NORTH CAROLINA STATE BUREAU OF INVESTIGATION

TRAINING PROGRAM FOR DNA CASEWORK ANALYSIS

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1 PURPOSE AND SCOPE

The purpose of this manual is to provide a consistent training program for the analysis of forensic DNA utilizing PCR based technology by the Molecular Genetics Section at the North Carolina State Bureau of Investigation. This program provides individuals with the theoretical background and the working knowledge to conduct independent casework analysis and effective expert witness testimony. Heavy emphasis shall be placed on quality assurance of all tests performed, data integrity via thorough documentation, and excellence in obtaining consistent and congruous results.

The training program detailed in this document provides the following:

- Theoretical knowledge of the principles of PCR based technology.
- Working knowledge of the principles and practices of STR technology as they relate to the forensic analysis of DNA.
- The ability to perform independent, accurate, and consistent forensic analysis on forensic case material.
- The ability to provide effective expert witness testimony that includes, but is not limited to the presentation of PCR and STR technology and the defense of analytical conclusions.

2 REQUIREMENTS FOR QUALIFICATION

2.1 Prerequisites

Individuals must possess a strong scientific background and have extensive course work in biology, chemistry, biochemistry, statistics, and genetics.

2.2 Competency Tests

Individuals must pass a series of well defined competency tests. These tests are to determine the trainee's ability to consistently analyze DNA from a variety of sources. Proficiency tests will include blood, mixed fluids, and simulated cases.

2.3 Written Examination

A written examination shall examine the trainee's understanding of the theoretical and working knowledge of DNA, PCR, STR, and the following subject areas:

2.3.1 Interpretation of gel scans.
2.3.2 Defense of the PCR and STR technology in court.
2.3.3 Understanding of population frequencies generated.
2.3.4 Understanding of validation studies.
2.3.5 Knowledge of the technical literature associated with the procedures and loci under study.

2.4 Case Internship

An analyst in training shall be allowed to work cases under the close supervision of a trained DNA Analyst or Analysts. The trained analyst will supervise the trainee by reviewing and initialing all notes and results from the analysis and ensuring that proper procedures and quality control measures are maintained. The trainee may write the report, but the finalized report must have the signature of the trained DNA Analyst, who will testify to the results in court. The total number of cases worked by the trainee during the case internship will be decided by the Training Officer. Criteria such as prior experience and the comfort level of the trainee in performing the tasks may be considered in the decision. A recommended minimum number of 10 cases should be completed during the Case Internship.

2.5 Mock Trial

A mock trial shall be used to determine the trainee's ability to provide effective expert witness testimony.

3 INSTRUCTIONS FOR THE TRAINING OFFICER

3.1 This program is designed to provide each trainee with the theoretical background and working knowledge to reliably analyze forensic material utilizing PCR based technology. Every topic listed in this manual is equally important; a deficiency in one area can lead to the failure of a successful analysis and/or defense of the analysis in a court of law. Therefore, the training officer must pay very close attention to detail and ensure that all quality assurance guidelines are being followed for every sample processed in the training program. By ensuring each trainee maintains a high degree of concentration and awareness during the performance of his/her training, the proper techniques will be learned and later successfully applied to actual casework.

3.2 The order of topics listed in this manual are not necessarily in the chronological order that the tests will be performed. It may be necessary to learn and perform some techniques out of order. In this case, it is the responsibility of the training officer to provide the trainee with a clear explanation of any missing points or steps and later logically tie everything together.

3.3 It is the responsibility of the Training Officer to point out pertinent scientific literature and technical manuals included in the bibliography to the trainee so that they may become familiar with these readings.

3.4 It is the responsibility of the Training Officer to explain potential safety hazards to each Trainee BEFORE performing a task that may involve said potential safety hazard.

4 INSTRUCTIONS FOR THE TRAINEE

4.1 The trainee is required to keep files on all work completed. These files should include but are not limited to the Training Manual Log Sheet, worksheets, and gel scans. These files will be checked periodically by the Training Officer, Technical Leader and/or SAC.

