

Department of Forensic Science

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**FORENSIC BIOLOGY SECTION
TRAINING MANUAL
OF
DNA DATA BANK SAMPLE
ACCESSIONING,
POWERPLEX[®] FUSION ANALYSIS
AND Y-STR ANALYSIS**

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1 OVERVIEW OF TRAINING PROGRAM

1.1 Purpose

To provide a uniform training program for the analysis of DNA Data Bank samples using PCR-based STR fluorescence analysis, and in so doing, to adhere to current version of the “FBI Quality Assurance Standards for DNA Data basing Laboratories”

1.2 Scope

This training program applies to DNA Data Bank analysts in the Forensic Biology Section at the Virginia Department of Forensic Science (VADFS).

1.2.1 Technical support staff in the DNA Data Bank must complete only the training specified in Sections 2 and 3 of this document.

1.3 Goals

1.3.1 To develop theoretical knowledge of the principles of PCR-based technology.

1.3.2 To develop a thorough understanding of the principles and practices of STR technology as they relate to the forensic analysis of DNA Data Bank samples.

1.3.3 To develop a thorough understanding of the theory and application of instrumentation and specialized techniques used to analyze DNA Data Bank samples.

1.3.4 To develop the ability to perform independent, accurate analysis of database samples and to accurately document the results of all analyses in accordance with Department and Section policies and procedures.

1.4 Coordination of the Program

1.4.1 Responsibilities of DNA Data Bank Analyst Trainee

1.4.1.1 The trainee will keep a notebook containing all work completed. The notebook will include copies of all original electropherogram data, landscape printout(s), plate files, comments on problems that arose and how the problems were corrected, etc. A copy of the training coordinator’s monthly report will also be included in the notebook.

1.4.1.2 To provide for exposure to the pertinent literature available in the field, required readings will be assigned by the Training Coordinator. A list of readings associated with the training (with documented approval by the Biology Program Manager) is to be included in the trainee’s notebook.

1.4.1.3 The trainee will assist with DNA Data Bank sample analysis throughout the training period, only under the direct supervision of a qualified DNA Data Bank analyst.

1.4.1.4 Prior to beginning independent analysis, DNA Data Bank analyst trainees will satisfactorily complete both an oral and practical competency test.

1.4.2 Responsibilities of Training Coordinator

1.4.2.1 The training coordinator will be a DNA Data Bank analyst experienced in the use of DNA PCR-based STR analysis. The coordinator may delegate certain duties and blocks of instruction to other qualified analysts, but will be responsible for the overall training.

- 1.4.2.2 The training coordinator will instruct and monitor the trainee, assign required readings (e.g., scientific literature and technical manuals), and ensure the trainee has an understanding of required concepts and procedures.
 - 1.4.2.2.1 The list of required readings will be approved by the Biology Program Manager for each trainee.
- 1.4.2.3 The training coordinator will verify through the course of training that the trainee understands and follows Department and Section policies and protocols. This will be done through frequent communication and monitoring, as well as a monthly check of the trainee's notebook.
- 1.4.2.4 The training coordinator will provide all samples to the trainee for analysis. Once the sample set is complete, the training coordinator will verify the results and document the completion of each required task for each aspect of training.
- 1.4.2.5 The training coordinator shall closely monitor the trainee's progress and evaluate the trainee's performance during the course of the program. The training coordinator must submit monthly written evaluations of the trainee's progress to the Biology Program Manager in accordance with policy set forth in the Quality Manual, Section 19 Personnel and Training. An exemplar of the monthly report format is provided in the Quality Manual appendix. The monthly training report is due within five business days of the beginning of the month.
- 1.4.2.6 The training coordinator must maintain continual, open communication with the DNA Data Bank Supervisor and/or Section Supervisor regarding the trainee's progress.

1.4.3 Training Period

It is estimated that this training program can be completed in four to six months, which includes successful completion of the final oral and practical competency examinations. Some individuals may require more or less time than others to complete the training, depending on prior experience and education. The training program may be altered with the documented approval of the Biology Program Manager.

