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## 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	<b>Revised:</b> CO-TM-03-03, CO-TM-03-04, CO-TM-03-05, CO-TM-04-03



## 01 OVERVIEW

### CO-TM-01-01 OVERVIEW OF CODIS TRAINING PROGRAM

#### 1 Introduction

This manual is used to train CODIS analysts, CODIS technicians, and CODIS interagency liaisons. The manual is intended for use by new personnel and any personnel attempting to become qualified in a procedure for which they are not currently qualified.

The primary trainer will determine the successful completion of each module. Not all modules may be necessary for all employees. Each employee will be trained based on their position and/or specialized duties assigned for those employees.

Each employee should be given sufficient time to review required reading materials before being tested on a module. Supplemental reading materials may be assigned at the discretion of the trainer.

CODIS personnel employed by the Texas Department of Public Safety must meet the standards outlined in The FBI Quality Assurance Standards for DNA Databasing Laboratories before receiving approval to perform independent DNA testing. These qualifications consist of educational requirements, DNA experience, and training requirements. These qualifications are reviewed by the Technical Leader prior to beginning work in the CODIS section.

#### 1.1 Definition of Personnel

- A. CODIS Analysts include individuals that conduct and/or direct the analysis of databasing samples.
  - 1. These individuals perform secondary (technical) review for other analysts performing similar functions.
  - 2. CODIS analysts are audited to both the FBI's Quality Assurance Standards and the standards of the accrediting body.
- B. CODIS Technicians/CODIS Interagency Liaisons include individuals that perform CODIS sample handling
  - 1. These individuals complete intake of the databasing samples and prepare samples for CODIS analyst processing.
  - 2. Because these individuals do not perform DNA analysis, they are not audited to the FBI's Quality Assurance Standards, but are audited to the standards of the accrediting body.

#### 1.2 Experience/Training Requirements

- A. CODIS Analysts shall have a minimum of six (6) months of human- DNA laboratory experience with at least three months in a forensic or database DNA laboratory. Prior to independent work using DNA technology, CODIS analysts shall complete the successful analysis of a range of samples routinely encountered in database analysis.
- B. CODIS Analysts shall successfully complete a competency test before beginning independent DNA analysis.
- C. CODIS personnel shall successfully complete documented training specific to his or her job function(s).



## 2 Purpose

- A. The CODIS training program is intended to provide the trainee with sufficient background, laboratory skills, education, competency, and supervised hands-on experience to competently perform their duties with minimal supervision and meet the FBI Quality Assurance Standards for DNA Databasing Laboratories.
- B. Trainees having prior forensic or DNA database experience may complete a modified training program provided that the Technical Leader evaluates, approves, and documents the adequacy of the previous training. Any adjustments to the training program must be justified in writing in the trainee's notebook and approved by the System Quality Manager and Technical Leader. Specific modifications to the training program should be indicated on the applicable training checklist. Regardless of previous experience, analysts must successfully complete a competency test covering the routine DNA methodologies to be used prior to participating in independent database analysis.

## 3 Program Format

The training program is divided into four CODIS units and two General Laboratory Units, each consisting of a set of modules.

- A. General Laboratory Training Manual - Introduces the trainee to general laboratory practices, forensic science, quality assurance, general laboratory safety, legal issues, and department and laboratory policy.
  1. The Fundamentals Unit within the General Laboratory Training Manual must be completed *in its entirety* before the trainee can handle any unknown substances in the laboratory, per the Safety Manual.
  2. The General Laboratory Training Manual Legal Unit must be completed *before* approval for supervised work is granted.
- B. CODIS Technician Unit - Introduces the trainee to sample receiving and handling, data entry, AFIS verification, sample preparation and storage, and blood tube destruction.
- C. CODIS Technician Equipment Maintenance Unit – Introduces the trainee to maintenance of Capillary Electrophoresis equipment within the laboratory. The CODIS Technician Equipment Maintenance Unit is optional.
- D. CODIS Analyst Unit - Introduces the trainee to the history of CODIS, introduction to forensic DNA analysis, basic extraction, amplification, capillary electrophoresis, and data interpretation.
- E. CODIS Advanced Robotics Unit – Introduces the trainee to the operation of robotic instruments. The CODIS Advanced Robotics Unit is optional. Approval is granted individually for each module completed within the unit.
  1. Automated blood spotting
  2. Automated buccal lysis set-up
  3. QIA Symphony SP Extraction
  4. BSD 600 Operation
- F. Not all CODIS training modules will be necessary for every employee. Each employee will be trained based on their position and/or specialized duties assigned.



- G. Samples listed in the competency sections for each unit will be in addition to and separate from any samples worked during the practice portion of the training modules within each unit.
  - 1. Competency samples from the extraction competency test may be used to satisfy competency requirements for amplification, capillary electrophoresis, and robotic platform. The same sample may be used multiple times as a competency test sample for these modules.
- H. The requirement for successful completion of written examinations given as part of training listed in the CODIS training manual is a score of 75% or greater.
  - 1. If a score of less than 75% is obtained, the remediation process outlined in the CLS Employee Training Program chapter will be followed.

#### 4 Safety

- A. Specific safety precautions are described within each module of the training manual.
- B. In general, body fluids encountered during training in DNA may contain infective agents. Use universal precautions during sample handling.

#### 5 Responsibilities

##### 5.1 Technical Leader and/or Supervisor Responsibilities

- A. The Technical Leader is responsible for overseeing all training in the CODIS Section.
- B. The Technical Leader may assign a trainer(s) to the trainee for general laboratory assistance, instruction in procedures, and laboratory practicals and assessments.
- C. Meetings between the trainee, the trainer, Technical Leader, and/or Supervisor should be held routinely in order to evaluate the trainee's progress, plan future study/practical assignments and discuss any deficiencies which require additional training.

##### 5.2 Trainer Responsibilities

- A. The trainer(s) conducts training on assigned modules, reviews the training notebook, training records, and the completion of training requirements.
- B. The trainer will be qualified by DPS and proficiency tested in the category of work/relevant testing procedure for which they provide training.
- C. **CODIS Analysts only-** The trainer recommends that the analyst be approved for supervised analysis to the Technical Leader.

##### 5.3 Trainee Responsibilities

- A. The trainee will be required to keep a training notebook and successfully complete all exams and competency samples (where applicable).
  - 1. Completion of modules and/or practicals will be recorded on the appropriate training checklist.
  - 2. The following is a list of items maintained in the training notebook by the trainee:
    - a) *Training Record (LAB-303) (optional)*
    - b) *List of any in-house training videos, demonstrations and lectures attended that are not already listed on the appropriate unit training checklist.*
    - c) *Sample identification results and other practical exams*



- d) *Competency tests and results (analysts only)*
  - e) *Special project assignments with summary reports (optional)*
  - f) *Written exams (analysts only)*
  - g) *Workshop or lecture certificates (copy)*
- B. The training program requires the trainee to keep up with reading assignments on a self-study basis.
- C. The trainee is responsible for informing his/her trainer or Technical Leader when problems arise at any time during the training period.

## **6 Review and Authorization**

### **6.1 General Laboratory Training Unit**

General Laboratory Training Unit requirements will conclude when the following are met:

- A. The trainee successfully completes General Laboratory Training Manual Fundamentals Units
- B. The trainee successfully completes General Laboratory Training Manual Legal Units
- C. Completion of the general laboratory training is documented via the Certificate of Completion (LAB-308) with recognition by the Laboratory Director.

### **6.2 CODIS Analyst Unit**

- A. CODIS Analyst unit training will conclude with examiner approval to conduct mentored/supervised work when the following are met:
  - 1. Competency samples for the category of work/relevant testing procedure are correctly analyzed.
  - 2. Successful completion of six months of forensic DNA laboratory experience.
- B. The training notebook and other training records documenting completion of training requirements are reviewed by the trainer and the Technical Leader. Quality Assurance and the Section Supervisor (if applicable) may also review the training notebook and other training records.
- C. The training notebook is submitted along with a Work Authorization form (LAB-309) to System QA for review and Laboratory Director authorization.
- D. The newly qualified analyst is enrolled in the proficiency testing program once the Work Authorization form (LAB-309) has been completed.

### **6.3 CODIS Advanced Robotics**

- A. CODIS Advanced Robotics unit training will conclude with approval to operate the appropriate robotic workstation when the following are met:
  - 1. Competency samples for the category of work/relevant testing procedure are correctly analyzed
- B. The training notebook and other training records documenting completion of training requirements are reviewed by the trainer and the Technical Leader. Quality Assurance and the Section Supervisor (if applicable) may also review the training notebook and other training records.



- C. The training notebook is submitted along with a Work Authorization form (LAB-309) to System QA for review and Laboratory Director authorization.

#### **6.4 CODIS Technician Unit**

- A. CODIS technician unit training will conclude with technician authorization to handle databasing samples when the following are met:
  - 1. Supervised performance relating to sample receiving and handling.
- B. The training notebook and other training records documenting completion of training requirements are reviewed by the trainer and the Technical Leader. Quality Assurance and the Section Supervisor (if applicable) may also review the training notebook and other training records.
- C. The training notebook is submitted along with a Work Authorization form (LAB-309) to System QA for review and Laboratory Director authorization.
- D. There is no proficiency or mentored/supervised work requirement for CODIS technicians who only participate in the handling of databasing samples.

#### **6.5 CODIS Technician Equipment Maintenance Unit**

- A. CODIS Technician Equipment Maintenance unit training will conclude with technician authorization to perform maintenance on capillary electrophoresis equipment when the following are met:
  - 1. Supervised performance relating to capillary electrophoresis maintenance.
- B. The training notebook and other training records documenting completion of training requirements are reviewed by the trainer and the Technical Leader. Quality Assurance and the Section Supervisor (if applicable) may also review the training notebook and other training records.
- C. The training notebook is submitted along with a Work Authorization form (LAB-309) to System QA for review and Laboratory Director authorization.
- D. There is no proficiency or mentored/supervised work requirement for CODIS technicians who only participate in the maintenance of capillary electrophoresis equipment.

#### **6.6 Supervised Work Requirements**

- A. Upon completion of training requirements, analysts new to the laboratory will enter a period of supervised work following authorization by the Laboratory Director.
- B. Those analysts previously qualified to conduct work in the laboratory may either perform an abbreviated period of supervised work or be authorized for independent work by the Quality Manager.
- C. At least one mentor that was either previously qualified or is currently qualified in the category of work that they are overseeing is assigned to the new analyst.
- D. Analysts will document the plates on which supervised work was performed using Supervised Case Log (LAB-307) or electronic equivalent.
- E. Trainer must initial the Analysis Forms (LAB-CO-05) indicating that they have reviewed the results with the trainee.
- F. Once all supervised work requirements are successfully completed, the mentor(s) can recommend the analyst for independent work to the Technical Leader. The authorization



for independent work is granted through completion of the Work Authorization form (LAB-309) by the Quality Manager and Technical Leader.

- G. CODIS DNA Analysis: successful completion of analyzing a minimum of three full plates of databasing samples.

## **7 Evaluation of Training Program**

The trainee will complete an evaluation of each module content, the unit content, or the overall program, including the trainer, using the LAB-304. The trainee and trainer will complete a checklist and sign-off sheet.



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## CO-TM-01-02 INTRODUCTION TO CODIS / HISTORY OF CODIS PROGRAM

**Duration** 2 to 4 days

**Purpose** To introduce the trainee to the history of the CODIS program and the laws that impact CODIS sample collection

**Prerequisite** Overview of CODIS Training Program

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### 1 Objectives

#### 1.1 Theoretical

Texas DPS implemented the Combined DNA Index System (CODIS) on September 1, 1995, in accordance with Texas Government Code Title 4 Subtitle B §411.142. The principal purpose of this system is to assist federal, state, and local criminal justice and law enforcement agencies in the investigation and/or prosecution of CODIS eligible offenses in which biological evidence is recovered.

The State CODIS Program is responsible for establishing and maintaining a computerized database that serves as the State's central depository for DNA records. The State DNA Database, consisting of several authorized categories of DNA records, is housed and maintained by the State CODIS Laboratory at the Texas Department of Public Safety Crime Laboratory in Austin.

#### 1.2 Practical

Following the completion of training, the trainee will be able to:

- A. List the three major components of the criminal justice system and how they interact with each other.
- B. Understand the process of developing a DNA profile from biological material.
- C. Explain the importance of using comparisons in forensic DNA analysis.
- D. List the major developments in forensic DNA typing.
- E. Explain why DNA databases are effective.
- F. Define and explain the difference between database, databank, and population database.
- G. List the four components that make a database effective.
- H. Describe how forensic databases work.
- I. List and describe the three tiers that make up the CODIS database.
- J. Define CODIS and describe its purpose.
- K. Define the following terms and explain the differences between them: convicted offender index, forensic index and arrestee index.
- L. List the information stored and not stored in CODIS.
- M. Describe how NDIS ensures that quality data is being put into the database.
- N. Explain who has access to CODIS.
- O. Explain how the success of CODIS is measured.
- P. Describe the difference between a forensic hit and an offender hit and what information they provide to the investigation.



- Q. Describe the process of following up a “cold hit.”
- R. Explain why “hit confirmation” is so important.
- S. Describe other uses for DNA databases.
- T. Understand the federal and state laws that impact CODIS.
- U. List concerns people have with DNA databases.
- V. Describe how privacy concerns are addressed in CODIS.
- W. List the policies in place that ensure privacy is maintained within CODIS.
- X. Explain the justification for sample retention.

## **2 Training Outline**

### **2.1 Lesson Plan**

- A. Overview and History of DNA Typing (Butler)
  - 1. Overview of the Criminal Justice System
  - 2. Overview of Forensic DNA Analysis
    - a) *Basic principles*
    - b) *Steps in DNA sample processing*
    - c) *Profile comparisons*
    - d) *Major historical events*
- B. DNA Databases (Butler)
  - 1. Value of DNA Databases
  - 2. Database vs. Databank
  - 3. Aspects of a National DNA Database
  - 4. The U.S. National Database
    - a) *Three tiers*
    - b) *CODIS software*
    - c) *Indices*
    - d) *Stored information*
    - e) *Assurance of quality data*
    - f) *CODIS users*
    - g) *Measurement of success*
    - h) *Searches*
    - i) *Hit confirmation*
  - 5. Issues and Concerns with DNA Databases
    - a) *Privacy concerns*
    - b) *Sample retention*



6. Other uses for DNA Databases
  - a) *Missing persons*
  - b) *Partial matches and familial searching*

### C. DNA Database Laws

1. Federal Laws
  - a) *DNA Identification Act of 1994*
  - b) *DNA Analysis Backlog Elimination Act of 2000*
  - c) *Justice for All Act of 2004*
  - d) *DNA Fingerprint Act of 2005*
  - e) *Katie Sepich Enhanced DNA Collection Act of 2012*
2. State Laws
  - a) *House Bill 40*
  - b) *House Bill 1188*
  - c) *Senate Bill 638*
  - d) *Senate Bill 1380*
  - e) *House Bill 588*
  - f) *House Bill 562*
  - g) *House Bill 1068*
  - h) *House Bill 867*
  - i) *Senate Bill 727*
  - j) *House Bill 1399*
3. Legal Challenges to DNA Database Laws
  - a) *Maryland v. King*
  - b) *People v. Buza*

## 2.2 Required Readings

- A. CODIS Manual, Texas Department of Public Safety Crime Laboratory.
  1. Overview
  2. Sample Collection and Handling, *Sample Collection* section
- B. Butler, John M. *Fundamentals of Forensic DNA Typing*. Elsevier Academic Press. 2010.
  1. Chapter 1: Overview and History of DNA Typing
  2. Chapter 12: DNA Databases
- C. . Texas DPS Crime Laboratory Service Manual: *CODIS DNA Procedural Guidelines* chapter

## 2.3 Suggested Readings

Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. Chapter 8: DNA Databases: Uses and Issues.