4.2 The readings assigned are very important. While it is not necessary to memorize protocols and reagent recipes, it is necessary to become familiar with each and be able to perform all duties independently. The trainee is expected to become familiar with the literature that pertains to the forensic analysis of DNA using PCR based technology that is included in the bibliography.

5 SAFETY

5.1 There are many potential hazards that exist in the laboratory. While the exposure to all hazards can be minimized or avoided, it is the responsibility of the Training Officer to ensure the Trainee is aware of all potential hazards. These potential hazards include but are not limited to the following:

5.1.1 Infectious Agents

5.1.1.1 Viral agents, including HIV and Hepatitis
5.1.1.2 Bacteria, including sexually transmitted diseases
5.1.1.3 Fungi
5.1.1.4 Parasites

5.1.2 Hazardous Materials

5.1.2.1 Caustic Agents (Acids and Bases)
5.1.2.2 Carcinogens/Mutagens
5.1.2.3 Teratogens
5.1.2.4 Organic Chemicals
5.1.2.5 Flammable Materials
5.1.2.6 Oxidizers

5.1.3 Electrical Hazards

5.1.3.1 Electrophoresis units
5.1.3.2 Laboratory Equipment
5.1.3.3 Grounding

5.1.4 Burn Hazards

5.1.4.1 Autoclaves
5.1.4.2 Thermocyclers

5.2 Laboratory Safety Procedures

5.2.1 Individuals must be trained in laboratory safety by the Section Safety Officer prior to the commencement of training. Various manuals are provided that must be followed to ensure safety of all laboratory personnel. The following manuals are to be used for reference and guidance for laboratory safety: MSDS Notebook, Molecular Genetics

Section Manual, and the DOJ Safety Manual.

5.2.2 It is the responsibility of the training officer to alert the trainee to safety hazards specific to this laboratory, including all items mentioned above.

6 ASEPTIC TECHNIQUE AND CONTAMINATION

6.1 The Polymerase Chain Reaction (PCR) is a powerful tool that allows very small amounts of DNA to be amplified over a million times. Because of the sensitivity of this technique, contamination control is a very serious issue that must be emphasized and practiced with every sample. The Decontamination and Clean-up protocols must be strictly followed

6.1.1 All items used in the identification, transfer and isolation of forensic DNA must be sterile and/or free of contaminate DNA.

6.1.2 Gloves must be worn at all times while handling samples.

6.1.3 A fresh, sterile pipet tip must be used for each transfer of DNA or chemical to be used for DNA analysis.

6.1.4 All isolations, extractions, and amplifications shall be performed on a clean work bench except for organic extractions. Because of the danger of damaging HEPA filters by phenol and chloroform, and since the materials used pose a health hazard to analysts, this procedure shall be performed in a chemical fume hood.

6.1.5 Scissors, tweezers, and other instruments used for cuttings or extractions shall be sterilized in between each sample.

6.1.6 The extraction of the Known and Unknown samples shall be separated by time. Between extraction of the Known and Unknowns samples, the work space and instruments shall be decontaminated.

6.2 In addition to the Decontamination protocol, special attention must be paid to the work area(s) where samples will be examined, extracted, and amplified.

· The Examination Work area(s) must be separated in time or space

from the amplification setup areas.

- The Extraction Work area(s) must be physically separated from the amplified DNA work area and be separated in time or space from the PCR setup area.

- The PCR Setup Work area must be physically separated from the amplified DNA work area.

- The Amplified DNA Work area must be physically separated from all other areas to contain the amplified DNA product. All equipment and reagents used in this area shall be dedicated and must not be used in either extraction or PCR setup.

7 DOCUMENTATION

7.1 Goals

7.1.1 To provide protocols for the preparation of reagents and performance of tests to ensure consistent, reliable results.

7.1.2 To provide a thorough record of events for each case analysis.

7.2 Protocols

STR protocols shall be made available to each analyst. The analyst shall not deviate from any protocol without permission from the SAC. Any deviation (purposely or by mistake) from the protocol shall be thoroughly documented on the worksheet at the time of occurrence.

7.3 STR Recipe Book

The working copy of the STR Recipe Book will be maintained by the Quality Control agent.

