate scenario and click "OK".
 14. Scan the appropriate reagents and appropriate daughter plate(s).
 15. **Optional:** To print a plate layout, select "Plate Content" and "Layout View" and then "Print."
 16. In the "Master Mix Addition" module in STaCS, click "Process".
 17. Exit the Excel workbook. This file will be used to create the plate layout for the amplification worksheet.
 18. To print the amplification worksheet, go to the Start menu, navigate to "M:\STaCS Production" and open "STaCS pickSample.xls".
 19. Press "Ctrl+W" and select the pickSample.csv file from the "C:\PickSample" folder.
 20. Enter the number of reactions. To ensure enough PCR reaction mix is prepared, include controls and additional reactions in the calculation.
 21. Select "Print".
 22. Exit and do not save.
 23. Prepare PCR reaction mix:
 - a) *Vortex the PCR components well and centrifuge briefly prior to use.*
 - b) *Prepare the PCR reaction mix by adding the following volumes in a microcentrifuge tube. To ensure enough PCR reaction mix is prepared, factor additional reactions to the calculation.*



- i. Master Mix 6 μ L X # of reactions
 - ii. Primer Set 6 μ L X # of reactions
 - c) Vortex reaction mix and spin briefly.
24. Aliquot 12 μ L of PCR reaction mix into appropriate wells on the 96-well amplification plate.
 25. Add 3 μ L buccal lysis to each well containing PCR reaction mix.
 26. For each amplification set, add 3 μ L of the positive control to the appropriate wells in the amplification plate.
 27. Cover the amplification plate with an appropriate seal being careful not to cover the barcode and centrifuge for 1 minute.
 28. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
 29. Click "Close" to exit the "Master Mix Addition" module.
 30. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.

C. Amplification

1. Transport the amplification plate into the PCR room.
2. Open STaCS. Select the "Amplification" module. Place amplification plate on thermal cycler. Scan the thermal cycler and plate. Close the thermal cycler cover.
3. Select the 25 cycle thermal cycler program and begin method.
4. In STaCS, click on "Start Process". Click "Close" to exit the "Amplification" module.
5. The thermal cycler is programmed according to the chart below:

PCR Thermal Cycler	Times and Temperatures for GlobalFiler Express Amplification Kits				
	Initial Incubation Step	25 cycles each		Final Extension	Final Hold
Veriti	Hold	Denature	Anneal / Extend	Hold	Hold
	95°C 1 min	94°C 3 sec	60°C 30 sec	60°C 8 min	4°C ∞ hold

6. When PCR has completed, open STaCS. Select the "Amplification" module. Scan the appropriate plate in "Available Plates" and click "Complete Process". Click "Close" to exit the "Amplification" module.
7. Amplification plates may be stored in the refrigerator until analysis is complete. If plates need to be stored longer than 2 weeks, they should be stored in the freezer.

6.7 Amplifying Extracted Samples

A. Master Mix Addition

1. In STaCS, select the "Master Mix Addition" module.
2. Select "Create Plate".
3. In the "Plate Layout" drop-down menu, select the appropriate layout.



4. In the "Plate Source" drop-down menu, select the appropriate source.
5. Select "GlobalFiler Express" in the "Amplification Plate Process Type" drop-down menu.
6. Select the appropriate plate from the "Plate Create Worklist" and click "Allocate". Samples from multiple plates may be allocated when amplifying rework samples.
7. Select "Create".
8. Place barcode on amplification plate with plate name facing up and fold excess underneath the plate edge. Scan plate.
9. Click "Close".
10. Scan the appropriate amplification plate.
11. **Optional:** To print a plate layout, select "Plate Content" and "Layout View" and then "Print."
12. Select "Get Scenario(s)".
13. Scan the appropriate reagents and appropriate extraction plate(s).
14. **Optional:** To print a plate layout, select "Plate Content" and "Layout View" and then "Print."
15. In the "Master Mix Addition" module in STaCS, click "Process".
16. Exit the Excel workbook. This file will be used to create the plate layout for the amplification worksheet.
17. To print the amplification worksheet, go to the Start menu, navigate to "M:\STaCS Production" and open "STaCS pickSample.xls".
18. Press "Ctrl+W" and select the pickSample.csv file from the "C:\PickSample" folder.
19. Enter the number of reactions. To ensure enough PCR reaction mix is prepared, include controls and additional reactions in the calculation.
20. Select "Print".
21. Exit and do not save.
22. Prepare PCR reaction mix:
 - a) *Vortex the Master Mix and Primer Set and centrifuge briefly prior to use.*
 - b) *Prepare the PCR reaction mix by adding the following volumes per reaction in a microcentrifuge tube. To ensure enough PCR reaction mix is prepared, factor additional reactions to the calculation.*
 - i. *Master Mix 6 μ L X # of reactions*
 - ii. *Primer Set 6 μ L X # of reactions*
 - c) *Vortex reaction mix and spin tube briefly in a microcentrifuge.*
23. Aliquot 12 μ L of PCR reaction mix into appropriate wells on the 96-well amplification plate.
24. Add 3 μ L of DNA extract to each well containing PCR reaction mix. 1 and 2 μ L of DNA extract may also be used. Keep in mind, the total DNA input must be 3 μ L. Add appropriate amount of TE⁻⁴ to bring the volume up to 3 μ L (for example, 1 μ L extract + 2 μ L TE⁻⁴ or 2 μ L extract + 1 μ L TE⁻⁴).



25. For each amplification set, add 3 µL of positive control to the appropriate wells in the amplification plate.
26. Add 3 µL TE⁻⁴ to the amplification negative control.
27. Cover the amplification plate with an appropriate seal being careful not to cover the barcode and centrifuge for 1 minute.
28. In the “Record Activity Completion Result” window, select the appropriate result and click “OK”.
29. Click “Close” to exit the “Master Mix Addition” module.
30. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.

B. Amplification

1. Transport the amplification plate into the PCR room.
2. Open STaCS. Select the “Amplification” module. Place plate on thermal cycler. Scan the thermal cycler and plate. Close the thermal cycler cover.
3. Select the 25 or 26 cycle thermal cycler program and begin method.
4. In STaCS, click on “Start Process”. Click “Close” to exit the “Amplification” module.
5. The thermal cycler is programmed according to the chart below:

PCR Thermal Cycler	Times and Temperatures for GlobalFiler Express Amplification Kits				
	Initial Incubation Step	25 or 26 cycles each		Final Extension	Final Hold
Veriti	Hold	Denature	Anneal / Extend	Hold	Hold
	95°C 1 min	94°C 3 sec	60°C 30 sec	60°C 8 min	4°C ∞ hold

6. When PCR has completed, open STaCS. Select the “Amplification” module. Scan the appropriate plate in “Available Plates” and click “Complete Process”. Click “Close” to exit the “Amplification” module.
7. Amplification plates may be stored in the refrigerator until analysis is complete. If plates need to be stored longer than 2 weeks, they should be stored in the freezer.

7 Interpretation

Refer to the *Data Interpretation Guidelines* chapter

8 Records

Processing information is recorded in STaCS.

9 Literature References and Supporting Documentation

Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. Chapter 4 PCR Amplification: Capabilities and Cautions

Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. Chapter 5 Short Tandem Repeat (STR) Loci and Kits



Flores, Shahida, et al. "Internal validation of the GlobalFiler Express PCR Amplification Kit for the direct amplification of reference DNA samples on a high-throughput automated workflow." *Forensic Science International: Genetics*. 2014. 10:33-39

Wang, Dennis, et al. "Developmental validation of the GlobalFiler Express PCR Amplification Kit: A 6-dye multiplex assay for the direct amplification of reference samples." *Forensic Science International: Genetics*. 2015. 19:148-155

Applied Biosystems. GlobalFiler Express PCR Amplification Kit User Guide.

Tecan. Freedom EVO Operating Manual.

Tecan. Freedom EVOware Standard Getting Started Guide.

BSD 600-Duet Semi-Automated Dried Sample Punch Instrument Operator Guidelines.



CO-04-05 YFILER PLUS AMPLIFICATION

1 Scope

The Yfiler Plus amplification kit is a 25 Y-STR marker multiplex PCR kit designed to allow direct amplification from blood or buccal cells.

2 Related Chapters

EZ1 Advanced XL Extraction

QIA Symphony SP Extraction

Capillary Electrophoresis

Partial Matches and Familial Searches

Match Verification

3 Safety

Wear lab coat and gloves when working in the laboratory. Face masks may be used as appropriate.

4 Equipment and Materials

- STaCS computer
- Thermal cycler
- Centrifuge
- Microcentrifuge tubes, 1.5 mL
- Vortex
- 96-well amplification plate
- Amplification cover
- Pipettes, adjustable
- Pipette tips
- Yfiler Plus PCR Amplification Kit
- Prep-n-Go™ Buffer
- BSD 600 (instrument, computer, and appropriate software)
- Tecan Freedom EVO 100 (instrument, computer, and appropriate software)
- TYPE I Water
- TE Buffer

5 Standards, Controls, and Calibration

- A. A positive control must be included with each amplification set. However, more than one positive control may be used if necessary.
- B. An amplification negative control must be included with each amplification set. This negative control will consist of all amplification reagents without the addition of template DNA.
- C. Reagent blanks are denoted as “Negative” and amplification negatives as “Ampblank” in STaCS.



- D. Blood plates have a combined reagent blank/amplification negative control.
- E. For buccal plates and extracted plates, the reagent blank must be amplified using the most sensitive template volume.

6 Procedures

6.1 Amplifying FTA Blood Samples

A. Plate Create

1. In STaCS, select the "Plate Create" module.
2. Select "FTA-96 Wells" from the "Plate Type" drop-down menu. Ensure the "Protocol" and "Sample Nature" are "Yfiler Plus" and "Blood" respectively.
3. Select "Create Plate".
4. Scan sample barcodes to allocate onto the plate.
5. Select "Save".
6. If applicable, click "Yes" to move controls to next available well.
7. Place barcode on PCR plate with plate name facing up and fold excess underneath the plate edge.
8. Click "Close". To exit "Create Plate" module.

B. Manual Plate Preparation for FTA samples

1. Open the "Plate Preparation" module in STaCS.
2. Scan the appropriate PCR plate.
3. Click "Get Scenario(s)".
4. Select the appropriate scenario.
5. Scan the consumables.
6. Optional: To print a plate layout, select "Plate Content" and "Layout View" and then "Print."
7. In the "Plate Preparation" module of STaCS, click "Process".
8. Exit the excel file. This file is used to create the plate layout for the amplification worksheet.
9. To print the amplification worksheet, go to the Start menu, navigate to "M:\STaCS Production" and open the appropriate pickSample.xls file.
10. Press "Ctrl+W" and select the pickSample.csv file from the "C:\PickSample" folder.
11. Enter the number of reactions. To ensure enough PCR reaction mix is prepared, factor additional reactions in the calculation.
12. Select "Print".
13. Exit and do not save.
14. Prepare PCR reaction mix:
 - a) *Vortex the PCR components and centrifuge briefly prior to use.*



- b) *Prepare the PCR reaction mix by adding the following volumes per reaction in a microcentrifuge tube.*
 - i. *Master Mix* 10 μ L X # of reactions
 - ii. *Primer Set* 5 μ L X # of reactions
 - iii. *TE⁻⁴ Buffer* 10 μ L X # of reactions
 - c) *Vortex the reaction mix and spin briefly.*
 15. Aliquot 25 μ L of PCR reaction mix into appropriate wells on the 96-well amplification plate.
 16. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
 17. Click "Close" to exit the "Plate Preparation" module.
- C. Punching FTA Samples Using the BSD 600:
1. Turn on the BSD robot and pressure pump. Ensure enough TYPE I water is in the Humidifier Bottle and adjust flow control screws for appropriate air pressure.
 2. Open STaCS and select the "Punch" module.
 3. Scan the appropriate instrument and plate.
 4. Click "Create Input File".
 5. STaCS will launch the "BSD Duet Menu" software. Type the appropriate user name and password.
 6. The BSD will automatically initialize.
 7. Select "Continue" and follow additional prompts by clicking "Continue."
 8. Select the "STaCS plate" or other appropriate plate layout. Make sure "Samples" and "Cleaning" are selected before continuing.
 9. Place the amplification plate on the appropriate location on the BSD plate table and scan plate. Select "Continue" and follow additional prompts by clicking "Continue."
 10. Scan appropriate archive card and follow prompts for punching and cleaning steps.
 11. Repeat for each additional sample.
 12. Respond to query boxes as necessary.
 13. When punching is complete, pull the amplification plate off the BSD plate table and place in the PCR hood.
 14. Select "End Run".
 15. Exit the BSD software. Select "Yes" to end run and "No" to print.
 16. Exit the "BSD Main Menu" by selecting "Exit".
 17. Select "Yes" when prompted that "File Verification has completed, continue saving files?"
 18. For each amplification set, add 1 μ L of the positive control for 28 cycles **OR** 2 μ L of the positive control for 27 cycles to the appropriate wells in the amplification plate.



19. Cover the amplification plate with seal being careful not to cover the barcode and centrifuge for 1 minute.
20. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
21. Select "Close" to exit "Punch Module"
22. When finished for the day, turn off pump and robot and clean out the chute mechanism when appropriate.
23. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.

D. Amplification

Proceed to Section 6.3, Part B

6.2 Amplifying Non-FTA Blood Samples

A. Plate Create

1. In STaCS, select the "Plate Create" module.
2. Select "FTA-96 Wells" from the "Plate Type" drop-down menu. Ensure the "Protocol" and "Sample Nature" are "YFiler Plus" and "Blood" respectively.
3. Select "Create Plate".
4. Scan sample barcodes to allocate samples onto the plate.
5. Select "Save".
6. Click "Yes" to move controls to the next available well.
7. Place barcode on PCR plate with plate name facing up and fold excess barcode underneath the plate edge.
8. Click "Close" to exit "Create Plate" module.

