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Department of Forensic Science

VIRGINIA

DEPARTMENT

OF

FORENSIC BIOLOGY SECTION

TRAINING MANUAL

FORENSIC SCIENCE

**ANALYSIS OF FORENSIC CASEWORK
USING PCR-BASED STR
FLUORESCENCE IMAGING ANALYSIS
AT THE POWERPLEX[®] 16 LOCI
AND Y-STR ANALYSIS**

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TABLE OF CONTENTS

- 1 Overview of Training Program
 - 2 Safety
 - 3 Introduction to DNA PCR-Based Typing
 - 4 DNA Isolation
 - 5 DNA Quantitation – Yield Gel
 - 6 Operating the BIOMEK[®] NX^P Automation Workstation
 - 7 DNA Quantitation
 - 8 Normalization Wizard and Amplification Process
 - 9 Product Gel
 - 10 Capillary Electrophoresis
 - 11 Capillary Electrophoresis Data Analysis and Interpretation
 - 12 Statistics, Report Writing and CODIS
 - 13 Testimony and Expert Witness Qualification
 - 14 Hair Root Evaluation for STR Analysis
 - 15 Y-STR Analysis
- Appendix A Bibliography
- Appendix B Glossary
- Appendix C Additional Training Aids

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1 OVERVIEW OF TRAINING PROGRAM

1.1 Purpose and Scope

- 1.1.1 The purpose of this document is to provide a uniform training program for the analysis of forensic casework using DNA PCR-based STR fluorescence imaging analysis, and in so doing, to adhere to the national DNA standards. This training program supplements successful completion of college course work in biochemistry, molecular biology, and genetics. The Department of Forensic Science requires all DNA examiners to complete course work (graduate or undergraduate) in genetics, biochemistry, molecular biology (molecular genetics or recombinant DNA technology) or other courses which provide a basic understanding of the foundation of forensic DNA analysis, as well as course work and/or training in statistics as it applies to forensic DNA analysis. These courses must be completed before an individual can be deemed qualified to perform DNA analysis on casework by the Department.
- 1.1.2 The program will provide exposure to methods, techniques and procedures presently used and accepted by the courts and forensic DNA examiners. Additionally, it will provide for exposure to the pertinent literature available in the field and a review of applicable court rulings on the admissibility of forensic DNA analysis. The training will focus on the methods currently used in the Virginia Department of Forensic Science to allow for proficiency to be developed using both known and case materials. The training will also provide exposure to court procedures and assistance in developing the skills necessary for effective expert witness testimony.
- 1.1.3 Throughout the DNA training, oral and practical examinations and/or informal mock trials related to case approach and identification of biological substances will continue to ensure that the information learned remains fresh and the skills honed.
- 1.1.4 The sequence in which the tasks are presented in the outline should not necessarily be considered as a mandatory order of instruction. Exposure to legal aspects and testimony will be continuous throughout the training.
- 1.1.5 Oral and practical examinations and/or mock trials encompassing several topics will be staged periodically.
- 1.1.6 Upon completion of DNA training, the trainee will undergo a comprehensive oral competency examination, followed approximately two weeks later by a formal video taped mock trial. The oral competency examination is used to ascertain the trainee's technical knowledge of case approach, identification of biological substances, and DNA analysis, with the emphasis on DNA and how case approach and identification of biological substances integrates with DNA analysis. The practical examination, analysis of a validated fabricated case, is used to ascertain the trainee's technical skills and abilities in case approach, identification of biological substances, and DNA analysis. Finally, the trainee will testify to the examinations performed on the fabricated case at a mock trial, thus likening this test to an actual courtroom situation. Satisfactory performance in all areas is required prior to upgrading a trainee to a qualified examiner in the Forensic Biology Section.

1.2 Coordination of the Program

The training coordinator will be an experienced examiner. The coordinator may delegate certain duties and blocks of instruction to other qualified examiners, but will be responsible for the overall training.

1.3 Training Period

It is estimated that this training program can be completed in four to six months, which is to include successful completion of the formal mock trial. Some individuals may require less time than others, depending on such factors as experience and education. The qualifications of the trainee will be evaluated and modifications will be made to this training program as appropriate. The length of the training period is a matter which will be left to the discretion of the Biology Program Manager, the trainee's supervisor, and the training coordinator.

1.4 Location of Training

Whenever practical, the bulk of an individual's training will occur in the laboratory to which he/she will be assigned. If this is not possible, the training will be conducted at the most convenient laboratory. Such arrangements will be made through the Biology Program Manager. Oversight and direction of the training will be provided by the Program Manager.

1.5 Mock Trials

- 1.5.1 Each case a forensic examiner analyzes has the potential of involving him/her as an expert witness in courtroom testimony. The trainee must never underrate this important aspect of the work. It is the training coordinator's responsibility to ensure that the trainee is thoroughly prepared for legal questioning. This can be done by a combination of mock trials, prearranged as well as impromptu question and answer sessions, pertinent literature review, and observation of courtroom testimony given by experienced examiners.
- 1.5.2 A mock trial may take place after the trainee has completed a block of this training protocol and a practical examination of a case incorporating that block of the training. The case will be fabricated so that the training coordinator knows the correct answers. The fabricated case thus serves as a monitor of the trainee's proficiency in applying techniques and procedures to actual casework examinations.
- 1.5.3 The final mock trial will incorporate all aspects of training (case approach, identification of biological substances, and DNA analysis) and will be held subsequent to the final practical examination of a fabricated case. THE TRAINEE WILL NOT RECEIVE THE FINAL MOCK CASE UNTIL ALL PHASES OF THIS TRAINING PROTOCOL HAVE BEEN SATISFACTORILY COMPLETED.
- 1.5.4 If the individual has no prior testimony experience, a minimum of 2 mock trials with attendant practical examinations are required prior to the final mock trial.
- 1.5.5 Other related legal training will be integrated into the program on a continual basis.
- 1.5.6 It cannot be overemphasized that testimony training is just as important as the analytical training. The trainee must successfully meet acceptable performance standards in both areas before he/she is deemed to be qualified to conduct forensic examinations on evidential material.

1.6 Guidelines for Comprehensive Oral Examination and Final Comprehensive Mock Trial and Oral Examination

- 1.6.1 Approximately two weeks prior to the final mock trial, an informal oral examination of the trainee will be conducted by the section supervisor, the Biology Program Manager, and the Laboratory Director or designee to ascertain the technical knowledge of the individual. This will be limited to two (2) hours. Questions should be confined to technical aspects of the training and should be used to ascertain whether the goals, as set forth in each technical portion of the training program, have been achieved.
- 1.6.2 Immediately following the oral examination the trainee may be released while the supervisor, the Biology Program Manager, and the Laboratory Director or designee evaluates the trainee's performance.
- 1.6.3 The outcome of the oral examination evaluation will be:
- 1.6.3.1 Satisfactory.
- 1.6.3.2 Not satisfactory.
- 1.6.3.2.1 If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.

- 1.6.4 The final mock trial will not exceed four (4) hours. Prior to trial, the "prosecutor" and the "defense attorney" may reach an agreement as to selected items to be introduced at trial in order to remain within the set time constraints.
 - 1.6.5 The atmosphere of the trial will be formal. That is, it will be conducted in the same manner as a real courtroom situation. This includes conduct, protocol, and all other aspects.
 - 1.6.6 Harassment of the expert witness by defense counsel or prosecutor will be kept to the minimum necessary to achieve the desired goal. Questioning by both the prosecutor and defense attorney(s) should be relevant and realistic.
 - 1.6.7 There may be two defense lawyers at the trial, one of whom must be a qualified examiner in the Forensic Biology Section.
 - 1.6.8 The trial may be stopped at any time upon the request of any of the involved parties.
 - 1.6.9 Immediately following the trial, the trainee may be released while the Department Director or his designee, the Biology Program Manager, section supervisor, and trial participants evaluate the trainee's performance.
 - 1.6.10 The outcome of the trial evaluation will be:
 - 1.6.10.1 Satisfactory.
 - 1.6.10.2 Not satisfactory.
 - 1.6.10.2.1 If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.
 - 1.6.11 This evaluation may be followed by a short performance critique.
 - 1.6.12 The training coordinator will review the videotape with the individual as soon as possible. Other comments should be gathered by the individual from trial participants/observers as soon as possible.
 - 1.6.13 Satisfactory performance on technical aspects and testimony must be achieved before the individual is qualified to perform the duties of an examiner.
- 1.7 Transition from Trainee to Examiner
- 1.7.1 After the individual has successfully completed all training (case approach, identification of biological substances, and DNA analysis), there follows a somewhat awkward period of adjustment. The supervisor must ensure that the transition from trainee to qualified examiner takes place as smoothly as possible. A newly qualified examiner cannot function without some guidance.
 - 1.7.2 For a period of time, all of the newly qualified examiner's reports must be reviewed by the supervisor or designee prior to release. Casework must be monitored closely for at least six (6) months.
 - 1.7.3 The supervisor, or designee, will accompany the newly qualified examiner to court for the first few cases.
- 1.8 Instructions for the Training Coordinator
- 1.8.1 The intent of the training program is to ensure that each and every trainee is provided with certain basic principles and fundamentals necessary for the complete education of an examiner in the Forensic Biology Section. All of the listed topics must be incorporated into the program. However, education and prior experience of the trainee will be used as a guide to determine the amount of time devoted to each topic. Some of the topics will suggest an order of events and this ranking should be followed. ANY

DEVIATION FROM THE CONTENTS OF THIS PROTOCOL MUST BE CLEARED WITH THE BIOLOGY PROGRAM MANAGER.