1.5 Analyst Qualification

- 1.5.1 The sequence in which the tasks are presented in the outline should not necessarily be considered as a mandatory order of instruction. Oral and practical examinations may be staged periodically.
- 1.5.2 The final qualification of a trainee as a Data Bank analyst will be based upon the following:
 - 1.5.2.1 Successful completion of the required training samples.
 - 1.5.2.2 Satisfactory completion of an oral competency exam to include technical questions pertaining to all aspects of examination of data bank samples.
 - 1.5.2.3 Satisfactory completion of a practical exam which will consist of blood and/or buccal samples.

1.6 Guidelines for Final Oral Competency Examination

- 1.6.1 A formal oral examination of the trainee will be conducted by the DNA Data Bank Supervisor, Biology Program Manager, and the Technical Services Director or designee to ascertain the technical knowledge of the individual. This will be limited to two (2) hours. Questions will be confined to technical aspects of the training as well as accreditation questions. The performance of the trainee will be used to ascertain whether the goals of the training program have been achieved.

- 1.6.2 The outcome of the oral examination evaluation will be:
 - 1.6.2.1 Satisfactory
 - 1.6.2.2 Not satisfactory
- 1.6.3 Satisfactory performance on both the practical and the oral examination must be achieved before the individual is qualified to independently perform the duties of a DNA Data Bank analyst.
- 1.6.4 When the trainee has satisfactorily completed all of the requirements of the program, a letter will be issued by the Biology Program Manager to the Technical Services Director stating that the person is qualified to perform the duties of a DNA Data Bank analyst. If the trainee cannot meet the criteria expected of him/her during the period allowed for training, appropriate steps will be taken.

1.7 References

- 1.7.1 Quality Manual
- 1.7.2 Safety Manual
- 1.7.3 DNA Data Bank Operating Policies and Procedures Manual
- 1.7.4 Forensic Biology Section Data Bank Procedures Manual Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® Fusion System
- 1.7.5 Forensic Biology Section Qiagen BioRobot® Universal System Procedures Manual
- 1.7.6 Forensic Biology Section Data Bank Procedures Manual Fluorescent Detection PCR-Based STR DNA Protocol: AmpFℓSTR® Yfiler™ System
- 1.7.7 CODIS Operating Policies and Procedures Manual
- 1.7.8 Forensic Biology Section Procedures Manual Section VI Quality Assurance Program DNA Typing of Biological Materials

2 SAFETY**2.1 Hazards**

Each individual working in the DNA Data Bank must be acutely aware of the potential hazards inherent in his/her work. Refer to the Safety Manual for descriptions of potential hazards in the laboratory.

2.2 Safety Procedures

Reference: Safety Manual. All trainees are required to read, understand and follow all policies and procedures set forth in the Safety Manual.

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3 SAMPLE ACCESSIONING AND HIT VERIFICATION

3.1 Goals

- 3.1.1 To understand the workflow of the sample accessioning and processing areas.
- 3.1.2 To understand Department and Section policies regarding contamination control.
- 3.1.3 To understand the need for confidentiality and security of genetic profiles/personal identifying data and the relevant Code of Virginia sections governing the release of DNA Data Bank information.
- 3.1.4 To understand and correctly perform the procedures described in the DNA Data Bank Operating and Procedures Manual including accessioning, handling, processing and retention of DNA samples.
- 3.1.5 To understand all laws in the Code of Virginia (COV) governing the DNA Data Bank.
- 3.1.6 To understand hit verification requests and respond with the appropriate information.
- 3.1.7 To understand the processing of the weekly clearance report for Arrestee samples.