B. Plate Preparation for non-FTA samples

1. Open the "Plate Preparation" module in STaCS.
2. Scan the appropriate PCR plate.
3. Click "Get Scenario(s)".
4. Select the appropriate scenario.
5. Scan the consumables.
6. Optional: To print a plate layout, select "Plate Content" and "Layout View" and then "Print."
7. In the "Plate Preparation" module of STaCS, click "Process".
8. Exit the excel workbook. This file creates the plate layout for the amplification worksheet.
9. To print the amplification worksheet, go to the Start menu, navigate to "M:\STaCS Production" and open the appropriate pickSample.xls file.
10. Press "Ctrl+W" and select the pickSample.csv file from the "C:\PickSample" folder.



11. Enter the number of reactions. To ensure enough PCR reaction mix is prepared, factor additional reactions in the calculation.
 12. Select "Print".
 13. Exit and do not save.
 14. Add 2 μ L Prep-n-Go Buffer to the appropriate wells.
 15. In the "Record Activity Completion Result" window, select the appropriate result.
 16. Click "Close" to exit the "Plate Preparation" module.
- C. Punching non-FTA samples using the BSD 600
1. Turn on the BSD robot and pressure pump. Ensure enough TYPE I water is in the Humidifier Bottle and adjust flow control screws for appropriate air pressure.
 2. Open STaCS and select the "Punch" module.
 3. Scan the appropriate instrument and plate.
 4. Click "Create Input File".
 5. STaCS will launch the "BSD Duet Menu" software. Type the appropriate user name and password.
 6. The BSD will automatically initialize.
 7. Select "Continue" and follow additional prompts.
 8. Select the "STaCS plate" or other appropriate plate layout. Make sure "Samples" and "Cleaning" are selected before continuing.
 9. Place the amplification plate on the appropriate location on the BSD plate table and scan plate, Select "Continue" and follow additional prompts by clicking "Continue."
 10. Scan appropriate archive card and follow prompts for punching and cleaning steps.
 11. Repeat for each additional sample.
 12. Respond to query boxes as necessary.
 13. When punching is complete, pull the amplification plate off the BSD plate table and place in the PCR hood.
 14. Select "End Run".
 15. Exit the BSD software. Select "Yes" to end run and "No" to print.
 16. Exit the "BSD Main Menu" by selecting "Exit".
 17. Select "Yes" when prompted that "File Verification has completed, continue saving files?"
 18. Spin down plate to submerge punches in Prep-n-Go buffer.
 19. Preparing PCR reaction mix:
 - a) *Vortex the PCR components and centrifuge briefly prior to use.*



- b) *Prepare the PCR reaction mix by adding the following volumes per reaction in a microcentrifuge tube.*
 - i. *Master Mix* *10 µL X # of reactions*
 - ii. *Primer Set* *5 µL X # of reactions*
 - iii. *TE⁻⁴ Buffer* *8 µL X # of reactions*
- c) *Vortex the reaction mix and spin briefly.*
20. Aliquot 23 µL of PCR reaction mix into appropriate wells on the 96-well amplification plate.
21. For each amplification set, add 2 µL of the positive control for 27 cycles **OR** 1 µL of the positive control and 1 µL of TE⁻⁴ Buffer for 28 cycles to the appropriate wells in the amplification plate.
22. Cover the amplification plate with seal being careful not to cover the barcode and centrifuge for 1 minute.
23. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
24. Select "Close" to exit "Punch Module".
25. When finished for the day, turn off pump and robot and clean out the chute mechanism when appropriate.
26. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.

D. Amplification

Proceed to Section 6.3, Part B

6.3 Amplifying Extracted Samples

A. Master Mix Addition

1. In STaCS, select the "Master Mix Addition" module.
2. Select "Create Plate".
3. In the "Plate Layout" drop-down menu, select the appropriate layout.
4. In the "Plate Source" drop-down menu, select the appropriate source.
5. Select "Yfiler Plus" in the "Amplification Plate Process Type" drop-down menu.
6. Select the appropriate plate from the "Plate Create Worklist" and click "Allocate".
7. Select "Create".
8. Place barcode on amplification plate with plate name facing up and fold excess underneath the plate edge. Scan plate.
9. Click "Close".
10. Scan the appropriate amplification plate.
11. Optional: To print a plate layout, select "Plate Content" and "Layout View" and then "Print."
12. Select "Get Scenario(s)".



13. Scan the appropriate reagents and appropriate extraction plate(s).
14. Optional: To print a plate layout, select "Plate Content" and "Layout View" and then "Print."
15. In the "Master Mix Addition" module in STaCS, click "Process".
16. Exit the excel workbook. This file will be used to create the plate layout for the amplification worksheet.
17. To print the amplification worksheet, go to the Start menu, navigate to "M:\STaCS Production" and open "STaCS pickSample.xls".
18. Press "Ctrl+W" and select the pickSample.csv file from the "C:\PickSample" folder.
19. Enter the number of reactions. To ensure enough PCR reaction mix is prepared, factor additional reactions in the calculation.
20. Select "Print".
21. Exit and do not save.
22. Prepare PCR reaction mix:
 - a) *Vortex the PCR components and centrifuge briefly prior to use.*
 - b) *Prepare the PCR reaction mix by adding the following volumes per reaction in a microcentrifuge tube.*
 - i. *Master Mix* *10 µL X # of reactions*
 - ii. *Primer Set* *5 µL X # of reactions*
 - iii. *TE⁻⁴ Buffer* *7 µL X # of reactions*
 - c) *Vortex reaction mix and spin tube briefly.*
23. Aliquot 22 µL of PCR reaction mix into appropriate wells on the 96-well amplification plate.
24. Add 3 µL of DNA extract to each well containing PCR reaction mix. **Note:** Volumes of 4 µL, 5 µL, 6 µL, 8 µL and 10 µL of DNA extract may also be used. Keep in mind, the total reaction volume must be 25 µL. Add appropriate amount of TE⁻⁴ to bring the volume up to 25 µL (for example, 3 µL extract + 7 µL TE⁻⁴ or 8 µL extract + 2 µL TE⁻⁴).
25. For each amplification set, add 2 µL of the positive control and 1 µL of TE⁻⁴ Buffer for 27 cycles **OR** 1 µL of the positive control and 2 µL of TE⁻⁴ Buffer for 28 cycles to the appropriate wells in the amplification plate.
26. Add 3 µL TE⁻⁴ to the amplification negative control.
27. Cover the amplification plate with seal being careful not to cover the barcode and centrifuge for 1 minute.
28. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
29. Click "Close" to exit the "Master Mix Addition" module.
30. Decontaminate the bench top of the laminar flow hood with a 10% bleach solution.



B. Amplification

1. Transport the amplification plate into the PCR room.
2. Open STaCS. Select the “Amplification” module. Place plate on thermal cycler. Scan the thermal cycler and plate. Close the thermal cycler cover.
3. Select the 27 **OR** 28 cycle thermal cycler program and begin method.
4. In STaCS, click on “Start Process”. Click “Close” to exit the “Amplification” module.
5. The thermal cycler is programmed according to the chart below:

PCR Thermal Cycler	Times and Temperatures for YFiler Plus Amplification Kits				
	Initial Incubation Step	27 OR 28 cycles each		Final Extension	Final Hold
Veriti/9700	Hold	Denature	Anneal / Extend	Hold	Hold
	95°C	94°C	61.5°C	60°C	4°C
	1 min	4 sec	1 min	22 min	∞ hold

6. When PCR has completed, open STaCS. Select the “Amplification” module. Scan the appropriate plate in “Available Plates” and click “Complete Process”. Click “Close” to exit the “Amplification” module.
7. Amplification plates may be stored in the refrigerator until analysis is complete. If plates need to be stored longer than 2 weeks, they should be stored in the freezer.

7 Interpretations

Refer to the *Data Interpretation Guidelines* chapter

8 Records

Processing information is recorded in STaCS.

9 Literature References and Supporting Documentation

Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. **Chapter 4: PCR Amplification: Capabilities and Cautions**

Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. **Chapter 5: Short Tandem Repeat (STR) Loci and Kits**

Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. **Chapter 13: Y-Chromosome DNA Testing**

Applied Biosystems. *Yfiler Plus PCR Amplification Kit User's Manual*.

Gopinath, Siddhita, et al. “Developmental validation of the Yfiler Plus PCR Amplification Kit: An enhanced Y-STR multiplex for casework and database applications.” *Forensic Sci Int Genet*. 2016. 24:164-175.

BSD 600-Duet Semi-Automated Dried Sample Punch Instrument Operator Guidelines.



CO-04-06 CAPILLARY ELECTROPHORESIS

1 Scope

Amplified samples are analyzed by injection into a genetic analyzer. 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 genotyping software analyzes collected data and assigns allele designations. Set-up procedures may be performed manually or using an approved robotic platform.

2 Related Chapters

Data Interpretation Guidelines

Investigator 24plex GO! Amplification

GlobalFiler Express Amplification

Yfiler Plus Amplification

3 Safety

- A. Some robotic workstations utilize large moving arms with sharp tips which could easily pierce the skin. While some safeguards exist to protect the operator, injury may still occur if not properly operated. Decks should not be accessed while an arm is in motion. Physical safety features built into robots should never be compromised for the purpose of expedience.
- B. Formamide is harmful if absorbed through the skin and is considered an irritant. Polymer is considered an irritant. Appropriate personal protective equipment (PPE) must be worn.
- C. The CE contains a laser. Operating this instrument without precautions, or in any manner that is not in compliance with recommended procedures, may be unsafe and can cause physical harm from radiation exposure.

4 Equipment and Materials

- Genetic Analyzers (instrument, computer, and appropriate software)
- TECAN Freedom EVO 100 (instrument, computer, and appropriate software)
- Capillary arrays
- Conditioning reagent
- Reservoirs and reservoir septa
- Buffer (10x) w/EDTA
- Prepackaged anode/cathode buffer reservoirs
- Polymer
- Matrix standard kits
- Internal size standards
- Allelic ladders
- Hi-Di formamide
- 96-well semi-skirted sequencer plates and plate septa
- Plate cassette
- Vortex



- Centrifuge
- Microcentrifuge tubes – 1.5 mL
- Pipettes and tips

5 Standards, Controls, and Calibration

- A. Appropriate reagent blanks, ladders, positive and amplification negative controls are included with each CE instrument run.
- B. A reagent blank must be included with a subsequent run if samples are run on different instrument models or amplified with different primers. In addition, any changes in the injection and/or volume conditions for a sample must also be performed on the reagent blank if the changes are more sensitive than the initial run.
- C. An internal size standard must be added to each sample.
- D. Standard injection times should be used for the initial injection.
 1. Investigator 24plex GO!
 - a) *Initial injection time is 30 seconds on the 3500xl.*
 - b) *Samples may be re-injected for 15 and/or 30 seconds on the 3500xl.*
 2. GlobalFiler Express
 - a) *Initial injection time is 24 seconds on the 3500xl.*
 - b) *Samples may be re-injected for 12 or 24 seconds.*
 3. Yfiler Plus
 - a) *Initial injection time is 24 seconds on the 3500xl.*
 - b) *Samples may be re-injected for 12 or 24 seconds.*

6 Procedure

6.1 Logging into STaCS using CE Computers

1. Hold the shift button and right click on the STaCS icon.
2. Click “Run as different user”.
3. Type in the user’s CODIS login name and password.

6.2 Weekly/Biweekly maintenance

1. Under the “Configuration” tab in STaCS, select “Sequencer Configuration”.
2. Scan the appropriate CE instrument and consumables.
3. Select “Save”.
4. Click “Close” to exit the module.
5. Perform the water wash wizard, flush the water trap, and replenish the polymer, buffer and water reservoirs (if applicable).
6. Under the “Utilities” tab in STaCS, select the “Instrument Maintenance” module.
7. Scan the appropriate CE instrument.
8. Click “Run Scheduler”.



9. Verify the weekly/biweekly maintenance schedule is highlighted.
10. In the "Maintenance Result" window, select the appropriate result. Add appropriate comments as needed (e.g. # of injections, changed array, etc.).
11. Click "Save".
12. Click "Close" to exit the "Run Schedule" screen.
13. Click "Close" to exit the "Instrument Maintenance" module.

6.3 Electrophoresis Plate Prep

1. In STaCS, open the "Electrophoresis Plate Prep" module and click "Create Plate".
2. Select the appropriate plate options including the "Plate Layout", "Plate Source", and "Amplification Plate Processing Type" (if applicable).
3. Select the appropriate "New Plate Subtype" (sequencer model).
4. Select the appropriate amplification plate and click "Allocate".
5. Click "Create".
6. Place barcode on sequencer plate and scan.
7. Click "Close".

6.4 Sample Preparation

- A. For Investigator 24plex GO! Manual Set-up:
 1. Select the appropriate sequencer plate.
 2. Select "Get Scenario(s)".
 3. If prompted, select the appropriate scenario and click "OK".
 4. Scan the appropriate amplification and sequencer plates and consumables.
 5. Prepare a deionized formamide and size standard master mix in a 1.5 mL microcentrifuge tube using the following volumes:
 - a) 12.0 μ L Hi-Di formamide
 - b) 1.0 μ L BTO size standard
 6. In the "Electrophoresis Plate Prep" module in STaCS, select "Process".
 7. If the "Select Transfer Map Directory" Screen appears, select "c:", double click "C:\\" to expand the folder, and double click the "PickSample" folder. Click "OK". Exit the notepad screen. This file will be used to create the plate layout for the sequencer set-up worksheet.
 8. To print the sequencer set-up worksheet, go to the Start menu, navigate to "M:\STaCS Production" and open "EPP STaCS pickSample.xls".
 9. Press "Ctrl+W" and select the pickSample.csv file from the "C:\PickSample" folder.
 10. Select "Print".
 11. Exit and do not save.