- 1.8.2 The training coordinator or designated examiner will document the completion of each required training task by the trainee on the checklist for that aspect of training.
- 1.8.2.1 The completed checklists will be retained by the trainee in the appropriate sections of his/her training notebook.
 - 1.8.2.2 One copy of all completed checklists will accompany the Training Coordinator's final report to the Biology Program Manager stating that all aspects of the training program have been completed satisfactorily.
- 1.8.3 The trainee will be evaluated on his/her performance during the course of the program. The training coordinator must submit monthly written evaluations of the trainee's progress to the Biology Program Manager. The monthly training report is due on each trainee within five working days of the end of the month.
- 1.8.4 The monthly training report must include:
- 1.8.4.1 A summation of the progress made during the month.
 - 1.8.4.2 An evaluation of the trainee's notebook.
 - 1.8.4.3 An evaluation of the progress during the month, to include:
 - 1.8.4.3.1 Problem areas, as applicable, and their solutions or proposed solutions.
 - 1.8.4.3.2 Trainee's strong points.
 - 1.8.4.3.3 Trainee's weak points and suggested remedies.
 - 1.8.4.3.4 Statement concerning trainee's overall performance.
 - 1.8.4.3.5 Plans for the upcoming month.
- 1.8.5 This report will be in memorandum format, one memorandum per trainee. Each memorandum will become a part of the training history of the trainee and will be used to document the trainee's progress toward qualification. The monthly report format is located at the end of this section.
- 1.8.6 The coordinator is to discuss the monthly evaluation with the trainee and the trainee's supervisor prior to forwarding it to the Biology Program Manager. Any comments by the trainee, coordinator, or supervisor are to be included with the report.
- 1.8.7 When the trainee has satisfactorily completed all training requirements (case approach, identification of biological substances, and DNA analysis), a memorandum will be issued by the Biology Program Manager to the Department Director recommending that the person be qualified to perform the duties of an examiner in the section. If the trainee cannot meet the criteria expected of him/her during the period allowed for training in each of the areas, steps will be taken to effect the appropriate action.
- 1.8.8 The training should culminate so that the trainee has the following:
- 1.8.8.1 Knowledge of the principles and practices of forensic serology and DNA as these relate to the analysis of case material.
 - 1.8.8.2 Knowledge of the theory and application of instrumentation and specialized techniques used to examine biological evidence.

1.8.8.3 The ability to perform accurate forensic analyses independently and proficiently, to accurately document the findings of all analyses in accordance with Department and Section policies and procedures, and to accurately report those findings in a Certificate of Analysis.

1.8.8.4 The ability to perform accurate forensic analyses independently and proficiently, to accurately document the findings of all analyses in accordance with Department and Section policies and procedures, and to accurately report those findings in a Certificate of Analysis.

1.9 Instructions for the Trainee

1.9.1 The trainee is expected to keep a loose-leaf notebook on all work completed. The completed checklist for each training topic and the training coordinator's monthly report will also be included in the notebook.

1.9.2 The notebook should be organized by subject. Within each subject category, the types of tests or examinations observed and performed, notes and comments on each type of test, and the review of pertinent literature should be included. For each procedure performed, comments/notes should include the following, as appropriate: principle, procedural outline (to include the purpose of critical reagents), sensitivity, specificity, interpretation of results, possible interferences/problems, and comments, including comparison to other methods.

1.9.3 The DNA PCR-based STR fluorescence imaging analysis and quality control procedures can be found in the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedures Manual, Section III - Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 BIO System, Section IV - The Biomek®2000 Automation Workstation Program Manual, Section VIII - Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 System, Section IX - Biomek® NX^P Automation Workstation Procedures Manual, and Section VI - The Quality Assurance Program DNA Typing Of Biological Materials.

1.9.4 A list of study questions is located at the end of each section in this manual. The trainee is encouraged to write out the answers to the questions after completing the required tasks and readings for the section.

1.9.5 The training program provides the trainee with exposure to various types of samples. Similar samples have been grouped together. Each group of samples can be worked simultaneously, although they may be at different stages of the procedure.

1.9.6 The trainee will assist with casework throughout the training only under the direct supervision of a qualified examiner.

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MONTHLY TRAINING REPORT FORMAT

(on letterhead stationary)

MEMORANDUM

TO: (Name), Biology Program Manager

FROM: (Name), Training Coordinator

DATE:

SUBJECT: Training Report: (Name)

This report reviews and evaluates the forensic DNA PCR training of (Name) for the month of _____.

1. Progress during the month
2. Evaluation of trainee's notebook
3. Evaluation of progress
4. Plans for the upcoming month

cc: (Trainee)
(Director of Technical Services)
(Regional Director)
(Supervisor, if different than the Training Coordinator)

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2 SAFETY

2.1 Bloodborne Pathogen

All trainees will attend a course on bloodborne pathogen training organized by the Department's Safety Coordinator.

2.2 Hazards

Each individual working in the laboratory of the Forensic Biology Section must be acutely aware of the potential hazards inherent in his/her work. These hazards include, but are not limited to:

2.2.1 Infectious agents, such as those associated with:

- Hepatitis
- AIDS
- Sexually transmitted diseases
- Parasitic infections
- Bacterial infections

2.2.2 Hazardous materials, such as:

- Ethidium bromide
- Acids and bases
- Organic chemicals

2.3 Safety Procedures

2.3.1 Reference: Department of Forensic Science Safety Manual

2.3.1.1 All trainees are required to read and be familiar with the Department of Forensic Science Safety Manual.

2.3.2 Personal protection

2.3.2.1 Use gloves, safety glasses and other protective clothing and equipment.

2.3.2.2 Avoid production of aerosols.

2.3.2.3 No mouth pipetting.

2.3.2.4 Material Safety Data Sheets (MSDS)

2.3.2.4.1 Read and be familiar with the prescribed precautions for the handling of all chemical used in a particular procedure before performing the procedure.

2.3.3 Biosafety Practices

2.3.3.1 Follow prescribed cleaning procedures for yourself, your work areas, and equipment.

2.3.3.2 All biological materials and containers/supplies that have come in contact with biological materials and/or hazardous chemicals will be placed in biohazard bags, which will be disposed of according to approved guidelines.

2.3.3.3 All glassware for disposal will be placed in the broken glass containers, which will be disposed of according to approved guidelines.

- 2.3.3.4 Organic and other hazardous chemicals (e.g., phenol, ethidium bromide) will be retained in appropriately labeled containers in a hood until picked up by a disposal company.

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3 INTRODUCTION TO DNA PCR-BASED TYPING

The original PCR method, invented by Kary Mullis, was used to diagnose prenatal sickle-cell anemia.^{1,3} Since the first report in 1985 of DNA amplification using the process known as polymerase chain reaction (PCR), many advances and modifications have been made to the basic method. PCR is an *in vitro* method for the enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA. A repetitive series of cycles involving template denaturation, primer annealing, and extension of the annealed primers by DNA polymerase results in the exponential accumulation of a specific fragment whose termini are defined by the 5' ends of the primers. Because the primer extension products synthesized in one cycle can serve as a template in the next, the number of target DNA copies approximately doubles at every cycle. Thus, 20 cycles of PCR yields about a million-fold amplification. With advances in the polymerase chain reaction process, analyzing forensic casework has been made more efficient. The ability to extract and type DNA from forensic evidentiary samples has revolutionized the field of forensic DNA. Previously, genetic marker typing was limited to the analysis of blood group markers and soluble polymorphic protein markers. Because the number of suitable markers expressed in particular fluids and tissues is relatively small, and because mixtures of fluids cannot be separated for conventional genetic marker typing, a suspect frequently cannot be included or excluded as a fluid donor in a case. However, the development of methods to extract DNA from virtually all biological specimens has greatly expanded the potential for individual identification.³

3.1 *Taq* DNA Polymerase

Initially, PCR amplification used the Klenow fragment of *E. coli* DNA polymerase I to extend the annealed primers. This enzyme was inactivated by the high temperatures required to separate the two DNA strands at the outset of each PCR cycle. Fresh enzyme had to be added constantly during every cycle. To make the process more time efficient, molecular biologists began to use an enzyme from a thermophilic eubacterial microorganism, *Thermus aquaticus* (*Taq*), capable of growth at 70-75°C.^{1,2} Although *Taq* DNA polymerase has a very limited ability to synthesize DNA above 90°C, the enzyme is relatively stable and is not denatured irreversibly by exposure to high temperatures. Preliminary results indicated retention of 65% activity after a 50-cycle PCR amplification when the upper limit temperature was 95°C.¹ Under normal reaction conditions, the amount of *Taq* DNA polymerase becomes limiting after 25-30 cycles of amplification.⁴ Two of the forms of *Taq* DNA polymerase that are now available are genetically engineered forms of the native enzyme synthesized in *E. coli*. One of these DNA polymerases, AmpliTaq Gold™ (PE Applied Biosystems), is provided in an inactive state. Heat activates the enzyme. This feature allows flexibility in reaction setup. Both forms of the polymerase carry a 5'→3' polymerization-dependent exonuclease activity, but they lack a 3'→5' exonuclease activity.^{4,5}

3.2 PCR Primers

In order to amplify a sequence of DNA, oligonucleotide primers flanking the target DNA sequence of interest are used. These oligonucleotides typically have different sequences and are complementary to sequences that lie on opposite strands of the template DNA with their 3' ends oriented toward each other. The template DNA is first denatured by heating in the presence of a large molar excess of each of the two oligonucleotides and the four dNTPs. The reaction is then cooled to a temperature that allows the oligonucleotide primers to anneal to their target sequences, after which the annealing primers are extended with the enzyme *Thermus aquaticus* (*Taq*) DNA polymerase in an automated series of heating and cooling cycles. With each cycle the number of copies of DNA will approximately double. The primers are responsible for the sequence specificity of the PCR reaction. Typically the length of the primers used in the PCR amplification should be at least 16 nucleotides and preferably 20 to 30 nucleotides and single stranded.⁴

References

1. Gelfand, D. H. and Erlich, H. A., PCR Technology: Principles and Applications for DNA Amplification, Stockton Press, New York, New York, 1989.
2. Engelke, D. R., Krikos, A., Brick, M. E., and Ginsburg, D., "Purification of *Thermus aquaticus* DNA Polymerase Expressed in *Escherichia coli*," *Analytical Biochemistry*, Vol. 191: 396-400, 1990.