### **3 Practice**

#### **3.1 Safety**

None

#### **3.2 Standards, Controls Reagent Preparation**

None

#### **3.3 Equipment**

None

#### **3.4 Observed Performance**

- A. Discuss Chapter 1 in *Fundamentals of Forensic DNA Typing* by John M. Butler
- B. Discuss Chapter 12 in *Fundamentals of Forensic DNA Typing* by John M. Butler
- C. Discuss Federal and State Legislation regarding DNA Databases
- D. Discuss Government Code, Chapter 411, Subchapter G

### **4 Assessment**

#### **4.1 Competency and Qualifying Examination**

The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee.

#### **4.2 Evaluation of Training**

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer.



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## 02 CODIS TECHNICIAN UNIT

### CO-TM-02-01 BLOOD SAMPLE COLLECTION AND HANDLING

**Duration** 2 to 4 days

**Purpose** The trainee will learn how to properly receive and evaluate blood samples for acceptance into the CODIS laboratory.

**Prerequisite** General Safety (General Laboratory Training, Part I), Introduction to CODIS / History of CODIS Program (CODIS TM)

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#### 1 Objectives

##### 1.1 Theoretical

All CODIS samples should be collected in accordance with the *CODIS DNA Procedural Guidelines* chapter of the CLS Manual and subjects shall be collected in accordance with State laws. This module presents the minimum standards for accepting samples in the CODIS laboratory.

Following the completion of training, the trainee will be able to:

- A. List the information that is recorded on the DNA Database Card during collection
- B. Describe the reasons for rejecting a blood sample and how to document the reject
- C. List the information collected when taking orders for blood collection kits

##### 1.2 Practical

Following the completion of training, the trainee will be able to:

- A. Print CODIS barcode labels using STaCS
- B. Prepare labeled blood tube racks for storage
- C. Process and screen blood kits for acceptance
- D. Properly package and ship blood kit orders
- E. Scan/save court orders/paperwork submitted with blood kit

#### 2 Training Outline

##### 2.1 Lesson Plan

- A. Blood Receiving and Storage
  1. Printing CODIS barcode labels
  2. Preparing blood tube racks for storage
  3. Processing blood kits
    - a) *Checking for appropriate seal*
    - b) *Checking contents of kit*
    - c) *Determining whether a kit is accepted/rejected*
      - i. *Blood tube labeled appropriately*
      - ii. *Blood collection tube has at least 0.5 cm of blood*
      - iii. *Readable thumbprints on DNA Database Card*





### 3.3 Observed Performance

- A. The trainee will perform the following activities with the trainer
  1. Discuss information listed on DNA Database Card
  2. Describe reasons for rejecting blood samples
- B. Trainee will observe trainer opening a set of 72 blood collection kits including:
  1. Printing CODIS barcode labels
  2. Preparing blood tube racks for storage
  3. Processing blood kits
    - a) *Checking for appropriate seal*
    - b) *Checking contents of kit*
    - c) *Determining whether a kit is accepted/rejected*
      - i. *Blood tube labeled appropriately*
      - ii. *Blood collection tube has at least 0.5 cm of blood*
      - iii. *Readable thumbprints on DNA Database Card*
      - iv. *Blood tube information matches DNA Database Card*
    - d) *Placing barcode label on DNA Database Card and blood tube*
  4. Storing blood tubes
  5. Scanning/saving court orders and other additional paperwork submitted with the collection kit

### 3.4 Supervised Performance

Under the supervision of the trainer, trainee will open a set of 72 blood collection kits (at a minimum) including all of the tasks listed in the Observed Performance.

## 4 Assessment

### 4.1 Competency and Qualifying Examination

Competency is not required for this module.

### 4.2 Evaluation of Training

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer and is a prerequisite for remaining CODIS technician modules.



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## CO-TM-02-02 BUCCAL SAMPLE COLLECTION AND HANDLING

**Duration** 2 to 4 days

**Purpose** The trainee will learn how to properly receive and evaluate buccal samples for acceptance into the CODIS laboratory

**Prerequisite** General Safety (General Laboratory Training, Part I), Introduction to CODIS / History of CODIS Program (CODIS TM)

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### 1 Objectives

#### 1.1 Theoretical

All CODIS samples should be collected in accordance with the *CODIS DNA Procedural Guidelines* chapter of the CLS Manual and subjects shall be collected in accordance with State laws. This module presents the minimum standards for accepting samples in the CODIS Laboratory.

Following the completion of training, the trainee will be able to:

- A. List the information that is recorded on the buccal DNA Database Card during collection
- B. Describe the reasons for rejecting a buccal sample and how to document the reject
- C. List the information collected when taking orders for buccal collection kits

#### 1.2 Practical

Following the completion of training, the trainee will be able to:

- A. Print CODIS barcode labels using STaCS
- B. Prepare labeled buccal sample boxes for storage
- C. Process and screen buccal kits for acceptance
- D. Properly package and ship buccal kit orders
- E. Scan/save court orders/paperwork submitted with buccal kit

### 2 Training Outline

#### 2.1 Lesson Plan

- A. Buccal Receiving and Storage
- B. Printing CODIS barcode labels
- C. Preparing buccal storage boxes
- D. Processing Buccal Kits
  1. Checking for appropriate seal
    - a) *Checking contents of kit*
    - b) *Determining whether a kit is accepted/rejected*
      - i. *Buccal swab storage envelope is labeled appropriately*
      - ii. *Buccal swab storage envelope contains two swabs*
      - iii. *Readable thumbprints on DNA Database Card*





- Buccal storage boxes
- Court Order stamp

### 3.3 Observed Performance

- A. The trainee will perform the following activities with the trainer
1. Discuss information listed on buccal DNA Database Card
  2. Describe reasons for rejecting buccal samples
- B. Trainee will observe trainer setting up and opening a set of 72 buccal kits including:
1. Printing CODIS barcode labels
  2. Preparing buccal boxes for storage
  3. Processing buccal kits
    - a) *Checking for appropriate seal*
    - b) *Checking contents of kit*
    - c) *Determining whether a kit is accepted/rejected*
      - i. *Buccal swab storage envelope labeled appropriately*
      - ii. *Buccal swab storage envelope contains two buccal swabs*
      - iii. *Readable thumbprints on DNA Database Card*
      - iv. *Information on buccal swab storage envelope matches DNA Database Card*
    - d) *Placing barcode label on DNA Database Card and buccal swab storage envelope*
  4. Storing buccal samples
  5. Scanning/saving court orders and other additional paperwork submitted with the collection kit

### 3.4 Supervised Performance

- A. Under the supervision of the trainer, trainee will open a set of 72 buccal collection kits (at a minimum) including all of the tasks listed in the Observed Performance.
- B. Under the supervision of the trainer, trainee will package and ship a buccal swab collection kit order.

## 4 Assessment

### 4.1 Competency and Qualifying Examination

Competency is not required for this module.

### 4.2 Evaluation of Training

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer and is a prerequisite for remaining CODIS technician modules.



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## CO-TM-02-03 DATA ENTRY

**Duration** 2 to 4 days

**Purpose** The trainee will learn which information is required for STaCS Data Entry Submission as well as Redraws/Rejects, and how/why cards are transported to AFIS for print verification.

**Prerequisite** Blood Sample Collection and Handling, Buccal Sample Collection and Handling

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### 1 Objectives

#### 1.1 Theoretical

Samples meeting the qualifying criteria for sample receiving will be entered into the STaCS Data Entry module. Rejected samples (those not meeting the qualifications) or samples requiring redraws are entered into Data Entry module as rejects which are not put into processing.

#### 1.2 Practical

Following the completion of training, the trainee will be able to:

- A. Enter information from DNA Database Cards into STaCS
- B. Enter rejects/redraws into STaCS

### 2 Training Outline

#### 2.1 Lesson Plan

- A. Submission Information (qualifying samples)
  - 1. Barcode Labels
    - a) *CODIS number*
    - b) *Storage Unit*
  - 2. Required Fields (highlighted)
    - a) *Sample Nature (Blood vs. Buccal)*
    - b) *Last Name – No suffixes*
    - c) *SID number*
    - d) *Contributor Type*
    - e) *Gender*
    - f) *Agency*
  - 3. Fields that carryover to the next record
    - a) *Gender*
    - b) *Race*
    - c) *Sample Nature*
    - d) *Contributor Type*
    - e) *Qualifying Reason*
  - 4. Blood vs. Buccal



- a) *Rack Identifier*
- b) *Rack Position*
- c) *Contributor Type*
5. Agency Types
  - a) *Community Supervision and Correction Departments (CSCD) and alternate names*
  - b) *Juvenile Probation*
  - c) *Police Departments*
  - d) *Sheriff's Office*
  - e) *Texas Department of Criminal Justice (TDCJ) unit*
  - f) *Texas Department of Criminal Justice – parole (TDCJ-parole)*
  - g) *Unknown/PD/SO/CSCD*
6. Problem Kits
  - a) *Reasons for rejection*
- B. Redraws/Rejects
  1. Computerized Criminal History (CCH)
    - a) *Confirm/Verify Information from the data card*
    - b) *Use CCH to fill out Reject information*
    - c) *If not in CCH, use data card to fill out Reject information*
  2. Required Fields (highlighted)
    - a) *Last Name – No suffixes*
    - b) *SID number*
    - c) *Sample Nature*
    - d) *Contributor Type*
    - e) *Gender*
    - f) *Agency*
  3. Reasons for rejection
- C. Card Storage locations
  1. Data Entry completion
  2. Reject/Redraw designated location

## **2.2 Required Readings**

CODIS Manual, Texas DPS Crime Laboratory: Sample Collection and Handling, *Recording DNA Database Card Information* section



### **3 Practice**

#### **3.1 Equipment**

- STaCS computer
- DNA database cards
- CCH access
- Barcode scanner
- Agencies list

#### **3.2 Observed Performance**

- A. Trainee will discuss with trainer the importance of ensuring the accuracy of each data submission in STaCS
- B. Trainee will observe trainer enter information from a set of 72 DNA Database Cards including:
  1. Starting the STaCS software and entering DNA Database Card information into STaCS
  2. Ensuring Data Entry record and DNA Database Card match before saving
  3. Entering Reject/Redraw information into STaCS
  4. Verifying all information is correct before saving a Reject/Redraw

#### **3.3 Supervised Performance**

Under supervision of the trainer, trainee will enter information from a set of 72 DNA Database Cards (at a minimum) into STaCS, including all the tasks listed in the Observed Performance.

### **4 Assessment**

#### **4.1 Competency and Qualifying Examination**

Competency is not required for this module.

#### **4.2 Evaluation of Training**

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer and is a prerequisite for remaining CODIS technician modules.



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## CO-TM-02-04 AFIS VERIFICATION

**Duration** 2 to 4 days

**Purpose** The trainee will learn how to update STaCS to reflect which cards have been accepted by AFIS through print verification.

**Prerequisite** Data Entry

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### 1 Objectives

#### 1.1 Theoretical

All non-rejected submissions received in CODIS will be sent to AFIS for verification. Once the DNA Database Cards have returned from AFIS, personal information and sample status for each sample will be updated in STaCS and the sample will be queued for processing if acceptable. Duplicate samples and rejected samples will not be processed.

Following the completion of training, the trainee will be able to:

- A. List reasons why AFIS would reject a sample
- B. Explain the difference between Redraw/Reject and a Problem Kit in STaCS
- C. Explain the importance of sorting out duplicate samples
- D. Explain the storage protocol of both acceptable and rejected cards
- E. Describe how a duplicate check is activated in STaCS

#### 1.2 Practical

Following the completion of training, the trainee will be able to:

- A. Identify acceptable, rejected, duplicate, problem, and redraw samples
- B. Create an AFIS Send File
- C. Update STaCS database accordingly
- D. Store cards in the appropriate location
- E. Navigate within STaCS to update information after print verification

### 2 Training Outline

#### 2.1 Lesson Plan

- A. AFIS Send worklist
  - 1. Generate an AFIS Send file
  - 2. File transfer and manual upload
  - 3. Send the DNA Database Cards to AFIS for verification
- B. Receive cards from AFIS once verified
- C. AFIS Verification Worklist
  - 1. Login and start AFIS Verification module in STaCS
  - 2. Transfer submissions from AFIS Verification list to AFIS Verification Personal list
  - 3. Edit existing information in STaCs using AFIS summary sheet or CCH sheet



4. Correct Problem kit and Redraw fields using AFIS verification (when applicable)  
Sort out rejected submissions from AFIS (when applicable)
  - a) *Send to Problem Kit worklist or Redraw Management worklist*
  - b) *File card with CODIS Liaison*
5. For buccal samples, change the “Contributor Type” from “Arrestee” to “Convicted” (when applicable)
6. Duplicate Check performed by STaCs once information is saved
  - a) *Submission Match (Duplicate)*
  - b) *Potential Submission Match (Potential Duplicate)*
  - c) *No Match (Original Submission)*
  - d) *BOLO*
7. Submission status is updated appropriately in STaCS

D. Storage of verified cards

## 2.2 Required Readings

CODIS Manual, Texas DPS Crime Laboratory: Sample Collection and Handling, *AFIS Verification* section

## 3 Practice

### 3.1 Safety

None

### 3.2 Standards, Controls, Reagent Preparation

None

### 3.3 Equipment

- STaCS database computer
- DNA Database Cards

### 3.4 Observed Performance

- A. The trainee will perform the following activities with the trainer
  1. Discuss reasons why AFIS would reject a sample
  2. Explain the difference between Redraw/Reject and Problem Kit in STaCS
  3. Discuss the importance of sorting out duplicate samples
  4. Explain the storage protocol for both the accepted and rejected cards
  5. Describe how a duplicate check is activated in STaCS
- B. Trainee will observe trainer verify a stack of 100 DNA Database Cards including:
  1. Navigating through the STaCS software
  2. Creating an AFIS Send file and delivering DNA Database Cards for AFIS verification



3. Selecting samples to move from the AFIS Verification list to AFIS Verification Personal list
4. Making necessary amendments to the print verification information
5. Sorting through acceptable/rejected samples, Problem Kit and Redraw lists and performing the Duplicate Check
6. Storing DNA Database Cards after print verification

### **3.5 Supervised Performance**

Under the supervision of the trainer, trainee will verify a stack of 100 DNA Database Cards (at a minimum), including navigating through the STaCS software in order to perform the tasks in the Observed Performance, as well as storing the DNA Database Cards.