12. Aliquot 12 μL master mix into appropriate wells of the sequencer plate. **Note:** Every well of an injection must contain solution. Therefore, if a well does not contain a sample, add formamide/BTO master mix or formamide only.
 13. Add 1.0 μL sample or Investigator 24plex GO! allelic ladder to the designated wells.
 14. Cover the plate with the appropriate septa and centrifuge.
 15. Denature for 3 min at 95°C.
 16. Snap cool for 3 min.
 17. In the “Record Activity Completion Result” window, select the appropriate result and click “OK”.
 18. Click “Close” to exit the “Electrophoresis Plate Prep” module.
- B. For Investigator 24plex GO! Tecan Freedom Evo Set-up:
1. Select the appropriate sequencer plate.
 2. Select “Get Scenario(s)”.
 3. If prompted, select the appropriate scenario and click “OK”.
 4. Scan the robot and appropriate consumables.
 5. Prepare a deionized formamide and size standard master mix in a 1.5 mL microcentrifuge tube using the following volumes:
 - a) 12.0 μL Hi-Di formamide
 - b) 1.0 μL BTO size standard
 6. Place master mix, sequencer plate, and amplification plate in appropriate locations on the robot deck.
 7. Scan the appropriate amplification and sequencer plates.
 8. Turn on the Tecan robot.
 9. In the “Electrophoresis Plate Prep” module in STaCS, click “Process”.
 10. STaCS will launch the EVOware software.
 11. Select “Run Direct” from the “Run” drop-down menu.
 12. Select the appropriate amplicon volume.
 13. When the Tecan has completed the process, click “Cancel”.
 14. In the “File” drop-down menu, select “Exit, unload drivers”.
 15. Select “Yes” when prompted to move arms to home position.
 16. Add 1 μL ladder to the designated well(s).
 17. Cover the plate with the appropriate septa and centrifuge.
 18. Denature for 3 min at 95°C.
 19. Snap cool for 3 min.
 20. In the “Record Activity Completion Result” window, select the appropriate result and click “OK”.



21. Click "Close" to exit "Electrophoresis Plate Prep" module.
- C. For GlobalFiler Express Manual Set-up:
1. Select the appropriate sequencer plate.
 2. Select "Get Scenario(s)".
 3. If prompted, select the appropriate scenario and click "OK".
 4. Scan the appropriate amplification and sequencer plates and consumables.
 5. In the "Electrophoresis Plate Prep" module in STaCS, select "Process".
 6. If the "Select Transfer Map Directory" Screen appears, select "c:", double click "C:\\" to expand the folder, and double click the "PickSample" folder. Click "OK". Exit the notepad screen. This file will be used to create the plate layout for the sequencer set-up worksheet.
 7. To print the sequencer set-up worksheet, go to the Start menu, navigate to "M:\STaCS Production" and open "EPP STaCS pickSample.xls".
 8. Press "Ctrl+W" and select the pickSample.csv file from the "C:\PickSample" folder.
 9. Select "Print".
 10. Exit and do not save.
 11. Prepare a deionized formamide and size standard master mix in a 1.5 mL microcentrifuge tube using approximately the following volumes:
 - a) 9.5 μ L deionized formamide X # of reactions (including controls and ladders)
 - b) 0.5 μ L size standard X # of reactions (including controls and ladders)
 12. Aliquot 10 μ L master mix into appropriate wells of the sequencer plate. **Note:** Every well of an injection must contain solution. Therefore, if a well does not contain a sample, add formamide/size standard master mix or formamide only.
 13. Add 1 μ L sample or GlobalFiler Express ladder to the designated wells.
 14. Cover the plate with the appropriate septa and centrifuge.
 15. Denature for 3 min at 95°C.
 16. Snap cool for 3 min.
 17. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
 18. Click "Close" to exit the "Electrophoresis Plate Prep" module.



D. For GlobalFiler Express Tecan Freedom Evo Set-up:

1. Select the appropriate sequencer plate.
2. Select "Get Scenario(s)".
3. If prompted, select the appropriate scenario and click "OK".
4. Scan the robot and appropriate consumables.
5. Prepare a deionized formamide and size standard master mix in a 1.5 mL microcentrifuge tube using approximately the following volumes:
 - a) 9.5 μ L deionized formamide X # of samples (including controls and ladders)
 - b) 0.5 μ L size standard X # of samples (including controls and ladders)
6. Place master mix, sequencer plate, and amplification plate in appropriate locations on the robot deck.
7. Scan the appropriate amplification and sequencer plates.
8. Turn on the Tecan robot.
9. In the "Electrophoresis Plate Prep" module in STaCS, click "Process".
10. STaCS will launch the EVOware software.
11. Select "Run Direct" from the "Run" drop-down menu.
12. Select the appropriate amplicon volume.
13. When the Tecan has completed the process, click "Cancel".
14. In the "File" drop-down menu, select "Exit, unload drivers".
15. Select "Yes" when prompted to move arms to home position.
16. Add 1 μ L GlobalFiler Express ladder to the designated well(s).
17. Cover the plate with the appropriate septa and centrifuge.
18. Denature for 3 min at 95°C.
19. Snap cool for 3 min.
20. In the "Record Activity Completion Result" window, select the appropriate result and click "OK".
21. Click "Close" to exit "Electrophoresis Plate Prep" module.

E. For Yfiler Plus Manual Set-up:

1. Select the appropriate sequencer plate.
2. Select "Get Scenario(s)".
3. If prompted, select the appropriate scenario and click "OK".
4. Scan the appropriate amplification and sequencer plates and consumables.
5. Prepare a deionized formamide and size standard master mix in a 1.5 mL microcentrifuge tube using approximately the following volumes:
 - a) 9.5 μ L deionized formamide X # of samples (including controls and ladders)
 - b) 0.5 μ L size standard X # of samples (including controls and ladders)



6. In the “Electrophoresis Plate Prep” module in STaCS, select “Process”.
7. If the “Select Transfer Map Directory” Screen appears, select “c:”, double click “C:\” to expand the folder, and double click the “PickSample” folder. Click “OK”. Exit the notepad screen. This file will be used to create the plate layout for the sequencer set-up worksheet.
8. To print the sequencer set-up worksheet, go to the Start menu, navigate to “M:\STaCS Production” and open “EPP STaCS pickSample.xls”.
9. Press “Ctrl+W” and select the pickSample.csv file from the “C:\PickSample” folder.
10. Select “Print”.
11. Exit and do not save.
12. Aliquot 10 µL master mix into appropriate wells of the sequencer plate.
Note: Every well of an injection must contain solution. Therefore, if a well does not contain a sample, add formamide/size standard master mix or formamide only.
13. Add 1 µL sample or Yfiler Plus ladder to the designated wells.
14. Cover the plate with the appropriate septa and centrifuge.
15. Denature for 3 min (or 10 min) at 95°C.
16. Snap cool for 3 min.
17. In the “Record Activity Completion Result” window, select the appropriate result and click “OK”.
18. Click “Close” to exit the “Electrophoresis Plate Prep” module.

6.5 Post PCR

1. In STaCS, open the “Post PCR” module and scan the appropriate sequencer.
2. Scan the appropriate sequencer plate(s).
3. Click “Create Sample Sheet(s)”.
4. In the Data Collection software, import the appropriate sample sheet(s) through the library tab. Make sure to select “.txt” from the drop down menu to view the sample sheets.
5. Place plate in Plate Base and cover with Plate Retainer. Place assembled plate(s) on the autosampler deck.
6. Link plate to appropriate location(s) on the autosampler deck
7. If applicable, click the Run icon in the Data Collection software to begin the run.
8. In STaCS select “Start Process”.
9. Click “Close” to exit the “Post PCR” module.
10. When the run has completed, open the “Post PCR” module in STaCS.
11. Scan the appropriate plate to complete the process.
12. In the “Record Activity Completion Result” window, select the appropriate result and click “OK”.
13. Click “Close” to exit the “Post PCR” module.



7 Records

Processing information is recorded in STaCS.

8 Literature References and Supporting Documentation

Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. **Chapter 6 Capillary Electrophoresis: Principles and Instrumentation.**

Applied Biosystems. 3500/3500xL Genetic Analyzer User Guide.

Tecan. Freedom EVO Operating Manual.

Tecan. Freedom EVOware Standard Getting Started Guide.



CO-04-07 DATA INTERPRETATION GUIDELINES

1 Scope

These guidelines are not intended to limit an analyst's discretion on data interpretation, but to ensure that conclusions are scientifically supported based upon validation studies, scientific literature, and experience. Some situations may occur that require an analyst to vary from the stated guidelines; however, these situations must be documented and approved by the Technical Leader.

2 Related Chapters

Standard Abbreviations List

Analytical Controls

Analytical Review

Capillary Electrophoresis

3 Standards, Controls, and Calibration

- A. Controls are required to assess the effectiveness, accuracy, and/or precision of the analytical procedures.
- B. Appropriate controls must be analyzed with each sample set and may include reagent blanks, amplification negative controls, amplification positive controls, and allelic ladders.
- C. The analyst's written name or initials on the Analysis Form (LAB-CO-05) indicate the positive control, negative control(s), and allelic ladder are acceptable.

4 Preliminary Evaluation of Data

The first step in data evaluation is to determine whether the results are of sufficient quality for interpretation purposes using methods appropriate for a CE Instrument. These criteria have been determined through validation studies and by evaluating data generated by the laboratory.

4.1 Thresholds

- A. All unknown data shall be analyzed at analytical thresholds determined by appropriate validation studies.
 1. Investigator 24plex GO!
 - a) 100 rfu for the 3500xl
 2. GlobalFiler Express
 - a) 90 rfu for the 3500xl
 3. Yfiler Plus
 - a) 80 rfu for the 3500xl
- B. Data shall be analyzed with stochastic thresholds determined by appropriate validation studies.
 1. Investigator 24plex GO!
 - a) 175 rfu for the 3500xl
 2. GlobalFiler Express
 - a) 200 rfu for the 3500xl



3. Yfiler Plus (DYS385 and DYF387S1 only)
 - a) 190 rfu for the 3500xl
- C. Data exceeding the detection threshold of the CE instrument is noted as an off-scale flag in the genotyping software. This setting is automatic through the collection software and is indicated as a red line through the off-scale allele. Off-scale alleles may display as flat-topped peaks in the raw data.
- D. Off-scale data is acceptable provided that split peaks are not labeled.
- E. Off-scale samples may be reworked using the following methods:
 1. Decrease the injection time.
 2. Dilute the amplified product.
 3. Re-amplify the sample with less template DNA.
- F. To increase peak height, the sample may be reworked using the following methods:
 1. Re-amplify the sample with increased template DNA.
 2. Increase the injection time, if applicable.

4.2 Internal size standard evaluation

- A. Appropriate internal size standards must be run with every sample.
- B. All sizing peaks must be above the established threshold.

4.3 Allelic ladder evaluation

- A. When interpreting STR results, genotypes are assigned to sample alleles by comparing their sizes to those obtained for the known alleles in the allelic ladders.
- B. Each ladder used for analysis is analyzed at the analytical threshold.
- C. At a minimum, one acceptable ladder must be present within each run.
- D. Each ladder used for analysis must have the appropriate number of properly labeled alleles present for each locus when analyzed.
- E. For weak ladders, an increased injection time, if applicable, is allowed to ensure all peaks are properly labeled.

4.4 Analytical Controls

Analytical controls must be evaluated according to the *Analytical Controls* chapter.

5 Data Interpretation

5.1 DNA Analysis software

- A. The DNA analysis software will be set to use specific analysis methods depending on the amplification kit and instrument used. It is up to each analyst to ensure the appropriate analysis method has been selected.
- B. The DNA analysis software assesses the quality of the data and assigns Process Quality Values (PQV's) which are represented as color coded flags. PQVs are not intended to replace data interpretation by an analyst.



- C. 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.
- D. Samples will be run through DNA analysis software without manipulation of the imported data. **Exception:** Correction of typographical errors will be noted in the comments section of the Analysis Form (LAB-CO-05).

5.2 Allele Identification

- A. True alleles are defined as peaks recognized by the DNA analysis software that meet validated threshold values and are not artifacts.
- B. Peaks below the analytical threshold should be considered unreliable as they may be indistinguishable from baseline noise.
- C. Stochastic effects or allelic dropout can result from low levels of DNA template or degraded DNA specimens and may result in false homozygous results if one of the alleles fails to be detected or falls below threshold.
- D. For all autosomal loci, heterozygous loci with alleles in the stochastic region are considered complete genotypes. For all autosomal loci, an apparent homozygote with an allele under stochastic threshold may be exhibiting allelic drop-out. These homozygous loci below the stochastic threshold must be re-amplified. Upon re-amplification, homozygous loci repeatedly below the stochastic threshold may be accepted provided the results are consistent with the previous amplification.
- E. If visible peaks are below analytical threshold and the sample concentration is assumed to be low, the sample must be re-amplified.
- F. If no peaks for a given locus are detected (i.e. null alleles), the sample must be re-amplified. This procedure may confirm null alleles.
- G. If the software is unable to differentiate peaks that are 1 base pair apart due to poor resolution, the sample must be re-run. Example, (9.3,10) at TH01.
- H. Samples with large spikes causing true alleles to be unlabeled because they are less than 20% must be re-run.