3. Reynolds, R. and Sensabaugh, G., "Analysis of Genetic Markers in Forensic DNA Samples Using the Polymerase Chain Reaction," *Analytical Chemistry*, Vol. 63: 1-15, 1990.
4. Sambrook, J., Fritsch, E. F., and Maniatis, T., Molecular Cloning: A Laboratory Manual, Vol. 2, Cold Spring Harbor Laboratory Press, New York, 1989.
5. Longley, M. J., Bennett, S. E., and Mosvaugh, D. W., "Characterization of the 5'→3' Exonuclease Associated With *Thermus aquaticus* DNA Polymerase," *Nucleic Acid Research*, Vol. 18: 7317-7322, 1990.

General DNA Study Questions:

1. What is DNA?
2. Where is DNA found?
3. What is the role of DNA in the field of forensics?
4. What is the structure of DNA?
5. What is the composition of DNA?
6. What is a nucleotide?
7. What is a purine?
8. What is a pyrimidine?
9. What type of bonds holds the chains together?
10. What is complementary pairing?
11. What is a chromosome? gene? codon?
12. How many base pairs are there in a DNA molecule?
13. When would you expect two individuals to have the same DNA profile?
14. What is an allele?
15. What is meant by a genotype? phenotype?

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4 DNA ISOLATION

4.1 Goals

- 4.1.1 To develop a basic understanding of the theory and procedures of DNA isolation from blood stains, other biological stains, tissue, bone, teeth, hair and mixtures.
- 4.1.2 To become acquainted with the sensitivity of the isolation procedure.
- 4.1.3 To become familiar with the limitations of the isolation procedure.
- 4.1.4 To become familiar with the use of controls incorporated at this stage of the procedure.
- 4.1.5 To become familiar with the reagents used for DNA isolation and the function of each.
- 4.1.6 To become familiar with proper documentation of DNA isolation.

4.2 Tasks

- 4.2.1 Prepare reagents necessary for DNA isolation.
- 4.2.2 Perform DNA isolation on at least 28 blood stains. Use the organic extraction method for 14 of the samples, the manual DNA IQ™ extraction method for 7 samples, and the automated DNA IQ™ extraction method (addressed in Chapter 6 of this manual) for the remaining 7 samples. Initially use large stains (200 µl) and gradually move to smaller stains (5 µl) to test the ability to obtain DNA from smaller stains. These stains are prepared on a variety of different substrates commonly encountered in casework. Continue on to Chapter 5, DNA QUANTITATION - YIELD GEL and Chapter 7, DNA QUANTITATION. Note: All 28 samples will be quantitated using the yield gel and the Plexor® HY human quantitation methods to allow for a comparison of these quantitation techniques to be made. Complete the entire DNA analysis of these 28 samples before proceeding to task 4.2.3 below.

ATTENTION: Ensure that all appropriate controls are isolated with the training samples.

- 4.2.3 Perform DNA isolation on at least 28 other unmixed biological stains, including semen, vaginal fluid, saliva, vasectomized semen samples, urine and feces. These stains vary from large stains (200 µl) to smaller stains (5 µl) and are prepared on a variety of different substrates commonly encountered in casework. Use the organic extraction method for 14 of the samples and the manual DNA IQ™ extraction method for the remaining 14 samples.
 - 4.2.3.1 Extract semen stains (excluding vasectomized semen samples) using the “Organic/DNA IQ™ Extraction Method for Mixed Body Fluid Stains (Differential Procedure)” starting at the step which begins “Add to each sperm fraction”.
 - 4.2.3.2 Extract vaginal fluid, saliva, urine, and feces using the “Organic Extraction Method for Blood Stains and Tissue Samples or the DNA IQ™ Extraction Method for Buccal Cells, Blood Stains, Hairs, Envelopes, Stamps, and Cigarette Butts (Non-Differential Procedure)”.
 - 4.2.3.3 Extract vaginal fluid, saliva, urine, and feces using the “Organic Extraction Method for Blood Stains and Tissue Samples or the DNA IQ™ Extraction Method for Buccal Cells, Blood Stains, Hairs, Envelopes, Stamps, and Cigarette Butts (Non-Differential Procedure)”.
 - 4.2.3.4 Continue on to Chapter 7, DNA QUANTITATION. Complete the entire DNA analysis of these 28 samples before proceeding to task 4.2.4 below.

ATTENTION: Ensure that all appropriate controls are isolated with the training samples.

- 4.2.4 Perform DNA isolation on at least 20 mixed biological stains. Each stain will consist of a mixture of two biological fluids, to include semen, vaginal fluid, blood or saliva. Use the manual differential extraction methods. Ten of the mixed body fluid stains should be extracted using the organic differential extraction method. Five of the mixed body fluid stains should be extracted using the manual DNA IQ™ extraction method and the remaining 5 mixed body fluid stains should be extracted using the automated DNA IQ™ extraction method addressed in Chapter 6 of this manual. As above, stains are prepared on a variety of different substrates commonly encountered in casework and vary in size and quantity of fluid present. Continue on to Chapter 7, DNA QUANTITATION. Complete the entire DNA analysis of these 20 samples before proceeding to task 4.2.5 below.

ATTENTION: Ensure that all appropriate controls are isolated with the training samples.

- 4.2.5 Perform DNA isolation on the following validation samples using either an organic extraction method or the DNA IQ™ extraction method (manual or robotic).
- 4.2.5.1 Two blood/bone/tissue sample sets, each set from a different individual and each set containing 1 blood sample, 2 bone samples, and 4 tissue samples (total of 14 samples - 2 blood, 4 bone, and 8 tissue samples)
 - 4.2.5.2 Five animal samples
 - 4.2.5.3 Ten contaminated stains (contaminants such as bacteria, soil, grass, cleaning agents, etc.)
 - 4.2.5.4 Five blood/semen/hair sample sets, each set from a different individual (total of 15 samples - 5 blood, 5 semen, and 5 hair samples)
 - 4.2.5.5 A family study (at least 5 samples)
 - 4.2.5.6 Old stains - at least 5 years old (10 samples)
 - 4.2.5.7 Two buccal swab/teeth sample sets, each set from a different individual (total of 4 samples - 2 buccal swabs and 2 teeth)
 - 4.2.5.8 Continue on to Chapter 7, DNA QUANTITATION

ATTENTION: Ensure that all appropriate controls are isolated with the training samples.

- 4.2.6 Read applicable literature and become familiar with the glossary terms. Refer to Appendices A, B, and C.

4.3 Training Evaluation

4.3.1 Knowledge

- 4.3.1.1 Review of notes and worksheets in training notebook by training coordinator.
- 4.3.1.2 Mini -mock trials/oral and practical examinations.

4.3.2 Skills

- 4.3.2.1 The trainee should perform DNA isolation on a sufficient variety and number of samples to develop and exhibit an unquestionably sound technique for successfully isolating DNA. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

STUDY QUESTIONS:

1. Using the following methods, how do you extract DNA from a blood sample? a semen sample?
 - a. Organically
 - b. DNA IQ™ extraction method (manual and robotic)
2. What is stain extraction buffer and how does it work? DNA IQ™ Lysis Buffer?
3. What is TNE? When is it used and how does it work?
4. What is the function of SDS in the isolation procedure?
5. What is the function of Proteinase K in the isolation procedure?
6. What is the function of Sarkosyl in the isolation procedure?
7. What is the function of the organic extraction portion of the procedure? What is the function of phenol, chloroform, and isoamyl alcohol in the extraction procedure?
8. Explain the use of DTT in isolation.
9. What are histones? protamines?
10. Why is it important to autoclave reagents and certain supplies?
11. Explain the use of Microcon filters.
12. Why is the chelex extraction method not used by the DFS?
13. Does the DNA IQ™ System isolate only human genomic DNA? Please explain your answer.
14. Is the DNA obtained using the DNA IQ™ System single-stranded or double stranded? Why?
15. What is the purpose for heating the DNA sample once the DNA IQ™ Elution Buffer has been added to the sample?
16. What may be some of the reasons why inconsistent DNA yields may be obtained with the DNA IQ™ System?
17. Which method is more efficient in isolating DNA from samples containing a low level of DNA versus a higher level of DNA and why?

5 DNA QUANTITATION – YIELD GEL

5.1 Goals

- 5.1.1 To become familiar with the theories of electrophoresis as they apply to submarine gels used for DNA quantitation.
- 5.1.2 To learn the parameters used for electrophoresis of the yield gel.
- 5.1.3 To become familiar with the photographic procedures used to document yield gel results.
- 5.1.4 To develop an understanding and working knowledge of the use of yield gels, including interpretations, limitations, and proper documentation.
- 5.1.5 To become familiar with the controls run on yield gels.

5.2 Tasks

- 5.2.1 Prepare reagents and gels necessary to quantitate DNA samples using a yield gel.
- 5.2.2 Run yield gels.
- 5.2.3 Interpret the results of yield gels.
- 5.2.4 Look at degraded samples on a yield gel to become familiar with the appearance of a degraded sample versus a sample with high molecular weight DNA present. Note: These previously isolated samples will be provided to the training coordinator by either the Biology Program Manager or the Forensic Molecular Biologist.
- 5.2.5 Compare the yield gel results to the Plexor[®] HY System quantitation results obtained.
- 5.2.6 Read applicable literature and become familiar with the glossary terms. Refer to Appendices A, B, and C.