7.4 Worksheets

The purpose of the worksheets are to provide a means to thoroughly document each step of the analytical process. Each worksheet is to be completed either during or as soon as possible following the step.

8 RECEIVING AND HANDLING OF EVIDENCE

8.1 Goals

8.1.1 To obtain a working knowledge of factors and conditions that influence the deterioration of evidence as it relates to packaging, handling, and storage conditions and time.

8.1.2 To develop a thorough understanding of evidence handling procedures.

8.1.3 To develop a thorough understanding of the necessity for detailed comprehensive notes and adequate labeling of evidential materials.

8.2 Tasks

Read and become thoroughly familiar with the Molecular Genetics section manual regarding receiving and handling of evidence.

9 DNA EXTRACTION

9.1 Goals

9.1.1 To develop skill that will allow the trainee to independently and successfully isolate DNA from forensic samples for PCR analysis

9.1.2 To develop a thorough understanding of the methodology and theory of DNA isolation from bloodstains, saliva stains, vaginal fluid stains, semen stains, epithelial cells, and stain mixtures.

9.1.3 To become familiar with the sensitivity and limitations of isolation procedures.

9.1.4 To develop a thorough understanding of contamination issues during isolation and the steps necessary to avoid contamination.

9.1.5 To understand the use of controls during this procedure.

9.1.6 To become familiar with and understand the function of the reagents used

for DNA isolation.

9.1.7 To become familiar with the methods of reconcentrating DNA and removing inhibitors.

9.1.8 To become familiar with all documentation required for DNA isolation.

9.2 Tasks

9.2.1 Attend lecture and watch demonstration of procedure from a qualified analyst.

9.2.2 Prepare all reagents necessary for DNA isolation.

9.2.3 Perform DNA isolation on at least 100 bloodstains. At least 50 bloodstains shall be from simulated known bloodstains and at least 50 shall be from simulated "case samples".

9.2.4 Perform DNA isolation on at least 25 saliva and/or vaginal fluid stains.

9.2.5 Perform Differential extractions on at least 30 mixed stain samples, including semen and vaginal secretions, semen and blood, semen and saliva, and semen alone.

9.2.6 Perform DNA isolation for competency tests on samples of various types of samples which will include:

9.2.6.1 Bloodstains (≥32 samples)
9.2.6.2 Mixed fluids (≥32 samples
9.2.6.3 Simulated cases (2-4 cases)

9.2.7 Complete documentation for all DNA isolation procedures above.

10 QUANTITATION OF DNA

10.1 Goals

10.1.1 To develop skill that will allow the trainee to independently and successfully perform the slot blot technique and the chemiluminescent

detection and interpret the results.

10.1.2 To develop a thorough understanding and working knowledge of the use of the slot blot technique and chemiluminescent detection so the analyst can independently perform the test.

10.1.3 To understand limitations of the slot blot technique and chemiluminescent detection and to successfully interpret results of the test.

10.1.4 To understand the importance and use of the controls used during the procedures.

10.1.5 To become familiar with the theory of the chemiluminescent procedure. This includes understanding the purpose and use of chemicals in each step of the process.

10.1.6 To learn how to use the film processor and dark room techniques.

10.2 Tasks

10.2.1 Attend lecture and watch demonstration of procedure from a qualified analyst.

10.2.2 To prepare reagents necessary to complete the slot blot technique and chemiluminescent detection of isolated and control DNA.

10.2.3 Perform and complete at least 5 slot blot membranes using appropriate controls on DNA samples prepared by the trainee. The trainee should quantitate DNA Samples isolated that are discussed above.

10.2.4 Develop each membrane and interpret the results.

10.2.5 Complete documentation for all tests performed above.

11 AMPLIFICATION OF DNA

11.1 Goals

11.1.1 To develop skill that will allow the trainee to independently and

successfully perform all of the tasks required for the amplification of isolated DNA.

11.1.2 To develop a thorough understanding and working knowledge of the STR PCR systems used in the laboratory.

11.1.3 To understand the importance and use of the controls used during the procedure.

11.1.4 To understand and avoid any issue of contamination.

11.1.5 To become familiar with the limitations and problems associated with amplification.

11.1.6 To understand the purpose for each reagent used in the amplification process as well as each step performed by the thermocycler.