5.3 Peak Imbalance

- A. Peak imbalance refers to the condition where the peak height of the allele with the smaller RFU at a heterozygous locus is less than 40% of the peak height of the allele with the larger RFU.
- B. If the only locus that is imbalanced is Amelogenin, then sample analysis may proceed.
- C. The entire profile is fully evaluated for overall peak imbalance. If the overall profile peak height balance is above 40%, the sample does not need to be re-processed. If the sample is within the stochastic range or the overall peak heights of the sample are below 40%, the sample must be re-amplified.
- D. Samples with a reproducible peak imbalance where the lower RFU value is at least 20% of the higher RFU value shall be recorded as heterozygous.
- E. Samples with a reproducible peak imbalance where the lower RFU value is less than 20% of the higher RFU value shall be recorded as homozygous (i.e. only the highest RFU value will be labeled).



5.4 Artifacts

A. General Guidelines

1. Artifacts or anomalies may be detected on the electropherogram. Labeled artifacts that occur within the analysis range must be documented either on the Analysis Form (LAB-CO-05) or the DNA analysis software.
2. Examine raw data to determine if a peak is a true allele or an artifact.
3. Analysts are encouraged to consult with the technical leader if the identity of an artifact is in question.

B. Spikes

1. Spikes are typically present in at least two dye colors and have the same data points. Spikes also have distinct peak morphology (i.e. sharp, narrow peaks) different from that of a true allele.
2. If a spike occurs within the allele typing areas and is labeled by the genotyping software, the analyst may re-inject that sample or delete the labeled peaks associated with that spike provided that they check the raw data to verify the spike. Spikes are not cause for rejection.

C. Stutter

1. A stutter peak is a minor product peak generally one repeating unit shorter (N-4) or longer (N+4) than the corresponding main allele peak that is produced during amplification of STR loci.
2. Suspected stutter peaks that are more than 20% (or 22% at D22 for 24plex GO!) of the highest allele's RFU must be re-amplified for samples not approaching the saturation point of the CE instrument. Re-amplification of the sample may help clarify whether a peak is stutter or a true allele. Special care and attention should be taken for off-scale samples with suspected stutter. Suspected or elevated stutter peaks in off-scale samples does not necessitate re-amplification.
3. Upon re-amplification, if a stutter peak is reproducibly labeled, it may be deselected with the approval of the Technical Leader.

D. Non-Template Addition (Minus A)

1. Minus A is a minor peak one base pair shorter than the main allele peak and is the result of incomplete adenylation during PCR. In order to prevent mistyping a microvariant allele as Minus A, the complete profile must be observed during an assessment for the presence of minus A peaks.
2. Minus A may occur when excess amplification product is present. If this occurs, the analyst may re-amplify the sample using less template DNA.
3. Peaks in a minus A position that are more than 75% of the true allele's RFU must be re-amplified. **Note: A true heterozygous pairing 1 base pair apart is not cause for re-amplification.**

E. Pull-up

1. Pull-up is the result of spectral overlap from the spectral region of one dye into another and may result from either off-scale (OS) peaks from the amplification of



excessive template DNA or when there is a decrease in spectral separation. Evidence of pull-up peaks in a sample is not cause for a sample to be rejected.

2. Examine raw data to determine if a peak is a true allele or the result of pull-up.

F. Dye Blobs

1. Dye blobs are broad or asymmetrical peaks caused by either dye molecules in the primer mix or instrument related issues. Evidence of dye blobs in a sample is not cause for a sample to be rejected.
2. Examine raw data to determine if a peak is a true allele or a dye blob.

5.5 Triple Alleles

Special care and attention should be taken to prevent misidentification of triple alleles from low level contamination or artifacts (pull-up etc). Consideration should also be given to the possible cumulative effect of n-4 and n+4 stutter.

- A. Triple alleles at various loci may be imbalanced (Type I) or balanced (Type II).
- B. Type I alleles, where the allele with the lowest RFU is less than 60% of the allele with the highest RFU, must be re-amplified.
- C. Type II alleles, where the allele with the lowest RFU is at least 60% of the allele with the highest RFU, are acceptable if there is no reason to believe that one allele is an out of range allele from a neighboring locus.
- D. Type I alleles with a reproducible peak imbalance where the allele with the lowest RFU is at least 20% of the allele with the highest RFU shall be recorded as triple alleles at that locus.
- E. Type I alleles with a reproducible peak imbalance where the allele with the lowest RFU is less than 20% of the allele with the highest RFU shall be recorded as heterozygous at that locus (i.e. the allele with the lowest RFU will be unlabeled).

5.6 Microvariants and Off-ladder Alleles

- A. Microvariants are alleles that contain incomplete repeating units. The number designation for alleles containing an incomplete repeat unit falling within the range of the ladder alleles should include the number of complete repeats, a decimal point, and the number of base pairs in the incomplete repeat (i.e., 9.3 for TH01).
- B. Off-ladder alleles are peaks that fall outside the designated range of allelic values for a locus. If an allele falls outside the smallest or largest ladder allele at a locus, it will be designated as greater than or less than the appropriate ladder allele. For GlobalFiler Express, if a peak falls between 33.2 and 42.2 at FGA, it will be designated as >33.2.
- C. To determine the presence of a microvariant or off-ladder allele, examine the allele and rule out any migration anomalies. If the peak is questionable because of sizing concerns, the analyst may re-inject or re-run the sample.
- D. If a peak falls between loci, the analyst should use their best judgment and experience to determine the appropriate allelic designation. The analyst should examine peak height ratios, repeating units from adjacent loci, and stutter percentages to help identify the correct locus. The analyst may consult the Technical Leader or Section Supervisor for guidance. Mark the sample as "ILA" on the analysis form. The sample should be amplified with another kit, if possible, to confirm the allelic designation.



5.7 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 that fails to meet laboratory quality requirements.
- B. The DNA data cannot be used for CODIS upload and will be re-worked depending on the data re-work reason.
 - 1. Profiles with autosomal loci in the stochastic region
 - 2. Profiles with allelic drop-out or null alleles
 - 3. Profiles with potential contamination
- C. Once the profile has been re-worked, as referenced in the above sections, and the criteria for an acceptable profile is met, the profile is considered interpretable.

5.8 Anomalies

- A. Amelogenin may have an X null allele due to primer binding site mutations. The profile exhibits only a Y allele. This anomaly is not cause for rejection.
- B. Amelogenin may have a Y null allele due to deletions on the Y chromosome or primer binding site mutations. The profile exhibits only an X allele. The Amelogenin Cross-check PQV in GeneMapper ID-X may confirm the null Y allele. This anomaly is not cause for rejection.

6 Interpretation

None

7 Records

Processing information is recorded in STaCS

Analysis Form (LAB-CO-05)

8 Literature References and Supporting Documentation

Butler, John M. *Advanced Topics in Forensic DNA Typing: Interpretation*. Elsevier Academic Press. 2015. Chapter 1 Data Interpretation Overview.

Butler, John M. *Advanced Topics in Forensic DNA Typing: Interpretation*. Elsevier Academic Press. 2015. Chapter 3 Data, Models, Thresholds.

Butler, John M. *Advanced Topics in Forensic DNA Typing: Interpretation*. Elsevier Academic Press. 2015. Chapter 3 STR Alleles and Amplification Artifacts.

Butler, John M. *Advanced Topics in Forensic DNA Typing: Interpretation*. Elsevier Academic Press. 2015. Chapter 4 STR Genotypes.

Butler, John M. *Advanced Topics in Forensic DNA Typing: Interpretation*. Elsevier Academic Press. 2015. Chapter 5 STR Profiles.



Butler, John M. *Advanced Topics in Forensic DNA Typing: Interpretation*. Elsevier Academic Press. 2015. Chapter 8 Troubleshooting Data Collection.

Applied Biosystems. GeneMapper ID User Manual.

Applied Biosystems. GeneMapper ID-X User Manual.

SWGAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories.

SWGAM Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Testing Laboratories.

Qiagen. Investigator 24plex GO! Handbook.

Applied Biosystems. GlobalFiler Express PCR Amplification Kit User Guide.

Applied Biosystems. Yfiler Plus Amplification Kit User Guide.



CO-04-08 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, from other samples during sample preparation, or from previously amplified DNA product. Reagent blanks and negative amplification controls are used to detect contamination. This chapter serves as a contingency plan, should contamination occur.

For preventive measures, refer to the *Facilities* chapter.

2 Related Chapters/Documents

Facilities

CLS Manual: Quality Incident (QI) and Quality Action Plan Process (QAP)

3 Practices

3.1 Staff Profiles

- A. All CODIS Section laboratory personnel should have a DNA profile on file for the purpose of identifying possible sources of contamination. Personally identifiable information will be retained with the Technical Leader.
- B. In the event that a staff member is suspected to be the contamination source, the Technical Leader must be notified.

3.2 Contamination and Sample Switch Assessment

- A. Suspected quality issues must be investigated.
- B. Contamination must be documented either in STaCS or the supporting plate documentation.
- C. Determining the appropriate actions to be taken will depend on which step the suspected issue occurred or in which sample/control it was observed.
- D. To help determine at which step the issue might have transpired, analysts may repeat the typing procedure in which contamination/sample switch was detected and work backwards through the sample processing steps (i.e. electrophoresis plate prep → re-amplification → re-extraction) until the issue is resolved. If the issue is resolved during this process, no further action is needed.
- E. If contamination or the sample switch is still evident after the above actions have been taken, the Technical Leader must be notified. The Technical Leader will define and direct the actions to take. Examples may include the following:
 1. 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.
 2. If contamination is suspected, suspected buffers and prepared reagents may be discarded and replaced with fresh reagents and buffers.
 3. Suspected laboratory equipment may be decontaminated.
 4. A redraw request may be made if it is determined that the source material is contaminated.



- F. The Technical Leader may initiate a Quality Incident (LAB-510 or electronic equivalent). Preventive measures taken to minimize reoccurrence of the issue should be addressed in the Quality Incident.
- G. The Technical Leader (or designee) will document contamination events using the contamination log workflow in QualTrax. See the [Contamination Log Workflow](#) chapter in the DNA Manual for instructions.

3.3 Interpreting Data Potentially Affected by Contamination

Typically, data will not be interpreted if it is potentially affected by contamination. Attempts must be made to resolve the issue as described above. If a resolution cannot be made, data may be interpreted as long as the contamination is minimal and it is approved by the Technical Leader.



CO-04-09 ANALYTICAL REVIEW

1 Scope

This chapter covers the requirements for the primary (1st read), technical review (2nd read), and administrative review. Analysts are responsible for independently analyzing all of the samples from the original raw data. The resolution of any discrepancies between the primary reader and technical reviewer will be documented on the Analysis Form (LAB-CO-05). In the event that there is an unresolved discrepancy, the Technical Leader or Section Supervisor will be notified and the resolution will be documented on the Analysis Form.

Quality control measures are in place to ensure information is being entered/recorded accurately in STaCS.

2 Related Chapters/Documents

Data Interpretation Guidelines

Match Verification

CLS Manual: Review of Laboratory Records

CLS Manual: Laboratory Records

3 Qualifications for Review

- A. An analyst who performs technical reviews on DNA database samples shall be an individual authorized to perform technical reviews in the specific DNA method, technology, typing test kit and platform that the review encompasses.
- B. The analyst shall possess a current forensic analyst license issued by the Texas Forensic Science Commission.
- C. The technical reviewer must have completed a competency test prior to participating in the technical review of DNA data.
- D. The technical reviewer is required to successfully complete an external proficiency test to the full extent in which they participate in database analysis twice each year from an approved provider. An analyst proficiency-tested in the specific DNA methodology is qualified to serve as a technical reviewer without needing to take an additional proficiency test as a technical reviewer.
- E. The administrative reviewer for CODIS offender letters is not required to be a current or former qualified DNA analyst.

4 Primary Analytical Review

1. In the "Analysis" module in STaCS, select the appropriate plate in the "Analysis" queue.
2. Click "Analyze/Review" to hold the plate for analysis. This will ensure the plate will not be analyzed by someone else. Select "OK" to exit out of the STaCS prompt.
3. Open GeneMapper ID-X and analyze samples according to the procedures outlined in the *Data Interpretation Guidelines* chapter and document the review on the Analysis Form (LAB-CO-05). The review should also include the following elements:
 - a) *Review all the controls, internal size standards, and allelic ladders to verify that the expected results were obtained.*



- b) *If applicable, confirm that reworked samples have appropriate controls.*
 - c) *Review all the DNA types to verify that they are supported by the raw or analytical data.*
 - d) *If applicable, review the supporting plate documentation associated with the plate and document the review on the notes/worksheets.*
4. Select the appropriate samples to be exported including all ladders and controls. Conversely, de-select or delete any duplicate samples so that they will not be imported. STaCS does not allow duplicated CODIS numbers to be processed on a plate.
 5. Select "Report Manager" and ensure "STaCS" is selected under "Report Settings." Select "File" and then select "Export." Navigate to M:\DataAnalysis\Output. Delete the "_STaCS" suffix in the file name (e.g. trSEQ-000000-00_STaCS to trSEQ-000000-00). Select "Export."
 6. In STaCS, open the "Analysis" module. Highlight the plate just analyzed and select "Analyze/Review." Verify the negative control(s) and at least one positive control passed. Click "Process." The genotyping report/table will now be imported into STaCS.
 7. Indicate samples needing to be reworked by selecting a rework point and a rework reason from the drop-down menu. Add a comment if needed.
 8. Verify the profiles that appear in the various filtered screens (e.g. Trisomy, Hit Confirmation/QA/QC Runs, Problem Alleles, Mismatch, Missing) by selecting "Next." When filtering is complete, the "Save" button will be activated. Double-check rework points/reasons before saving.
 9. Verify results in the "Data Analysis Outcome" window correspond with information recorded on the Analysis Form. Notify the appropriate personnel if any discrepancies are discovered. Click "Close" to exit the "Data Analysis Outcome" window. Click "Close" to exit the "Analysis" module.
 10. Ensure the project is exported to the appropriate folder on Macshare and is deleted from GeneMapper ID-X.
 11. The primary review is documented on the Analysis Form and in STaCS.