5.3 Training Evaluation

- 5.3.1 Knowledge
 - 5.3.1.1 Review of notes, photographs, and worksheets in training notebook by training coordinator.
 - 5.3.1.2 Mini-mock trials/oral and practical examination.
- 5.3.2 Skills
 - 5.3.2.1 The trainee should demonstrate an unquestionably sound technique for quantitating DNA by running a sufficient number of yield gels and accurately interpreting the associated results. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

STUDY QUESTIONS:

1. What information about a sample can be obtained from a yield gel? What information cannot be obtained?
2. What is the purpose of lambda Hind III?
3. What calibration standards are used? How are the calibration standards made?
4. What is in loading buffer and why?

5. What percent agarose is used for the yield gel and why?
6. Why aren't acrylamide gels used to obtain better resolution?
7. How does ethidium bromide work to aid in visualizing DNA?
8. What are some problems associated with the use of ethidium bromide? What would happen if ethidium bromide was left out of the gel? What would the analyst have to do?
9. Why might an analyst run a yield gel in addition to the Plexor[®] HY System?
10. Explain the appearance of degraded vs. non-degraded DNA on a yield gel.

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6 OPERATING THE BIOMEK[®] NX^P AUTOMATION WORKSTATION

6.1 Introduction

- 6.1.1 Training on the operation of the Biomek[®] NX^P Automation Workstation will be divided into two sections. Section I covers the training requirement for a new Project Coordinator. Section II covers the training of the non-Project Coordinator DNA trainee and casework examiner.

6.2 Section I

6.2.1 Goals

- 6.2.1.1 To be familiar with how to initiate the Biomek[®] NX^P Software.
- 6.2.1.2 To understand how to set up the deck for isolation of DNA.
- 6.2.1.3 To be able to make modifications to the Biomek[®] NX^P Software.
- 6.2.1.3.1 To be familiar with the Biomek[®] Automation Workstation and associated programs to troubleshoot problems.
- 6.2.1.3.2 To assist the examiners by becoming the Project Coordinator of the Biomek[®] Automation Workstation and providing oversight to the Forensic Laboratory Specialist who will serve as the primary operator.

6.2.2 Tasks

- 6.2.2.1 Read and become familiar with the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedures Manual, Section IX - Biomek[®] NX^P Automation Workstation Procedures Manual.
- 6.2.2.2 Learn about each step of the Biomek[®] NX^P Software and the operation of the Biomek[®] Automation Workstation, including required documentation.
- 6.2.2.3 Observe the Forensic Molecular Biologist and/or Qualified Project Coordinator perform isolation, quantitation, dilution, and amplification setup on a plate of casework samples. Observe the entire DNA isolation, quantitation, dilution, and amplification setup process, deck setup, and program design.
- 6.2.2.4 Initiate and perform the Biomek[®] Workstation calibration programs (e.g., deck framing). Also perform the Biomek[®] Tool calibration procedures.
- 6.2.2.5 Run a checkerboard and a zebra stripe training set.
Checkerboard: One set of samples consisting of 48 samples (24 blood and/or buccal samples and 24 blanks).
Zebra Stripe: One set of samples consisting of 48 samples (24 blood and/or buccal samples and 24 blanks).
- 6.2.2.6 The trainee will prepare and perform a sensitivity series using the Biomek[®] Automation Workstation and the Plexor[®] HY System.
- 6.2.2.7 Perform the deselection of data points on the standard curve for the five sets of problem data in order to improve the quality of the Plexor[®] HY System Standard Curves used to estimate the concentration of the sample DNA.

6.2.2.8 Observe the Project Coordinator abort a 16 sample water run and perform the Recover Dispense Only and Recovery methods on the run.

6.2.2.9 The trainee will conduct a minor validation.

6.2.2.9.1 Checkerboard: Two sets: one set of samples consisting of at least 32 samples (16 blood and/or buccal samples and 16 blanks) and one set of samples consisting of 40 samples (20 blood and/or buccal samples and 20 blanks) will be taken through the DNA isolation, quantitation, and amplification steps using the Biomek[®] 2000 Automation Workstation in conjunction with the Plexor[®] HY System, Normalization Wizard, and PCR Amplification Set Up programs. One of these checkerboard runs will be aborted and the Recovery Dispense Only and Recovery Methods will be performed.

16.2.2.9.1.1 These samples will be carried through the typing steps. Note: each set of samples will be run independently of the other sets.

6.2.2.9.2 Perform DNA isolation, quantitation, and amplification using the Biomek[®] Automation Workstation in conjunction with the DNA IQ[™] Extraction, Plexor[®] HY System, Normalization Wizard, and PCR Amplification Set Up programs on two sets of competency samples assigned. The samples will be loaded as they would be if they were actual casework samples. The first set will include at least 10 samples that are typically encountered in casework analysis, such as blood samples, buccal swabs, cigarette butts, etc. The second set of samples will include at least 12 mixture samples requiring a differential extraction. All samples will be carried through the typing steps.

6.2.3 Training Evaluation

6.2.3.1 Evaluation of documentation skills by the Forensic Molecular Biologist and/or Qualified Project Coordinator.

6.2.3.2 The trainee should understand and be able to independently operate the Biomek[®] Automation Workstation. This will be evaluated and monitored throughout the training.

STUDY QUESTIONS:

1. How far in advance can the Biomek[®] Automation Workstation setup be performed before the 96 deep well plate containing samples is loaded onto the deck of the robot?
2. What is the purpose of performing the “home all axes” function? What is the purpose of framing the deck? What effect will increasing/decreasing the values for the X, Y, and Z axes have on labware and deck positions? What is the purpose of the tool calibration? How often is each quality control measure performed?
3. In the case of an emergency, what is the best way to shut the Biomek[®] Automation Workstation down?
4. What is the purpose of the recovery dispense only and recovery methods?
5. Why is it imperative to ensure that no air bubbles exist in the sample during the Plexor[®] HY procedure and Normalization Wizard and amplification setup steps?
6. What instrumentation is used to detect the Plexor[®] HY System data?
7. What happens to the fluorescent signal during the real-time PCR process when a sample contains DNA?
8. Can the Plexor[®] HY System detect PCR inhibitors? If so, what effect do PCR inhibitors have on the amplification plot?

6.3 Section II

6.3.1 Goal

- 6.3.1.1 To be familiar with how to set up the samples for isolation on the Biomek[®] Automation Workstation, including the required documentation.

6.3.2 Tasks

- 6.3.2.1 Read and become familiar with the Commonwealth of Virginia Department of Forensic Science Forensic Biology Procedures Manual, Section IX - Biomek[®] NX^P Automation Workstation Procedures Manual.
- 6.3.2.2 At a minimum isolated DNA from the 7 blood stains and 5 mixed biological stains addressed in Section 4, DNA Isolation, plus controls. Note: In accordance with the instruction provided in Section 4 of this manual, the blood stain set and mixed biological stain set will be isolated on the Biomek[®] Automation Workstation at different times.
- 6.3.2.3 Observe the Project Coordinator run the blood stains and mixed biological stains through the entire DNA isolation process, deck setup, and initiation of the Biomek[®] NX^P Software.

Continue on to Chapter 7, DNA QUANTITATION.

STUDY QUESTIONS:

1. Please explain the 96 deep well plate setup when more than one DNA examiner's evidence samples are isolated using the Biomek[®] Automation Workstation.
2. How does the 96 deep well setup differ when evidence and known samples are isolated at the same time using the Biomek[®] Automation Workstation?
3. How does the 96 deep well setup differ once the known samples have been loaded into the deep well plate and subsequently a different examiner wants to load evidence samples into the deep well plate?
4. If a signal is detected in a reagent blank, how does this affect the rest of the samples isolated during that particular run?
5. What instrumentation is used to detect the Plexor[®] HY System data?
6. What happens to the fluorescent signal during the real-time PCR process when a sample contains DNA?
7. Can the Plexor[®] HY System detect PCR inhibitors? If so, what effect do PCR inhibitors have on the amplification plot?

7 DNA QUANTITATION

7.1 Goals

- 7.1.1 To develop an understanding and working knowledge of the Plexor[®] HY quantitation method, including limitations and interpretation of results and proper documentation.
- 7.1.2 To become familiar with the controls used with the Plexor[®] HY System.
- 7.1.3 To develop an understanding and working knowledge of the amplification reaction used in the Plexor[®] HY System.

7.2 Tasks

- 7.2.1 Read the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedures Manual, Section IX - Biomek[®] NX^P Automation Workstation Procedures Manual.
- 7.2.2 Quantitate the DNA from the 28 blood stains, 28 unmixed biological stains, 20 mixed biological stains, and validation sample sets addressed in Section 4, DNA Isolation. Note: In accordance with the instruction provided in Section 4 of this manual, the blood stain, unmixed biological stain, mixed biological stain, and the validation study sets will be isolated on the Biomek[®] Automation Workstation at different times.
- 7.2.3 Observe the Project Coordinator run the blood stains and unmixed biological stains through the entire Plexor[®] HY quantitation process, deck setup, and initiation of the Biomek[®] NX^P Software and the Plexor[®] data collection and data analysis programs.
- 7.2.4 Interpret the results from the Plexor[®] Analysis Software.
- 7.2.5 Look at the results of degraded samples using the Plexor[®] method in order to become familiar with the appearance of human DNA and bacterial DNA. Note: These previously isolated samples will be provided to the training coordinator by either the Biology Program Manager or the Forensic Molecular Biologist.
- 7.2.6 Compare the Plexor[®] HY System results to the yield gel results obtained in Chapter 5 for the 28 blood stains isolated in Chapter 4.
- 7.2.7 Read applicable literature and become familiar with the glossary terms. Refer to Appendices A, B, and C.
- 7.2.8 Continue on to Chapter 8, NORMALIZATION WIZARD AND AMPLIFICATION PROCESSES.

