# 1`NORTH CAROLINA STATE BUREAU OF INVESTIGATION

# TRAINING PROGRAM FOR DNA TECHNICIANS

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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 assist Qualified Analysts conducting scientific tests on forensic evidence using validated procedures. DNA Technicians will be under the direct supervision of a Qualified Body Fluid Analyst. 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 accurate and consistent forensic analysis on forensic case material.

# 2 REQUIREMENTS FOR QUALIFICATION

## 2.1 Prerequisites

Individuals must have earned a B.S. degree and possess a strong scientific background and should 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 perform the

specific technique in which they are being trained.

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 Proficiency Testing

The Qualified Technician will participate in a Proficiency Testing Program in the specific area in which they are trained as soon as possible after training has been completed.

# 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 assist in the analysis of forensic material utilizing DNA technology. 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 can be learned and later successfully applied to actual casework.

3.2 Each of the tasks (i.e. "Quantitation of DNA" or "Gel Preparation") listed in this manual do not have to be learned before a DNA Technician can begin working with samples. Once a Technician is trained and has successfully completed the competency test on a specific task, he/she may begin assisting the Casework Analyst with that task.

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.

## 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, Chemical Hygiene Program, the Bloodborne Pathogen Program and the DOJ Safety Manual.

5.2.2 The trainee will be briefed on the fire evacuation plan for the laboratory.

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

## 5.3 Laboratory Orientation

The trainee may be taken throughout the laboratory and shown areas of interest to their work. They will also be provided with a written job description, an organizational chart, and various manuals including the Crime Laboratory Procedures Manual, SBI Policy Manual and the Section Quality Assurance Manual.

# 6 ASEPTIC TECHNIQUE AND CONTAMINATION (wording for Techs?)

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, DNA organic extractions 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 PREPARATION AND QC OF REAGENTS AND EQUIPMENT

9.1 Goals

9.1.1 To develop the skills to accurately prepare and/or QC reagents that are used in Extraction, DNA Quantitation, and Electrophoresis.

9.1.2 To learn to document routine QC checks on refrigerators, freezers, waterbaths, balances, and thermalcyclers.

9.2 Tasks

9.2.1 To learn how to prepare reagents by accurately weighing dry chemicals and measuring liquids while utilizing the Recipe book.

9.2.2 To learn how to correctly standardize and use the pH meter.

9.2.3 To learn how to accurately take temperature measurements.

9.2.4 To learn how to accurately use the weight standards to check the calibration on the analytical balances.

9.2.5 Perform the quality control checks on the various models of thermocyclers

# 10 DNA EXTRACTION

10.1 Goals

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

10.1.2 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.

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

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

10.1.5 To understand the use of controls during this procedure.

10.1.6 To become familiar with and understand the function of the reagents used for DNA isolation.

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

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

10.2 Tasks

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

10.2.2 Prepare all reagents necessary for DNA isolation.

10.2.3 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”.

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

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

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

10.2.6.1 Bloodstains (≥32 samples)
10.2.6.2 Mixed fluids (≥32 samples
10.2.6.3 Simulated cases (2-4 cases)

10.2.7 Complete documentation for all DNA isolation procedures above.

# 11 QUANTITATION OF DNA

## 11.1 Goals

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

11.1.2 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..

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

11.1.4 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.

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

## 11.2 Tasks

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

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

11.2.3 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.

11.2.4 Develop each membrane.

11.2.5 Complete documentation for all tests performed above.

11.2.6 Successfully complete a defined competency test.

## 12 GEL PREPARATION (AND ELECTROPHORESIS?????)

12.1 Goals — To develop the skills to successfully prepare and run analytical gels.

12.2 Task

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

12.2.2 Prepare **at least** two gels while under the supervision of a qualified analyst.

# 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.

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

# 14 REPORT WRITING

## 14.1 Goals

14.1.1 To develop the skill necessary to effectively report STR analysis.

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

14.1.3 To develop a working knowledge of the LIMS system.

14.1.4 To develop a working knowledge of the Word Perfect Macros used in report writing.

## 14.2 Tasks

14.2.1 To understand the format of DNA reports.

14.2.2 To understand how reports are generated and saved utilizing the LIMS system.

| Revision History | | |
|---|---|---|
| Effective Date | Revision Number | Reason |
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| APPROVAL SIGNATURES | Date |
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| Author/Title (Print) | |
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| 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. | | |

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|---|---|---|
| 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 | | |
| | | |

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|---|---|---|
| **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:<br>Bloodstains (≥32 samples)<br>Mixed fluids (≥32 samples<br>Simulated cases (2-4 cases) | | |
| Completion of assigned reading | | |
| Successful completion of a written test. | | |
| Successful completion of Case | | |

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|---|---|---|
| Internship | | |
| Successful completion of moot courts. | | |