## **4 Assessment**

### **4.1 Competency and Qualifying Examination**

Competency is not required for this module.

### **4.2 Evaluation of Training**

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer and is a prerequisite for remaining DNA technician modules.



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## CO-TM-02-05 SAMPLE PREPARATION AND STORAGE

**Duration** 5 to 10 days

**Purpose** The trainee will learn how to properly prepare samples for processing and archiving.

**Prerequisite** AFIS Verification

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### 1 Objectives

#### 1.1 Theoretical

CODIS samples are typically processed in large groups. Accurate labeling and placement of samples is necessary to preserve sample identity throughout processing.

Following the completion of training, the trainee will be able to:

- A. List the precautionary measures taken when handling biohazardous material
- B. Explain the importance of shaking blood tubes prior to blood spotting

#### 1.2 Practical

Following the completion of training, the trainee will be able to:

- A. Prepare samples for a blood spotting run
- B. Prepare buccal swabs for processing
- C. Utilize STaCS to transfer buccal samples from temporary storage into permanent storage
- D. Properly store archive samples

### 2 Training Outline

#### 2.1 Lesson Plan

- A. Preparing samples for a blood spotting run
  - 1. Setting up blood tubes on Tecan racks
    - a) *Pulling samples from blood tube racks (BTR)*
    - b) *Placing samples in the appropriate order and position on Tecan rack*
  - 2. Preparing Archive cards
    - a) *Labeling stain cards*
    - b) *Labeling envelopes*
  - 3. Blood tube opening
    - a) *Proper shaking techniques*
    - b) *Uncapping blood tubes*
    - c) *Bubble removal*
  - 4. Recapping and blood tube rack (BTR)
    - a) *Recapping tubes*
    - b) *Returning blood tubes to blood storage racks*



5. Archive Storage
  - a) *Stuffing stain cards into labelled envelopes*
  - b) *Sealing envelopes*
  - c) *Storing sealed stain cards in archive boxes*
- B. Preparing buccal samples for processing
  1. Pulling samples from storage
    - a) *Pulling envelopes from storage location*
    - b) *Placing envelopes in appropriate order*
    - c) *Transferring samples from temporary storage to permanent storage in STaCS*
  2. Storing buccal swab envelopes

## 2.2 Required Readings

- A. CODIS Manual, Texas DPS Crime Laboratory.
  1. CODIS Records
  2. Blood Sample Preparation and Storage
  3. Buccal Sample Preparation and Storage.

## 3 Practice

### 3.1 Safety

- A. A lab coat and gloves are required when handling samples. Eye protection and face masks are provided and may be used as well.
- B. Extra precaution should be taken to avoid contact with the aerosol spray of blood droplets when removing blood tube tops.

### 3.2 Standards, Controls, Reagent Preparation

None

### 3.3 Equipment

- Blood tube racks (BTR)
- Tecan racks
- Blood Spotting Sample Selection / LBV sheets
- Biohazard bags
- Cotton squares
- Rubber stopper lids
- Barcoding software / computer
- Archive cards
- Blood / buccal envelopes
- Archive storage boxes
- Envelope Sealer



### **3.4 Observed Performance**

- A. Trainee will discuss with trainer precautionary measures when handling biohazardous material
- B. Trainee will observe the trainer prepare buccal swabs for processing including:
  - 1. Pulling samples from the sample selection sheet
  - 2. Transferring samples from temporary to permanent storage in STaCS
  - 3. Placing buccal swab storage envelopes into archive storage boxes
- C. Trainee will observe a Forensic Scientist perform a blood spotting run.
- D. Trainee will observe the trainer store blood samples after processing.

### **3.5 Supervised Performance**

- A. Under the supervision of the trainer, the trainee will store blood samples after processing, including all of the tasks listed in the Observed Performance.
- B. Under the supervision of the trainer, the trainee will prepare buccal swabs for processing, including all of the tasks listed in the Observed Performance.

## **4 Assessment**

### **4.1 Competency and Qualifying Examination**

Competency is not required for this module.

### **4.2 Evaluation of Training**

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer and is a prerequisite for remaining CODIS technician modules.



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## CO-TM-02-06 BLOOD TUBE DESTRUCTION

**Duration** 3 to 5 days

**Purpose** The trainee will learn the process for blood tube destruction.

**Prerequisite** Sample Preparation and Storage

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### 1 Objectives

#### 1.1 Theoretical

Blood samples should not be destroyed until a suitable profile has been developed. Once a sample has been uploaded to CODIS and has been Administratively Reviewed, the original liquid blood tube will be destroyed in order to make room for incoming samples. Tubes to be destroyed in blood tube racks (BTR) are removed and disposed. Remaining blood tubes are consolidated into racks and are stored. All biohazardous material must be properly disposed.

Following the completion of training, the trainee will:

- A. Recognize which blood samples should be destroyed and which should be kept
- B. Be familiar with proper biohazard handling and disposal procedures

#### 1.2 Practical

Following the completion of training the trainee will be able to:

- A. Pull appropriate Blood Tube Racks (BTR) for destruction
- B. Place appropriate tubes into biohazard bags for destruction
- C. Save and store samples not on disposal list

### 2 Training Outline

#### 2.1 Lesson Plan

- A. Blood Tube Disposal
  - 1. Sample Disposal module in STaCS
    - a) *Locating blood tubes*
    - b) *Scanning blood tubes*
    - c) *Verifying the List Count decreases by the number of tubes scanned*
  - 2. Storage Subsystem module in STaCS
    - a) *View Contents*
      - i. *Long Term Storage location*
      - ii. *Noting Item Count number*
    - b) *Store Items*
      - i. *Scanning Long Term Storage location*
      - ii. *Scanning blood tubes*
    - c) *Item Count Update*
      - i. *View Contents*
      - ii. *Verifying Item Count was updated*



3. Biohazard Disposal
  - a) *Container and bags*
  - b) *Emptying racks of tubes*
  - c) *Securing bag*
  - d) *Moving to appropriate location*
- B. Samples not on Sample Disposal
  1. Error with tubes not ready to be disposed
  2. Clearing Error
  3. Setting aside tube to put in Awaiting Disposal
  4. Awaiting Disposal Racks
    - a) *Storage Subsystem module in STaCS*
    - b) *Scanning Awaiting Disposal Rack barcode*
    - c) *Scanning tubes*
    - d) *Placing tubes in order scanned into rack*
- C. Washing racks
  1. Removing barcodes
  2. Cleaning with bleach solution
  3. Air-drying racks

## 2.2 Required Readings

(CODIS Manual, Texas DPS Crime Laboratory: Sample Destruction, *Scope* and *Blood Tube Disposal* sections)

## 3 Practice

### 3.1 Safety

A lab coat and gloves are required when handling samples. Eye protection and face masks are provided and may be used as well.

### 3.2 Standards, Controls, Reagent Preparation

None

### 3.3 Equipment

- STaCS computer
- Blood Tube Racks (BTR)
- Biohazard bags

### 3.4 Observed Performance

- A. The trainee will perform the following activities with the trainer
  1. Discuss when samples may be destroyed and how to determine which samples are ready to be destroyed
  2. Discuss proper handling and disposal of biohazardous material



- B. Trainee will observe trainer disposing of a rack of blood tubes including:
  - 1. Navigating the appropriate modules in STaCS
  - 2. Properly disposing blood tubes
  - 3. Consolidating and storing remaining blood tubes
  - 4. Washing the emptied racks so they can be reused

### **3.5 Supervised Performance**

Under the supervision of the trainer, trainee will dispose of one rack of blood tubes (at a minimum) including all of the tasks listed in the Observed Performance.

## **4 Assessment**

### **4.1 Competency and Qualifying Examination**

Competency is not required for this module.

### **4.2 Evaluation of Training**

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer.



## 03 CODIS ANALYST UNIT

### CO-TM-03-01 INTRODUCTION TO FORENSIC DNA ANALYSIS

**Duration** 2 to 4 days

**Purpose** Enable a trainee to communicate with appropriate forensic DNA terms. Educate a trainee on the earlier procedures and advances in forensic DNA typing.

**Prerequisite** None

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#### 1 Objectives

##### 1.1 Theoretical

Studying the history of human identification testing will provide the trainee with a foundation of concepts and methodologies used in forensic DNA laboratories. By studying the development of DNA analysis in forensic science, the analyst will understand the methods and theory behind the work being performed in the CODIS laboratory.

##### 1.2 Practical

Following the completion of training the trainee will be able to:

- A. Describe the two main purposes of DNA
- B. List the three components of a nucleotide unit and the four nucleobases
- C. Describe why hybridization is a fundamental property of DNA
- D. Define denaturation and renaturation
- E. Define gene, allele, locus, exon, intron, and polymorphic
- F. Define diploid, haploid, homozygous, heterozygous, and genotype
- G. Describe the two primary forms of measuring DNA variation
- H. Describe the law of segregation and the law independent assortment
- I. Describe the significance of Hardy-Weinberg equilibrium
- J. Describe the RFLP process
- K. List some of the limitations of RFLP
- L. List and describe some of the early PCR-based protocols
- M. Define short tandem repeat (STR) and STR multiplex system
- N. List both advantages and disadvantages for using PCR based methods
- O. Describe the steps involved in processing forensic DNA samples
- P. List and describe some of the early DNA detection methods
- Q. List some methods/guidelines that are followed during sample collection to ensure sample preservation
- R. Describe some of the methods used to collect reference samples
- S. Describe optimal storage conditions for preserving DNA



## 2 Training Outline

### 2.1 Lesson Plan

- A. Basics of DNA Biology and Genetics (Butler)
  - 1. Basic DNA Principles
    - a) *DNA structures and definitions*
    - b) *Base pairing and hybridization*
    - c) *Chromosomes, genes, and DNA markers*
    - d) *Nomenclature for DNA markers*
  - 2. Population Variation
    - a) *Types of DNA polymorphisms*
    - b) *Genetic variability*
    - c) *Recombination*
    - d) *Methods for measuring DNA variation*
  - 3. Introductory Genetic Principles
    - a) *Laws of Mendelian genetics*
    - b) *Hardy-Weinberg equilibrium and linkage equilibrium*
- B. Historical Methods (Butler)
  - 1. A Comparison of DNA Typing Methods
  - 2. The Pre-DNA Years (1900-1985)
    - a) *Blood group testing*
    - b) *Forensic protein profiling*
  - 3. The First Decade of DNA Testing (1985-1995)
  - 4. RFLP-Based DNA Testing
    - a) *Multi-locus VNTR probes*
    - b) *Single-locus VNTR probes*
    - c) *Restriction enzyme differences between laboratories*
    - d) *Speed and sensitivity*
    - e) *Quality concerns and 'the DNA wars'*
  - 5. Early PCR-Based DNA Testing
    - a) *HLA DQ alpha/DQA1*
    - b) *PolyMarker (PM + DQA1)*
    - c) *D1S80: a PCR-amplified VNTR*
    - d) *Short tandem repeats (STRs)*
  - 6. The Second Decade of DNA Testing (1995-2005)
    - a) *Fluorescent detection STR kits*



7. Advantages and Limitations of DNA Typing Methods
- C. Sample Collection, Storage, and Characterization (Butler)
  1. Steps in DNA testing process
  2. Sample Collection
    - a) *DNA sample sources*
    - b) *Biological evidence at crime scenes*
    - c) *Evidence collection and preservation*
    - d) *Collection of reference DNA samples*
  3. Sample Storage and Transport of DNA Evidence
- D. Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories (SWGDM)

## 2.2 Required Readings

- A. National Research Council. *The Evaluation of Forensic DNA Evidence*. National Academy Press. 1996. Chapter 1
- B. Butler, John M. *Fundamentals of Forensic DNA Typing*. Elsevier Academic Press. 2010.
  1. Chapter 2: Basics of DNA Biology and Genetics
  2. Chapter 3: Historical Methods
- C. Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. Chapter 1: Sample Collection, Storage, and Characterization
- D. Cushwa, William T. and J.F. Medrano. "Effects of Blood Storage Time and Temperature on DNA Yield and Quality." *Biotechniques*, **14:204-205**
- E. SWGDAM Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories.

## 3 Practice

### 3.1 Safety

None

### 3.2 Standards, Controls Reagent Preparation

None

### 3.3 Equipment

None

### 3.4 Observed Performance

The trainee will perform the following activities with the trainer

- A. Discuss The Evaluation of Forensic DNA Evidence
- B. Discuss Chapter 2 in *Fundamentals of Forensic DNA Typing* by John M. Butler
- C. Discuss Chapter 3 in *Fundamentals of Forensic DNA Typing* by John M. Butler



- D. Discuss Chapter 1 in *Advanced Topics in Forensic DNA Typing: Methodology* by John M. Butler
- E. Discuss “Effects of Blood Storage Time and Temperature on DNA Yield and Quality” by Cushwa and Medrano

## **4 Assessment**

### **4.1 Competency and Qualifying Examination**

The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee.

### **4.2 Evaluation of Training**

- A. The trainee and trainer will complete the appropriate training checklist
- B. Successful completion of this module is determined by the trainer.



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## CO-TM-03-02 EZ1 ADVANCED XL EXTRACTION

**Duration** 3 to 5 days

**Purpose** Trainee will recover and isolate DNA using the Qiagen EZ1 Advanced XL

**Prerequisite** Introduction to Forensic DNA Analysis

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### 1 Objectives

#### 1.1 Theoretical

Several DNA extraction methods are used in the forensic community. In the past, many of these methods were taxing of both time and labor and some involved the use of hazardous chemicals. With the necessity of high throughput assays, magnetic separation has become an integral tool for forensic laboratories with highly automated and cost effective systems.

Silica-coated magnetic particles are introduced into a lysate resulting in DNA molecules binding to the silica in the presence of chaotropic salts. A magnet is then used to separate the bound molecules from the lysate into a series of washes and finally into an elution solution, yielding a purified DNA extract. The amount and concentration of the yielded product can be influenced by varying the amount of magnetic particles and the volume of elution solution used respectively.

