CMPD Biology Section

Training Manual

Approved by: _____ Date: _____
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(Laboratory Director)

Provisions for Modification and Updating of This Manual

Any updates, modifications, additions, or deletions to this manual will have the following information and an updated issue date located at the bottom of each page.

Summary of Revisions

Date Issued	Summary of Changes Made
4/15/2009	Modified Sec. 2; 6.1.6; 9.2.8; 13; 14.2.7; Appendix II and III
9/8/2011	Updated instruments

TRAINING PROGRAM FOR PCR BASED STR ANALYSIS

CHARLOTTE-MECKLENBURG POLICE DEPARTMENT

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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 Biology Section at the Charlotte-Mecklenburg Police Department Crime Laboratory. 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:

1. Theoretical knowledge of the principles of PCR based technology.

2. Working knowledge of the principles and practices of STR technology as they relate to the forensic analysis of DNA.

3. The ability to perform independent, accurate, and consistent forensic analysis on forensic case material.

4. 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 and genetics; specifically: Molecular Biology, Genetics, Biochemistry and Statistics

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 a variety of sources. Proficiency tests will include blood, mixed fluids, and simulated cases.

2.3 Written Examination

A written examination shall test the trainee's understanding of the theoretical and working knowledge of DNA, PCR, STRs, and statistical interpretation of PCR results.

2.4 Mock Trial

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

2.5 The training program may be modified based on the analyst's previous experience. Sufficient experience of an individual will be determined by the technical leader.

3. INSTRUCTIONS FOR THE TRAINER

3.1 The 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 trainer 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 trainer 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 trainer 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 trainer 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 electropherograms. These files will be checked periodically by the trainer.

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 trainer 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
A. Electrophoresis Units
B. 3130 Unit

Burn Hazards
A. Autoclaves
B. Thermal cyclers
C. Sterilizers
D. Heat blocks

5.2 Laboratory Safety Procedures

5.2.1 Individuals must be trained in laboratory safety, have knowledge of safety precautions available in the laboratory, and know who is the 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 and CMPD Safety Manual.

5.2.2 It is the responsibility of the trainer to alert the trainee to safety hazards specific to this laboratory.

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 times. Because of the sensitivity of this technique, contamination control is a very serious issue that must be emphasized and practiced with every sample.

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 Amplification setup shall be performed under a biological safety cabinet or fume hood. Special precautions 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.

6.1.6 The extraction of the Known and Unknown samples shall be separated by time or space. Between extraction of the Known and Unknown 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.

6.2.1 The Examination Work area(s) must be separated in time or space from the amplification setup areas.

6.2.2 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.

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

6.2.4 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 Protocol Notebook

Each analyst shall have access to the section copy of the STR protocols as well as a link to the online version. The analyst shall not deviate from any protocol without permission from the DNA Technical Leader. Any deviation (purposely or by mistake) from the protocol shall be thoroughly documented on the worksheet at the time of occurrence.

7.3 STR Chemicals

The working copy of the appropriate QC forms, which includes procedures for preparing solutions, will be maintained in the QC notebook.

7.4 Worksheets

The purpose of the worksheets is 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 related to packaging, handling, 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

8.2.1 Read the Laboratory policies regarding receiving and handling of evidence.

9. DNA ISOLATION

9.1 Goals

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

9.1.2 To develop a basic understanding of the methodology and theory of DNA isolation from bloodstains, saliva stains, hairs, vaginal fluid stains, semen stains, stain mixtures, bone samples, and tooth samples.

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.

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 30 bloodstains by each extraction method. Concentrate the isolated DNA, if necessary.

9.2.3 Perform DNA isolation on at least 10 saliva stains by each extraction method. Concentrate the isolated DNA, if necessary.

9.2.4 Perform differential extractions on 10 mixed stain samples. Concentrate the isolated DNA, if necessary.

9.2.5 Perform DNA isolation on at least 5 hairs by each extraction method. Concentrate the isolated DNA, if necessary.

9.2.6 Perform DNA isolation on at least 5 bone samples. Concentrate the isolated DNA, if necessary.

9.2.7 Perform DNA isolation on at least 5 tooth samples. Concentrate the isolated DNA, if necessary.

9.2.8 Perform DNA isolation on at least 5 mock cases. Concentrate the isolated DNA, if necessary.

9.2.9 Perform DNA isolation for proficiency tests on samples of various types which will include:

1. Bloodstains (10 samples)
2. Mixed fluids (10 samples)
3. Hairs (10 samples)
4. Saliva (10 samples)
5. Bone (3 samples)
6. Teeth (3 samples)
7. Mock cases (2-4 cases)

9.2.10 Complete documentation for all DNA isolation procedures above.

10. QUANTITATION OF DNA

10.1 Goals

10.1.1 To develop skills that will allow the trainee to independently and successfully perform the Quantifiler and Quantifiler Y tests and interpret the results.