7.3 Training Evaluation

- 7.3.1 Knowledge
 - 7.3.1.1 Review of notes and worksheets in training notebook by training coordinator.
 - 7.3.1.2 Mini-mock trials/oral and practical examinations.
- 7.3.2 Skills
 - 7.3.2.1 The trainee should demonstrate an unquestionably sound technique for quantitating DNA and accurately interpreting the associated results. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

STUDY QUESTIONS:

1. Why would an analyst run the Plexor[®] HY quantitation method versus running a yield gel?

2. What DNA standards are used for the Plexor[®] HY quantitation method?
3. What primers are used in the Plexor[®] HY quantitation method and why?
4. What dye is utilized to detect the human male DNA concentration? The human autosomal DNA concentration? The IPC? The background?
5. For what is the IPC useful?
6. Explain how the Plexor[®] HY Quantitation System works.
7. Can the Plexor[®] HY Quantitation System quantitate highly degraded DNA samples? Why or why not?
8. Can the Plexor[®] HY Quantitation System quantitate DNA with inhibitors present? Why or why not?

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8 NORMALIZATION WIZARD AND AMPLIFICATION PROCESS

8.1 Goals

- 8.1.1 To develop an understanding and working knowledge of the amplification process, including proper documentation.
- 8.1.2 To become familiar with problems associated with amplification.
- 8.1.3 To understand the importance of an amplifying environment that has no contamination.
- 8.1.4 To understand the importance of quality control associated with the amplification process.

8.2 Tasks

- 8.2.1 Work in an environment free of contamination and follow proper guidelines to prevent contamination.
- 8.2.2 Program a thermal cycler and perform the quality control test on the thermal cycler, completing all appropriate documentation.
- 8.2.3 Perform manual amplification setup on half of the training samples addressed in Section 4, DNA Isolation, using the PowerPlex[®] 16 System. Refer to the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedures Manual, Section VIII - Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex[®] 16 System for the procedure.
- 8.2.4 Observe the Project Coordinator run the second half of the training samples addressed in Section 4, DNA Isolation, through the entire Normalization Wizard, amplification setup and deck setup using the Biomek[®] Automation Workstation.
- 8.2.5 Read applicable literature and become familiar with glossary terms. Refer to Appendices A, B, and C.
- 8.2.6 Continue on to Chapter 9, PRODUCT GEL or chapter 10, CAPILLARY ELECTROPHORESIS

8.3 Training Evaluation

8.3.1 Knowledge

- 8.3.1.1 Review of notes and worksheets in training notebook by training coordinator.
- 8.3.1.2 Mini-mock trials and/or question and answer sessions.

8.3.2 Skills

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

STUDY QUESTIONS:

1. What is a nuclease?
2. What is an endonuclease?
3. What is an exonuclease?
4. Explain the amplification process.

5. What is a DNA polymerase?
6. What is the name of the DNA polymerase that is used by the DFS and how does it work? Why do we use this DNA polymerase?
7. What is a primer?
8. What is the function of the primer?
9. What is the origin of the primer?
10. What is the function of $MgCl_2$?
11. What impact does the use of AmpliTaq Gold™ have on the PCR process?
12. What is primer - dimer?
13. How can primer - dimer affect the results?
14. Explain denaturation, annealing, and extension of the DNA.
15. 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?
16. What precautions are used to ensure that allelic drop out has not occurred?
17. What is plateau effect and how does it affect the DNA sample?
18. What are the components of the PowerPlex® 16 reaction mix? What is the purpose of each component?
19. What are some of the factors that inhibit amplification and why? What steps can the analyst take to overcome inhibition problems?
20. What are the amplification conditions for the PowerPlex® 16 System kit?
21. How many primers are used in the PowerPlex® 16 System?
22. What precautions are used to prevent contamination of the sample DNA with a foreign source?
23. What measures are taken to ensure that the thermal cycler is working properly? What is the purpose of each quality control test?

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9 PRODUCT GEL

9.1 Product Gel

9.1.1 Goals

- 9.1.1.1 To develop an understanding and working knowledge of the use of the product gel, including limitations, interpretation of results, and proper documentation.
- 9.1.1.2 To become familiar with the use of controls on the product gel.
- 9.1.1.3 To become familiar with the photographic procedures used to document product gel results.

9.2 Tasks

- 9.2.1 Prepare reagents and product gels necessary to evaluate the success of the amplification process. Refer to the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedures Manual, Section III - Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex[®] 16 BIO System for the procedure.
- 9.2.2 Prepare and run product gels.
- 9.2.3 Photograph the product gels.
- 9.2.4 Interpret the results of the product gels and use this information to determine if the samples should be typed and the volume of sample to load on the CE injection plate.
- 9.2.5 Read applicable literature and become familiar with glossary terms. Refer to Appendices A, B and C.
- 9.2.6 Continue on to Chapter 10, CAPILLARY ELECTROPHORESIS

9.3 Training Evaluation

9.3.1 Knowledge

- 9.3.1.1 Review of notes, photographs, and worksheets in training notebook by training coordinator.
- 9.3.1.2 Mini-mock trials and/or question and answer sessions.

9.3.2 Skills

- 9.3.2.1 The trainee should demonstrate an unquestionably sound technique for running product gels and accurately interpreting and properly documenting the results. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

STUDY QUESTIONS:

1. What is the purpose of a product gel?
2. What control is used on the product gel and why?
3. How large are the regions of interest that the analyst is trying to detect for the PowerPlex[®] 16 loci?
4. On the yield gel and/or Plexor[®] HY System, the analyst finds 10 ng/ul of DNA, but after amplification finds no DNA or primer-dimer on the product gel. What could be the reason?

10 CAPILLARY ELECTROPHORESIS

10.1 Goals

- 10.1.1 To become familiar with the theories of electrophoresis as they apply to capillary electrophoresis used in STR typing analyses.
- 10.1.2 To learn the parameters used for electrophoresis of DNA amplified with the PowerPlex® 16 typing System.
- 10.1.3 To develop an understanding and working knowledge of the use of the capillary electrophoresis instrumentation, including the limitations and proper documentation.
- 10.1.4 To become familiar with the controls associated with capillary electrophoresis.

10.2 Tasks

- 10.2.1 Prepare reagents and gels necessary to perform capillary electrophoresis of the DNA samples. Refer to the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedures Manual, Section VIII - Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 System for the procedure.
- 10.2.2 Run the PowerPlex® 16 System samples on the capillary electrophoresis instrument.
- 10.2.3 Read applicable literature and become familiar with glossary terms. Refer to Appendices A, B, and C.
- 10.2.4 Observe bubble remove wizard and common maintenance tasks: changing the polymer, running the water wash wizard, flushing the water trap, and defragmenting the hard drive.
- 10.2.5 Continue on to Chapter 11, CAPILLARY ELECTROPHORESIS DATA ANALYSIS AND INTERPRETATION.

10.3 Training Evaluation

10.3.1 Knowledge

- 10.3.1.1 Review of notes and worksheets in training notebook by training coordinator.
- 10.3.1.2 Mini-mock trials and/or question and answer sessions.

10.3.2 Skills

- 10.3.2.1 The trainee should demonstrate an unquestionably sound technique for running consistently interpretable PowerPlex® 16 samples on the capillary electrophoresis instrument using proper documentation. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

STUDY QUESTIONS:

1. What is capillary electrophoresis (CE)?
2. What is the function of the Hi-Di™ Formamide?
3. What is the function of the spectral calibration?
4. What is the function of the spatial calibration?

5. What controls are used on the CE? Why are these used?
6. Describe the signal processing from when a DNA fragment passes the detection window until a peak is observed on the electropherogram.
7. Describe the sample injection into the capillary. What is the formal name for this type of injection?
8. What type of polymer is used to separate DNA fragments on the CE? What is it composed of?

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11 CAPILLARY ELECTROPHORESIS DATA ANALYSIS AND INTERPRETATION

11.1 Goals

- 11.1.1 To develop a working knowledge of the GeneMapper™ ID software used for the analysis of the electropherogram data.
- 11.1.2 To become familiar with the interpretation of the electropherograms.
- 11.1.3 To become familiar with the base pair size range of the different PowerPlex® 16 loci.
- 11.1.4 To understand the use of controls and the internal lane standard.
- 11.1.5 To understand the problems that may be encountered with regard to interpretation.
- 11.1.6 To become familiar with the proper documentation of the results.

11.2 Tasks

- 11.2.1 Analyze the CE data using the GeneMapper™ ID software. Refer to the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Manual, Section VIII -Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 System for the procedure.
- 11.2.2 Interpret all results successfully and properly document the results.
- 11.2.3 Read applicable literature and become familiar with glossary terms. Refer to Appendices A, B, and C.

11.3 Training Evaluation

11.3.1 Knowledge

- 11.3.1.1 Review of notes, copies of all electropherograms (including the landscape printout) and other data related to the analytical process in the training notebook by the training coordinator.
- 11.3.1.2 Mini-mock trials and/or question and answer sessions.