Following the completion of training, the trainee will be able to:

- A. Describe the purpose of DNA extraction.
- B. Describe, compare and contrast various methods of DNA extractions (organic, FTA, solid-phase).
- C. Describe the purpose of a chaotropic salt.
- D. Describe the components of the EZ1 Advanced XL instrument and the reagents used in the EZ1 DNA Investigator Kit.
- E. Explain the various sample types and controls associated with an EZ1 run.
- F. Describe the instrument decontamination process.
- G. Explain measures used to limit contamination.
- H. Describe the Plate Create process and EZ1 Processing modules used in STaCS.

#### 1.2 Practical

Following the completion of training, the trainee will be able to:

- A. Prepare the necessary reagents used in the EZ1 Advanced XL extraction protocol.
- B. Perform the necessary precautions to prevent contamination during the extraction process.
- C. In STaCS, create a plate in the Plate Create module and process it in the EZ1 Processing module.
- D. Isolate DNA from blood archive cards or buccal swabs using the EZ1 Advanced XL extraction protocol.
- E. Perform post-process decontamination and workspace clean up.



## 2 Training Outline

### 2.1 Lesson Plan

- A. Discuss contamination and steps taken to avoid it
  - 1. Maintaining sample integrity
  - 2. Types of contamination
    - a) *Sample-to-sample*
    - b) *Extraneous*
  - 3. Precautions
    - a) *Decontaminate workspace before and after processing*
      - i. *Appropriate cleaning solutions*
      - ii. *Avoid using bleach*
    - b) *Clean cutting and punching tools between samples*
    - c) *Properly seal the samples after use*
    - d) *Appropriate Personal Protective Equipment (PPE)*
      - i. *Room specific lab coats*
      - ii. *Gloves*
      - iii. *Face mask*
- B. Qiagen EZ1 Advanced XL
  - 1. Identifying parts
    - a) *Exterior*
      - i. *Door*
      - ii. *Control panel*
      - iii. *Card slot*
      - iv. *Status LEDs*
    - b) *Interior*
      - i. *Pipettor head*
      - ii. *Cartridge rack*
      - iii. *Tube/tip rack*
      - iv. *Magnets*
- C. Qiagen EZ1 DNA Investigator Kit
  - 1. Components
    - a) *Buffer G2*
      - i. *Water dilution*
    - b) *Proteinase K*
    - c) *Reagent cartridges*



2. Receiving in STaCS (Qiagen Kit Receiving & Qiagen Container Breakdown)
  - a) *Receiving*
  - b) *Container breakdown*
- D. Plate Create module in STaCs
  1. Plate Type
    - a) *EZ1 plate*
  2. Protocol
    - a) *Not Defined*
  3. Sample Nature
    - a) *Leave menu blank*
- E. EZ1 Processing module in STaCS
  1. Available Plates
  2. Consumables
  3. Sets
    - a) *Add Set*
  4. Scanned Sets
- F. EZ1 sample setup and processing with STaCS
  1. Sample types
    - a) *Blood cards*
      - i. *FTA cards*
      - ii. *Non-FTA cards*
    - b) *Buccal swabs*
    - c) *Long term storage (LTS) plates*
  2. Isolate DNA from blood archive cards
  3. Isolate DNA from buccal swab cutting
  4. Isolate DNA from LTS plate (lecture only)

## 2.2 Required Readings

- A. Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. Chapter 2: DNA Extraction Methods.
- B. Tan, Siun Chee and Yiap, Beow Chin. "DNA, RNA, and Protein Extraction: The Past and the Present." *Journal of Biomedicine and Biotechnology*. 2009. 2009:574398.
- C. Montpetit, Shawn A., Fitch, Ian T., O'Donnell, Patrick T.O. "A Simple Automated Instrument for DNA Extraction in Forensic Casework." *Journal of Forensic Sciences*. 2005 50:03.
- D. Berensmeier, Sonja. "Magnetic particles for the separation and purification of nucleic acids." *Applied Microbiology and Biotechnology*. 2006 73:495-504.



- E. CODIS Manual, Texas DPS Crime Laboratory: *EZ1 Advanced XL Extraction* chapter
- F. Qiagen. EZ1 DNA Investigator Handbook.
- G. Qiagen. EZ1 Advanced XL User Manual.
- H. Texas DPS and/or laboratory specific validation study.

### **3 Practice**

#### **3.1 Safety**

- A. Wear lab coat and gloves when working in the laboratory.
- B. Face masks may be used as appropriate.
- C. Avoid using bleach to decontaminate work areas due to the guanidinium compounds present in the EZ1 DNA Investigator Kit.

#### **3.2 Standards, Controls Reagent Preparation**

STaCS modules for tracking and processing records

#### **3.3 Equipment**

- STaCS computer
- Qiagen EZ1 Advanced XL
- Qiagen EZ1 DNA Investigator Kit
- Single hole puncher
- Heat block
- Scissors
- Pipettes, adjustable
- Pipette tips
- Tweezers
- Ethanol
- Kimwipes
- Blank FTA card
- Reagent blanks for each set of extractions

#### **3.4 Observed Performance**

- A. The trainee will perform the following activities with the trainer
  1. Discuss topics dealing with contamination including maintaining sample integrity and methods used to avoid contamination
  2. Discuss the parts and use of the EZ1 Advanced XL robot
  3. Discuss the components and use of the EZ1 DNA Investigator kit including reagent preparation
  4. Discuss and observe kit receiving and container breakdown in STaCS
  5. Discuss and observe the use of Plate Create module in STaCS test server
  6. Discuss and observe the use of EZ1 Processing module in STaCS test server
  7. Discuss and observe the EZ1 Advanced XL extraction process



- B. Trainee will observe trainer extract a set of samples on the EZ1 Advanced XL including:
1. Creating a plate in STaCS
  2. Preparing samples for extraction
    - a) *Reagent preparation (if needed)*
    - b) *Creating sample barcodes*
    - c) *Incubation*
    - d) *Instrument preparation*
      - i. *Cartridges*
      - ii. *Elution tubes*
      - iii. *Tips and tip holders*
      - iv. *Selecting appropriate elution protocol*
  3. Utilizing EZ1 Processing module in STaCS
  4. Post-process decontamination

### **3.5 Supervised Performance**

Under the supervision of the trainer, trainee will extract a set of at least five samples on the EZ1 Advanced XL, including all of the tasks listed in the observational exercises.

- A. These extractions will be stored and used for the amplification exercises in further training.
- B. The exercises will be utilizing the STaCS test server.

## **4 Assessment**

### **4.1 Competency and Qualifying Examination**

- A. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainer.
- B. The trainee will independently extract a minimum of 5 samples on the EZ1 Advanced XL utilizing the STaCS test server. These extractions will be stored and used for the amplification competency set in further training.

### **4.2 Evaluation of Training**

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer.



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## CO-TM-03-03 AMPLIFICATION

**Duration** 1 to 2 weeks

**Purpose** Educate trainee about the polymerase chain reaction and short tandem repeats as well as the manual and automated DNA amplification protocols using validated amplification kits.

**Prerequisite** EZ1 Advanced XL Extraction

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### 1 Objectives

#### 1.1 Theoretical

The discovery of the polymerase chain reaction has paved the way for many other important tools and technologies in the forensic science community. The polymerase chain reaction (PCR) allows for the replication of specific regions of DNA in order to yield millions of copies. Short tandem repeats (STRs) are small, repeatable fragments of DNA that are highly variable among individuals. Using PCR to amplify multiple STR markers simultaneously allows for more rapid analysis and greater discrimination. Coupling PCR and multiplex STR technology with automation has yielded greater accuracy and higher throughput in the CODIS laboratory.

Following the completion of training the trainee will be able to:

- A. Define polymerase chain reaction (PCR) and describe the steps involved
- B. Describe the different components in an amplification mix as well as the purpose of each component
- C. Describe the importance of Taq polymerase
- D. Describe “hot-start” PCR
- E. Define short tandem repeats (STR) and discuss their importance in forensic science
- F. Define multiplex PCR
- G. List and describe the amplification kits currently used in the lab
- H. Describe the significance of using multiple kits in the lab
- I. Define the CODIS core STR loci
- J. Describe the appropriate controls used in the amplification of DNA samples and their purposes
- K. Describe the relationship between the reagent blank and samples
- L. Define PCR template ranges as stated in the CODIS SOP
- M. Describe possible causes of PCR inhibition and how to remove PCR inhibitors
- N. Define the amplification thermal cycling parameters for each kit
- O. Describe the differences between direct amplification and standard amplification



## 1.2 Practical

Following the completion of training the trainee will be able to:

- A. Create an amplification plate in STaCS Master Mix Addition module.
- B. Prepare the amplification mix and perform either the manual or automated robotic amplification set-up of samples.
- C. Complete the STaCS Master Mix Addition and Amplification modules as well as the appropriate documentation for quality control.

## 2 Training Outline

### 2.1 Lesson Plan

- A. Discuss contamination and steps taken to avoid it
  1. Maintaining sample integrity
  2. Types of contamination
    - a) *Sample-to-sample*
    - b) *Extraneous*
  3. Technological advances
    - a) *Direct kits*
    - b) *Increased kit sensitivity*
  4. Precautions
    - a) *Separating pre- and post-PCR sample processing*
    - b) *Appropriate Personal Protective Equipment (PPE)*
      - i. *Room specific lab coats*
      - ii. *Gloves*
      - iii. *Face masks*
    - c) *Decontaminating work surfaces*
      - i. *Bleach*
    - d) *Equipment*
      - i. *Laminar flow hood*
      - ii. *Aerosol-barrier tips*
    - e) *Staff profiles*
    - f) *Contamination log*
    - g) *Limiting consumable and reagent contamination*
      - i. *STaCS tracking*
      - ii. *Reagent aliquots*



- B. Discuss how STaCS workflows lead into amplification
  - 1. Master Mix Addition module
    - a) *EZ1/QIA Symphony*
    - b) *Buccal*
  - 2. Plate Preparation module followed by Punch module
    - a) *Blood*
- C. Current kits used
  - 1. Investigator 24plex GO! kit
    - a) *Components*
    - b) *Volumes used*
      - i. *Buccal*
      - ii. *Blood*
      - iii. *Extract*
    - c) *Thermocycler parameters*
  - 2. GlobalFiler Express
    - a) *Components*
    - b) *Volumes used*
      - i. *Buccal*
      - ii. *Blood*
      - iii. *Extract*
    - c) *Thermocycler parameters*
  - 3. Yfiler Plus
    - a) *Components*
    - b) *Volumes used*
      - i. *Blood*
      - ii. *Extract*
    - c) *Thermocycler parameters*
- D. Amplification plate set-up using STaCS
  - 1. Manual or automated process
    - a) *Buccal plate*
    - b) *Blood plate*
    - c) *EZ1/QIA Symphony plate*
    - d) *Rework plate*
  - 2. Supporting plate documentation
    - a) *PickSample sheet*
    - b) *Plate layout*



3. Master Mix Addition module
  - a) *Create Plate*
    - i. *Plate Layout*
    - ii. *Plate Source*
    - iii. *Amplification Plate Analytical Process*
    - iv. *Plate Create Worklist*
    - v. *Allocate samples to plate*
    - vi. *Proper barcode placement*
  - b) *Get Scenario*
    - i. *Manual*
    - ii. *Tecan EVOware*
  - c) *Process*
  - d) *“Record Activity Completion Result” window*
- E. QA/QC measures
  1. STaCS
    - a) *Controlling reagents*
      - i. *Workflow specific*
      - ii. *Expiration dates*
  2. Quality control runs for amplification kits
  3. Amplification Controls
    - a) *Negative Control*
      - i. *Directly reflecting the most sensitive volume of the sample(s) used*
      - ii. *Rework scenarios*
    - b) *Amplification Blank*
      - i. *Detects contamination in amplification reagents*
      - ii. *Workflow differences*
    - c) *Positive Control*
      - i. *Amplification indicator*
      - ii. *Software check*
- F. Sample preparation for amplification
  1. Bench top decontamination (optional)
  2. Master Mix preparation and aliquotting
    - a) *Manual process*
    - b) *Automated process*



3. Sample addition
    - a) *Manual process*
    - b) *Automated process*
  4. Controls
    - a) *Negative Control*
    - b) *Amplification Blank*
    - c) *Positive Control*
  5. Plate transport preparation
    - a) *Clear seal*
    - b) *Centrifuge*
  6. Bench top decontamination (required)
  7. Replenishing consumables
    - a) *Restocking consumables*
    - b) *STaCS Storage Subsystem*
- G. Amplification module
1. Thermocycler – PCR plate assignment
  2. Thermocycler program for specific kit

## 2.2 Required Readings

- A. Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012.
  1. Chapter 4: PCR Amplification: Capabilities and Cautions.
  2. Chapter 5: Short Tandem Repeat (STR) Loci and Kits.
  3. Chapter 13: Y-Chromosome DNA Testing.
- B. Butler J. "Genetics and genomics of core short tandem repeat loci used in human identity testing." *J For Sci*. 2006. 51:253-265.
- C. Qiagen. Developmental Validation of the Investigator 24plex GO! Kit. June 2015. <https://www.qiagen.com/us/resources/>
- D. 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.
- E. Qiagen. Investigator 24plex GO! Handbook.
- F. CODIS Manual, Texas DPS Crime Laboratory:
  1. Investigator 24plex GO! Amplification
  2. GlobalFiler Express Amplification
- G. 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



- H. 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
- I. Applied Biosystems. GlobalFiler Express PCR Amplification Kit User Guide.
- J. Texas DPS and/or laboratory specific validation studies for approved amplification kits

### 2.3 Suggested Readings

- A. 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.
- B. Applied Biosystems. Yfiler Plus PCR Amplification Kit User Manual.
- C. CODIS Manual, Texas DPS Crime Laboratory: *Yfiler Plus Amplification*

## 3 Practice

### 3.1 Safety

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

### 3.2 Standards, Controls, Reagent Preparation

- A. STaCS modules for tracking and processing records
- B. Amplification controls added to each amplification plate
- C. New amplification kit lots are quality control tested prior to use

### 3.3 Equipment

- STaCS computer
- Thermocycler
- Centrifuge
- Microcentrifuge tubes, 1.5 mL
- Vortex
- 96-well amplification plate
- Amplification cover
- Appropriate plate seal
- Pipettes, adjustable
- Pipette tips
- Investigator 24plex GO! PCR Amplification Kit
- Investigator STR GO! Punch Buffer
- Investigator STR GO! Lysis Buffer
- GlobalFiler Express PCR Amplification Kit
- Prep-n-Go Buffer
- Yfiler Plus PCR Amplification Kit
- TE<sup>-4</sup> buffer
- Tecan Freedom EVO 100 (instrument, computer, and appropriate software)
- TYPE I water