11.1.7 To understand the purpose and become familiar with the validation of the thermocyclers.

11.1.8 To develop knowledge that will allow the trainee to independently and successfully perform all of the tasks required for the amplification of isolated DNA for the STR system or loci under study.

11.2 Tasks

11.2.1 Attend lecture and watch demonstration of procedure from a qualified analyst.

11.2.2 Perform the amplification process using DNA previously isolated from the samples above.

11.2.3 Prepare an environment that minimizes the risk of contamination and follow proper procedures that will prevent contamination.

11.2.4 Perform the quality control tests on the various models of thermocyclers.

11.2.5 Complete documentation for all amplification tests performed above.

12 GEL ELECTROPHORESIS

12.1 Goals

12.1.1 To develop the skills to successfully run analytical gels.

12.1.2 To understand the importance and use of controls used during the procedures.

12.1.3 To develop a thorough understanding and working knowledge of fluorescently tagged primer systems.

12.2 Task

12.2.1 Attend lecture and watch demonstration of procedure from a qualified analyst.

12.2.2 Prepare and run analytical gels using samples previously amplified.

12.2.3 Prepare analytical gels for samples to be electrophoresed.

13 SCANNING OF ANALYTICAL GELS

13.1 Goals

13.1.1 To develop the skill required to successfully analyze analytical gels using the imaging system and imaging software.

13.1.2 To understand the use of and the limitations of the imaging system and imaging system software.

13.2 Tasks

13.2.1 Scan the analytical gels produced using the imaging system.

13.2.2 Using the imaging system, optimize the scanned gels described above.

13.2.3 Produce clear printouts of the optimized gels using the imaging system software.

14 GEL SCAN INTERPRETATION

14.1 Goals

14.1.1 To obtain the skills and practice necessary to independently, successfully, and consistently interpret data obtained from gel scans.

14.1.2 To develop a thorough working knowledge of the use of controls in the interpretation.

14.1.3 To develop and understanding of the use and necessity of controls used throughout the entire analysis process.

14.1.4 To become familiar and understand the effects of sample concentration on the interpretation and process.

14.1.5 To understand the limitations of the process and become familiar with the problems that may be encountered during the interpretation.

14.1.6 To develop a working knowledge of the StaRCall Software including sizing of bands, image grey-scale manipulation, and labeling of gel scans.

14.1.7 To understand the use of CODIS in forensic casework and how to enter samples (including mixtures), perform searches, and complete PopStats.

14.2 Tasks

14.2.1 Attend lecture and watch demonstration of procedure from a qualified analyst.

14.2.2 Interpret all gel scans successfully completed from the tests above.

14.2.3 Examine any unsuccessful gels (e.g. the sample did not align with a ladder marker) and determine, if possible, the reason for the problem.

14.2.4 Examine and make allelic calls on complex mixtures from actual forensic cases to compare to those calls made from a Qualified Case-working Analyst. At least three (3) cases will examined.

14.2.5 Successfully complete the exercises on gel scans found in a directory set up in the DNA imaging station/software. These samples will include a variety of simulated cases may include but not be limited to mixtures, weak samples, overblown samples, and samples with allelic dropout.

14.2.6 Attend a hands-on demonstration and lecture of the NCSBI CODIS system.

15 REPORT WRITING

15.1 Goals

15.1.1 To develop the skill necessary to effectively report STR analysis and provide expert witness testimony in a court of law.

15.1.2 To become familiar and develop a working knowledge of the terminology and presentation of PCR analysis and results.