3.2 Tasks

- 3.2.1 Read, understand and follow DNA Data Bank Operating and Procedures Manual.
- 3.2.2 Receive an overview/tour of sample accessioning and processing workflow.
- 3.2.3 To articulate, to the satisfaction of the DNA Data Bank Supervisor, VADFS policy and relevant COV sections governing the release of genetic/personal identifying data.
- 3.2.4 Retrieve and accession arrestee samples. The trainee must use proper contamination control techniques and personal protective equipment. The trainee must correctly identify and rectify any issues associated with the samples.
- 3.2.5 Retrieve and accession convicted offender samples. The trainee must use proper contamination control techniques and personal protective equipment. The trainee must correctly identify and rectify any issues associated with the samples.
- 3.2.6 Enter personal identifying data into VADFS DNAweb database.
- 3.2.7 Gain access to and use multiple databases/agencies to research samples with information issues or to verify lawful inclusion of the sample in the DNA Data Bank. Such databases include, but are not limited to, Virginia Criminal Information Network (VCIN), courts website, Local Inmate Data System (LIDS) database, Department of Juvenile Justice (DJJ) records, and/or Department of Corrections (DOC) records.
- 3.2.8 Read, understand and follow all laws in the COV governing the DNA Data Bank.
- 3.2.9 Process hit verification requests by collecting and distributing the correct information to DNA Data Bank analysts/CODIS administrator and Casework examiner staff.
- 3.2.10 Understand the laws governing the weekly clearance report for Arrestee samples and process the report.

3.3 Training Evaluation (Knowledge and Skills)

The trainee should perform DNA accessioning, problem sample follow-up, and hit verification requests on a sufficient variety and number of samples to develop and exhibit an unquestionably sound technique. This will be

monitored by administrative review of the work product and continual observation by the DNA Data Bank Supervisor and/or other qualified DNA Data Bank staff member.

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4 DNA ISOLATION

4.1 Goals

- 4.1.1 To develop a basic understanding of the theory and procedure for DNA isolation from blood and buccal samples using the QIAGEN® BioRobot Universal System.
- 4.1.2 To develop a basic understanding of the theory and procedure of DNA isolation from blood and buccal samples using the Manual QIAamp® extraction procedure.
- 4.1.3 To become acquainted with the operation of the semi-automated BSD puncher instrument.
- 4.1.4 To become acquainted with the sensitivity of the isolation procedure.
- 4.1.5 To become familiar with the limitations of the isolation procedure.
- 4.1.6 To become familiar with the use of controls incorporated at this stage of the procedure.
- 4.1.7 To become familiar with the function of each reagent used for DNA isolation.
- 4.1.8 To become familiar with the operation of the QIAGEN® BioRobot Universal System.
- 4.1.9 To become familiar with proper documentation for DNA isolation.

4.2 Tasks

- 4.2.1 Prepare reagents necessary for DNA isolation.
- 4.2.2 Perform DNA isolation on a minimum of 10 dried blood stains using the manual QIAamp® extraction procedure. These 10 samples will be divided into two sets of 5 samples. Complete the entire DNA analysis process for these 10 samples before proceeding to the next set of samples.
- 4.2.3 Perform DNA isolation on a minimum of 20 buccal samples using the manual QIAamp® extraction procedure. These 20 samples will be divided into two sets of 10 samples. Complete the entire DNA analysis process for these 20 samples before proceeding to the next set of samples.
- 4.2.4 Under the direct supervision of a qualified Data Bank Analyst, the trainee will set up and operate the BSD semi-automated puncher for 5 plates of buccal samples (approx 90 samples per plate). These plates may be punched for either QIAGEN extraction or direct amplification.
- 4.2.5 Under the direct supervision of a qualified Data Bank Analyst, the trainee will set up and operate the QIAGEN® BioRobot Universal System for 2 runs (approx 90 samples per plate). Each set will be carried through the entire extraction process before proceeding to the next set of samples. Necessary reagents to perform the extraction will be prepared by the trainee, if applicable.
- 4.2.6 Perform DNA isolation on a minimum of 80 buccal samples provided by the training coordinator using the QIAGEN® BioRobot™ Universal System. These samples will be divided into two sets of 40 samples. Each set will be carried through the entire DNA analysis process before processing the next set of samples.
- 4.2.7 Read applicable literature and technical manuals.