### 3.4 Observed Performance

- A. The trainee will perform the following activities with the trainer
  1. Discuss topics dealing with contamination including maintaining sample integrity and methods to avoid contamination
  2. Discuss how STaCS workflows lead into amplification
  3. Discuss the amplification kits currently used in the laboratory, including components, volumes used, and thermocycler parameters
  4. Discuss and observe the simulation of amplification plates through the STaCS test server including workflow determination, Master Mix Addition (MMA) module, and manual and automated scenarios
  5. Discuss the QA/QC measures taken for amplification
  6. Discuss and observe the amplification set-up for manual amplification with GlobalFiler Express
  7. Discuss and observe the amplification set-up for manual and automated amplification with Investigator 24plex GO!
  8. Discuss and observe post amplification cleanup including decontamination and replenishing consumables
  9. Discuss and observe the Amplification module in STaCS and selecting a thermocycler program
- B. The trainee will observe the trainer set-up and amplify a set of known samples with Investigator 24plex GO! including:
  1. Creating an amplification plate using the Master Mix Addition module in STaCS
  2. Amplification plate set-up
    - a) *Generate PickSample or plate layout sheet (if needed)*
    - b) *Master Mix preparation and aliquoting*
      - i. *Manual scenario*
      - ii. *Automated scenario*
    - c) *Sample addition*
      - i. *Manual scenario*
      - ii. *Automated scenario*
    - d) *Controls*
      - i. *Negative Control*
      - ii. *Amplification Blank*
      - iii. *Positive Control*
    - e) *Plate transport*
  3. Assigning plate to thermocycler using the Amplification module in STaCS
  4. Post-process decontamination



- C. The trainee will observe the trainer set-up and amplify a set of known samples with GlobalFiler Express including:
1. Creating an amplification plate using the Master Mix Addition module in STaCS
  2. Amplification plate set-up
    - a) *Generate PickSample or plate layout sheet (if needed)*
    - b) *Master Mix preparation and aliquotting*
      - i. *Manual scenario*
    - c) *Sample addition*
      - i. *Manual scenario*
    - d) *Controls*
      - i. *Negative Control*
      - ii. *Amplification Blank*
      - iii. *Positive Control*
    - e) *Plate transport*
- D. **Optional:** The trainee will observe trainer set-up and amplify a set of known samples with Yfiler Plus.

### 3.5 Supervised Performance

The exercises will utilize the STaCS test server.

- A. Under supervision, the trainee will manually set-up and amplify a set of at least five known samples from EZ1 extracts with Investigator 24plex GO! using the manual scenario, including all of the applicable tasks listed in the Observed Performance.
- B. Under supervision, the trainee will set-up and amplify a plate of known samples with Investigator 24plex GO! using the automated scenario, including all of the applicable tasks listed in the Observed Performance.
- C. Under supervision, the trainee will manually set-up and amplify a set of at least five known samples from EZ1 extracts with GlobalFiler Express using the manual scenario, including all of the applicable tasks listed in the Observed Performance.
- D. **Optional:** Under supervision, the trainee will manually set-up and amplify a set of at least five known samples with Yfiler Plus using the manual scenario, including all of the applicable tasks listed in the Observed Performance.

## 4 Assessment

### 4.1 Competency and Qualifying Examination

- A. The trainee will independently amplify a set of at least five known samples from an EZ1 extraction with Investigator 24plex GO! using the manual scenario in the STaCS test server. This plate will be stored and used for the capillary electrophoresis competency set in Capillary Electrophoresis (CODIS TM).
- B. The trainee will independently amplify a plate of known samples with Investigator 24plex GO! using the automated scenario in the STaCS test server. This plate will be stored and used for the capillary electrophoresis competency set in Capillary Electrophoresis (CODIS TM).



- C. The trainee will independently amplify a set of at least five known samples from an EZ1 extraction with GlobalFiler Express using the manual scenario in the STaCS test server. This plate will be stored and used for the capillary electrophoresis competency set in Capillary Electrophoresis (CODIS TM).
- D. **Optional:** The trainee will independently amplify a set of at least five known samples from an EZ1 extraction with Yfiler Plus using the manual scenario in the STaCS test server. This plate will be stored and used for the capillary electrophoresis competency set in Capillary Electrophoresis (CODIS TM).
- E. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee.

#### 4.2 Evaluation of Training

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer.



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## CO-TM-03-04 CAPILLARY ELECTROPHORESIS (CE)

**Duration** 2 to 4 weeks

**Purpose** Educate trainee on the proper use of the genetic analyzers, how to troubleshoot problems with the instrument and make recommendations on fixing any encountered problems.

**Prerequisite** Amplification

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### 1 Objectives

#### 1.1 Theoretical

Multiplex short tandem repeat (STR) markers can be amplified using fluorescent dye-labeled primers. The PCR products are then separated by size using capillary electrophoresis (CE). Genetic analyzers gather data by detecting signals from fluorescently labeled DNA fragments after separation.

Multicomponent analysis is the process that separates the different fluorescent dye colors into distinctive spectral components. Although each dye emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra. The precise spectral overlap is measured by analyzing DNA fragments labeled with each of the dyes. These dye-labeled fragments are matrix standards.

Spectral files vary between instruments, virtual filter sets and run conditions on a single instrument. However, the spectral overlap between the dyes is reproducible under constant run conditions.

Following the completion of training the trainee will be able to:

- A. Compare and contrast different DNA separation methods
- B. Define capillary electrophoresis (CE)
- C. Label and describe the components of the various genetic analyzers
- D. Describe how genetic analyzer platforms differ from one another
- E. Describe the maintenance process and frequency for each platform
- F. Describe the steps taken in troubleshooting a problem with the CE
- G. Define multicomponent analysis
- H. Define filter set
- I. Define spectral and spatial calibrations and describe when each is needed
- J. Describe capillary orientation of each of the array types
- K. Describe the sample set-up needed for a CE run
- L. Describe the STaCs workflows that are associated with CE

#### 1.2 Practical

Following the completion of training the trainee will be able to:

- A. Clean and maintain the genetic analyzer
- B. Document the weekly/biweekly maintenance in STaCs



- C. Navigate through the data collection software
- D. Navigate through the Electrophoresis Plate Prep and Post PCR modules in STaCs
- E. Change an array from the various genetic analyzers and complete the appropriate documentation
- F. Run and evaluate a spatial/spectral file and complete the appropriate documentation
- G. Perform manual and automated plate set-up and capillary electrophoresis on previously analyzed samples. Complete the STaCs Electrophoresis Plate Prep and Post PCR modules
- H. Complete the STaCs Electrophoresis Plate Prep and Post PCR modules
- I. Troubleshoot problems with the genetic analyzer
- J. Archive data and maintain the CE computer

## 2 Training Outline

### 2.1 Lesson Plan

The lesson plans will be covered for each appropriate genetic analyzer platform.

- A. Computer and instrument start up
- B. Genetic analyzer
  - 1. Platforms
    - a) *3500xl*
  - 2. Parts of the instrument
    - a) *Laser*
      - i. *Solid state (3500xl)*
    - b) *Charge-coupled device (CCD)*
    - c) *Autosampler*
    - d) *Polymer delivery pump*
    - e) *Oven*
    - f) *Capillary array*
    - g) *Detection cell*
    - h) *Radio frequency identification (RFID) - 3500xl*
- C. Data collection software (3500/3500xl)
  - 1. Common Operations tiles
    - a) *Quick Start Run*
    - b) *Create New Plate*
    - c) *Create Plate from Template*
    - d) *Edit Existing Plate*
    - e) *View Run Results*
    - f) *Wizards*



2. Dashboard Panel Tab
  - a) *Component Gauges*
    - i. *Conditioner/Polymer*
    - ii. *ABC (Anode Buffer Container)*
    - iii. *CBC (Cathode Buffer Container)*
    - iv. *Array*
  - b) *Instrument Status Panel*
    - i. *Oven pre-heat*
  - c) *Consumables Information*
  - d) *Calendar Reminders*
3. Workflow Panel Tab
  - a) *Plate Set-up*
  - b) *Run Instrument*
  - c) *Review Results*
4. Library Panel Tab
  - a) *Manage Plates*
  - b) *Manage Assays*
    - i. *Instrument protocols*
    - ii. *Dye sets*
    - iii. *Size standards*
    - iv. *Size calling protocols*
    - v. *QC protocols*
    - vi. *HID Analysis protocols*
  - c) *Manage File Name Conventions*
  - d) *Manage Results Group*
5. Maintenance Panel Tab
  - a) *Calibrate spatial*
  - b) *Calibrate spectral*
  - c) *Maintenance wizards*
    - i. *Install capillary array*
    - ii. *Fill array with polymer*
    - iii. *Remove bubbles*
    - iv. *Replenish polymer*
    - v. *Wash pump and channels*
    - vi. *Change polymer type*
    - vii. *Shutdown the instrument*
    - viii. *Reactivate the instrument*



- D. Instrument maintenance and cleaning
  - 1. Purpose
  - 2. Annual planned maintenance (PM)
  - 3. Biweekly (3500xl) maintenance
    - a) *Sequencer Configuration module (STaCS)*
    - b) *Water trap flush*
    - c) *Water wash wizard (data collection software)*
    - d) *Water/buffer reservoirs and septa*
    - e) *Array port flush*
    - f) *Instrument Maintenance module (STaCS)*
  - 4. Instrument shutdown
  - 5. Long-term capillary storage
  - 6. Maintenance log
    - a) *Capillary changes*
    - b) *Spatial files*
    - c) *Spectral files*
    - d) *Instrument service*
  - 7. Performance check
- E. Rebooting the instrument
  - 1. Soft reboot - Resetting the instrument only
    - a) *Reset instrument using reset button*
    - b) *Reset instrument using On/Off button*
  - 2. Hard reboot – Computer and instrument reboot
- F. Sample CE set-up (covered for all STaCS scenarios)
  - 1. STaCS automatically assigns all TECAN scripts by scenario
  - 2. Electrophoresis Plate Prep (EPP) module (STaCS)
    - a) *EPP Daughter Plate Creation*
      - i. *Plate layout*
      - ii. *Plate source*
      - iii. *New plate sub-type*
      - iv. *Allocating samples to plate*
    - b) *Scenarios*
      - i. *Manual set-up*
      - ii. *Number of plates for manual set-up*
      - iii. *Automated set-up*
      - iv. *Amplification Kit*





- e) *Start process in STaCS*
  - f) *Evaluate data prior to Post PCR module completion*
    - i. *Evaluate ladders and controls*
    - ii. *Assign samples/controls for reinjection (if needed)*
  - g) *Record Activity Completion Results window*
- G. Data transfer
- 1. Run conditions in data folder
  - 2. Data folder with run conditions into appropriate folder in Macshare
- H. Post run cleanup
- 1. Delete sample sheet form Plate Manager queue
  - 2. Remove plates from autosampler
    - a) *Clean septa*
    - b) *Discard plate*
- I. Troubleshooting
- 1. STaCS error messages
    - a) *Wrong plate subtype*
    - b) *Maintenance is needed on instrument*
    - c) *Instrument is locked with another application*
  - 2. Identifying Problems with the Instrument
    - a) *Status light*
    - b) *Error messages*
    - c) *Bad data*
      - i. *Poor resolution*
      - ii. *Capillary issues*
      - iii. *Excessive pull-up*
      - iv. *Migration problems*
  - 3. Determining Causes of Problems
    - a) *Bubbles*
    - b) *Leaks*
    - c) *Arcing*
    - d) *Dirty detection cell window*
    - e) *Bad reagents*
    - f) *Array*
      - i. *Detection cell orientation*
      - ii. *Silica lining deterioration*



4. Determining Solutions to Problems
  - a) *Water flush*
  - b) *Formamide flush*
  - c) *Wizards*
  - d) *Array replacement*

## 2.2 Required Readings

- A. Butler, John M. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2012. Chapter 6: *Capillary Electrophoresis: Principles and Instrumentation*
- B. CODIS Manual, Texas DPS Crime Laboratory: *Capillary Electrophoresis*
- C. Applied Biosystems. *3500/3500xL Genetic Analyzer User Guide*.
- D. Saferstein, R. *Forensic Science Handbook, Volume. I, 2nd edition*. 2002. pp. 69-110.
- E. Buel, E, et al. "Capillary Electrophoresis STR Analysis: Comparison to Gel-Based Systems." *J For Sci*. 1998. 43:164-1701.
- F. Texas DPS and/or laboratory specific validations

## 3 Practice

### 3.1 Safety

Appropriate personal protective equipment (PPE) must be worn. Formamide is harmful if absorbed through the skin and is considered an irritant. Polymer is considered an irritant.

### 3.2 Standards, Controls, Reagent Preparation

- A. STaCS modules for tracking and processing records
- B. Appropriate amplification controls, in-lane size standard, and allelic ladder with each instrument run

### 3.3 Equipment

- 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 plates and plate septa
- Plate cassette



- Vortex
- Centrifuge
- Microcentrifuge tubes – 1.5 ml
- Pipettes
- Pipette tips

### 3.4 Observed Performance

- A. The trainee will perform the following activities with the trainer
1. Discuss the genetic analyzer platforms and their components
  2. Discuss and navigate through the data collection software for the genetic analyzer platforms
  3. Discuss the instrument maintenance for the genetic analyzer platforms
  4. Discuss the process of changing an array on the genetic analyzer platforms
  5. Discuss the process of completing a spatial and spectral
  6. Discuss and observe the simulation of CE runs through the STaCS test server including the Electrophoresis Plate Prep module, both manual and automated scenarios for all platforms, and the Post PCR module
  7. Discuss sample CE set up including aliquoting formamide/size standard, sample transfer, and addition of allelic ladder using the manual and automated methods
  8. Discuss the evaluation of ladders, controls, troubleshooting, and Post PCR module completion
  9. Discuss the archiving and transfer of data
- B. Trainee will observe the trainer perform maintenance on the 3500xl genetic analyzer platforms including:
1. Water trap flush
  2. Water wash wizard
  3. Buffer dilution if applicable
  4. Water/buffer reservoirs
  5. Array flush
  6. Run spectral/spatial
  7. Instrument shut-down (short-term)
- C. Trainee will observe the trainer set-up and run a set of previously analyzed Investigator 24plex GO! samples on the genetic analyzer including:
1. Electrophoresis Plate Prep module (STaCS)
  2. Preparing the master mix
    - a) *Formamide*
    - b) *Size standard*



3. Setting up the plate with the appropriate scenario
    - a) *Automated*
    - b) *Manual*
  4. Samples/control transfer
  5. Adding the allelic ladder
  6. Centrifuge plate
  7. Post PCR module (STaCS)
    - a) *Instrument/plate assignment*
    - b) *Sample sheet creation and import*
    - c) *Post run examination of ladders and controls, troubleshooting, and re-injections if needed*
    - d) *Module completion*
  8. Data transfer
  9. Post run cleanup
- D. Trainee will observe the trainer set-up and run a set of previously analyzed GlobalFiler Express samples on the genetic analyzer including:
1. Electrophoresis Plate Prep module (STaCS)
  2. Preparing the master mix
    - a) *Formamide*
    - b) *Size standard*
  3. Setting up the plate with the appropriate scenario
    - a) *Automated*
    - b) *Manual*
  4. Samples/control transfer
  5. Adding the allelic ladder
  6. Centrifuge plate
  7. Post PCR module (STaCS)
    - a) *Instrument/plate assignment*
    - b) *Sample sheet creation and import*
    - c) *Post run examination of ladders and controls, troubleshooting, and re-injections if needed*
    - d) *Module completion*
  8. Data transfer
  9. Post run cleanup
- E. **Optional:** The trainee will observe the trainer set up and run a set of previously analyzed Yfiler Plus samples on the 3500xl using the manual scenario.