5 Technical Review

1. In the "Analysis" module in STaCS, select the appropriate plate in the "Technical Review" queue.
2. Click "Analyze/Review" to hold the plate for analysis. This will ensure the plate will not be analyzed by someone else. Select "OK" to exit out of the STaCS prompt.
3. Open GeneMapper ID-X and analyze samples according to the procedures outlined in the *Data Interpretation Guidelines* chapter and document the review on the Analysis Form (LAB-CO-05). The review should also include the following elements:
 - a) *Review all the controls, internal size standards, and allelic ladders to verify that the expected results were obtained.*
 - b) *If applicable, confirm that reworked samples have appropriate controls.*



- c) *Review all the DNA types to verify that they are supported by the raw or analytical data.*
 - d) *If applicable, review the supporting plate documentation associated with the plate and document the review on the notes/worksheets.*
4. Select the appropriate samples to be exported including all ladders and controls. Conversely, de-select or delete any duplicate samples so that they will not be imported. STaCS does not allow duplicated CODIS numbers to be processed on a plate.
5. Select "Report Manager" and ensure "STaCS" is selected under "Report Settings." Select "File" and then select "Export." Navigate to M:\DataAnalysis\Output. Delete the "_STaCS" suffix in the file name (e.g. trSEQ-000000-00_STaCS to trSEQ-000000-00). Select "Export."
6. In STaCS, open the "Analysis" module. Highlight the plate just analyzed and select "Analyze/Review." Verify the negative control(s) and at least one positive control passed. Click "Process." The genotyping report/table will now be imported into STaCS.
7. Indicate samples needing to be reworked by selecting a rework point and a rework reason from the drop-down menu. Add a comment if needed.
8. Review the Analysis Form from the primary reviewer. **Note:** An independent review of the data must be completed before reviewing the primary reviewers analysis form.
 - a) *Verify information was entered correctly into STaCS and note any discrepancies.*
 - i. *Rework points/reasons*
 - ii. *Comments (if applicable)*
 - b) *Compare the rework points/reasons between the primary and technical review (if applicable). Note any discrepancies.*
 - c) *Note any discrepancies highlighted in STaCS*
 - d) *If corrections to the genotyping table are needed, send the plate back to the primary reader for re-analysis by selecting "Re-Analyze". Add a description describing the reason for re-analysis in the comments field.*
 - e) *Discrepancies between the primary and technical review must be go through the review resolution process defined in CLS. The outcome must be documented on the Analysis Form.*
 - f) *Initial and date the primary reviewer's Analysis Form to indicate the technical review.*
9. Verify the profiles that appear in the various filtered screens (e.g. Trisomy, Hit Confirmation/QA/QC Runs, Problem Alleles, Mismatch, Missing) by selecting "Next." When filtering is complete, the "Save" button will be activated. Double-check rework points/reasons before saving.
10. Verify results in the "Data Analysis Outcome" window correspond with information recorded on the Analysis Form. Notify the appropriate personnel if any discrepancies are discovered. Click "Close" to exit the "Data Analysis Outcome" window. Click "Close" to exit the "Analysis" module.



11. Ensure the project is exported to the appropriate folder in Macshare and is deleted from GeneMapper ID-X.
12. The technical review is documented in STaCS and on both Analysis Forms.

6 Administrative Review of Technical Records

1. Open the “Administrative Review” module in STaCS and select the appropriate plate to review.
2. Review the Analysis Forms and supporting plate documentation (if applicable) for inconsistencies.
3. Review the data to ensure the reagent blank was treated appropriately and to confirm re-injection times with supporting plate documentation (if applicable).
4. To acknowledge the completion of the administrative review, verify the appropriate plate is selected in the “Administrative Review Worklist” window and click “Complete.”
5. In Macshare, transfer the appropriate run folder from the “Archive” folder into the “Completed” folder.
6. Run folders containing raw data, projects, and genotyping reports/tables are electronically archived.
7. The administrative review is documented on the Analysis Forms, supporting plate documentation (if applicable), and in STaCS.
8. For Familial Search batches, also review the batch folder to ensure the appropriate records are included (refer to LAB-CO-23 Familial Search Document Checksheet).

7 Administrative Review of CODIS Offender Letters

For the administrative review of official correspondence related to database hits containing personally identifiable information, refer to the *Match Verification* chapter.

8 Records

Analysis Form (LAB-CO-05)

Processing information recorded in STaCS

Run folders containing raw data, projects, and genotyping reports/tables are electronically archived



CO-04-10 CODIS UPLOAD

1 Scope

After the conclusion of the technical review, samples are uploaded into CODIS according to the following procedures. All CODIS core loci are required for acceptance into the National DNA Index System (NDIS).

2 Procedure

2.1 Preparation for upload

1. Open the “CODIS Upload” module in STaCS.
2. Highlight the appropriate samples to be uploaded.
3. Select the appropriate CODIS operator from the drop-down menu and click “Create File”.
4. Select the “CODIS Upload” folder in Macshare to save the CODIS upload files. Click “OK”. STaCS will generate the CODIS xml upload file.
5. Click “Close” to exit the “CODIS Upload” module.

2.2 Data Upload

1. Open the CODIS software.
2. Import the xml file in “Specimen Manager”.
3. Open “Message Center” and check that the files were imported. Validate the file first then check the validation report to ensure there were no errors. Any discrepancies should be resolved prior to importing the data.
4. Import the data and check the import report for any errors. Save the import summary and report to M:\CODIS Upload.

2.3 CODIS Confirmation

1. In STaCS, open the “CODIS Confirmation” module.
2. Click “Connect to CODIS”.
3. Click “Close” to exit the “CODIS Confirmation” module.

3 Records

CODIS import files

CODIS upload information is recorded and maintained in STaCS

SDIS Import Reconciliation Reports



05 DATABASING PRACTICES

CO-05-01 CODIS SOFTWARE AND DATABASE

1 Scope

It is the responsibility of the State CODIS Administrator to oversee offender data generated by the CODIS Laboratory, as well as data uploaded by the local CODIS laboratories. Data from convicted offender/arrestee samples may have been generated internally by the CODIS Laboratory or may have been provided through outsourcing to a contract laboratory.

Once CODIS receives samples or profiles and enters them into the STaCS or CODIS database, it is the responsibility of the CODIS Program Manager, or designee, to ensure the security and integrity of the database. This involves backup of critical data, safeguarding against computer viruses, and limiting access to CODIS computers. Only CODIS personnel or authorized CODIS IT personnel are granted login access to the CODIS network.

2 Related Documents

CLS Manual: Records Requests and Release of Laboratory Records and Information

3 Procedures

TLE domain computers will be accessed, used, and protected as per the DPS General Manual. The following procedures refer only to those computers in the CODIS Laboratory not connected to the TLE Domain.

3.1 General Precautions and Procedures

A. User Accounts

1. User accounts are set up by an administrator.
2. A login ID and password are required for access.
3. Locking screen savers and proper log on and log off procedures shall be followed.
4. Screen saver and desktop preferences may be customized to specific user names.

B. Data Backup

1. Backups will be performed on the CODIS network by the CODIS Program Manager or their designee.
2. Backups of stand-alone computers must be performed by the personnel using the equipment.
3. Data should be backed up in appropriate file format as dictated by the volume of changes made to files.

C. File strategies

1. Save your work often.
2. Save files in appropriate directory. Files not kept in appropriate directories are subject to periodic purging.

D. Passwords

1. Do not password protect any files except your own personal, non-DPS files.
2. Observe proper password security with the network.



3. When using network computers, log off each and every time you leave the computer.

E. Security

CODIS personnel are responsible for ensuring that only authorized personnel have access to section computers.

3.2 CODIS Network Computers

- A. The security and integrity of the CODIS network data are of the utmost importance and are therefore regulated by the NDIS Procedures Manual & CODIS Administrator's Handout, the NDIS DNA Data Acceptance Standards (FBI) and NDIS Security Requirements.
- B. Analysts are responsible for the data they enter into the CODIS database and the CODIS Administrators are responsible for the system as a whole.
- C. All laboratory personnel are responsible for ensuring that only authorized personnel have access to section computers.
- D. Users are also responsible for ensuring that viruses are not introduced into the computers.
- E. All computers will use a locking screen saver after a period of inactivity.

3.3 Data Back Up

- A. The CODIS database is incrementally uploaded to NDIS daily and periodically fully uploaded as requested by FBI.
- B. The CODIS server should be backed up at least once a week, if possible.
- C. Each new back up will be stored off-site.

3.4 Stand-alone Computers

- A. These computers are separate from the network.
- B. They may be password-protected.

4 Data Management

4.1 Ownership of Data

All data generated from analysis of databasing samples are the property of the State of Texas and will be maintained by the Texas DPS CODIS Databasing Laboratory.

4.2 Electronic Storage of Data

- A. Analytical data files along with associated CODIS upload files will be stored on recordable media and be retained following the Texas Department of Public Safety Records Retention Schedule.
- B. All data on the CODIS server, which includes offender identification data and the CODIS DNA profile data, will be backed-up at least once per week.

4.3 Storage of Hard Copy Data

- A. All convicted offender/arrestee data and/or records received in hard copy form from a contract laboratory will be archived by the DPS and maintained for inspection.
- B. All convicted offender/arrestee data and/or records generated in hard copy form by the CODIS laboratory during analysis will be archived and maintained for inspection.



5 Data Security

5.1 Policy

- A. Never insert any recordable media or copy any files onto the CODIS network unless the media has been scanned for viruses with a current version of the DPS virus protection software.
- B. No freeware or shareware may be loaded onto a CODIS computer.
- C. Protect your password. Do not share it with others or write it down. The system will prompt you to change your password every three months.
- D. Do not change any program or network settings on “community” workstations without the permission of the CODIS Program Manager or designee.
- E. Be sure to save all data onto the server so that it is backed-up by the scheduled back-up.
- F. All persons accessing the CODIS network will log off or lock out their account after use to prevent unauthorized access.
- G. Workstations will automatically lock after 10 minutes of inactivity.

5.2 Network Security – Tasks

- A. Data Backup
 - 1. The network servers are used to back up the CODIS network. The software is set to perform a full back up once a week and incremental backups every other night of the week.
 - 2. A three recordable media rotation is used with the most recent media being delivered to off-site storage. The media are labeled as: CODIS A, B, and C as well as STaCS A, B, and C.
 - 3. Log into the server and open the backup software to verify that the backup operation was performed correctly.
 - 4. Remove the current backup media and replace with the next one in the weekly sequence noting the media change in the backup software.
 - 5. Ensure that the backup is delivered to the appropriate person for off-site storage.

6 Acceptable Indexes/Specimen Categories

New indexes will be added as permitted by State or Federal legislation and with the approval of the CODIS Program Manager. Specific Specimen Categories for each index are indicated. References to Moderate Match Estimate (MME) and Match Rarity Estimate (MRE) calculations only include the original 13 CODIS Core Loci unless otherwise specified. The following indexes will be maintained in the DPS State CODIS database:

6.1 Arrestee

Profiles from samples properly collected under Texas Government Code Title 4 Subtitle B Chapter 411 Subchapter G Section §411.1471 are entered in this index. Arrestee samples will consist of single source profiles with the CODIS core loci. Profiles may contain no more than three alleles at any one locus.



6.2 Offender

Single source profiles with the CODIS core loci originating from convicted offender samples will be entered into this index. Profiles may contain no more than three alleles at any one locus. Specimen Categories include “Convicted Offender” and samples previously labeled as “Juvenile”. Samples, including those from juveniles, are placed into the specimen category of “Convicted Offender.”

6.3 Multi-Allelic Offender

An Offender/Arrestee sample having three or more alleles at two or more loci will be entered into this index.

6.4 Forensic Unknown

Single source profiles obtained from evidentiary samples attributed to the putative perpetrator(s), whose source is unknown, or which match a suspect known, are entered in this index. For inclusion into NDIS, profiles must contain the CODIS core loci. No profile from an evidentiary sample which matches the victim known or an exclusionary known (i.e. husband, boyfriend) shall be entered into SDIS or NDIS. Obtaining a profile matching the suspect from an item belonging to the suspect (e.g. clothing or other article that would be expected to yield the owner’s profile) is generally not considered an evidentiary sample and shall not be entered into CODIS.

6.5 Forensic Mixture

Forensic profiles that are not from a single source but contain DNA contributed from more than one source and contain alleles that can be attributed to a putative perpetrator(s) are entered into this index. The profiles must contain at least 8 of the original 13 CODIS core loci and have a Moderate Match Estimate (MME) not less than 1.000E+007. An effort should be made to deduce the probative or suspect profile from a mixture by eliminating alleles that could only have come from the victim. In situations where it is ambiguous and there is potential for the suspect and victim to have a shared allele, the allele in question should be included in the mixture. There may not be more than four alleles at any locus for these profiles. These profiles must meet all other requirements of forensic unknown profiles.

6.6 SDIS Forensic Mixture

Forensic Mixture profiles that do not meet the NDIS requirements for upload may be placed in the SDIS Forensic Mixture category. This category will contain Forensic Mixture DNA records that have a minimum of 7 loci from a combination of the CODIS core loci including D2S1338 and D19S433. SDIS Forensic Mixture DNA records submitted to SDIS shall have a minimum MME value of 1.00E+005 (equivalent to 10 matches per a database of 1 million samples).

