TRAINING PROGRAM FOR THE POLYMERASE CHAIN REACTION ANALYSIS

NORTH CAROLINA STATE BUREAU OF INVESTIGATION - DNA DATABASE

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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 analysis of DNA Database samples. 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 analysis of DNA.
- The ability to perform independent, accurate, and consistent analysis on DNA Database samples.

2. REQUIREMENTS FOR QUALIFICATION

2.1 Prerequisites

Individuals shall possess a minimum of a bachelor's degree in a biological science. Individuals are encouraged to take the following courses at NCSU or their equivalent: Genetics, Biochemistry, and Genetics 501, 502, and 560 or 561, if they lack similar courses from the university they graduated from..

2.2 Proficiency Tests

Individuals must pass a series of well defined proficiency tests. These tests are to determine the trainee's ability to consistently analyze DNA from DNA Database samples. Proficiency tests will be from known and unknown blood samples. Individuals will complete a competency test prior to starting independent analysis of DNA Database samples.

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 DNA Database samples 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. 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 samples.

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 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 should become familiar with the literature that pertains to the 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:

Infectious Agents

A. Viral agents, including HIV and Hepatitis
B. Bacteria, including sexually transmitted diseases
C. Fungi
D. Parasites

Hazardous Materials

A. Caustic Agents (Acids and Bases)
B. Carcinogens/Mutagens
C. Teratogens
D. Organic Chemicals

Electrical Hazards

Electrophoresis units

Burn Hazards

Autoclaves
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 in 5.1.

6. ASEPTIC TECHNIQUE AND CONTAMINATION CONTROL

6.1 The Polymerase Chain Reaction (PCR) is a powerful tool that allows very small amounts of DNA to be amplified over a million fold. 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 protocol and the STR Quality Protocol (section 12.1) must be strictly followed.

6.1.1 All items used in the identification, transfer and isolation of 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 under a biological safety cabinet except for organic extractions. Because of the danger of damaging HEPA filters by phenol and chloroform, this procedure shall be performed on the bench. Therefore, special precaution must be taken to ensure that the bench and surrounding areas have been properly decontaminated.

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

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

7.2 To provide a thorough record of events for each sample set .

7.2 Protocol Notebook

Each analyst shall be given a copies of the STR protocols. 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. DNA DATABASE SAMPLING HANDLING PROCEDURES

8.1 Goals

8.1.1 To become familiar with and be able to receive samples from Correctional Officers and the U.S. Mail.

8.1.2 To properly record the chain of custody on samples received within the Unit and to enter the sample into the SBI Specimen Management Program.

8.1.3 To become skilled at making bloodstains from the liquid blood samples received, and to do so following all proper safety guidelines.

8.1.4 To develop a knowledge of how bloodstains and DNA Database cards are stored within the DNA Databank.

8.1.5 To fully understand the need for confidentiality and security of genetic profiles as governed by N.C. General Statute § 15A-266. To understand that authorization is needed to disseminate such records.

8.2 Tasks

8.2.1 To properly record the receipt of DNA Database samples, filling out all necessary chain of custody documents.

8.2.2 To log samples into the Specimen Management Program, produce the necessary bar codes, and properly store the sample in the DNA Databank.

8.2.3 To prepare bloodstains from the liquid blood samples received.

8.2.4 To be able to articulate to the satisfaction of the Training Officer what the policy is on release of genetic information from individuals within the Database.

9. DNA ISOLATION

9.1 Goals

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

9.1.2 To develop a basic understanding of the methodology and theory of DNA isolation from bloodstains.

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

9.1.4 To develop a cognizant 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 various methods of DNA concentration utilizing microcons and EtOH precipiatation.

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

9.2 Tasks

9.2.1 Prepare all reagents necessary for DNA isolation.

9.2.2 Perform DNA isolation on at least 200 bloodstains. The Chelex method shall be used for a few blood stains and the organic extraction methods for the remainder.

9.2.3 Perform DNA concentration methods using EtOH precipitation and the microcon method.

9.2.7 Perform DNA isolation for proficiency tests on known and unknown blood stains

9.2.8 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.

10.2 Tasks

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

10.2.2 Perform and complete at least 5 slot blot membranes using appropriate controls on DNA samples prepared by the trainee.

10.2.3 Develop each membrane and interpret the results.

10.2.4 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 Powerplex system.

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.

11.2 Tasks

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

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

11.2.3 Perform the validation on the thermal cycler.

11.2.4 Complete documentation for all amplification tests performed above.

12. GEL ELECTROPHORESIS

12.1 Goals

12.1.1 To develop the skills to successfully complete and stain product gels if necessary.

12.1.2 To develop a thorough understanding and working knowledge of the use of product gels.

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

12.1.4 To develop a thorough understanding and working knowledge of the types of non-radioisotopic systems.

12.2 Tasks

12.2.1 Prepare reagents necessary to perform product gels.

12.2.2 Prepare and run product gels using previously amplified samples.

12.2.3 Stain the product gels that were run above, if applicable.

13. GEL SCAN INTERPRETATION

13.1 Goals

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

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

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

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

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

13.2 Tasks

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

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

13.2.3 Successfully interpret at least 200 samples from known Database samples.

14. REPORTING PCR RESULTS

14.1 Goals

14.1.1 To develop the skill necessary to effectively report STR type Database samples.

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

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

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

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15. CODIS OPERATING SYSTEMS

15.1 Goals

15.1.1 To understand the goals of CODIS and how it operates at a state and national level.

15.1.2 To become skilled at entering DNA profiles data into CODIS and searching against the database.

15.1.3 To be able to troubleshoot problems independently which may arise when

using CODIS.