### 3.5 Supervised Performance

The exercises will be utilizing the STaCS test server.

- A. Under the supervision of the trainer, the trainee will perform maintenance on the 3500xl genetic analyzer platforms.
- B. **Optional:** Under the supervision of the trainer, the trainee will change an array.
- C. Under the supervision of the trainer, the trainee will use the manual scenario to set up and run a set of at least five previously analyzed Investigator 24plex GO! samples on the 3500xl, including all of the applicable tasks listed in the Observed Performance.
- D. Under the supervision of the trainer, the trainee will use the automated scenario to set up and run a plate of previously analyzed Investigator 24plex GO! samples on the 3500xl, including all of the applicable tasks listed in the Observed Performance.
- E. Under the supervision of the trainer, the trainee will use the manual scenario to set up and run a set of at least five previously analyzed GlobalFiler Express samples on the 3500xl, including all of the applicable tasks listed in the Observed Performance.
- F. Under the supervision of the trainer, the trainee will use the automated scenario to set up and run a plate of previously analyzed GlobalFiler Express samples on the 3500xl, including all of the applicable tasks listed in the Observed Performance.
- G. **Optional:** Under the supervision of the trainer, the trainee will use the manual scenario to set up and run a set of at least five previously analyzed Yfiler Plus samples on the 3500xl, including all of the applicable tasks listed in the Observed Performance.

## 4 Assessment

### 4.1 Competency and Qualifying Examination

- A. Using the extracted samples amplified with Investigator 24plex GO! from the previous assessment, the trainee will independently set-up and run the samples on the 3500xl using the manual scenario on the STaCS test server. The data generated will be used for the Data Interpretation competency.
- B. Using the plate of known samples amplified with Investigator 24plex GO! from the previous assessment, the trainee will independently set-up and run the samples on the 3500xl using the automated scenario on the STaCS test server. The data generated will be used for the Data Interpretation competency.
- C. Using the extracted samples amplified with GlobalFiler Express from the previous assessment, the trainee will independently set-up and run the samples on the 3500xl using the manual scenario on the STaCS test server. The data generated will be used for the Data Interpretation competency.
- D. Using a plate of known samples amplified with GlobalFiler Express, the trainee will independently set-up and run the samples on the 3500xl using the automated scenario on the STaCS test server. The data generated will be used for the Data Interpretation competency.
- E. **Optional:** Using the extracted samples amplified with Yfiler Plus from the previous assessment, the trainee will independently set-up and run the samples on the 3500xl using the manual scenario on the STaCS test server. The data generated will be used for the Data Interpretation competency.



- F. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee.

#### **4.2 Evaluation of Training**

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer.



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## CO-TM-03-05 DATA INTERPRETATION

**Duration** 6 to 8 weeks

**Purpose** Educate trainee on the proper use of GeneMapper ID-X, how to analyze and interpret short tandem repeat (STR) data, how to troubleshoot problems with data, and how to review and assign rework points/reasons in the STaCS Analysis module report window.

**Prerequisite** Capillary Electrophoresis

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### 1 Objectives

#### 1.1 Theoretical

Following the processes of PCR amplification and capillary electrophoresis coupled with detection, a data file is generated for each sample and control. These files are now waiting to be analyzed and interpreted by a qualified analyst. When problems arise during the processing of DNA samples, the analysts must be able to systematically reason through the possible causes and solutions utilizing the resources and guidelines covered during their training.

Following the completion of training the trainee will be able to:

- A. Describe what information can be gathered from the Sample View tabs.
- B. Define Process Quality Values (PQVs).
- C. List the different PQV levels of assessment.
- D. List and define the flags associated with the Sample-Level Quality Assessments.
- E. Describe how to customize table settings to display certain PQVs.
- F. Discuss the different analysis settings found in the Analysis Method Editor and describe their function.
- G. Discuss the difference between the Advanced and Classic Peak Detection Algorithm.
- H. Define the thresholds for each genetic analyzer platform with regards to analysis, ladder evaluation and internal size standard evaluation.
- I. Describe the guidelines that have been established for data interpretation.
- J. Develop a systematic approach for data interpretation and troubleshooting.

#### 1.2 Practical

Following the completion of training the trainee will be able to:

- A. Navigate through GeneMapper ID-X.
- B. Use the various editors and managers in the software to customize views and settings.
- C. Use and interpret the appropriate supporting plate documentation.
- D. Navigate through the STaCS Analysis module.
- E. Examine and interpret size standards, allelic ladders, controls, and sample data using GeneMapper ID-X.
- F. Identify problems with data and give the possible causes and recommend solutions to remedy the problem.



- G. Perform the report analysis through STaCS.
- H. Archive data and maintain the analysis computer.

## 2 Training Outline

### 2.1 Lesson Plan

- A. GeneMapper ID-X software
  - 1. GeneMapper ID-X login
  - 2. Project window
    - a) *Navigation pane*
    - b) *Toolbar icons*
      - i. *Add Samples to Project*
      - ii. *Display Plots*
      - iii. *Report Manager*
      - iv. *Label Edit Viewer*
      - v. *Size Match Editor*
      - vi. *Analysis Method Editor*
      - vii. *Panel Manager*
      - viii. *GeneMapper ID-X Manager*
      - ix. *Analyze*
      - x. *Low Quality on Top*
      - xi. *Table Setting*
      - xii. *Table Setting Editor*
    - c) *Sample view*
      - i. *Sample columns view*
      - ii. *Info tab*
      - iii. *Raw Data tab*
      - iv. *EPT (Electrophoresis, Power, and Temperature) Data tab*
    - d) *Sample columns*
      - i. *Sample File*
      - ii. *Sample Type*
      - iii. *Size Standard*
      - iv. *Panel*
      - v. *Analysis Method*
    - e) *Process Quality Values - PQVs*
      - i. *Sizing Quality Assessment*
      - ii. *Allelic Ladder Quality Assessment*





- d) *Plot Settings*
- e) *Size Standards*
- f) *Report Settings*
- 5. Report Manager
  - a) *Report Setting*
    - i. *STaCS*
    - ii. *Export*
    - iii. *Allele call*
- B. Supporting plate documentation
  - 1. Analysis Form (LAB-CO-05)
  - 2. PickSample amp sheets and run sheets (STaCS)
    - a) *DNA template/amplicon volumes*
    - b) *Re-injections if applicable*
  - 3. Plate Detail Sheets (STaCS)
    - a) *Re-injections*
    - b) *Plate set-up information*
- C. STaCS Analysis module
  - 1. Selecting plate from the appropriate queue
    - a) *Analysis*
    - b) *Technical Review*
  - 2. Establishing plate ownership
- D. Preliminary examination of data
  - 1. Analytical threshold
    - a) *Investigator 24plex GO!*
      - i. *100rfu for the 3500xl*
    - b) *GlobalFiler Express*
      - i. *90rfu for the 3500xl*
    - c) *Yfiler Plus*
      - i. *80rfu for the 3500xl*
  - 2. Stochastic Threshold
    - a) *Investigator 24plex GO!*
      - i. *175rfu for the 3500xl*
    - b) *GlobalFiler Express*
      - i. *200rfu for the 3500xl*
    - c) *Yfiler Plus (DYS385 and DYF387S1)*
      - i. *190rfu for the 3500xl*



3. Internal size standard
    - a) *Reviewing the internal size standard for all data*
      - i. *Threshold*
      - ii. *Observe PQVs – SQ*
      - iii. *Raw data*
    - b) *Plot setting*
      - i. *Check LIZ Size Standard*
    - c) *Size match editor*
      - i. *Examine sizing quality*
    - d) *Size standard shift*
      - i. *Observe data points*
  4. Allelic ladder
    - a) *Evaluate the alleles for correct labeling*
      - i. *Allele shift*
      - ii. *Resolution*
    - b) *Examine peak morphology of alleles*
    - c) *Check documentation if ladder(s) are reinjected*
  5. Off-scale data
    - a) *Review PQVs - SOS*
    - b) *Observe Relative Fluorescence Units (RFU)*
      - i. *CE instrument specific*
    - c) *Review raw data*
- E. Interpretation of analyzed data
1. Allele Identification
    - a) *True alleles*
    - b) *Peaks below the analytical threshold*
    - c) *Stochastic effects or allelic dropout*
    - d) *Low signal*
    - e) *Inhibited samples*
    - f) *No signal / null alleles*
    - g) *Poor resolution*
    - h) *Spikes*
  2. Controls: ( ) denotes STaCS nomenclature
    - a) *Positive (STaCS barcode for control)*
    - b) *Amplification negative (Ampblank)*
    - c) *Reagent blank (Negative)*



3. Artifacts
  - a) *Pull-up*
  - b) *Spikes*
  - c) *Stutter*
  - d) *Dye blobs*
  - e) *Minus A*
  - f) *Kit related artifacts*
  - g) *Non-specific amplification*
  - h) *Capillary issues*
4. Contamination
  - a) *True contamination*
  - b) *Chimeras*
5. Off-ladder (OL) alleles
  - a) *Microvariants*
    - i. *Designation*
  - b) *Out of Marker Range (OMR)*
  - c) *Inter Locus Allele (ILA)*
6. Triple alleles
  - a) *Balanced – Type II triple alleles*
  - b) *Imbalanced – Type I triple alleles*
7. Peak imbalance
  - a) *Peak height ratios*
  - b) *Overall sample health*
  - c) *Primer mutations*
8. Stochastic effect and allelic dropout
  - a) *Degraded DNA*
  - b) *Low copy number DNA*
  - c) *Null alleles*
9. Re-injections
  - a) *Validation constraints*
  - b) *Reagent blank (Negative)*
    - i. *Most sensitive injection time*
    - ii. *Changing capillary electrophoresis platform*



- F. Troubleshooting – developing a systematic approach
1. Identifying problems with data
    - a) *Established data interpretation guidelines (CODIS SOP)*
    - b) *GeneMapper ID-X software tools*
      - i. *PQVs*
      - ii. *Raw data*
      - iii. *Sample info*
      - iv. *EPT data*
    - c) *Pattern recognition*
      - i. *Amplification Kits*
      - ii. *Capillary electrophoresis platform*
      - iii. *Reagents*
    - d) *Analyst discretion*
  2. Assigning proper rework points based on workflow level
    - a) *Capillary electrophoresis (Post PCR)*
      - i. *Injection / run-time issues*
    - b) *Capillary electrophoresis (Electrophoresis Plate Prep - EPP)*
      - i. *Injection / run-time issues*
      - ii. *TECAN / manual set-up issues*
    - c) *Amplification (Master Mix Addition - MMA)*
      - i. *TECAN / manual set-up issue*
      - ii. *Template volume*
      - iii. *Peak imbalance confirmation*
    - d) *Pre-amplification (Plate Preparation)*
      - i. *Re-punch*
      - ii. *Water wash*
    - e) *Extraction (Plate Create)*
      - i. *EZ1 / QIAsymphony plate create*
  3. Assigning proper rework reason
    - i. *Utilizing the Analysis Form (LAB-CO-05) legend*
    - ii. *Resolving discrepancies between primary and secondary reviewer*
    - iii. *Technical leader resolution*
- G. Generating and exporting a STaCS report
1. Selecting samples/controls for report
    - a) *Single CODIS number representative*



- b) *Controls*
  - c) *Allelic ladder*
  - 2. Exporting report from Genemapper ID-X
    - a) *Report manager*
      - i. *Report setting – STaCS*
      - ii. *M:\DataAnalysis\Output*
- H. STaCS report analysis
- 1. Analysis module (STaCS)
    - a) *Selecting plate from appropriate queue*
    - b) *Analyze/Review button*
      - i. *Review control profiles*
      - ii. *Process sample profiles*
    - c) *Rework assignments*
      - i. *Proper rework point*
      - ii. *Proper rework reason*
      - iii. *Additional information in “Comments” field*
    - d) *Verify consistency of rework information with Analysis Form (LAB-CO-05)*
    - e) *Verify rework points/reasons of primary reviewer, if applicable*
    - f) *Verify profile consistency with previous runs, if applicable*
    - g) *Review all of the filtered sample screens*
    - h) *Save*
    - i) *Verify information in Data Analysis Outcome Window*
  - 2. Export project from Genemapper ID-X
    - a) *Archive in run folder*
      - i. *M:\DataAnalysis\Working folder for First reads*
      - ii. *M:\DataAnalysis\Archive folder for Second reads*

## 2.2 Required Readings

- A. Butler, John, M. *Advanced Topics in Forensic DNA Typing: Interpretation*. Elsevier Academic Press. 2015.
  - 1. Chapter 1: Data Interpretation Overview.
  - 2. Chapter 2: Data, Models, Thresholds.
  - 3. Chapter 3: STR Alleles and Amplification Artifacts.
  - 4. Chapter 4: STR Genotypes.
  - 5. Chapter 5: STR Profiles.
  - 6. Chapter 8: Troubleshooting Data Collection.



- B. CODIS Manual, Texas DPS Crime Laboratory.
  - 1. Data Interpretation Guidelines
  - 2. Response to Quality Issues
  - 3. Analysis Form (LAB-CO-05)
- C. SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories (excluding mixture interpretation).
- D. Applied Biosystems. GeneMapper ID-X Software Getting Started Guide.
- E. Applied Biosystems. GeneMapper ID Software User Guide.

### 2.3 Suggested Readings

SWGDAM Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Testing Laboratories (excluding mixture interpretation).