6.7 Forensic Partial

Single source profiles obtained from a single source (or fully deduced profile originating from a mixture) with either locus or allelic dropout. A minimum of 8 of the 13 original CODIS core loci are required for submission to NDIS. These profiles shall not have more than 3 alleles at one locus while the remaining loci can have up to 2 alleles and an MME value of at least 1.000E+007.

6.8 SDIS Forensic Partial

Forensic Partial profiles that do not meet the NDIS requirements for upload may be placed in the SDIS Forensic Partial category. This category will contain Forensic Partial DNA records that have a minimum of 7 loci from a combination of the CODIS core loci including D2S1338 and D19S433. SDIS Forensic Partial DNA records submitted to SDIS shall have a minimum MME value of 1.00E+005 (equivalent to 10 matches per a database of 1 million samples).



6.9 Legal

The Legal Index contains DNA profiles of persons whose samples are collected under applicable legal authorities. Like all single source “reference” samples, they must have the CODIS core loci and contain no more than three alleles at any one locus.

6.10 Missing Person

Single source profiles which originate from an intimate sample (e.g., toothbrush) belonging to the missing person (deduced victim known) or from a sample known to have been collected from the missing person (e.g. medical specimens) can be entered into this index. Specimen Categories include “Missing Person”, “Deduced Missing Person”, and “Deduced Victim Known”.

6.11 Relatives of Missing Person

Single source profiles from relatives of missing persons may be included in this index. These profiles may only be searched against the Missing Person and Unidentified Human (Remains) indexes. If a match is made and verified to be the missing person for whom the samples were included, the profiles must be removed from CODIS. Specimen Categories include Biological Child, Biological Father, Biological Mother, Biological Sibling, Maternal Relative, and Paternal Relative.

6.12 Spouse

Single source profiles from samples obtained from a presumptive parent of a common child of a missing person are entered into this index. If a match is made and verified to be the missing person for whom the samples were included, the profiles must be removed from CODIS.

6.13 Suspect

The Suspect index contains legally obtained known reference samples submitted for comparison to forensic evidence. This index is not allowable for upload to NDIS. Suspect known reference samples that contain the CODIS core loci may be uploaded to NDIS as Legal samples.

6.14 Unidentified Human (Remains)

Single source profiles from an unidentified deceased person (including body parts and tissue) or from individuals who are unable to provide their identity (e.g., children). Specimen Category is Unidentified Person.

6.15 Pedigree Tree

A pedigree tree is a chart showing the graphical representation of the relationships among family members and one or more missing persons. The Pedigree Tree index consists of DNA records of relatives and spouses of missing persons that are associated with a pedigree. Pedigree Trees are searched against unidentified human remains. Specimen Categories include Biological Child, Biological Father, Biological Mother, Biological Sibling, Maternal Relative, Paternal Relative, and Spouse.

6.16 Forensic Targeted

Specimens that have an MME value less than the NDIS MME upload acceptance threshold may be eligible to be re-categorized into the Forensic Targeted Category.



7 Analyst Workbench/CODIS Message Center

- A. The Analyst Workbench (AWB) is the interface used to perform functions related to CODIS. All CODIS functionality is accessed through the AWB.
- B. The CODIS Message Center is the main module on the Analyst Workbench and is the internal electronic messaging system within CODIS.
 1. The CODIS Message Center provides users with the capability to view, refresh, filter, delete, execute/process, validate, transmit, and archive/unarchive messages associated with specific CODIS modules and processes.
 2. All messages generated by CODIS modules are received and executed through the Message Center.
 3. Messages are capable of being sent and received without the Analyst Workbench or Message Center running; however, AWB should be opened daily to monitor message traffic.
 4. The options should be set to allow automatic processing of disposition messages and match messages.
- C. Local labs are encouraged to send their uploads daily. Upload reports should be reviewed to look for unusual occurrences such as large numbers of deleted samples, rejected specimens or loci, or rejected user changes. The NDIS upload report should be available daily.

8 Documentation of CODIS Upload

All data generated from analysis of databasing samples will be uploaded into CODIS using the Import function. Samples imported into CODIS will be documented in STaCS and by saving the import report.



CO-05-02 PROFILE REMOVAL/EXPUNCTION PROCEDURES

1 Scope

The DPS recognizes the need to remove from CODIS all DNA profiles which may have been improperly included in the database, or the DNA profile of an individual who has 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 ANN. § 411.1471(e).

2 Requirements

Federal law requires that the laboratory, as a participant in NDIS, expunge the DNA records of persons whose qualifying convictions had been overturned. 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.” 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. See Federal DNA Identification Act at 42 U.S.C. §14132(d)(2).

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. 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).

3 Administrative Removal

Administrative removal can be requested by letter from the collecting agency and signed by the appropriate Corrections or Probation and Parole official stating a blood or buccal sample was erroneously collected from the individual in question. The letter should further certify that a criminal history check was performed and no qualifying offense exists to justify inclusion in the database. If the letter does not contain this certification, the CODIS Liaison, or designee, will ensure a criminal history check is performed and no qualifying offenses exist. The inadvertently taken sample may be retained if it is determined that there is a different offense that does qualify the individual for CODIS inclusion and no satisfactory sample otherwise exists.

The DPS Laboratory Director, or designee, can order removal of a DNA profile from the database if it is determined no qualifying offense exists to justify inclusion in CODIS. 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. If needed, DPS Legal Counsel may be contacted to provide a recommended course of action to assure that DPS and CODIS are in compliance with applicable statutes.



4 Court Ordered Expunction

In the event of acquittal or if 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. The DPS must be notified, in writing, of the need to expunge. This may be accomplished by:

- A. 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. The CODIS Liaison or designee will also certify no other qualifying offense exists justifying inclusion in CODIS, or
- B. 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. The CODIS Liaison or designee will verify through a criminal history check no other qualifying offense exists which would justify retention of the individual's profile in CODIS.

5 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 CODIS Program Manager or designee will ensure the expunction or administrative removal of all information related to the DNA profile in question. This will include:
 1. Removal of all identifying information from the STaCS database associated with the sample number assigned to the individual in question. The sample number record will be amended to indicate the identifying information was removed.
 2. Removal, if applicable, of all DNA profile information from all levels of the CODIS DNA database, including the DPS offender sample number assigned to the individual in question.
- B. Documentation of destruction of the original offender sample and bloodstain card will be retained on file by the DPS.

6 Notification and Records

Notification of removal or expunction will be in writing from the CODIS Program Manager or designee to the requestor on official DPS letterhead. The DPS CODIS Lab may retain documents relating to the administrative removal but shall destroy any records relating to court ordered expunction of a sample.



CO-05-03 SEARCHES

1 Scope

The following policies address the various searches that are performed in the CODIS software.

2 Related Chapters

Partial Matches and Familial Searches

Match Dispositioning

Match Verification

3 Policy

3.1 Records

- A. The data in the DNA database are confidential and are governed by provisions contained in Government Code 411 Subchapter G and NDIS DNA Data Acceptance Standards (FBI).
- B. Names or other personal identifying information may not be stored in the state CODIS database.
- C. A file or reference number to another information system may be included in the state CODIS database only if the information is necessary to:
 - 1. Generate an investigative lead or exclusion;
 - 2. Support the statistical interpretation of a test result; or
 - 3. Allow for the successful implementation of the DNA database.

3.2 Autosearches (Index to Index Searches)

- A. The following search combinations are permissible:

Index	Forensic	Convicted Offender, Arrestee, and Legal	Unidentified Human (Remains)	Missing Person	Relatives of Missing Person
Forensic	X	X	X	X	
Convicted Offender, Arrestee, and Legal	X		X	X	
Unidentified Human (Remains)	X	X	X	X	X
Missing Person	X	X	X		
Relatives of Missing Person			X		
Forensic Targeted		X			

- B. Additional configurations can be added by the CODIS Program Manager as needed or as new indexes are permitted according to state or federal statute. Autosearches will be performed Monday – Friday as detailed below with the exception of a Missing Persons Database search which is performed monthly or as instructed by NDIS.



C. Conducting CODIS Searches

1. The State CODIS laboratory will conduct Autosearches Monday – Friday, unless otherwise specified by the CODIS Program Manager.
2. Searches may also be performed periodically to ensure no hits are missed or to search batch target files created internally or downloaded from the Criminal Justice Information System – Shared Enterprise Network (CJIS-SEN).
3. Searches involving Unidentified Human Remains samples against relative reference samples and pedigree trees are conducted monthly, or as instructed by NDIS.

D. Reporting Matches

1. Match detail reports are generated automatically by the State CODIS laboratory and transferred to the local CODIS laboratory as they occur. In the event that a match is identified between a submitted forensic evidentiary profile and a convicted offender profile, the local CODIS laboratory is notified automatically via the CODIS software.
2. A discussion between the CODIS Administrator for the local CODIS laboratory and the State or Local CODIS Administrator for the State CODIS laboratory will determine the significance of the match. Matches are followed up as indicated in the match dispositioning procedure. If a match verification is needed, the State CODIS laboratory will retest the archived offender sample to verify concordance. After verification, SDIS CODIS Offender Letters are issued to the local CODIS laboratory and NDIS CODIS Offender Letters are issued to the corresponding state or federal laboratory.
3. Local CODIS laboratories are responsible for communicating search results to the appropriate local law enforcement agency and/or prosecutors to determine the need for match verification and retesting of archived samples.

3.3 Manual Searches (Profile to Index Searches)

- A. Manual Searches are performed at the discretion of the CODIS Program Manager or their designee following the entry of a profile from the keyboard (Keyboard Search) or after receiving a request from an outside agency (Remote Search).
- B. Conducting a Manual Search
 1. Written requests from a local CODIS laboratory must be approved by the Technical Leader or CODIS Administrator for that laboratory or state.
 2. Keyboard searches will be conducted by the CODIS Program Manager or their designee as a courtesy to the requesting agency.
 3. Remote search requests may be executed at the discretion of the CODIS Program Manager for approved local CODIS laboratories.
 4. Searches will be made in a timely manner following the receipt of a search request.



C. Reporting Matches

1. A record of any search will be maintained by the CODIS Program Manager. Generally this will consist of a copy of the Data Entry Form or written request, a copy of the State Match Detail Short Report, and any generated notifications to the requesting agency.
2. Following a positive search result, the submitting agency will be notified of the search results by a written notification. Match verification procedures will be followed, if desired by the requesting agency.
3. Following a negative search result, the submitting agency will be notified of the search results by a written notification. Local CODIS laboratories are responsible for communicating negative search results to the appropriate local law enforcement agency and/or prosecutors.
4. Requests generating a negative search result may be added to a batch file for periodic automated searches. Agencies will not be notified of the results of subsequent searches unless a positive result (match) has been found.



CO-05-04 PARTIAL MATCHES AND FAMILIAL SEARCHES

1 Scope

Partial matches and familial searches may determine a potential familial relationship between an offender and a putative perpetrator. This policy serves as the guideline for providing investigative leads to law enforcement officials in unsolved cases where other investigative leads have been exhausted.

Because the information that is ultimately provided will be the name or names of an offender or offenders in Texas's DNA database who may be related to the actual perpetrator, the process developed requires special DNA testing and review of the offender's non-DNA information. The process specified in this policy was developed to keep privacy concerns in mind while providing information that may be useful in solving and preventing violent crimes.

2 Background

The Texas CODIS database was formally established in 1995 and consists of DNA profiles from offenders/arrestees and evidentiary profiles. When a crime scene profile is searched against the offender database, a match is declared if the crime scene profile cannot be excluded as having originated from the offender in question. In some instances, the profiles may not match directly, but may share similarities that based on statistical analysis, could have originated from closely related individuals. Advances in DNA technology can provide additional information as to whether individuals may be related. It is important that investigating agencies understand that although individuals identified by these procedures can be considered potential relatives, most of them will be unrelated to the offender. It should also be stressed to the investigating agency that a negative search does not mean that the true perpetrator does not have a relative in the CODIS database. Only those offenders with a high likelihood will be tested further and investigated.

3 Limitations

The following points should be made to agencies requesting information:

- A. These approaches should only be used where necessary, justifiable, and impartial, taking into account the impact of the sensitive ethical issues familial searching can generate.
- B. The number of names appearing on some familial searches can potentially be several thousand and prohibits the laboratory from testing all candidates.
- C. In order to ensure transparency and integrity, it is imperative that all agencies involved in the process communicate, work together, and understand the limitations of the information the search provides.
- D. These procedures only apply if the putative perpetrator is male.
- E. A consistent Y-STR profile between the evidentiary item and an offender does not confirm relatedness between the offender and the perpetrator. As in a regular CODIS match, the information given is only an investigative lead and should be treated as such and nothing more.

4 Review Committee

- A. In reviewing requests for Partial Match verifications and Familial Searches, a committee will be responsible for determining the necessity of granting the request. The committee should include the following individuals:
 1. CODIS Program Manager, CODIS Section Supervisor, Technical Leader, and CODIS Program Coordinator.



2. Should further consultation be needed, the involvement of a DPS General Counsel representative and/or a Texas Ranger representative may be requested.
- B. The committee will review the official request and forward to the DPS Division Chief or designee (through chain of command) for approval. A written response will be provided to the requesting agency if the request is denied at any step of the approval process.

5 Partial Profile Match Policy

5.1 General Information

- A. A **Partial Match** is a moderate stringency candidate match between two single source profiles having at each locus at least one allele in common. A partial match is not an exact match and occurs during a routine weekly search of the CODIS database using the current CODIS software.
- B. The name of the offender may be released to the law enforcement agency if the policy outlined below has been followed and all of the following conditions have been met.