4.3 Training Evaluation

- 4.3.1 Knowledge
 - 4.3.1.1 Review of notes and worksheets in training notebook by training coordinator.

4.3.1.2 Oral and practical examinations.

4.3.2 Skills

The trainee should perform DNA isolation on a sufficient variety and number of samples to develop and exhibit an unquestionably sound technique for successfully isolating DNA using the QIAGEN® BioRobot Universal System. This will be monitored by review of the documentation in the training notes and continual observation by the training coordinator and/or other qualified analysts.

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5 AMPLIFICATION

5.1 Goals

- 5.1.1 To develop an understanding and working knowledge of the amplification process and the purpose of each reagent involved.
- 5.1.2 To become familiar with problems associated with amplification.
- 5.1.3 To understand the importance of an amplification environment that produces no contamination.
- 5.1.4 To understand the importance of quality controls associated with the amplification process.

5.2 Tasks

- 5.2.1 Work in an environment free of contamination and follow proper procedure to prevent contamination.
- 5.2.2 Program a thermal cycler
- 5.2.3 Perform the quality control test on a thermal cycler.
- 5.2.4 Perform amplification using the PowerPlex[®] Fusion System.
 - 5.2.4.1 Amplification using extracted DNA
All QIAGEN (manual or robotic) extracted training samples
 - 5.2.4.2 Direct amplification
Perform direct amplification on a minimum of 80 buccal samples provided by the training coordinator using the PowerPlex[®] Fusion System. These samples will be divided into two sets of 40 samples. Each set will be carried through the entire DNA analysis process before processing the next set of samples.
- 5.2.5 Read applicable literature and technical manuals.

5.3 Training Evaluation

- 5.3.1 Knowledge
 - 5.3.1.1 Review of notes and worksheets in training notebook by training coordinator.
 - 5.3.1.2 Oral and practical examinations.
- 5.3.2 Skills

The trainee should demonstrate an unquestionably sound technique for DNA amplification by consistently achieving uncontaminated results on the electropherograms. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

6 CAPILLARY ELECTROPHORESIS - POWERPLEX® FUSION**6.1 Goals**

- 6.1.1 To become familiar with the theories of electrophoresis as they apply to capillary electrophoresis (CE) used in STR analysis.
- 6.1.2 To learn the parameters used for CE of the PowerPlex® Fusion System.
- 6.1.3 To develop an understanding and working knowledge of the use of the CE instrument, including the limitations and proper documentation.
- 6.1.4 To become familiar with the controls associated with CE.
- 6.1.5 To become familiar with the quality control tasks done to ensure the CE instrument stays in proper working order.

6.2 Tasks

- 6.2.1 Prepare the instrument with necessary reagents to perform CE of the DNA samples.
- 6.2.2 Run the PowerPlex® Fusion System samples on the CE instrument.
- 6.2.3 Under the direct supervision of a qualified Data Bank Analyst, the trainee will perform the monthly QC tasks on the CE instrument.
- 6.2.4 Under the direct supervision of a qualified Data Bank Analyst, the trainee will perform a capillary array change, spectral and spatial on the CE instrument.
- 6.2.5 Read applicable literature and technical manuals.

6.3 Training Evaluation

- 6.3.1 Knowledge
 - 6.3.1.1 Review of notes and worksheets in training notebook by training coordinator.
 - 6.3.1.2 Oral and practical examinations.
- 6.3.2 Skills

The trainee should demonstrate an unquestionably sound technique for running consistently interpretable PowerPlex® Fusion electropherogram profiles on the CE instrument using proper documentation. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

**7 CAPILLARY ELECTROPHORESIS DATA ANALYSIS AND INTERPRETATION
– POWERPLEX® FUSION**

7.1 Goals

- 7.1.1 To develop a working knowledge of the software used for the analysis of the CE data.
- 7.1.2 To become familiar with the visual interpretation of the electropherograms.
- 7.1.3 To become familiar with the base pair size range of the different PowerPlex® Fusion System loci.
- 7.1.4 To understand the use of controls and the internal lane standard.
- 7.1.5 To understand the problems that may be encountered with regard to interpretation.