## 3 Practice

### 3.1 Safety

None

### 3.2 Standards, Controls, Reagent Preparation

None

### 3.3 Equipment

None

### 3.4 Observed Performance

- A. The trainee will perform the following activities with the trainer
  - 1. Discuss and observe GeneMapper ID-X including using various editors and managers to customize views and settings
  - 2. Discuss and observe the use and completion of the appropriate supporting plate documentation
  - 3. Discuss and observe the use and completion of the Analysis Form (LAB-CO-05)
  - 4. Discuss and observe the report analysis through STaCS
  - 5. Discuss and observe the archiving of data post report analysis
  - 6. Discuss and observe the troubleshooting of problematic data using the appropriate guidelines, software tools, and pattern recognition
- B. The trainee will observe the trainer use Genemapper ID-X as well as STaCS to analyze and interpret data including:
  - 1. Establishing plate ownership in STaCS
  - 2. Internal size standard evaluation
  - 3. Ladder and control evaluation
  - 4. Sample interpretation, troubleshooting, and rework assignment
  - 5. Generating and exporting a report



6. Report analysis in STaCS
7. Data archiving

### 3.5 Supervised Performance

The exercises will be utilizing the STaCS test server.

- A. Under the supervision of the trainer, the trainee will analyze and interpret data from a set of at least five known Investigator 24plex GO! samples run in the previous CE exercise, including all of the tasks included in the Observed Performance.
- B. Under the supervision of the trainer, the trainee will analyze and interpret data from a full plate of known Investigator 24plex GO! samples run in the previous CE exercise, including all of the tasks included in the Observed Performance.
- C. Under the supervision of the trainer, the trainee will analyze and interpret data from a set of at least five known GlobalFiler Express samples run in the previous CE exercise, including all of the tasks included in the Observed Performance.
- D. Under the supervision of the trainer, the trainee will analyze and interpret data from a full plate of known GlobalFiler Express samples run in the previous CE exercise, including all of the tasks included in the Observed Performance.
- E. **Optional:** Under the supervision of the trainer, the trainee will analyze and interpret data from a set of at least five known Yfiler Plus samples run in the previous CE exercise, including all of the tasks included in the Observed Performance.

## 4 Assessment

### 4.1 Competency and Qualifying Examination

- A. Using the data generated from the previous assessment, the trainee will independently analyze the set of Investigator 24plex GO! samples utilizing the STaCS test server.
- B. Using the data generated from the previous assessment, the trainee will independently analyze the full plate of previously analyzed Investigator 24plex GO! samples utilizing the STaCS test server.
- C. Using the data generated from the previous assessment, the trainee will independently analyze the set of GlobalFiler Express samples utilizing the STaCS test server.
- D. Using the data generated from the previous assessment, the trainee will independently analyze the full plate of previously analyzed GlobalFiler Express samples utilizing the STaCS test server.
- E. **Optional:** Using the data generated from the previous assessment, the trainee will independently analyze the set of Yfiler Plus samples utilizing the STaCS test server.
- F. **Optional:** Using the data generated from the previous assessment, the trainee will independently analyze and interpret data from the set of at least five known Yfiler Plus samples utilizing the STaCS test server.
- G. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee.

### 4.2 Evaluation of Training

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer.



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## CO-TM-03-06 TECHNICAL AND ADMINISTRATIVE REVIEW

**Duration** 1 to 2 weeks

**Purpose** Provide trainee with guidelines for technical reviews and administrative reviews for analysis and hit confirmations.

**Prerequisite** Introduction to Forensic DNA Analysis, Data Interpretation

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### 1 Objectives

#### 1.1 Theoretical

The review process ensures that all results and conclusions are supported in such that any supervisor or independent forensic scientist would be able to draw the same conclusions after reviewing.

Upon completion of this training, the trainee will have knowledge of:

- A. How a thorough review of the raw data and supporting documentation, when applicable, is performed to ensure that conclusions are reasonable and within the constraints of scientific knowledge.
- B. The three separate review components to the review process
  1. Technical Review
    - a) *How to conduct an evaluation of raw data to check for consistency, accuracy, and completeness.*
    - b) *How to conduct an independent evaluation of raw data and other supporting documentation to ensure an appropriate and sufficient basis for the scientific conclusions.*
    - c) *The requirements for personnel who perform technical review (must be conducted by a second qualified analyst.)*
  2. Administrative Review of Technical Records
    - a) *How to conduct a review of the supporting documentation to check for consistency with laboratory policies and for editorial correctness.*
    - b) *The requirements for personnel who perform administrative review (must be conducted by someone other than the analyst completing the technical review.)*
  3. Administrative Review for Hit Confirmations
    - a) *How to conduct a review of the supporting documentation to check for consistency with laboratory policies and for editorial correctness.*
    - b) *The requirements for personnel who perform administrative review (must be conducted by someone other than the analyst completing the letter.)*

#### 1.2 Practical

Upon completion of this training, the trainee will be able to:

- A. Understand the purpose of technical and administrative reviews.
- B. Understand the requirements of the technical and administrative reviews.
- C. Perform technical review of raw data.



- D. Perform administrative review of plates for technical records and/or administrative review of hit confirmations.

## **2 Training Outline**

### **2.1 Lesson Plan**

#### **A. Technical Review**

1. All raw data and supporting documentation, when applicable, will be reviewed prior to availability of data for CODIS upload.
2. The results and conclusions must be reasonable and within the constraints of validated scientific knowledge.
3. Refer to the CODIS SOP chapter: Analytical Review and the Crime Laboratory Service Manual chapter: Review of Laboratory Records for the requirements of technical review

#### **B. Administrative Review**

1. The administrative review of technical records will be performed on plates that have been technically reviewed to ensure that the results are logical and accurate.
2. The administrative review of hit confirmations will be performed on the final draft to ensure that the reported results are accurate and there are no spelling or grammatical errors.
3. Refer to the CODIS SOP chapter: Analytical Review/Match Verification and the Crime Laboratory Service Manual chapter: Review of Laboratory Records for the requirements of administrative review.

### **2.2 Required Readings**

#### **A. Crime Laboratory Service Manual. Texas DPS Crime Laboratory.**

1. Document Management and Deviations;
2. Laboratory Records;
3. Laboratory Reports, Letters, and Certificates.

#### **B. CODIS Manual, Texas Department of Public Safety Crime Laboratory (most recent version).**

1. Analytical Controls;
2. Data Interpretation Guidelines;
3. Analytical Review.

#### **C. Federal Bureau of Investigation. Quality Assurance Standards Audit for DNA Databasing Laboratories (most recent version). Standard 12.**

## **3 Practice**

### **3.1 Safety**

None

### **3.2 Standards, Controls, Reagent Preparation**

None



### **3.3 Equipment**

None

### **3.4 Observed Performance**

For technical review, the trainer will demonstrate the technical review of at least one full plate of samples.

### **3.5 Independent Exercises**

- A. If authorization is sought for technical review of autosomal STR data, the trainer will provide a minimum of 5 CODIS analysis plates from the current validated autosomal amplification kit(s) on which the trainee will perform a preliminary technical review, prior to the actual plate review by a qualified analyst. These reviews shall be evaluated by the trainer, and the trainer will discuss any discrepancies found during the actual review with the trainee.
- B. If authorization is sought for technical review of Y-STR data, the trainer will provide a minimum of 1 CODIS analysis plate from the current validated Y-STR amplification kit in which qualification is sought. The trainee will perform a preliminary technical review, prior to the actual plate review by a qualified analyst. The review will be evaluated by the trainer (QA/QC samples may be prepared to satisfy this requirement).
- C. After an evaluation of the trainee's preliminary plate reviews, the trainer and/or technical lead may assign further preliminary plate reviews until several plates have been reviewed by the trainee without significant discrepancies between the preliminary review and the actual plate review.

## **4 Assessment**

### **4.1 Competency and Qualifying Examination**

The independent exercises described above will serve as the competency test in technical review of CODIS analytical data.

### **4.2 Evaluation of Training**

- A. The trainee and trainer will complete the CODIS Analyst Training Checklist.
- B. Successful completion of this module is determined by the trainer and is a prerequisite for independent technical review of CODIS analytical data.



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## 04 ADVANCED ROBOTICS UNIT

### CO-TM-04-01 AUTOMATED BLOOD SPOTTING

**Duration** 1 to 2 weeks

**Purpose** Trainee will become familiar with the proper procedure for spotting whole blood onto blood archive cards using the Tecan Freedom EVO-150.

**Prerequisite** Overview of CODIS Training Program

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#### 1 Objectives

##### 1.1 Theoretical

The Tecan Freedom EVO-150 Robot is the liquid handling robot used to automate the transfer of blood onto FTA archive cards. This automation increases production and efficiency, by increasing processing speed, improving accuracy and precision, and alleviating analyst fatigue.

Following the completion of training, the trainee will:

- A. Be familiar with the operational aspects of using the EVO-150 robotic device
- B. Understand the importance of maintaining proper accountability of the samples as they are handled
- C. Understand the requirements of the quality assurance standards which require the analyst to annotate successful completion of these steps and the proper annotation of any event which will affect the downstream handling or analysis of the samples
- D. Understand the precautionary measures associated with the BSD-600 punching instrument

##### 1.2 Practical

Following the completion of training, the trainee will be able to:

- A. Identify the CODIS system to identify/locate an offender sample
- B. Identify the major components of the Tecan robot
- C. Describe the blood archive card and define its purpose
- D. Describe hazards and precautions associated with handling blood products.
- E. Prepare blood samples for spotting onto archive cards
- F. Prepare Tecan robot for operation
- G. Identify commonly observed difficulties with Tecan robot operation
- H. Load correct software and assay for procedure
- I. Perform spotting of blood onto archive cards without incident
- J. Identify two software "crash" scenarios and responses
- K. Correctly end the assay and shut down the instrument
- L. Properly maintain the robotic device
- M. Demonstrate proper handling of samples and biohazardous materials generated
- N. Properly annotate batch records





## 2 Training Outline

### 2.1 Lesson Plan

- A. Identifying and locating an offender sample
  - 1. Numbering and barcode system used in the laboratory
  - 2. Location of a sample using BTR labels
- B. Major components of the Tecan robot
  - 1. PC computer and EVOware software
  - 2. Tecan robot platform and arm configurations
  - 3. Blood archive card racks
  - 4. Tip disposal container
  - 5. 16-position linear racks and rack positions
- C. Advantages of FTA paper
  - 1. Preservation/protection of DNA
  - 2. Neutralization of blood-borne pathogens
  - 3. Long term storage at room temperature
- D. Preparation of blood samples
  - 1. Print selection list from Blood Spotting STaCS module
  - 2. Safe methods in opening tubes
  - 3. Verify rack position of tubes
  - 4. Maintain vapor hood as necessary
  - 5. Verify blood archive cards labeling

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

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

- E. Blood Spotting with Tecan
  - 1. Properly seat all racks and tips on Tecan deck



2. Turning on the robot and computer
  3. Operate the EVOware software
  4. Double check the proper position of all components on the deck
  5. Choose appropriate script to be used
    - a) *Bed Scan*
    - b) *Archive spotting*
  2. Shut down the computer and turn off the robot
- F. Blood Spotting Manually
1. Low Blood Volume
- G. Commonly observed difficulties with Tecan robot operation
1. Double-tipping and tip stacking
  2. Tips towering in waste station
  3. Bent or clogged tips
  4. Blood clots in tubes
  5. Emergency assay halting procedure
- H. Load correct software and assay for procedure
- I. Spotting of blood onto archive cards without incident
1. Run the assay
  2. Observe barcode number of tubes and archive cards
  3. Replace used tips and re-cap each blood tube
  4. Perform manual spotting, as required
  5. Perform correct procedure for temporarily suspending assay operation
  6. Correctly restart procedure from an interruption
- J. Troubleshooting Software "Crash" Scenarios
1. Blue screen, registry loading error
  2. Program becomes completely unresponsive
- K. Ending the assay and shutting down the instrument
- L. Perform user level maintenance as required according to the Tecan Freedom EVO Operating Manual.
- 2.2 Required Readings**
- A. Smith, LM and Burgoyne L., "Collecting, archiving and processing DNA from wildlife samples using FTA databasing paper." *BioMed Central Ecology*. 2004. 4:4
  - B. Tecan Freedom EVO Operating Manual.
  - C. Tecan EVOware Standard Software.
  - D. CODIS Manual, Texas DPS Crime Laboratory: *Blood Sample Preparation and Storage*



### 3 Practice

#### 3.1 Safety

- A. Wear a lab coat and gloves when working in the laboratory. Safety glasses or goggles may be required during some operations, such as the opening of blood tubes where the blood may aerosolize.
- B. Use extreme caution when operating robots. Do not place arms or head in the path of a moving arm.

#### 3.2 Standards, Controls, Reagent Preparation

None

#### 3.3 Equipment

- Tecan Freedom EVO 150 robot
- Tecan EVOware software / Computer
- TXDPS Blood Archive staincards
- Blood archive card racks
- Electrically conductive (black) 1 mL tips
- Tip disposal container
- 16-position white linear racks and rack positions
- Biohazard bags
- Cotton squares
- Kimwipes
- Rubber stopper lids
- Wood applicators
- 6 gallon Nalgene jug

#### 3.4 Observed Performance

The trainee will perform the following activities with the trainer

- A. Discuss and observe sample preparation, processing, and disposal of waste.
- B. Discuss and observe the Blood Spotting module in STaCS.
- C. Discuss and observe the operation of the Tecan 150 robot.
- D. Observe the trainer transfer blood samples onto archive cards using the Tecan EVO 150 robot. The exercise will utilize the STaCS test server.

#### 3.5 Supervised Performance

*Under the supervision of the trainer, the trainee will transfer blood samples onto archive cards using the Tecan EVO 150 robot. The exercise will be utilizing the StaCS test server.*

### 4 Assessment

#### 4.1 Competency and Qualifying Examination

- A. A group of labeled offender blood samples will be assigned to the trainee. The trainee will independently transfer the blood samples onto archive cards using the Tecan EVO 150 robot utilizing the STaCS test server.
- B. A written exam is not required.



## 4.2 Evaluation of Training

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer.



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## CO-TM-04-02 AUTOMATED BUCCAL LYSIS SET-UP

**Duration** 1 to 2 weeks

**Purpose** Trainee will become familiar with the proper procedure for preparing buccal swabs for direct amplification

**Prerequisite** EZ1 Advanced XL Extraction

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### 1 Objectives

#### 1.1 Theoretical

Direct amplification kits contain enhanced buffers that are more tolerant to PCR inhibitors eliminating the need to extract and purify samples prior to amplification. Samples collected on FTA can immediately proceed to amplification since cells are already lysed. Unlike FTA, Omni Swabs contain untreated filter paper and require a lysis step prior to direct amplification.