5.2 Eligibility

- A. As a guiding policy, the offense of the case with the evidentiary profile should be from an unsolved homicide, sexual assault, or other violent crime that has significant public safety concerns. Property crimes will not be considered.
- B. The evidentiary profile should be from an item of evidence having unambiguous connection to the crime in question and a satisfactory level of confidence that the crime scene profile is relevant to the perpetrator (example: sperm cell fraction from vaginal swab of a sexual assault victim). Evidence that is ambiguous to the crime in question may be cause for denial of release of information (example: cigarette butt found near but not at the crime scene).
- C. The evidentiary profile must be a single source profile containing, at a minimum, 13 CODIS Core Loci with no allelic dropout. The evidentiary profile may be deduced from a mixture. Loci that contain ambiguous alleles will not be searched.
- D. A local CODIS laboratory that receives a partial match from a weekly search should determine if the case remains active, unsolved, and whether the investigator is interested in any possible investigative leads. The local CODIS laboratory must ensure that Y-STR testing is performed on the evidentiary sample, if not already completed.
- E. The local CODIS laboratory should instruct the law enforcement agency to contact the appropriate prosecutor's office.

5.3 Partial Match Review Request

- A. A request for information as a result of a partial match must be made as a joint request from the law enforcement agency and the corresponding district attorney's office and be submitted through the Local CODIS Administrator of the NDIS Participating Laboratory owning the evidentiary profile data.
- B. The request should include the following information:
 1. A statement that all standard investigative leads in the case have been exhausted; the case has significant public safety concerns; or a specific exception to the previous two conditions.
 2. The name of the local CODIS laboratory, the corresponding match ID(s), the "Specimen ID" of the profile, and a copy of the laboratory report.



3. The case summary, including all available information that would be helpful in identifying a relative of the perpetrator.
 4. A statement that the agency agrees to further investigate the case after the State CODIS laboratory releases the investigative information to the requesting agency.
 5. STR and Y-STR electropherograms of the profile should be attached to the request, or provided by the local CODIS laboratory.
- C. The local CODIS laboratory shall provide the State CODIS laboratory the statistical analysis used to conclude that there may be a potential familial relationship between the suspected perpetrator and the offender.

5.4 Reporting Results

- A. Once a determination has been made by the review committee to proceed with the process, Y-STR testing will be performed on the offender sample(s) involved in the partial match.
- B. If the Y-STR profiles have been determined to be consistent, DPS reserves the right to review non-DNA information in order to identify additional evidence bearing on relatedness.
- C. Prior to releasing an individual's identifying information, the State CODIS Laboratory shall verbally discuss the limitations and meaning of the results with the requesting agencies. This discussion should include all representatives involved in the request and may take place in person or via conference call.
- D. Results of the testing shall be made in writing to the requesting agencies and be emailed or delivered in person. Only secure and valid email addresses may be used. DPS will not send information to public domain email addresses. All sensitive Department material transmitted over any external network will be encrypted utilizing an encryption standard established by IMS.

6 Familial Search Policy

6.1 General Information

- A. A **Familial Search** is a deliberate search for biologically related individuals of a contributor of an evidentiary profile conducted using the CODIS software. The evidentiary profile is searched against the convicted offender and arrestee indexes.
- B. The name of the offender may be released to the law enforcement agency if the policy outlined below has been followed and all of the following conditions have been met.

6.2 Eligibility

- A. As a guiding policy, the offense of the case with the evidentiary profile should be from an unsolved homicide, sexual assault, or other violent crime that has significant public safety concerns. Property crimes will not be considered.
- B. Only profiles currently residing in CODIS at the NDIS level will be considered.
- C. The evidentiary profile should be from an item of evidence having unambiguous connection to the crime in question and a satisfactory level of confidence that the crime scene profile is relevant to the perpetrator (example: sperm cell fraction from vaginal swab of a sexual assault victim). Evidence that is ambiguous to the crime in question will be cause for denial for executing a familial search (example: cigarette butt found near but not at the crime scene).



- D. The evidentiary profile must be a single source profile containing, at a minimum, 13 CODIS Core Loci with no allelic dropout. The evidentiary profile may be deduced from a mixture. Loci that contain ambiguous alleles will not be searched.

6.3 Familial Search Request

- A. A request for a familial search in a case must be made as a joint request from a Texas law enforcement agency and the corresponding district attorney's office and be submitted through the Local CODIS Administrator of the Texas NDIS Participating Laboratory owning the evidentiary profile data.
- B. The request should include the following information:
 - 1. A statement that all standard investigative leads in the case have been exhausted; the case has significant public safety concerns; or a specific exception to the previous two conditions.
 - 2. The name of the local CODIS laboratory, the "Specimen ID" of the profile and a copy of the laboratory report.
 - 3. The case summary, including all available information that may be helpful in identifying a relative of the perpetrator.
 - 4. A statement that the agency agrees to further investigate the case after the State CODIS laboratory releases the investigative information to the requesting agency.
 - 5. A completed Local CODIS Laboratory Familial Search Request Checklist (LAB-CO-48).
 - 6. STR and Y-STR electropherograms of the profile should be attached to the request or provided by the local CODIS laboratory.
- C. An out of state request may be considered if circumstances show a connection to the State of Texas such as, but not limited to, an interstate forensic hit. These special circumstances should be outlined in the request. All other laboratory and profile requirements must still be observed. In addition, the out of state profile must be previously tested by the requesting state's familial search process (if one exists) and have no open investigative leads from the search.
- D. Upon approval, the State CODIS Laboratory shall perform the search.

6.4 Conducting a Familial Search

- A. The State CODIS laboratory shall perform the familial search using validated and approved software.
- B. Database samples to be tested are based on the candidate list generated from the familial search.
- C. Y-STR and/or STR testing of the selected sample(s) will be performed by the State CODIS laboratory.
- D. If the Y-STR profiles have been determined to be consistent and have met threshold or, if female, the profile met threshold without lineage testing, the DPS reserves the right to review non-DNA information in order to identify additional evidence bearing on relatedness.



6.5 Reporting Results

- A. Prior to releasing an individual's identifying information, the State CODIS Laboratory shall verbally discuss the limitations and meaning of the results with the requesting agencies. This discussion should include all representatives involved in the request and may take place in person or via conference call.
- B. Results of the testing shall be made in writing to the requesting agencies and be emailed or delivered in person. Only secure and valid email addresses may be used. DPS will not send information to public domain email addresses. All sensitive Department material transmitted over any external network will be encrypted utilizing an encryption standard established by IMS.
- C. Law enforcement agencies are encouraged to contact the State CODIS laboratory with any possible leads in order to assist in determining the validity of a possible suspect(s). Some suspects developed from the familial search may already have a sample in the CODIS database and therefore may be excluded, saving time and resources on the part of the law enforcement agencies and/or laboratories involved.

7 Literature References and Supporting Documentation

CODIS Bulletin BT072006. Interim Plan for the Release of Information In the Event of a "Partial Match" at NDIS. 2006.

Tactical Advice Using Familial DNA Intelligence Products in Serious Crime Investigations. Forensic Science Service Police Standards Unit and National Centre for Policing Excellence. 2006.

Information Bulletin #2008-BFS-01. DNA Partial Match (Crime Scene DNA Profile to Offender) Policy. California Department of Justice. 2008.

Colorado Bureau of Investigation DNA Familial Search Policy. CBI Policy Statement. 2009.

"SWGDM Recommendations to the FBI Director on the Interim Plan for the Release of Information in the Event of a Partial Match at NDIS, "Forensic Science Communications, v. 11, no. 4 (Oct 2009).

Clopper, C. and Pearson, E. The use of confidence or fiducial limits illustrated in the case of binomial, *Biometrika* (1934) 26:404-413.

Interpretation Guidelines for Y-Chromosome STR Typing, SWGDAM (2014).

Willuweit S., Roewer L. (2015), 'The new Y Chromosome Haplotype Reference Database.', *Forensic Sci. Int. Genet.* 15. 43-8.

Jianye Ge, Bruce Budowle, Arthur Eisenburg, Ranajit Chakraborty "Comparing DNA Based Familial Searching Policies."

S.P. Myers, et al. "Searching for first-degree familial relationships in California's offender DNA database: Validation of a likelihood ratio-based approach", *Forensic Sci. Int. Genet.* (2010).

Familial Searching Manual for CODIS 8.0, version 1.1, August 2018.



CO-05-05 MATCH DISPOSITIONING

1 Scope

Potential matches from the CODIS database must be reviewed to determine the value in the match. Each match must be given a designation (or disposition) referring to the potential value of the match.

2 Related Chapters

Match Verification

3 Policy

3.1 Dispositioning CODIS Matches

- A. The CODIS Program Manager or designee will review any potential CODIS matches as soon as practicable following an autosearch. Matches will be dispositioned appropriately after review.
- B. The State Laboratory will follow the NDIS model in expecting matches to be dispositioned within 30 days. When possible, a note should be added explaining the disposition once the permanent disposition has been assigned.
- C. Examples of possible dispositions can be found in the NDIS Procedures manual.

3.2 Pending Disposition

Any matches for which the disposition is unknown will be dispositioned as "Pending" until further information is obtained. Communications with the Local Lab should be initiated quickly to determine the status of "Pending" matches.

3.3 Conviction Matches

If a case is solved (via comparison to a suspect known, guilty plea, etc.) and the case suspect is the convicted offender/arrestee, disposition the match as "Conviction Match".

3.4 No matches

If a match is determined to be adventitious, disposition it as "No Match".

4 Overdue Dispositions

- A. Local laboratories that have a match that has been pending for more than 30 days will be notified that the match is still pending and will be asked to respond with the disposition within the following 15 days.
- B. If a reasonable attempt has not been made to disposition the match, by the end of the extension period, the matter will be brought to the attention of the CODIS Program Manager.

5 Forensic Hits

- A. The responsibility for case-to-case hits is shared equally between the two casework labs. If necessary, the CODIS laboratory should act as liaison between analysts at each lab by following the procedure described below:
 1. Notify all labs involved that there is a potential hit. Local labs should see these matches in their own Match Manager.



2. Advise both labs that they share the responsibility of determining the validity of the match.
3. Advise the labs to review their case files to determine if there was a known suspect that matched to the cases.
4. The local administrator in each lab should determine the case disposition and update CODIS appropriately. They should report back to SDIS once the validity of the match is determined.

6 Match Verifications

Matches that are deemed to be Offender/Arrestee Hits will be verified according to the *Match Verification* chapter.

7 Literature References and Supporting Documentation

CODIS Administrators Handbook

NDIS Procedures manual



CO-05-06 MATCH VERIFICATION

1 Scope

All matches should be evaluated to determine their value to investigating agencies. Once a match has been determined to provide an investigative lead, these procedures are used to verify the results of the match.

2 Related Chapters

Response Quality Issues

CODIS Upload

Profile Removal/Expunction Procedures

3 Policy

3.1 Offender/Arrestee Hits

Offender/Arrestee hits are generally initiated by the casework laboratory. When a match provides an investigative lead, it is the responsibility of the State Administrator, Local Administrator, or their designee to track the disposition of the match and to provide a letter containing the offender's identifying information to the requesting agency.

3.2 Remote Collection Hit Entry (LDIS)

1. Open Hit Tracking Module.
2. Set Hit Tracking Status to "Active", filter the appropriate date(s), then select "Search".

Note: If the CODIS number has been previously verified within the STaCS software, the Hit Tracking Status will be "HC Complete". Ensure all loci have been run (i.e. D2, D19, expanded CODIS core loci). If more loci need to be run, click the "Hit Conf. Run" button to put the sample in process.

3. Pull appropriate CODIS database cards.
4. Give stack of database cards to liaison or designee to review the qualifying offense.

3.3 Manual Hit Entry (NDIS)

1. Open Hit Tracking Module.
2. Click "New" button.
3. Enter appropriate Match ID and click "OK".
4. Enter the appropriate information into the fields. (i.e. Request Date, Lab Case #, Offense Date, City of Offense, Offense, Law Enforcement Agency (LEA), LEA Case #, and Item of Evidence.
5. For offense, if exact description is not listed, choose "Other" and enter "Other" into the comments section along with the offense.
6. If applicable, check the "Rush" box and select the appropriate level of urgency.
7. If applicable, check the "Additional Loci" box to run the sample for expanded loci.

Note: If the CODIS number has been previously verified within the STaCS software, the Hit Tracking Status will be "HC Complete". Ensure all loci have



been run (i.e. D2, D19, expanded CODIS core loci). If more loci need to be run, click the “Hit Conf. Run” button to put the sample in process.

8. Click “Save”.
9. Pull appropriate CODIS database cards.
10. Give stack of database cards to liaison or designee to review the qualifying offense.

3.4 Qualifying Offense Check

- A. The subject’s TDCJ and CCH records are checked by the liaison or designee to identify any possible qualifying offense(s) under any applicable statute.
 1. Open Hit Tracking Module.
 2. Query appropriate Hit Tracking Entry and double-click to open the record.
 3. In the Hit Tracking Case to Offender Entry window, click the “Submission” button.
 4. Review the CCH and any TDCJ databases for possible qualifying offense.
 5. Use dropdown to select appropriate offense and reason from the respective dropdown lists.
 - a) *Additional documentation (Court Orders and emails) stating the offense for subjects whose qualifying offense cannot be determined through CCH or TDCJ databases must be uploaded in PDF format. Name files according to section 6.1 File Nomenclature.*
 - b) *Click the “Files” button in the submission module to upload these files.*
 - c) *Click “Save” then “Close” to exit the module.*
 6. Click “Save” in Hit Tracking Case to Offender Entry window.
- B. The qualifying offense check will be administratively reviewed. See **6.4 Administrative Review**.