10.1.2 To develop a thorough understanding and working knowledge of the use of Sequence Detection System (SDS) 7500 so the analyst can independently perform the test.

10.1.3 To understand limitations of real-time PCR and the Quantifiler and Quantifiler Y Tests and to successfully interpret results of the tests.

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 real-time PCR. This includes understanding each step of the real-time PCR process.

10.2 Tasks

10.2.1 Prepare reagents necessary to complete the Quantifiler and Quantifiler Y detection of isolated and control DNA.

10.2.2 Perform and complete at least 5 Quantifiler and Quantifiler Y quantifications using appropriate controls on DNA samples and interpret the results.

10.2.3 Complete documentation for all tests performed above.

11. AMPLIFICATION OF DNA

11.1 Goals

11.1.1 To develop skills 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 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 thermal cycler.

11.2 Tasks

11.2.1 Perform the amplification process using DNA previously isolated from the samples in Section 9.

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

11.2.3 Complete documentation for all amplification tests performed in 11.2.1.

12. 3130 ELECTROPHORESIS

12.1 Goals

12.1.1 To develop the skills to successfully clean, set up, and operate the 3130.

12.1.2 To develop a thorough understanding and working knowledge of the use of the 3130.

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 STR systems.

12.2 Tasks

12.2.1 Prepare reagents necessary to set up the 3130.

12.2.2 Prepare and analyze amplified samples from Section 11.

12.2.3 Interpret the results for the runs.

13. GeneMapper ID

13.1 Goals

13.1.1 To develop the skills required to successfully analyze data using the system software, and to successfully and consistently interpret data obtained from the 3130.

13.1.2 To understand the use of and the limitations of the analysis and interpretation process, and problems that may arise.

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

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

13.2 Tasks

13.2.1 Using the GeneMapper ID system software, optimize adjustable parameters and perform QC checks.

13.2.2 Interpret all electropherograms successfully.

13.2.3 Examine any unsuccessful samples and determine, if possible the reason for the problem.

13.2.3 Successfully interpret mock case samples that were extracted in 9.2.8.

14. REPORT WRITING AND TESTIMONY

14.1 Goals

14.1.1 To develop the skills necessary to effectively report PCR analysis results, and provide expert witness testimony in a court of law.

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

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

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

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

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

14.2 Tasks

14.2.1 Read and understand pertinent scientific literature provided in the STR DNA reference list.

14.2.2 Thoroughly understand and be able to accurately and concisely answer the questions in Appendix III.

14.2.3 Read and thoroughly understand the Statistical Interpretation protocol in the CMPD SOP Manual.

14.2.4 Read and thoroughly understand the CMPD SOPs regarding report writing.

14.2.5 Develop a thorough understanding of the DNA testing guidelines set forth by the DNA Advisory Board (DAB), American Society of Crime Laboratory Directors (ASCLD), and the National Research Council (NRCII) report on DNA.

14.2.6 Draft reports on PCR analysis of proficiency tests and mock case results.

14.2.7 Complete statistical analysis on mock data sets to demonstrate understanding of the application of statistics to forensic evidence.

APPENDIX I. CMPD DNA LABORATORY: STR Training Manual Log Sheet

	Training Area	Date Completed/Initials	Trainer's Initials
I.	Education		
	A. Safety		
	1. Chemical Hazards/MSDS Sheets		
	2. Electrical Safety		
	3. Bloodborne Pathogen Training		
	4. Mandatory Readings*		
	B. The Polymerase Chain Reaction (PCR)		
	1. Lecture		
	2. Mandatory Readings*		
	C. STR Multiplex Systems		
	1. Lecture		
	2. Mandatory Readings*		
	D. STR SOP and QA/QC Manuals		
	1. Read and Understood		
	2. Mock STR Data Interpretation		
	E. Quality Control and Documentation		
	1. Use of STR QC Protocol Book		
	2. Use of STR QC Forms		
	3. Documentation using worksheets		
	4. QC of Reagents		
	F. Receiving and Handling of Evidence		
	1. Protocol Read and Understood		