11.3.2 Skills

- 11.3.2.1 The trainee should demonstrate a thorough understanding of all aspects of capillary electrophoresis interpretation by accurately interpreting PowerPlex® 16 results on all training samples and properly recording results. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

STUDY QUESTIONS:

1. What are the PowerPlex® 16 loci and on which chromosomes are they located?
2. The allelic ladder used to interpret the results at the PowerPlex® 16 loci consists of how many alleles?
3. The internal lane standard consists of how many peaks? What is the base pair size of each?
4. What is meant by a heteroduplex?
5. What is stutter? How is stutter differentiated from a true allele?
6. What is a spike? How is a spike differentiated from a true allele?

7. What is a non-template nucleotide addition?
8. What is allele/locus dropout and what can cause this to occur?
9. What is the genotype of the GM9947A cell line at each locus?
10. What is a microvariant? How does this differ from an off-ladder variant?
11. What is the base pair size range for each locus?
12. Some phenotypic XY males possess a deletion in the Y chromosome, resulting in the loss of AMEL Y sequence.
 - a. What is the estimated frequency of this deletion polymorphism?
 - b. What is the possible cause of this deletion polymorphism?
13. Please explain what a complex repeat unit means. Which of the PowerPlex[®] 16 loci are considered to have a complex repeat unit?

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12 STATISTICS, REPORT WRITING AND CODIS

12.1 Goals

- 12.1.1 To become familiar with the format and wording presently being used by DFS Forensic Biology examiners.
- 12.1.2 To become skilled in expressing the results of the PowerPlex® 16 typing of a sample in a clear, concise and technically correct fashion.
- 12.1.3 To become skilled in the computation and explanation of statistical information associated with PowerPlex® 16 typing results, using random match probabilities, likelihood ratios, and CPE.
- 12.1.4 To become familiar with the Combined DNA Index System applications/policies and procedures, and documentation required.

12.2 Tasks

- 12.2.1 Review Certificates of Analysis prepared by examiners and compare to the corresponding electrophoretic data and landscape printouts.
- 12.2.2 Draft reports on actual case data and compare these to the Certificates of Analysis issued by the examiners. Refer to the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedures Manual, Section VIII - Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 System for the procedure.
- 12.2.3 Compute statistical information on actual casework samples typed at the PowerPlex® 16 System loci. Compare this information to that reported by the examiner.
- 12.2.4 Read and become familiar with the Commonwealth of Virginia Department of Forensic Science CODIS Operating Policies and Procedures Manual.
- 12.2.5 Read applicable literature and become familiar with glossary terms. Refer to Appendices A, B, and C.
- 12.2.6 Perform CODIS searches under the direct supervision of qualified examiners and generate the proper documentation associated with the CODIS search.

12.3 Training Evaluation

12.3.1 Knowledge

- 12.3.1.1 Review of notes, computations, and reports prepared by the trainee in training notebook by training coordinator.
- 12.3.1.2 Mini-mock trials and/or question and answer sessions.

12.3.2 Skills

- 12.3.2.1 The trainee should demonstrate the ability to clearly, accurately and concisely set forth STR analytical results, including statistical information using random match probabilities, likelihood ratios, and combined probability of exclusion (CPE) in a report.
- 12.3.2.2 The trainee should demonstrate the ability to use CODIS.

13 TESTIMONY AND EXPERT WITNESS QUALIFICATION

13.1 Goals

- 13.1.1 To demonstrate technical knowledge by successfully completing an oral examination.
- 13.1.2 To successfully demonstrate skill in analysis and testimony by completing the analysis of a mock case and testifying on that case in a mock trial.
- 13.1.3 To become familiar with the legal aspects of DNA typing, including common challenges, controversial cases and case histories.

13.2 Tasks

- 13.2.1 Undergo an oral examination
- 13.2.2 Conduct analysis on a mock case, prepare a Certificate of Analysis and testify to the results of the analysis in a mock trial.
- 13.2.3 Become familiar with DNA testing guidelines/standards set forth by the following groups:
 - Scientific Working Group on DNA Analysis Methods (SWGDM)
 - American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB[®])
 - FBI Quality Assurance Standards
 - International Organization for Standardization (ISO) 17025
- 13.2.4 Observe examiners testify when possible.
- 13.2.5 Participate in at least one pretrial conference with a qualified examiner.
- 13.2.6 Give lectures on DNA analysis to law enforcement personnel and lay groups to practice explanations and public speaking skills.
- 13.2.7 Read applicable literature and become familiar with glossary terms. Refer to Appendices A, B, and C.

13.3 Training Evaluation

- 13.3.1 Knowledge
 - 13.3.1.1 Mini-mock trials and/or question and answer session.
- 13.3.2 Skills
 - 13.3.2.1 The trainee should demonstrate the ability to clearly and accurately testify to STR analytical results and conclusions, including statistical information.

STUDY QUESTIONS:

1. Are you certified or do you have a license to conduct forensic DNA analyses?
2. Are you an expert in the field of molecular biology?
3. How do you become a member of a professional organization such as the AAFS? Can anyone be a member? If I pay my dues can I (Defense Attorney) be a member too?

4. What is a proficiency test? Do you participate in proficiency testing? What is your error rate? Do you participate in blind proficiency testing?
5. What does SWGDAM stand for and what is this organization's function?
6. Have you had any statistics courses? Are you a statistician? population geneticist?
7. What is a DNA audit? How often is your laboratory audited? Who performs the audit?
8. What is the rationale for having different laboratory areas for isolation, PCR setup, amplification and typing?
9. What controls do you run during your PCR analyses?
10. What is a null allele?
11. What is the difference between a genotype and a phenotype?
12. What is the misincorporation rate of the *Taq* enzyme?
13. When was the first time PCR was used in this country? in Virginia?
14. How were the databases that are used by you created? Explain each step in detail.
15. How large is your database? How can you calculate 1 in billions or trillions from a database of only 100 people for each race?
16. What is linkage disequilibrium and how does this affect PowerPlex[®] 16 analyses? What does independence mean?
17. What is Hardy Weinberg Equilibrium? Is your database in Hardy Weinberg Equilibrium?
18. How do allele frequencies differ from genotypic frequencies? How are allele frequencies calculated? How are genotype frequencies calculated?
19. What are substructures or subpopulations and how does the existence of these affect your reporting of statistics?
20. Why do you report Caucasian, Black and Hispanic frequencies versus reporting only one general frequency? If my client were Asian, how would you determine the frequency?
21. What does the Power of Discrimination mean (Pd)? How is this computed?
22. What is a random match probability? How is it calculated? When is it used?
23. What is a likelihood ratio? How is it calculated? When is it used? Why is it used instead of a random match probability?
24. What is CPE? How is it calculated? When is it used? Why is it used instead of a random match probability or a likelihood ratio?
25. What does DAB stand for and what is its function? How was it created and why? Is it still in existence?
26. Explain the purpose of the FBI Quality Assurance Standards.
27. What does ASCLD/LAB[®] stand for and what is its function?

28. Who serves as the Department's Quality Assurance Officer?
29. Who serves as the Department's Safety Officer?

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14 HAIR ROOT EVALUATION FOR STR ANALYSIS

14.1 Goals

- 14.1.1 To become familiar with the microscopic structures of hair.
- 14.1.2 To become acquainted with the basic differences in human and animal hairs.
- 14.1.3 To understand the fundamentals of hair biology as it relates to hair growth, regression and rest.
- 14.1.4 To recognize the microscopic characteristics of human hair roots in the growth, regression and rest stages.
- 14.1.5 To recognize the hair root forms which may be suitable for STR typing based upon their microscopic morphology.
- 14.1.6 To become familiar with the preparation of temporary microscope slides containing hairs.
- 14.1.7 To become familiar with photomicroscopic documentation of hairs.

14.2 Tasks

14.2.1 Attend 4 lectures:

14.2.1.1 Hair Biology; Hair Microscopic Examination and Hair Root Evaluation for Nuclear DNA Typing

14.2.1.2 Stereomicroscopic Views of Human Hair Roots

14.2.1.3 Hair Evaluation for Nuclear DNA Typing (Examples)

14.2.1.3.1 Part I: Animal versus Human versus Textile Fiber

14.2.1.3.2 Part II: Root Suitability for STR Typing

14.2.1.4 Use of the digital camera with the compound microscope

14.2.2 Examine and sketch from reference slides the microscopic appearance of at least 2 dog hair telogen roots absent tissue and at least 2 cat hair telogen roots absent tissue.

14.2.3 Examine and sketch from reference human hair samples the microscopic appearance of:

- Anagen/catagen head hair root bulb with attached follicular tissue
- Anagen/catagen head hair root bulb absent attached follicular tissue
- Telogen head hair root club with attached follicular tissue
- Telogen head hair root club absent attached follicular tissue
- Anagen/catagen pubic hair root bulb with or without attached follicular tissue
- Telogen pubic hair root club with attached follicular tissue
- Telogen pubic hair root club absent follicular tissue

14.2.4 Trainee will gently remove several head hairs from their scalp, mount on temporary microscope slide; sketch and identify root forms. A microscopic root image will be captured with digital photography using a 20x objective; 10x ocular.

14.2.5 Trainee will forcibly remove several head hairs from their scalp, mount on temporary microscope slide; sketch and identify root forms. A microscopic root image will be captured with digital photography using a 20x objective; 10x ocular.

14.3 Training Evaluation

14.3.1 Knowledge

- 14.3.1.1 Review of trainee's sketches, notes and photographs by the training coordinator.
- 14.3.1.2 Trainee will complete a practical exercise determining hair root suitability for STR typing using the compound microscope. Hairs in Permound on microscope slides.
- 14.3.1.3 Trainee will complete a practical exercise determining hair root suitability for STR typing using the stereomicroscope. Hairs on post-it-notes.
- 14.3.1.4 Review and discussion of the practical exercises by the training coordinator.