7.2 Tasks

- 7.2.1 Analyze the CE data using the GeneMapper™ IDx software.
- 7.2.2 Interpret all results successfully and properly document the results.
- 7.2.3 Read applicable literature and technical manuals.

7.3 Training Evaluation

- 7.3.1 Knowledge
 - 7.3.1.1 Review of notes, copies of all electropherograms (including the landscape printout) in training notebook by training coordinator.
 - 7.3.1.2 Oral and practical examinations.

7.3.2 Skills

The trainee should demonstrate a thorough understanding of all aspects of CE data interpretation by accurately interpreting PowerPlex® Fusion results on all training samples and properly recording results. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

8 COMBINED DNA INDEX SYSTEM (CODIS)

8.1 Goals

Become familiar with the Combined DNA Index System applications/policies and procedures, and documentation required.

8.2 Tasks

8.2.1 Read and understand the CODIS Operating Policies and Procedures Manual.

8.2.2 Perform CODIS searches under the direct supervision of a qualified DNA Data Bank analyst and generate the proper documentation associated with the CODIS search.

8.2.3 Import DNA profiles into CODIS under the direct supervision of a qualified DNA Data Bank analyst and generate the proper documentation associated with the data import.

8.3 Training Evaluation

8.3.1 Knowledge

8.3.1.1 Review of notes prepared by the trainee in training notebook by training coordinator.

8.3.1.2 Oral and practical examinations.

8.3.2 Skills

The trainee should demonstrate the ability to use CODIS.

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9 TECHNICAL REVIEW OF DNA DATA BANK SAMPLES

9.1 Goals

- 9.1.1 To become familiar with all paperwork associated with the analysis of DNA Data Bank samples generated by the VADFS DNA Data Bank.
- 9.1.2 To become familiar with the technical review forms used to evaluate data generated by the VADFS DNA Data Bank.
- 9.1.3 To develop the ability to recognize a problem with the analytical data.

9.2 Tasks

- 9.2.1 Review all of the paperwork and electropherograms associated with the analysis of a minimum of 8 plates of DNA Data Bank samples generated by DNA Data Bank analysts.
 - 9.2.1.1 A qualified DNA Data Bank analyst will also review these plates for comparison against the trainee's review.
- 9.2.2 Complete the technical review forms used for evaluating data generated by the VADFS DNA Data Bank.
- 9.2.3 Successfully identify problem samples or problems with the controls associated with the samples reviewed in paragraphs 9.2.1 and 9.2.2, when appropriate.

9.3 Training Evaluation

- 9.3.1 Knowledge
 - Review the information reported on the technical review forms by the training coordinator.
- 9.3.2 Skills

The trainee should demonstrate the ability to identify problem samples and/or controls and determine when data is not acceptable and should be rejected.

10 DNA DATA BANK ANALYST QUALIFICATION

10.1 Goals

- 10.1.1 To demonstrate technical knowledge by successfully completing an oral examination.
- 10.1.2 To demonstrate skills and abilities by successfully completing all practical exercises, including a final competency examination.

10.2 Tasks

- 10.2.1 Undergo final oral and competency examinations.
- 10.2.2 Conduct analysis on blood and/or buccal samples as set forth in this training document.
- 10.2.3 Become familiar with DNA testing guidelines/standards set forth by the following groups:
 - Scientific Working Group on DNA Analysis Methods (SWGDM).
 - American Society of Crime Laboratory Directors/ Laboratory Accreditation Board-*International* (ASCLD/LAB[®] -*International*).
 - FBI Quality Assurance Standards for DNA Databasing Laboratories.
- 10.2.4 Read and understand the Department Quality Manual.

10.3 Training Evaluation

- 10.3.1 Knowledge
 - Question and answer sessions.