	Training Area (continued)	Date Completed/Initials	Trainer's Initials
II.	Laboratory Training		
	A. Aseptic Technique and Contamination Control		
	1. Cleaning of Equipment		
	2. Use of Biosafety Hood		
	3. Handling of Evidence		
	B. DNA Isolation		
	1. Qiagen and DNA IQ Extraction		
	1.1 Read and Understood		
	1.2 Demonstration of Extraction		
	1.3 Supervised Extraction of Known Samples		
	C. Quantification of DNA		
	1. Quantifiler and Quantifiler Y Tests		
	1.1 Read and Understand Protocols		
	1.2 Demonstration of ABI 7500 SDS set-up		
	1.3 Preparation of Human Quantification Standards		
	1.4 Demonstration of Quantifiler and Quantifiler Y set-up		
	1.5 Demonstration of Analysis and Interpretation		
	1.6 Supervised Analysis with the Quantifiler and Quantifiler Y Tests		
	D. STR Amplification		
	1. Amplification		
	1.1 Read and Understood Protocol		
	1.2 Amplification Demonstration		
	1.3 Supervised Amplification		
	E. 3130 Instrumentation		
	1. Cleaning and Maintenance		
	1.1 Read Protocol		

	Training Area (continued)	Date Completed/Initials	Trainer's Initials
	1.2 Instrument Cleaning Demonstration		
	1.3 Instrument Maintenance Demonstration		
	1.4 Supervised Cleaning		
	2. Instrument Preparation		
	2.1 Read and Understood Protocol		
	2.2 Set Up Demonstration		
	2.3 Prepare and Analyze a Matrix		
	3. ABI 3130 Collection Software		
	3.1 Read and Understood Protocol		
	3.2 File Set Up Demonstration		
	3.3 Supervised File Set Up		
	4. GeneMapper ID Software		
	4.1 Read and Understand Protocols		
	4.2 GeneMapper ID Demonstration		
	4.3 Supervised GeneMapper ID		
	4.4 Demonstration of Analysis Variables		
	4.5 Supervised Changing of Analysis Variables		
III.	Qualifying Test		
	A. Unsupervised STR Analysis		
	1. Extraction of Unknown Samples		
	2. Quantitation of Samples		
	3. Amplification of Samples		
	4. Electrophoresis		
	5. Interpretation of Results		
	B. Written Competency Exam		
	C. Mock trial		

Training Complete on date:_____ DNA Technical Leader:_____

APPENDIX II. CMPD DNA LABORATORY: STR Training Basic Reading List

This section contains basic information which should be covered during training. Of course, other information will be included especially specifics noted in the various laboratory manuals. Particular attention should be paid to the QA Manual and the DNA SOP Manual.

Section 1 – General DNA Laboratory Instruction

1. Laboratory Manuals. Read and understand all the laboratory manuals including the Safety Manual, DNA SOP, and QA Manual. The trainer need only be present to answer questions during this tutorial.

2. Introduction to the instruments. The trainer will instruct the trainee regarding all the components of the instruments and the function of each external part.

3. Discussion of the Amplification kits. The trainer will discuss the various loci, microvariants, single strand primer labeling, multi-color methodology, gender typing, and general characteristics of STR loci.

4. Discussion of the overall general approach to DNA analysis. The trainer will discuss the general concept of each step of the process including sample handling, extraction, quantitation, amplification, and electrophoresis.

Section 2 – Software Instruction and Literature

1. General explanation on the steps of analysis using prepared files. Receive an overview of what is happening from time of sample preparation to obtaining a genetic type.

2. Read from 3130 Users Manual. Be familiar with the contents of the 3130 Manual for future reference, especially sections on instrument hardware and buffer and water vial placement.

3130 Collection Software

1. Sample Sheets and Injection Lists. Utilize the 3130 Collection software to set up a mock sample sheet and injection list.

2. Understand all the parameters set in the injection list. Learn the function of each of the parameters and how they affect the run if changed.

GeneMapper ID Software

1. Read from GeneMapper ID Software Manual. Understand the material contained in the Manual especially sections on analysis of sample files, interpretation of results and troubleshooting.