Reference Materials:

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3. Evaluation of the human hair root for DNA typing subsequent to microscopic comparison. Linch CA, Smith SL, Prahlow JA. *J For Sci* 1998; 43(2): 305-314.
4. Postmortem microscopic changes observed at the human head hair proximal end. Linch CA, Prahlow JA. *J For Sci* 2001; 46(1): 15-20

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15 Y-STR ANALYSIS

15.1 Introduction

This is a guide designed to lead the qualified and practicing Forensic Biology Examiner (Forensic Scientist) through the procedures necessary to qualify as a Y-STR Examiner in the Virginia Department of Forensic Science (VA DFS) Forensic Biology Section. The guide is not inclusive of all the techniques and procedures that will be encountered during and after training. In addition, the order in which minimum sample sets are listed is arbitrary. Any or all of the samples may be processed in any order and concurrently if desired.

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

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

Because the Y-STR Examiner Trainee is a previously qualified and practicing Forensic Biology Examiner, areas such as General Lab Practice, Safety, Evidence Handling, Legal Aspects, Administration, etc. can be referred to in the Forensic Biology Section Training Manuals for Case Approach and Casework and are not specifically covered under this training program.

15.2 Examiner Qualification

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

15.2.1.1 Successful completion of the required training samples.

15.2.1.2 Satisfactory completion of an oral technical competency exam to include both technical questions as well as court-style questions pertaining to all aspects of Forensic Biology examination but focusing on aspects related specifically to Y-STR analysis and casework.

15.2.1.3 Satisfactory completion of a practical exam which will consist of mock case extracts/stains that must be examined as if these items were actual case submissions. This test will evaluate the trainee's skill in note taking, data reporting, accuracy in performance of Y-STR PCR-based DNA typing procedures and case file documentation practices.

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

15.2.3 A checklist of the required training will be maintained and reviewed by the training coordinator.

15.3 Required Knowledge and Skills

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

15.3.2 Knowledge areas specific to Y-STR analysis

15.3.2.1 Methods for assessing the quantity and quality of male DNA

15.3.2.2 Nature of the polymorphic regions examined by the VADFS on the Y chromosome

15.3.2.3 Methods for the reconstitution of dried extracts of DNA

- 15.3.2.4 Biochemistry of the Polymerase Chain Reaction (PCR)
- 15.3.2.5 Principles of detection of fragment-length polymorphisms – specifically capillary electrophoresis
- 15.3.2.6 Y-STR loci examined by the VADFS
- 15.3.2.7 Quality control/quality assurance measures used by the VADFS
- 15.3.2.8 Statistical calculations – particularly the counting method and application of an upper bounds 95% confidence interval
- 15.3.2.9 The trainee is required to read all articles and references listed in the Fluorescent Detection PCR-Based STR DNA Protocol: AMPFLSTR® YFILER™ - Forensic Biology Section Procedure Manual, Section VII
- 15.3.3 Instrument Operation
 - 15.3.3.1 Thermal Cycler
 - 15.3.3.2 Capillary electrophoresis instrumentation
- 15.3.4 DNA Typing Procedures
 - 15.3.4.1 Steps and procedures are defined in the Fluorescent Detection PCR-Based STR DNA Protocol: AMPFLSTR® YFILER™ - Forensic Biology Section Procedure Manual, Section VII.

15.4 Training Samples

- 15.4.1 The trainee will demonstrate the skills required of a Y-STR examiner by completing, at a minimum, the following sample sets from amplification through the typing/data analysis process:
 - 15.4.1.1 At least five (5) dilutions of male DNA
 - 15.4.1.2 At least five (5) non-probative casework samples from normalization wizard plates
 - 15.4.1.3 At least five (5) female DNA samples
 - 15.4.1.4 At least five (5) male:male mixtures of DNA of varying concentrations
 - 15.4.1.5 At least five (5) environmentally exposed samples
 - 15.4.1.6 At least five (5) male:female mixtures of varying concentrations with an excess of female DNA
- 15.4.2 It may be necessary that the trainee carry some or all samples from extraction on; however, if extracts are available, the trainee may choose to use them in lieu of extracting them him/herself. Plexor quantitation should be performed on all extracts prior to Y-STR amplification. Again, if plexor data is already available for accessible extracts, the trainee may use that data in lieu of having Plexor performed again.
- 15.4.3 During the completion of these minimum sample requirements, a total of at least five (5) separate amplifications and five (5) separate capillary electrophoretic runs should be conducted so as to demonstrate the skills necessary in conducting each of these portions of the analysis.
- 15.4.4 In addition, the trainee must perform manually the calculations performed by the US YSTR database for a minimum of three (3) different Y-STR profiles to be provided by the training coordinator.

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APPENDIX B - GLOSSARY

“A”:

Terminal nucleotide addition occurs when Taq DNA polymerase adds a nucleotide, generally adenine, to the ends of amplified DNA fragments in a template-independent manner. The efficiency with which this occurs varies with different primer sequences. Thus, an artifact peak one base pair shorter than expected (i.e., missing the terminal addition) is sometimes seen. Use of more template than recommended can generate incomplete terminal nucleotide addition at some loci.

ADENINE:

A purine base; one of the four molecules containing nitrogen present in the nucleic acids DNA and RNA; designated by letter A.

ALLELE:

One of two or more alternative forms of a gene.

ALLELE FREQUENCY:

The proportion of a particular allele among the chromosomes carried by individuals in a population.

ALLELIC LADDER:

An allelic ladder is an artificial mixture of common alleles present in the human population for a particular STR marker. They are generated with the same primers as tested samples and thus provide a reference DNA size for each allele included in the ladder.

AMINO ACIDS:

The building blocks of proteins. There are 20 common amino acids; they are joined together in a strictly ordered "string" that determines the character of each protein.

AMPLIFICATION:

Increasing the number of copies of a specific segment within a DNA chain. “Building” DNA. PCR DNA analysis uses this technique to amplify specimens that are too small to use for the RFLP method.

ANNEAL:

The process by which the complementary base pairs in the strands of DNA combine.

AUTOSOME:

A chromosome not involved in sex determination. The diploid human genome consists of 46 chromosomes, 22 pairs of autosomes, and one pair of sex chromosomes (the X and Y chromosomes).

BASEPAIR:

Two complementary nucleotides joined by hydrogen bonds; basepairing occurs between A and T and between G and C.

BASE SEQUENCE:

The order of nucleotide bases in a DNA molecule.

CHROMOSOME:

A discrete unit of the genome carrying many genes, consisting of proteins and a very long molecule of DNA, visible as a morphological entity only during the act of cell division. The entire human genome is tightly packaged into 23 pairs of chromosomes which are located within the nucleus of the cell.

CLONING:

The procedure for producing identical DNA sequences.

CODIS:

The COmbined DNA Index System. CODIS refers to the entire system of DNA indexes (convicted offender index, close biological relatives index, population file, forensic index, unidentified persons index, and missing persons index) maintained at the National, State, and Local levels.

CODON:

A group of three bases on the DNA molecule that will code for an amino acid, the chemical units of proteins.

CROSS-HYBRIDIZATION:

An interaction of a DNA sequence with another sequence (e.g., probe) that is not perfectly complementary to it. Cross-hybridization occurs at low stringency. See also DNA heteroduplex.

CYTOSINE:

A pyrimidine base; one of the four molecules containing nitrogen present in the nucleic acids DNA and RNA; designated by letter C.

DNA ADVISORY BOARD (DAB):

A board to develop standardized DNA quality assurance methods appointed by the FBI Director in accordance with the DNA Identification Act of 1994.

DEGRADATION:

Partial or complete deterioration of a biological substance by chemical or physical means.

DELETIONS:

Results from the removal of a sequence of DNA, the regions on either side being joined together.

DENATURATION:

Conversion of DNA from the double-stranded to the single-stranded state, usually accomplished by heating to destroy chemical bonds involved in base pairing.

DIFFERENTIAL AMPLIFICATION (ALSO REFERRED TO AS ALLELIC DROP OUT):

The preferential amplification of one allele over another, which can result from incomplete denaturation of the target molecules of one allele. Alternatively, this may occur when one of two alleles is replicated more readily than the other, as in amplification of genetic loci in which some alleles are much longer than others (VNTR region).

DNA HETERODUPLICATION:

Double stranded DNA molecule in which the two strands do not have completely complementary base sequences.

DNA PROFILE:

A DNA profile consists of a set of DNA identification characteristics, i.e., the particular chemical form at the various DNA locations (loci), which permit the DNA of one person to be distinguishable from that of another person.

ELECTROPHORESIS:

A technique in which molecules are separated by their velocity in an electric field.

ENZYMES:

Proteins that catalyze specific biochemical reactions, such as *Taq* polymerase, which cause the addition of bases.

EXPONENTIAL AMPLIFICATION:

Replication of DNA in which the copy number of the target sequence approximately doubles in each cycle or round of replication.

FLUORESCENCE:

Emission of or the property of emitting electromagnetic radiation usually as visible light resulting from and occurring only during the absorption of radiation from some other source.

GEL:

Semisolid matrix (usually agarose or acrylamide) used in electrophoresis to separate molecules.

GENE:

A stretch along a chromosome that codes for a functional product (either an RNA molecule or its translation product, a polypeptide).

GENOTYPE:

The total of the genetic information contained in the chromosomes of an organism; the genetic makeup of an organism.

GUANINE:

A purine base; one of the four molecules containing nitrogen present in the nucleic acids DNA and RNA; designated by letter G.

HARDY WEINBERG EQUILIBRIUM:

A principle of population genetics which states that population gene frequencies and population genotype frequencies remain constant from generation to generation if mating is random and if mutation, selection immigration and emigration do not occur. If these assumptions are true, it should be possible to calculate genotype frequencies from observed allele frequencies.