15.2 Tasks

15.2.1 To read and understand the Operating Manual provided for the CODIS system.

15.2.2 To practice entering profiles into CODIS and searching results, until proficient.

APPENDIX I. NORTH CAROLINA STATE BUREAU OF INVESTIGATION DNA LABORATORY: STR Training Manual Log Sheet

Training Area	Date Completed/Initials	Trainers Initials
1. Education		
A. Safety		
1. Chemical Hazards/MSDS Sheets		
2. Electrical Safety		
3. Blood borne Pathogen Training		
4. Readings		
B. The Polymerase Chain Reaction (PCR)		
1. Lecture		
2. Readings		
C STR Powerplex Multiplex Systems		
1. Lecture		
2. Readings		
D. NCSBI Quality Control and Documentation		
1. Use of STR QC Protocol Book		
2. Use of STR QC Recipe Book		
Training Area (continued)	**Date Completed/Initials**	**Trainers Initials**
3. Documentation using worksheets		

4. QC of Purchased Reagents		
5. QC of Prepared Reagents		
E. CODIS Operations		
1. Read Operation Manual		
2. Use of CODIS to upload profiles and search data		
F. Specimen Management		
1. Documentation of chain of custody of samples received		
2. Able to log in samples on Specimen Management Program		
2. Laboratory Training		
A. Aseptic Technique and Contamination Control		
1. Cleaning of Equipment		
2. Use of Biosafety Hood		
3. Handling of Database Samples		
B. DNA Isolation		
1. Chelex Extraction		
1.1 Read and Understood Protocol		
Training Area (continued)	**Date Completed/Initials**	**Trainers Initials**
1.2 Demonstration of Extraction		

1.3 Supervised Extractions of Known Samples		
2. Organic Extraction		
2.1 Read and Understood Protocol		
2.2 Demonstration of Extractions		
2.3 Supervised Extractions of Known Samples		
C. Quantitation of DNA		
1. Slot Blot		
1.1. Read and Understood Protocol		
1.2 Demonstration of Slot Blot		
2. Chemiluminescence		
2.1 Read and Understood Protocols		
2.2 Chemiluminescence Demonstration		
2.3 Supervised Chemiluminescence		
Training Area (continued)	**Date Completed/Initials**	**Trainers Initials**
2.4 Demonstration: Development of Chemiluminescent Image		
2.5 Supervised		

Development of Chemiluminescent Image		
3. Analysis of Quantitation		
3.1 Read and Understood Protocol		
3.2 Demonstration of Analysis		
3.3 Supervised Analysis		
D. STR Amplification and Typing		
1. PCR Amplification		
1.1 Read and Understood Protocols		
1.2 PCR Amplification Demonstration		
1.3 Supervised PCR Amplification		
Training Area (continued)	**Date Completed/Initials**	**Trainers Initials**
E. Electrophoresis and Analysis		
1. Polyacrylamide Gel Preparation		
1.1 Read and Understood Protocols		
1.2 Polyacrylamide Preparation Demonstration		
1.2 Polyacrylamide Preparation		

Demonstration		
1.3 Supervised Polyacrylamide Gel Pouring		
2. Gel Loading and Electrophoresis		
2.1 Read and Understood Protocols		
2.2 Gel Loading Demonstration		
2.3 Supervised Gel Loading		

Training Area (continued)	Date Completed/Initials	Trainers Initials
4. Scan and Analysis		
4.1 Read and Understood Protocol		
4.2 Gel Scan Demonstration		
4.3 Supervised Gel Scan		
4.5 Demonstration of Analysis and Typing		
4.6 Supervised Analysis and Typing		
5. Extraction Robot		
5.1 Read and Understood Protocol		
5.2 Robot Set-Up Demonstration(s)		
5.3 Robot Run Demonstration (s)		
5.4 Supervised Set-up and Run		
5.5 Ready for Independent Use		
6. Amplification Robot		
6.1 Read and Understand Protocol		
6.2 Robot Set-up and Demonstration(s)		
6.3 Robot Run Demonstration(s)		
6.4 Supervised Set-up and Run(s)		
6.5 Ready for Independent Use		

III. Competency Test		
Unsupervised Powerplex STR Analysis		
1. Organic Extraction of Unknown Samples		
2. Amplification of Samples		
3. Gel electrophoresis and typing		
4. Completion of competency test		

APPENDIX II. GENERAL QUESTIONS

1. What is DNA?

2. Where is it found?

3. What is DNA's importance to us as humans? As forensic scientists?

4. What is the structure of DNA?

5. What is a nucleotide? A purine? A pyrimidine? What are the 4 types of nucleotides?

6. Why does "A" always pair with "T" and "G" with "C"?

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

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?

9. Do any two people have the exact same DNA?

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

11. What is the function of proteinase K in the isolation procedure?

12. What is the function of SDS and DTT in the isolation procedure?

13. Why is it important to autoclave reagents?

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

15. What is Chelex and how is it used?

16. What information about a sample can be obtained from a slot blot? A product gel?

17. What is DNA polymerase? Which DNA polymerase are we using? How does it work?

18. What is a primer?

19. What is a probe?

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

21. What is allelic drop out?

22. What are some factors that inhibit amplification?

23. What is the purpose of the allelic ladder?

24. What is a homozygote? A heterozygote?

25. Explain why a person should have a maximum of two alleles at a single locus?

26. What are the limitations of PCR technologies for forensic use?

27. What is the amount of DNA that the forensic scientist needs for PCR analysis? For RFLP?

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

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

30. What are the advantages and disadvantages of current PCR technologies v. RFLP

technologies in forensic science?

31. What are STRs? Where are they found? How can they be used by the forensic scientist?

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

33. List the main ways contamination can be minimized.

34. Explain the three different major types of contamination.

35. What is BSA and how can it influence a PCR reaction?

36. What is the importance of mitochondrial DNA?

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