15.1.3 To become skilled in expressing written and oral PCR results simply, concisely, and accurately.

15.1.4 To develop a working knowledge of the LIMS system.

15.1.5 To become familiar with the legal aspects of PCR including appellate decisions and controversial cases.

15.1.6 To become familiar with pertinent scientific literature regarding PCR and STR typing.

15.1.7 To become skilled in expressing written and oral statistical results simply, concisely, and accurately.

15.2 Tasks

15.2.1 Read and understand pertinent scientific literature provided by the Training Officer.

15.2.2 Thoroughly understand and be able to accurately and concisely answer the questions in Appendix II.

15.2.3 Read and thoroughly understand the Statistical Interpretation protocol.

15.2.4 Read and thoroughly understand the Bureau and Section procedures that affect any casework.

15.2.5 Develop a thorough understanding of the current DNA Federal Standards (Quality Assurance Standards For Forensic DNA Testing Laboratories and American Society of Crime Laboratory Directors).

15.2.6 Develop a thorough understanding of the National Research Council (NRC II) report on DNA.

Revision History		
Effective Date	Revision Number	Reason
unknown	00	Original Document
7/17/03	01	Updating requirements for Training by making them more stringent (e.g. changing number and type of samples to be analyzed, specifying “computer simulation” training for gel scan analysis, specify analysis of mixtures {gel scans} from previously analyzed casework).

APPROVAL SIGNATURES	Date
Author/Title (Print)	
(Signature)	

Name/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	

APPENDIX I. NORTH CAROLINA STATE BUREAU OF INVESTIGATION DNA DNA Casework Analyst Training Manual Log Sheet

Training Area	Date Completed/Initials	Trainers Initials
1. Education		
1.1 Safety		
Chemical Hazards/MSDS Sheets		
Electrical Safety		
Blood borne Pathogen Training		
Mandatory Readings*		
1.2 The Polymerase Chain Reaction (PCR)		
Lecture		
Mandatory Readings*		
1.3 STR Multiplex Systems		
Lecture		
Mandatory Readings*		
1.4 NCSBI STR Interpretation Protocol		
Read and Understood		
1.5 NCSBI Quality Control and Documentation		
Use of STR QC Protocol Book		
Use of STR QC Recipe Book		
Documentation using worksheets		
QC of Purchased Reagents		
QC of Prepared Reagents		

Training Area (continued)	Date Completed/Initials	Trainers Initials
1.6 Receiving and Handling of Evidence		
Protocol Read and Understood		
2. Laboratory Training		
2.1 Aseptic Technique and Contamination Control		
Cleaning of Equipment		
Use of Biosafety Hood		
Handling of Evidence		
2.2 DNA Isolation (Organic Extractions)		
Read and Understood Protocol		
Demonstration of Organic Extractions		
Supervised Extractions		
Extractions of ≥50 Known Samples		
Extractions of ≥50 Unknown Samples		
Extractions of ≥25 saliva and or vaginal fluid stains		
Differential extractions of ≥30 mixture samples		
Demonstration of Differential Separations		
Supervised Differential Separations		
Demonstration of Sample Cleanup Procedure.		

Supervised Sample Cleanup.		
Training Area (continued)	**Date Completed/Initials**	**Trainers Initials**
2.3 Quantitation of DNA		
Read and Understood Protocol(s)		
2.3.1 Slot Blot		
Demonstration of Slot Blot		
Supervised Slot Blot		
2.3.2 Chemiluminescence		
Chemiluminescence Demonstration		
Supervised Chemiluminescence		
Demonstration: Development of Chemiluminescent Image		
Supervised Development of Chemiluminescent Image		
2.3.3 Analysis of Quantitation		
Demonstration of Analysis		
Supervised Analysis		
2.3.4 Quantitation of all Training Samples		
Training Area (continued)	**Date Completed/Initials**	**Trainers Initials**
2.4 STR Amplification and Typing		
2.4.1 PCR Amplification		
Read and Understood Protocols		
PCR Amp. Demonstration		

Supervised PCR Amp.		
Amp. of Training Samples		
2.5 Electrophoresis		
Read and Understood Protocol		
2.5.1 Polyacrylamide Gel Preparation		
Polyacrylamide Preparation Demonstration		
Supervised Polyacrylamide Gel Pouring		
2.5.2 Gel Loading and Electrophoresis		
Gel Loading Demonstration		
Supervised Gel Loading		
2.6 Gel Scan and Interpretation		
Read and Understood Protocol		
Lecture and demonstration of StaRCall software.		
Supervised analysis and interpretation(s) using StarCall software.		
Analysis and Interpretation of training sets		
Interpretation of Mixture Cases		
Training Area (continued)	**Date Completed/Initials**	**Trainers Initials**
Successful Interpretation of the data from the known mixture sets.		
Analysis and Interpretation of Computer Exercises		