2. Discussion of the Matrix run. View the Matrix results and verify proper values in the 'Reactions' screen. Learn the purpose and function of a matrix file, and how to recognize when one is faulty.

3. Projects. Learn the variables associated with a Project. Discuss the function of a matrix, sizing standard, and 'virtual filters'.

4. Analysis Parameters. Discussion of analysis parameters. Learn about each of the settings and their functions.

5. Importing of files into GeneMapper ID. Learn to transfer the raw data into a GeneMapper ID project.

6. Discuss steps of GeneMapper ID. Learn the mechanism of the conversion of raw data to a genetic profile. Learn to check the internal size standards and ladders for uniformity.

Section 3 – DNA Procedural Issues

1. DNA Related Issues. Understand the importance of certain regulations regarding DNA analysis such as specific efforts of contamination control and sample handling.

2. Extraction. Learn the various extraction methods and the advantages and disadvantages of each.

3. Quantitation. Learn the mechanism of DNA quantitation and understand any specific trouble shooting issues related to signal intensity.

4. Amplification. Learn how to deduce the optimum amplification parameters from the quantitation data. Learn the purpose, mechanism, and methodology of the PCR procedure.

5. 3130 Setup and Analysis. Learn the importance of each step of the electrophoresis process including sample setup, injection, separation, and signal detection.

Section 4 – Results Interpretation

1. Discussion of anomalies. Discuss factors associated with STR analysis such as stutter, microvariants, and –A peaks, and how to recognize them in an electropherogram.

2. Discussion of population statistics. Learn to use Pop Stats (or other appropriate statistics program) and understand the databases and calculations used in the software. Learn about the minimum allele frequency and calculations used by the program.

3. Discussion of mixtures. Review examples of mixtures and literature regarding mixture analysis. Pay particular attention to the SOP regarding mixture policies.

4. Reanalysis. Learn when and how to reanalyze samples which do not produce an optimum profile with the initial analysis.

5. Discussion of results of examples. Review the results of example files. Learn what records must be maintained on disk and in the case folder.

6. Discussion of trouble shooting. Discuss the more common resolvable error messages and learn how to access trouble shooting tips including the PE manuals and the PE Help Desk.

APPENDIX III. CMPD BIOLOGY LABORATORY: General Questions for the Trainee

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"? What type of bonds hold the chains together?
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 any pairs of chromosomes does each human have?
9. Do any two people have the exact DNA?
10. How many base pairs are there in a DNA molecule?
11. List the main ways contamination can be minimized.
12. What is aseptic technique and why is it important to use aseptic technique in the laboratory?
13. What is a homozygote? A heterozygote?
14. Explain why a person should have a maximum of two alleles at a single locus. What are the exceptions?
15. What is the rationale for having different laboratory areas of isolation, PCR set up, and amplification and typing?
16. What are STRs? Where are they found? How can they be used by the forensic scientist?
17. What is the difference between phenotype and genotype? Haploid versus diploid?
18. What is a proficiency test? Does CMPD participate in blind proficiency testing? How often do you take proficiency tests?
19. What is a DNA audit? How often is a DNA laboratory audited? Who performs the audit?
20. What does SWGDAM stand for and what is this organization's function?
21. What does DAB stand for and what is its function? How was it created and why? Is it still in existence?
22. What does ASCLD/LAB stand for and what is its function?
23. Explain the purpose of the FBI Quality Assurance Standards.

DNA ISOLATION

1. Using DNA IQ and Qiagen, how do you extract DNA from a blood sample? What type of extraction do you use to extract semen? Explain the procedure.
2. What is DNA IQ Lysis Buffer and how does it work?
3. What is the function of SDS in the isolation procedure?
4. What is the function of Proteinase K in the isolation procedure?
5. Explain the use of DTT in isolation.

6. What are histones and protamines?
7. What is it important to autoclave reagents and certain supplies?
8. Explain the use of Microcon filters.
9. Does the DNA IQ system isolate only human genomic DNA? Explain your answer.
10. Is the DNA obtained using the DNA IQ system single-stranded or double-stranded? Why?
11. What is the purpose for heating the DNA sample once the DNA IQ Elution Buffer has been added to the sample?
12. What may be some of the reasons why inconsistent DNA yields may be obtained with the DNA IQ system?