Following the completion of training, the trainee will:

- A. Explain the importance of lysing cells prior to direct amplification
- B. Describe the buccal lysis procedure
- C. List and explain the different components of the Multidrop DW plate filler
- D. Describe the proper maintenance of the Multidrop DW plate filler
- E. Describe the proper maintenance of the Tecan 200 robot
- F. List precautionary measures taken to limit contamination

#### 1.2 Practical

Following the completion of training, the trainee will be able to:

- A. Utilize STaCS to identify/locate available buccal plates for processing
- B. Perform quality control measures to prevent contamination
- C. Prepare a lysis plate using the Multidrop DW plate filler
- D. Incubate the lysis plate using the thermomixer/heat block
- E. Create a daughter plate using the Tecan 200 robot
- F. Complete the Lysis and Daughter Plate Create modules in STaCS

### 2 Training Outline

#### 2.1 Lesson Plan

- A. Identify buccal samples/plates for processing
  1. STaCS used in the laboratory
  2. Location of buccal samples/plates ready for processing
  3. Location of storage for processed lysis plates and daughter plates
- B. Buccal plate lysis procedure
  1. Lysis module in STaCS
    - a) *Scan plate*





6. Plate storage
  - C. Quality Control
    1. Reagent blank
- 2.2 Required Readings**
- A. CODIS Manual, Texas DPS Crime Laboratory
    1. Buccal Swab Preparation and Storage
    2. Buccal Lysis Set-up
  - B. Qiagen. Investigator 24plex GO! Handbook.
  - C. Tecan Freedom EVO Operating Manual.
  - D. Thermo Electron Corp. Multidrop DW User's Manual.

**2.3 Suggested Readings**

Applied Biosystems. GlobalFiler Express PCR Amplification Kit User Guide.

**3 Practice**

**3.1 Safety**

- A. Use extreme caution when operating robots. Do not place arms or head in the path of a moving arm.
- B. Wear proper personal protective equipment when working in the laboratory.
- C. Body fluids and lysate may contain infective agents. Use universal precautions when handling.

**3.2 Standards, Controls, Reagent Preparation**

- A. STaCS modules for tracking and processing records
- B. Reagent blanks (Negative) included in each lysis and daughter plate

**3.3 Equipment**

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



### 3.4 Observed Performance

- A. The trainee will observe the trainer prepare a buccal lysis plate using STR GO! Lysis Buffer including:
  - 1. Navigating the Lysis module in STaCS
    - a) *Operating the Multidrop DW plate filler*
    - b) *Operating the thermomixer*
  - 2. Navigating the Daughter Plate Create module
    - a) *Operating the Tecan 200 robot using the EVOware software*
    - b) *Creating a daughter plate*
  - 3. Proper plate storage
- B. The trainee will perform the following activities with the trainer
  - 1. Discuss the Lysis and Daughter Plate Creation modules in STaCS.
  - 2. Discuss the operation of the Multidrop DW plate filler.
  - 3. Discuss the operation of the Tecan 200 robot and EVOware software.
- C. **Optional:** The trainee will observe the trainer prepare a buccal lysis plate using Prep-n-Go Buffer with the Multidrop DW plate filler and the Tecan 200 robot.

### 3.5 Supervised Performance

- A. Under the supervision of the trainer, the trainee will prepare a buccal lysis plate using STR GO! Lysis Buffer with the Multidrop DW plate filler and Tecan 200 robot. The exercise will utilize the STaCS test server.
- B. **Optional:** Under the supervision of the trainer, the trainee will prepare a buccal lysis plate using Prep-n-GO Buffer with the Multidrop DW plate filler and Tecan 200 robot. The exercise will utilize the STaCS test server.

## 4 Assessment

### 4.1 Competency and Qualifying Examination

- A. The trainee will independently prepare a buccal lysis plate using STR GO! Lysis Buffer with the Multidrop DW plate filler and Tecan 200 robot utilizing the STaCS test server.
- B. **Optional:** The trainee will independently prepare a buccal lysis plate using Prep-n-Go Buffer with the Multidrop DW plate filler and Tecan 200 robot utilizing the STaCS test server.
- C. A written exam is not required.

### 4.2 Evaluation of Training

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer.



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## CO-TM-04-03 BSD-600 OPERATION

**Duration** 1 to 2 weeks

**Purpose** The trainee will become familiar with the proper procedure for punching substrates into a 96-well amplification plate using the BSD-600 instrument

**Prerequisite** EZ1 Advanced XL Extraction

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### 1 Objectives

#### 1.1 Theoretical

The BSD-600 applies semi-automation to punch substrates into a 96-well amplification plate. Coupled with the use of a barcode reader and STaCS, the BSD-600 helps streamline the direct amplification workflow.

Following the completion of training, the trainee will:

- A. Describe how the BSD-600 operates
- B. List the advantages of using FTA paper for archiving samples
- C. List precautions taken when handling biological samples
- D. Identify and explain the components of the BSD-600 robot
- E. Identify and explain the components of the BSD-600 Duet software
- F. List common BSD-600 errors and how to fix or navigate through the errors
- G. Describe the general maintenance procedures for the BSD-600

#### 1.2 Practical

Following the completion of training the trainee will be able to:

- A. Utilize STaCS to identify/locate available samples for processing
- B. Perform general maintenance on the BSD-600 instrument
- C. Perform quality control measures to prevent contamination
- D. Allocate samples to a plate using the Plate Create module in STaCS
- E. Prepare an amplification plate using the Plate Preparation module in STaCS
- F. Punch a plate of samples with the BSD-600 instrument using the Punch module in STaCS
- G. Complete the Amplification module in STaCS

### 2 Training Outline

#### 2.1 Lesson Plan

- A. Identify/locate available samples for processing
  1. STaCS used in the laboratory
  2. Location of samples ready for processing
  3. Long term storage of processed samples



- B. Blood FTA Archive Cards
  - 1. Advantages of FTA paper
    - a) *Preservation/protection of DNA*
    - b) *Neutralization of blood-borne pathogens*
    - c) *Long term storage at room temperature*
  - 2. Optimal punching locations
- C. Components of the BSD-600 robot
  - 1. PC computer
  - 2. BSD-600 Robotic Platform
  - 3. Air System
    - a) *Air pressure pump*
    - b) *Anti-static humidifier system*
    - c) *Dust extraction system*
  - 4. Light Targeting System
  - 5. Disk Detector System
  - 6. Barcode Reader
  - 7. Automatic punch switch and foot switch
- D. General BSD-600 maintenance
  - 1. Clean chute
  - 2. Clean instrument surface and work area
  - 3. Maintain appropriate amount of water in humidifier bottles
- E. Common BSD-600 errors
  - 1. Static affecting punches
  - 2. Barcode undetected by barcode reader
  - 3. Chute misalignment
  - 4. Punching errors
- F. Direct amplification protocol using the BSD-600 instrument
  - 1. Plate Create module in STaCS
    - a) *Selecting Plate Type, Protocol, and Sample Nature*
    - b) *Create Plate*
    - c) *Scan envelopes to allocate samples to plate*
    - d) *Move controls to next available well*
    - e) *Proper barcode placement*
  - 2. Plate Preparation module in StaCS
    - a) *Scan amplification plate*







### **3 Practice**

#### **3.1 Safety**

- A. Wear proper personal protective equipment when working with bloodborne pathogens in the laboratory.
- B. FTA fibers may become airborne during punching. Wear appropriate face masks.
- C. Use extreme caution when operating robots. Do not place hands or head in the path of moving parts.

#### **3.2 Standards, Controls, Prepared Reagents**

- A. Combined reagent blank and amplification negative for each set of punches
- B. STaCS modules for tracking and processing records

#### **3.3 Equipment**

- STaCS computer
- Centrifuge
- Microcentrifuge tubes
- 96-well amplification plate
- Pipette tips
- Pipettes, adjustable
- Vortex
- Thermal cycler
- Appropriate plate seal
- Amplification cover
- GlobalFiler Express PCR Amplification Kit
- Investigator 24plex GO! kit
- Investigator STR GO! Punch Buffer
- Yfiler Plus Amplification Kit
- TE buffer
- Tecan EVO 100 (instrument, computer, and appropriate software)
- BSD-600 (instrument, computer, and appropriate software)
- DI water

#### **3.4 Observed Performance**

- A. The trainee will perform the following activities with the trainer
  1. Discuss and observe the Plate Create, Plate Preparation, and Punch modules in STaCS.
  2. Discuss the operation of the BSD 600.
- B. The trainee will observe the trainer amplify a plate of known samples with the GlobalFiler Express kit using the Tecan 100 and the BSD-600 including:
  1. Creating and allocating samples to a plate in STaCS



2. Setting up the amplification PCR reaction mix utilizing the Plate Preparation module
  3. Operating the BSD-600 instrument utilizing the Punch module
  4. Adding the appropriate controls to the punch plate
  5. Navigating the Amplification module and selecting the appropriate thermal cycling program
  6. Proper plate storage
- C. **Optional:** The trainee will observe the trainer amplify a plate of known samples with the Investigator 24plex GO! kit using the Tecan EVO 100 and the BSD-600. The exercise will utilize the STaCS test server.
- D. **Optional:** Observe the trainer amplify at least five known samples with the Yfiler Plus kit using the BSD-600. The exercise will utilize the STaCS test server.

### 3.5 Supervised Performance

- A. Under the supervision of the trainer, the trainee will amplify a plate of known samples with the GlobalFiler Express kit using the Tecan EVO 100 and the BSD-600. The exercise will utilize the STaCS test server.
- B. **Optional:** Under the supervision of the trainer, the trainee will amplify a plate of known samples with the Investigator 24plex GO! kit using the Tecan EVO 100 and the BSD-600. The exercise will utilize the STaCS test server.
- C. **Optional:** Under the supervision of the trainer, amplify at least five known samples with the Yfiler Plus kit using the BSD-600. The exercise will utilize the STaCS test server.

## 4 Assessment

### 4.1 Competency and Qualifying Examination

- A. The trainee will independently amplify a plate of known samples with the GlobalFiler Express kit using the Tecan EVO 100 and the BSD-600 utilizing the STaCS test server.
- B. **Optional:** The trainee will independently amplify a plate of known samples with the Investigator 24plex GO! kit using the Tecan EVO 100 and the BSD-600 utilizing the STaCS test server.
- C. **Optional:** Independently amplify at least five known samples with the Yfiler Plus kit using the BSD-600 utilizing the STaCS test server.
- D. A written exam is not required.

### 4.2 Evaluation of Training

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer.



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## CO-TM-04-04 AUTOMATED DNA EXTRACTION USING THE QIASYMPHONY SP

**Duration** 3 to 5 days

**Purpose** The trainee will recover and isolate DNA using the QIASymphony.

**Prerequisite** Introduction of Forensic DNA Analysis

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### 1 Objectives

#### 1.1 Theoretical

Successful PCR amplification relies upon the isolation and purification of genomic DNA from forensic samples. Various robotic platforms are used in a high throughput laboratory setting to isolate and purify a large number of samples. Stringent precautionary and quality control measures are practiced during the extraction procedure.

Following the completion of training, the trainee will:

- A. Describe and become familiar with the protocol for extracting DNA from blood archive using the QIASymphony.
- B. Describe and become familiar with the protocol for extracting DNA from buccal swabs using the QIASymphony.
- C. Understand the quality control and precautionary measures associated with this extraction method.

#### 1.2 Practical

Following the completion of training, the trainee will be able to:

- A. Prepare the necessary reagents used in the QIASymphony extraction protocol, if necessary.
- B. Perform the necessary precautions to prevent contamination during the QIASymphony extraction process.
- C. Isolate DNA from blood archive cards or buccal swabs using the QIASymphony.
- D. Perform and document the necessary quality control measures taken during the QIASymphony extraction process.

### 2 Training Outline

#### 2.1 Lesson Plan

- A. Isolate DNA from blood archive
- B. Isolate DNA from buccal swab cutting
- C. Quality Control
  1. Worksheet documentation, including lot records
  2. Reagent blanks

#### 2.2 Required Readings

- A. Butler JM. *Advanced Topics in Forensic DNA Typing: Methodology*. Elsevier Academic Press. 2011. Chapter 2: DNA Extraction.



- B. CODIS Manual, Texas DPS Crime Laboratory: *QIA Symphony SP Extraction*
- C. Data Interpretation Guidelines. CODIS SOP. Texas DPS Crime Laboratory.
- D. Qiagen. QIA Symphony SP User Manual. October 2010.
- E. Qiagen. QIA Symphony DNA Investigator Handbook. Current Edition.

### **3 Practice**

#### **3.1 Safety**

- A. Appropriate personal protective equipment must be worn during reagent 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 properly.

#### **3.2 Standards, Controls, Reagent Preparation**

##### **A. Reagent Preparation**

Trainee will prepare any kit materials as needed.

##### **B. Quality Control**

1. Documentation and use of required worksheets.
2. Reagent blanks for each set of extractions as noted in the CODIS SOP.

#### **3.3 Equipment**

- QIA Symphony SP
- QIA Symphony DNA Investigator Kit
- Reagent cartridge
- Reagent cartridge holder
- Enzyme rack
- Sample prep cartridges
- Piercing lid
- 8-rod covers
- Disposable filter tips
- Spin baskets
- 1.5µL microcentrifuge tubes
- S-block (or other appropriate elution plate)
- Buffer ATL
- Proteinase K
- Biohazard bag
- Heat block
- Pipettors, adjustable
- Pipette tips
- Vortex
- Tape Pads
- Scissors
- Hole Punch
- Tweezers



### **3.4 Observed Performance**

- A. Trainer will give instructions and/or demonstrate the use of the QIASymphony with the STaCS test server.
- B. Trainer will explain the components in the QIASymphony DNA Investigator Kit.
- C. Trainer will discuss correct documentation and QC of samples

## **4 Assessment**

### **4.1 Competency and Qualifying Examination**

- A. Trainee will extract at least two blood and two buccal samples using the QIASymphony and the QIASymphony DNA Investigator Kit. The STaCS test server will be utilized.
- B. A written exam is not required.

### **4.2 Evaluation of Training**

- A. The trainee and trainer will complete the appropriate training checklist.
- B. Successful completion of this module is determined by the trainer.



## 05 FORMS

### TRAINING FORMS

	Document Name	FRN
1	CODIS Technician Training Checklist	<a href="#">LAB-CO-TM-01</a>
2	CODIS Technician Equipment Maintenance Training Checklist	<a href="#">LAB-CO-TM-02</a>
3	CODIS Analyst Training Checklist	<a href="#">LAB-CO-TM-03</a>
4	CODIS Advanced Robotics Training Checklist	<a href="#">LAB-CO-TM-04</a>