2.7 CODIS Operations		
CODIS system lecture		
2.7.1 Data entry		
Use of specimen management		
Entry of sample profile		
Second reading requirement		
Sample uploading and archiving		
2.7.2 PopStats		
Entry of sample profiles		
Calculation of frequencies		
Printing of the report		
2.7.3 Searches		
Entry of sample profiles		
Conducting a search		
Printing of the report		
Training Area (continued)	**Date Completed/Initials**	**Trainers Initials**
3. Competency		
Completion competency test samples: Bloodstains (≥32 samples) Mixed fluids (≥32 samples Simulated cases (2-4 cases)		
Completion of assigned reading		
Successful completion of a written test.		
Successful completion of Case		

Internship		
Successful completion of moot courts.		

APPENDIX II. GENERAL QUESTIONS

1.1_ What is DNA?

1.2_ Where is it found?

1.3_ What is DNA's importance to us as humans? As forensic scientists?

1.4_ What is the structure of DNA?

1.5_ What is a nucleotide? A purine? A pyrimidine? What are the 4 types of nucleotides?

1.6_ Why does "A" always pair with "T" and "G" with "C"?

1.7_ What is a gene? What is a locus? What do they do and how are they used by the forensic scientist?

1.8_ In general terms, what is a chromosome? How many pairs of chromosomes do each human have? Where are they found and are they the same in every location?

1.9_ Do any two people have the exact same DNA?

1.10_ How is DNA extracted from a blood sample? Other types of samples (semen, saliva, vaginal fluid, and other epithelial cells?

1.11_ What is the function of proteinase K in the isolation procedure?

1.12_ What is the function of SDS and DTT in the isolation procedure?

1.13_ Why is it important to autoclave reagents?

1.14_ What is aseptic technique and why is it important to use aseptic technique in a forensic lab.

1.15_ Explain why a 60°C extension hold time is added to the end of amplification.

1.16_ What information about a sample can be obtained from a slot blot?

1.17_ What is DNA polymerase? Which DNA polymerase are we using? How does it work?

1.18_ What is a primer?

1.19_ What is a probe?

1.20_ Explain denaturation, annealing, and extension of the DNA during the amplification process.

1.21_ What is allelic drop out?

1.22_ What are some factors that inhibit amplification?

1.23_ What is the purpose of the allelic ladder?

1.24_ What is a homozygote? A heterozygote?

1.25_ Explain why a person should have a maximum of two alleles at a single locus?

1.26_ What are the limitations of PCR technologies for forensic use?

1.27_ What is the amount of DNA that the forensic scientist needs for PCR analysis?

1.28_ What is the importance of controls throughout the process? Explain the controls we use during the analysis process.

1.29_ What is the rationale for having different laboratory areas for isolation, PCR set up, and amplification and typing?

1.30_ What are the advantages and disadvantages of current PCR technologies?

1.31_ What are STRs? Where are they found? How can they be used by the forensic scientist?

1.32_ What system do we use for typing via PCR? Where did we purchase our system? What genetic loci are used for our testing?

1.33_ List the main ways contamination can be minimized.

1.34_ Explain the three different major types of contamination.

1.35_ What is BSA and how can it influence a PCR reaction?

1.36_ What is the importance of mitochondrial DNA?

1.37_ What are the two type of fluorescent labels used for PowerPlex™ loci?

1.38_ Describe each GenBank® Locus and Locus Definition used in PowerPlex™. Know which fluorescent label is associated with each locus.

1.39_ Describe how the loci can be visualized on one gel using fluorescent technology .

1.40_ What is the fluorescent ladder (CXR) and how is it used?

1.41_ Explain how the Hitachi Software is used to aid in the determination of stutter bands.

1.42_ Why are gels pre-run before loading?