- 10.3.2 Skills

The trainee should demonstrate the ability to accurately complete the analysis on DNA Data Bank samples and document results in accordance with Department and Section policies and procedures.

11 Y-STR ANALYSIS

11.1 Introduction

This is a guide designed to lead the qualified and practicing Forensic Biology DNA Data Bank Analyst through the procedures necessary to qualify as a Y-STR Analyst in the VADFS DNA Data Bank. The guide is not inclusive of all the techniques and procedures that will be encountered during and after training. In addition, the order in which minimum sample sets are listed is arbitrary. Any or all of the samples may be processed in any order and concurrently if desired.

During the training period, the trainee will prepare and maintain an organized notebook which will serve as a ready-reference during the examiner's tenure in the VADFS Laboratory.

The training program will be monitored by a training coordinator who will provide monthly progress memos to the Biology Program Manager.

Because the Y-STR Analyst Trainee is a previously qualified and practicing DNA Data Bank Analyst, areas such as General Lab Practice, Safety, Administration, etc., can be referred to in the Forensic Biology Section Training Manual for DNA Data Bank Analysts and are not specifically covered under this training program.

11.2 Analyst Qualification

11.2.1 The final qualification of a trainee as a Y-STR Analyst will be based upon the following:

11.2.1.1 Successful completion of the required training samples.

11.2.1.2 Satisfactory completion of an oral technical competency exam to include both technical questions as well as court-style questions pertaining to all aspects of DNA Data Bank analysis that relate specifically to Y-STR analysis.

11.2.1.3 Satisfactory completion of a practical exam which will consist of blood and/or buccal samples. This test will evaluate the trainee's skill in accuracy in performance of Y-STR PCR-based DNA typing procedures.

11.2.2 The satisfactory completion of the oral technical competency exam and of the practical exam will be documented in a memo to be maintained in the training file of the trainee.

11.3 Required Knowledge and Skills

11.3.1 The trainee will possess a basic understanding of the principles of molecular biology and genetics that underlie and support both autosomal and Y-STR typing of DNA in the context of a forensic laboratory. Moreover, skills must be acquired in the operation of certain instruments and the evaluation of data derived during the analysis of Y-STRs. It is understood that the trainee already possesses the skills in operation of instruments and evaluation of data needed for autosomal STR analysis.

11.3.2 Required readings (with documented approval by the Biology Program Manager) will be assigned by the training coordinator and a list of the readings/approval will be kept in the training notebook.

11.3.3 Knowledge areas specific to Y-STR analysis

11.3.3.1 Nature of the polymorphic regions examined by the VADFS on the Y chromosome.

11.3.3.2 Biochemistry of the Polymerase Chain Reaction (PCR).

11.3.3.3 Principles of detection of fragment-length polymorphisms – specifically capillary electrophoresis.

11.3.3.4 Y-STR loci examined by the VADFS.

11.3.3.5 Quality control/quality assurance measures used by the VADFS.

11.3.4 Instrument Operation

11.3.4.1 Thermal Cycler.

11.3.4.2 Capillary electrophoresis instrumentation.

11.3.5 DNA Typing Procedures

Steps and procedures are defined in the Fluorescent Detection PCR-Based STR DNA Protocol: AMPF[®]STR[®] YFILER[™] - Forensic Biology Section Data Bank Procedures Manual.

11.4 Training Samples

11.4.1 The trainee will demonstrate the skills required of a Y-STR analyst by completing, at a minimum, the following sample sets from amplification through the typing/data analysis process:

11.4.1.1 At least forty (40) male DNA samples.

11.4.1.2 At least five (5) female DNA samples.

11.4.2 It may be necessary that the trainee carry some or all samples from extraction on; however, if extracts are available, the trainee may choose to use them in lieu of extracting them him/herself.

11.4.3 During the completion of these minimum sample requirements, a total of at least five (5) separate amplifications and five (5) separate capillary electrophoretic runs should be conducted so as to demonstrate the skills necessary in conducting each of these portions of the analysis.