QUANTIFICATION

1. Which loci do the Quantifiler human and male kits detect?
2. Explain the principles behind the Quantifiler reaction.
3. What is the significance of the C_T value?
4. What is the purpose of the IPC?
5. Explain the purpose of using Quantifiler Y on samples.
6. Are the Quantifiler reactions human specific?
7. How can you tell when a sample might be inhibited? How can inhibition be overcome?

AMPLIFICATION

1. What are a nuclease, endonuclease, and exonuclease?
2. Explain the amplification process. Include denaturation, annealing, and extension in your answer.
3. What is a DNA polymerase?
4. What is the name of the DNA polymerase used by CMPD and how does it work? Why do we use this polymerase?
5. What is a primer and what is its function?
6. What is the origin of the primer?
7. What is primer-dimer and how can it affect results?
8. What is preferential amplification (allelic drop-out) and why does this occur? Is this a problem when analyzing samples using the STR technology? Why or why not?
9. What precautions are used to ensure that allelic drop-out has not occurred?
10. What is plateau effect and how does it affect the DNA sample?
11. What are the components of the Identifiler kit? What is the purpose of each component?
12. What are some factors that inhibit amplification and why? What steps can an analyst take to overcome inhibition problems?
13. What are the amplification conditions for the Identifiler kit?
14. How many primers are used in the Identifiler kit?
15. What precautions are used to prevent contamination of the sample DNA with a foreign source?
16. What measures are taken to ensure that the thermal cycler is working properly? What is the purpose of each quality control test?
17. What are the limitations of PCR technologies for forensic use?
18. What is the amount of DNA that the forensic scientist needs for PCR analysis?

ELECTROPHORESIS

1. Explain in detail the detection process. Describe how the laser, CCD camera, DNA fragment, and the matrix work together to produce a signal.
2. Explain the purpose of the spectral.
3. Explain the purpose of a spatial
4. CMPD sets the minimum threshold at 75 RFU. What can you do to a sample that has peaks below this threshold in order to attempt to raise the peaks above the threshold?
5. What is the purpose of the extension step at the end of amplification? What kind of peaks would you see if this extension was not done?
6. How does GeneMapper ID analyze data?
7. What is a spike and how do you know a spike is not actually a true allele? How could you use the software to determine that something is a spike?
8. Explain the function of the ILS.
9. When the ILS is unusable, can the results of the run be accurately interpreted?
10. Explain the function of the electrodes in the analysis process.

RESULTS INTERPRETATION

1. What are the Identifiler loci and on which chromosomes are they located?
2. Name all of the dye labels and the loci that are labeled with each dye.
3. What is the purpose of the allelic ladder?
4. How many alleles are in the Identifiler allelic ladder?
5. The internal lane standard consists of how many peaks? What is the base pair size of each?
6. What is a stutter band and how is one differentiated from a true allele?
7. What is non-template nucleotide addition?
8. What is the genotype of the GM9947A cell line at each locus? What is this cell line derived from?
9. What is a microvariant? How does this differ from an off-ladder variant?
10. What is the base pair size range for each locus?
11. What is a complex repeat unit? Are any of the Identifiler loci considered to have a complex repeat unit?
12. What component in the amplification kit is designed to allow detection of only one DNA strand? What would be different in the electropherogram if the kit was not manufactured with this modification?
13. Give two characteristics of an electropherogram that would indicate you had a mixed sample.
14. What is the purpose of controls throughout the process? Explain the controls we use during the analysis process.

STATISTICS

1. How were the databases that are used by you created?
2. How large is your database (the one used for statistical calculations)?

3. How can you calculate 1 in billions or trillions from a database of only a few hundred people?
4. What is linkage disequilibrium and how does this affect Identifiler analyses? What does independence mean?
5. What is Hardy Weinberg Equilibrium (HWE)? Is the CMPD database in HWE and how do you know?
6. How do allele frequencies differ from genotypic frequencies? How are allele frequencies calculated? How are genotypic frequencies calculated?
7. What are substructures or subpopulations and how does the existence of these affect your reporting of statistics?
8. Why do you report Caucasian, African-American, and Hispanic frequencies versus reporting only one general frequency? If a suspect was of a race other than these, how could you determine the frequency?
9. What does the Power of Discrimination mean? How is this computed?
10. What is random match probability? How is it calculated? When is it used?
11. What is CPI? How is it calculated? When is it used?
12. What is the CPE?