3.5 Match Verification Laboratory Analysis

The match verification analyst will retest the archived offender sample(s) to verify the DNA profile.

3.6 Acknowledging Matches

1. Open Hit Confirmation Module.
2. For samples with Run Status, “Acknowledgement Required”, double-click the record to view profiles. Visually inspect the tables to ensure the profiles match.
3. From the Profile Status dropdown menu, select “Match” or “No Match”, as applicable.
 - a) *If there is a non-match between the CODIS DNA profile and the archive sample, the source of the discrepancy shall be investigated and documented per the Response to Quality Issues chapter. This may include additional collection of samples from individuals involved, if possible, and may require the initiation of a Quality Incident; see the Quality Incident (QI) and Quality Action Plan Process (QAP) chapter in the CLS Manual. Upon successful resolution, a CODIS Offender/Arrestee Letter may be released. Documentation will be maintained in STaCS.*



Note: *Inconclusive or incomplete results will not prevent the release of the CODIS Offender/Arrestee letter if the information within the profile is non-contradictory and a reasonable explanation or cause can be determined.*

- b) *If uploading additional loci to CODIS that were not present in the original DNA profile, ensure that all loci boxes are checked. Follow procedures outlined in the CODIS Upload chapter.*
- c) *Click "Save" and "Close".*

3.7 Generate CODIS Offender/Arrestee Letter (SDIS and NDIS)

1. Open Hit Tracking Module.
2. Set Hit Tracking Status to "Active" and enter the CODIS number into the CODIS Number field. Click "Search".
3. Set the Hit Tracking Status to "Active" and Select a record to review. Click the "View Profiles" button.
4. Open CODIS software to query the Match ID number and compare with the profile in STaCS. Close the window in STaCS with the profile when complete.
5. Double-click the match record to open the Hit Tracking Case to Offender Entry window.
 - a) *Open up CCH website to query the SID number associated with the CODIS number.*
 - b) *Check the CCH information against the Submission record, and CODIS database card, make updates as needed. If updates are made, click "Save", then "Close". If no changes are made, click "Close", then "Yes" to save without changes.*
 - c) *Click the "Letter" button to view the Offender Letter. Check the information on the letter against the CCH record, the CODIS match record, and the CODIS database card.*
 - d) *If all information is accurate, close the letter. In the pop-up window, choose "Yes" to approve.*
 - e) *Click "Save" to apply all changes and then "Close".*
 - f) *The letter will be administratively reviewed. See 6.4 Administrative Review.*

4 Release of Offender/Arrestee information without reanalysis

- A. In the case of an immediate public safety risk/national security issue, an offender's/arrestee's information can be released without reanalyzing the offender sample.
- B. The offender's/arrestee's information will be released, but re-analysis and verification of the qualifying offense will occur as soon as practicable.
- C. Approval by the State CODIS Administrator or designee is required prior to release of the offender's/arrestee's information.

5 Distributing CODIS Offender/Arrestee Hits

The laboratory will make a good faith effort to resolve both intrastate and interstate matches within 30 business days.



5.1 SDIS Hits

- A. SDIS CODIS Offender Letters will be distributed following verification and administrative review.
- B. Information provided shall include the offender's name, date of birth (DOB), Texas SID #, and FBI #.
- C. The letter will be signed by the Local CODIS SDIS Administrator or designee, and electronically distributed via STaCS Remote Collection on the CODIS network.

5.2 NDIS Hits

- A. NDIS CODIS Offender Letters will be distributed following verification and administrative review.
- B. Information provided shall include the offender's name, date of birth (DOB), Texas SID #, FBI #, and qualifying offense.
- C. The letter will be signed by the Local CODIS SDIS Administrator or designee, and will be faxed or emailed to the NDIS CODIS Administrator of the casework laboratory.
 1. Only secure and valid email addresses may be used. DPS will not send information to public domain email addresses.
 2. All sensitive Department material transmitted over any external network will be encrypted utilizing an encryption standard established by IMS.

6 Documentation

6.1 File Nomenclature

The following nomenclature is used to name supporting match documentation prior to upload into STaCS for archival purposes.

- A. For Court Orders: DNAO_#
- B. For Emails from TDCJ: TDCJ_#
- C. For Emails from TJJD: TJJD_#
- D. For Emails from Booking Agencies: # Qualifying Offense
- E. For Match Folders: MF #
- F. For Match Additional Documentation: # Match Info

Note: Where # is used above, type in the CODIS Number.

6.2 SDIS Hits

The following documentation will be maintained in STaCS: SDIS offender hit letters, supporting laboratory analysis, and amendment request documentation.

6.3 NDIS Hits

The following documentation will be maintained in STaCS: NDIS offender hit letters, supporting laboratory analysis, match verification requests, letter sent receipts, and amendment requests.



6.4 Administrative Review

The administrative review must include the following elements:

- A. Review of the supporting administrative documentation and the correspondence for clerical errors; and
- B. Review of the individual's biographical data, qualifying offense, and DNA profile generated from reanalysis, as applicable.
- C. The review will be documented and maintained within STaCS.

6.5 Qualifying Offense Administrative Review

1. Open Hit Tracking Module.
2. Set Hit Qualifying Offense Status to "Review".
3. Check the CCH information against Submission and make updates as needed.
Note: Any change made to the Qualifying Offense will need to be reviewed by either the liaison or designee.
4. If no qualifying offense exists, notify the CODIS Administrator. The offender sample should be removed from the CODIS database; see the Profile Removal/Expunction Procedures chapter.
5. This will not disqualify the match from being evaluated. Any match deemed investigative will be treated as such and all other procedures will continue as normal, including the issuance of a CODIS Offender letter. Document on the datacard the removal of the sample, initials, and date.
6. If the contributor type must be changed, make the change in STaCS Submission and notify the CODIS Administrator through email.

6.6 Hit Letter Administrative Review (CCH and CODIS access required)

1. Open Hit Tracking Module (STaCS).
2. Set the Hit Tracking Status to "Active" and enter the CODIS number into the CODIS Number field. Click "Search".
3. Select a record to review. Click the "View Profiles" button.
4. Open CODIS software to query the Match ID number and compare with the profile in STaCS. Close the window in STaCS with the profile when complete.
5. Double click the match record to open the Hit Tracking Case to Offender Entry window.
6. Open up CCH website to query the SID number associated with the CODIS number.
7. Check the CCH information against the Submission record, and CODIS database card, make updates as needed. If updates are made, click "Save", then "Close". If no changes are made, click "Close", then "Yes" to save without changes.
8. Click the "Letter" button to view the Offender Letter. Check the information on the letter against the CCH record, the CODIS match record, and CODIS database card.
Note: For NDIS hits, compare the information from the request documentation to the Hit Tracking Information, the CCH record, the CODIS match record, and the CODIS database card.



9. If all information is accurate, close the letter, and select the “OK” option in the pop-up window to release the letter.
10. If the information is not correct, select the “Revisions Required” option and enter into the comments the description of the revision needed. This option sends the letter back to the HC Complete status for review.
11. For NDIS hits, click the “Files” button to view letter.
12. Right click the “Hit Confirmation Letter” PDF, then select “Save As” to save to appropriate location with the format, DC0000#####.
13. Click “Save” in the Hit Tracking Case to Offender Entry window, then “Close” to complete the administrative review.

6.7 Previously Verified Matches (Match Folders)

Existing physical copies of Match Folders are scanned and stored in STaCS in the Submission module for the associated CODIS number.

6.8 Amended CODIS Offender Letters

- A. CODIS Offender Letters may be amended following a request by either the LDIS or NDIS laboratory.
 1. Open the Hit Tracking Module (STaCS).
 2. Query the applicable CODIS Offender Letter.
 3. Amend the information as applicable.
 4. Click the “Amend Letter” button, and check the amended changes.
 5. Click the “Letter” button to view the Offender Letter. Check the information on the letter against the CCH record, the CODIS match record, and the CODIS database card.
 6. If all information is accurate, close the letter. In the pop-up window, choose “Yes” to approve.
 7. Click “Save” to apply all changes and then “Close”.
- B. The letter will be administratively reviewed.

7 Hit Tracking

Hit tracking will be performed for both state (SDIS) and national hits (NDIS). Hit counts will be provided to the FBI in accordance with the NDIS Procedures.

8 Literature References and Supporting Documentation

NDIS Procedures Manual



CO-05-07 DATABASE MONITORING

1 Scope

In accordance with the Texas Government Code Title 4 Subtitle B Chapter 411 Subchapter G DNA Database System Section §411.142, the Department of Public Safety shall develop and maintain a monitoring system capable of identifying inaccurate or incomplete information. Data contained within the CODIS database server and in STaCS is routinely examined for accuracy in both the DNA data and in personally identifying information of subjects subjected to DNA Database testing.

2 Related Chapters

Sample Collection and Handling

Match Verification

3 Policy and Procedures

The following list of policies and procedures have been put into place to ensure the most accurate information is recorded in the CODIS Database and in STaCS.

- A. AFIS Verification (within *Sample Collection and Handling*) – This policy and procedure ensures that the fingerprints submitted on the CODIS DNA Database Card match the subject's Personally Identifiable Information (PII) on that same DNA Database Card. During this process, any minor discrepancies of the subject's PII are also corrected in STaCS with information taken directly from Texas Computerized Criminal History (CCH).
- B. Match Verification (CODIS SOP) – Prior to releasing a subject's PII to a Law Enforcement Agency (LEA) following a CODIS Hit, the subject's PII, qualifying offense and original DNA data are reviewed and the archived DNA sample is retested to confirm accuracy and concordance.
- C. Offender/Offender Searches – These searches occur on a monthly basis and look for any possible duplicates that may have entered the CODIS database. This search can also be useful in identifying any samples and/or data that may have been switched with another sample. In addition, the acknowledgment of twins in the database can also be checked.
- D. CODIS Sync Module in STaCS – This module compares sample profiles in STaCS with those in CODIS.
- E. Sex Data Reconciliation – This periodic search of STaCS is performed to investigate discrepancies between DNA data and the sex metadata recorded in STaCS at Data Entry. From this search, further inquiries are made into an offender/arrestee CCH record to ensure the PII is correct.
- F. Specimen Category Verification –
 1. Blood samples are always designated as "Convicted Offender" at Data Entry.
 2. Buccal samples are assigned as "Arrestee" at Data Entry. All buccal samples are checked against CCH and/or court orders are reviewed to determine if the subject has a felony conviction. If the individual has a felony conviction, the specimen category is updated to "Convicted Offender" after AFIS verification.
 3. The specimen category is verified again during the match verification process.
 4. Periodic searches are also performed in STaCS to determine discrepancies.



4 Records

Offender to Offender search results (CODIS)
Offender Dynamic Search results (STaCS)
Submission Audit History reports (STaCS)
SDIS Audit Trail Reports (CODIS)

5 Literature References and Supporting Documentation

Texas Government Code Title 4 Subtitle B Chapter 411 Subchapter G DNA Database System
Section §411.142



06 REAGENTS

CO-06-01 COMMERCIAL REAGENTS

1 Scope

This document includes policies and procedures for documenting commercial reagents and setting expiration dates when none are provided by the manufacturer.

2 Documenting Commercial Reagents

- A. Consumables, including commercial reagents, should be entered into the Receiving module in STaCS upon receipt into the laboratory.
- B. Commercial reagent entries must be verified by another individual prior to being saved. This is documented in the Comments field.
- C. Information recorded in STaCS includes, but is not limited to:
 1. Manufacturer/Supplier
 2. Material/Chemical/Reagent
 3. Lot Reference Number
 4. Received Date
 5. Expiry Date (expiration date)
 6. Created By (user creating entry)
 7. Current Status
 8. Activity History
- D. Once a consumable has been received into STaCS, it is given a unique barcode which is affixed to the container.
 1. The prefix indicates the identity of the consumable. Prefix codes are maintained in STaCS and can be referenced using the Consumable Configuration module.
 2. The numerical value following the prefix indicates the individual unit(s) being entered into STaCS.
- E. Commercial reagents must be labeled with the identity of the reagent and the expiration date and/or STaCS barcode label.
- F. Barcoded information may be retrieved using the Item Query module in STaCS.

3 Expiration date

- A. Commercial reagents that do not have expiration dates provided by the manufacturer shall be assigned an expiration date one year from the date the reagent is opened. This is documented using the Chemical Releasing module in STaCS.
- B. Expiration dates may be extended at one year intervals provided that a performance verification is performed, documented, and approved prior to use. The reagents must have been stored according to the manufacturer's guidelines. The new expiration date is entered into STaCS.
- C. A letter from the manufacturer may also be used to determine the expiration date.



07 FORMS

DIRECTORY OF FORMS

	Document Name	FRN
1	Analysis Form	LAB-CO-05
2	CODIS Collection Kit Order Form	LAB-CO-08
3	Amplification Kit Reagent Quality Control	LAB-CO-21
4	Quality Control for CE Instrument	LAB-CO-22
5	Familial Search Document Checksheet	LAB-CO-23
6	Quality Control for Robotic Workstations	LAB-CO-25
7	CODIS User Laboratory Application	LAB-CO-40
8	Analyst Authorization Form	LAB-CO-41
9	Familial Search Request Review	LAB-CO-43
10	Temperature Verification Log	LAB-CO-44
11	Biohazard Safety Cabinet Cleaning Log	LAB-CO-45
12	Solution 2000 Filter Replacement Log	LAB-CO-46
13	Local CODIS Laboratory Familial Search Request Checklist	LAB-CO-48
14	9700 Thermal Cycler Verification Record	LAB-CO-51
15	Veriti Thermal Cycler Verification Record	LAB-CO-52
16	Dry Bath/Thermomixer Quality Check	LAB-CO-53