HETEROZYGOTE:

A fertilized egg (zygote) with two different alleles at a designated locus. An individual organism that has different alleles of a particular gene on each member of a pair of chromosomes. An organism is heterozygous to a given gene if its two alleles are different.

HOMOZYGOTE:

A fertilized egg with two identical alleles at a designated locus. An individual organism having identical alleles of a particular gene on each member of a pair of chromosomes. An organism is homozygous to a given gene if its two alleles are the same.

IN VITRO:

Outside a living organism

JOE:

6-Carboxy-2',7'-dimethoxy-4',5'-dichlorofluorescein. Fluorescent dye used for STR allele labeling.

LINKAGE:

Describes the tendency of genes to be inherited together as a result of their location on the same chromosome; measured by percent recombination between loci.

LOCUS:

The position on a chromosome at which the gene for a particular trait resides; locus may be occupied by any one of the alleles for the gene.

MENDELIAN INHERITANCE:

The passing of genes from parent to progeny according to a pattern of independent segregation of alleles and the independent assortment of unlinked genes (e.g., genes on different chromosomes) during the formation of gametes.

MICROVARIANT:

Alleles that have a similar intensity to the other major bands for a locus but will not align with the allelic ladder. Alleles with one, two, or three nucleotides shorter than the common four base repeat alleles which are located between two alleles on the ladder are reported in accordance with the recommendations of the DNA Commission on the International Society of Haemogenetics. The number of complete repeat units is represented by an integer and any partial repeat is designated by a decimal followed by the number of bases in the partial repeat. Therefore a band occurring between 5 and 6 alleles and which is 1 bp from the 5 allele is designated as a 5.1.

MUTATION:

Any change in DNA sequence.

NUCLEIC ACID:

A nucleotide polymer of which DNA and RNA are major types.

NUCLEOTIDE:

A unit of nucleic acid composed of phosphate, ribose or deoxyribose, and a purine or pyrimidine base.

OLIGONUCLEOTIDE:

Single stranded DNA molecule of two or more nucleotide units in length.

PCR PRODUCT:

The double stranded DNA fragment of defined size and sequence which results from the PCR amplification process.

PLATEAU EFFECT:

A phenomenon of late stages of PCR amplification in which there is a progressive attenuation in the rate at which target sequence accumulates in each successive cycle.

POLYMERASE CHAIN REACTION (PCR):

An in vitro process that yields millions of copies of desired DNA through repeated cycling of a reaction involving the DNA polymerase enzyme.

POLYMORPHISM:

Difference in DNA sequence among individuals. Genetic variations occurring in more than 1% of a population would be considered useful polymorphisms for linkage analysis.

POPULATION:

A group of individuals residing in a given area at a given time.

PRIMERS:

Oligonucleotides which serve as growing points for polymerization of a new strand of DNA along a complementary template strand.

PULL-UP:

The phenomenon of "pull-up" (a.k.a. "bleed through") can occur with incomplete separation of colors. Care must be taken when evaluating electropherograms to determine if off-ladder peaks or minor peaks are not a result of bleed through. This is easy to determine by checking the results at the other dyes. This phenomenon occurs most often when the amplification reaction was overloaded or the spectral needs to be re-run.

SEX CHROMOSOMES (X AND Y CHROMOSOMES):

Chromosomes that are different in the two sexes and involved in sex determination.

SHORT TANDEM REPEATS (STR):

Multiple copies of an identical DNA sequence arranged in direct succession in a particular region of a chromosome.

STOCHASTIC FLUCTUATION:

A phenomenon occurring during the amplification of low levels of DNA resulting in an unequal sampling of the two alleles present from a heterozygous individual.

STUTTER:

Some STR loci have a tendency to produce one or more minor PCR products which are typically smaller than the major allele by 1 (n-1), 4 (n-4), or 10 (n-10) bases, depending on the locus. Generally peak height can readily distinguish true alleles from "stutter" peaks.

THYMINE:

A pyrimidine base; one of the four molecules containing nitrogen present in the nucleic acids DNA; designated by letter T.

URACIL:

A pyrimidine base with the same chemical composition as Thymine, but lacking a methyl group, found in RNA in place of thymine. Designated by the letter U.

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APPENDIX C - ADDITIONAL TRAINING AIDS

"Rapid and Efficient Resolution of Parentage by Amplification of Short Tandem Repeats," R. L. Alford, H. A. Hammond, I. Coto, and C. T. Caskey, American Journal of Human Genetics, Vol. 55: 190-195, 1994.

STR loci occur throughout the genome at an estimated frequency of one STR every 300-500 kb.

"Progress in a Genome Scan for Linkage in Schizophrenia in a Large Swedish Kindred," C. L. Barr, American Journal of Medical Genetics (Neuropsychiatric Genetics), Vol. 54: 51-58, 1994.

STRs are used in diagnostic, clinical, and genetic mapping of such medical disorders as Schizophrenia, as well as for linkage and linkage disequilibrium mapping.

"Instability of Short Tandem Repeats (Microsatellites) in Human Cancers," R. Wooster and A. M. Cleton-Jansen, Nature Genetics, Vol 6: 152-156, 1994.

The allele sizes of polymorphic microsatellite repeats in DNA from human cancers were compared to normal DNA from the same patients. In 16 out of 196 paired samples evidence of an extra allele of a different size in the tumor was found which was not present in the normal DNA.

Based on the study that was conducted the authors found that there is instability of microsatellite repeats in several human cancers (Myotonic Dystrophy, X-linked spinal and bulbar muscular atrophy [Kennedy's syndrome], Huntington's disease, breast cancer, soft tissue sarcoma, brain cancer, and ovarian cancer).

One of the loci that was examined included the tetranucleotide vWA (found within an intron of the gene encoding von Willebrand's factor).

Approximately 10% of breast cancers, sarcomas and ovarian cancers exhibited additional alleles.

Because PCR amplification can sometimes generate spurious bands, each experiment in which an extra allele was detected was repeated at least three times. The results were consistent in all the experiments.

"Slippage Synthesis of Simple Sequence DNA," Christian Schlotterer and Diehthard Tautz, Nucleic Acids Research, Vol. 20: 211-215, 1992.

Slippage synthesis occurs in vivo on a fixed template where only one strand is free to move, a situation which resembles chromosome replication. It seems therefore likely that slippage during replication is the cause of the observed length polymorphisms between individuals in a population.

As the size of the repeat unit increases from a di- or tri-, to a tetranucleotide repeat, the growth of the repeat products is slower, which suggests that the slippage rate is slower. Different possible combinations of nucleotide repeat motifs have been examined and it has been demonstrated that they all grow at different rates. AT repeats grow faster than GC repeats, indicating that slippage is potentially dependent on the AT-content of the sequences involved.

"The Evolutionary Dynamics of Repetitive DNA in Eukaryotes," Brian Charlesworth, Paul Sniegowski, and Wolfgang Stephan, Nature, Vol. 371, September 15, 1994.

The behavior of repetitive sequences can result in mutations that cause genetic diseases which confer significant fitness losses on the organism.

In vitro studies suggest that strand slippage during DNA replication is the major cause of the observed length polymorphism of microsatellites within populations.

Definitions:

Microsatellite sequences: arrays of short (2-5 bp) nucleotide repeats found in vertebrate, insect and plant genomes. At least 30,000 microsatellite loci are present in the human genome. Copy numbers are characteristically variable within a population.

Minisatellite sequences: arrays of longer (~ 15 bp) repeats, generally involving mean array lengths of 0.5-30 kb. They are found in the genome of vertebrates, fungi, and plants, and are highly variable in size.

"Substrate Nucleotide-Determined Non-Template Additions for Adenine by *Taq* DNA Polymerase: Implications of PCR-Based Genotyping and Cloning," V. L. Magnuson, D. S. Ally, S. J. Nylund, Z. E. Rayman, J. I. Knapp, A. L. Lowe, S. Ghosh, and F. S. Collins, *BioTechniques* 21: 700-709, 1996.

Certain terminal nucleotides can either inhibit or enhance adenine addition by *Taq* and the PCR primer design can be used to modulate this activity.

Since the 5' end of the forward primer carries the fluorescent label, it is only this strand that is detected.

For some PCR products, extended time at 4⁰C or room temperature is enough to allow the PCR to proceed further towards the allele + A (5%-25% after 2 days at 4⁰C).

As a general rule, it was demonstrated that when the 5' end nucleotide of the reverse primer was replaced with a "T", a 3' "A" terminal nucleotide would occur on the forward strand. The presence of an adenine on the 3' end of the PCR product is inhibitory to adenine addition by *Taq* DNA polymerase. The removal of "A" as terminal substrate nucleotide markedly enhances the PCR product to proceed to ≥ 70% allele + A.

"Sequence Analysis and Characterization of Stutter Products at the Tetranucleotide Repeat Locus vWA," P. Sean Walsh, Nicola J. Fildes, and Rebecca Reynolds, *Nucleic Acids Research*, Vol. 24: 2807-2812, 1996.

Definition:

Stutter: The PCR amplification of tetranucleotide short tandem repeat (STR) loci produces a minor product band 4 base pairs shorter than the corresponding main allele band. This is also referred to in the literature as shadow bands, DNA polymerase slippage product, or n-4 bands.

PCR amplification results from tetranucleotide repeat loci are easier to interpret than dinucleotide repeats because only a single band versus possible multiple bands for dinucleotides is observed in a position four bases shorter than each allele band, and the intensity of the stutter band is generally <10% of the main band.

The proportion of stutter product relative to the main allele increases as the number of uninterrupted core repeat units increases.

The most common repeat motif in the variable expansion region of a STR locus is referred to as the "core" repeat sequence.

The proportion of stutter peak can be overestimated for alleles that are 8 base pairs longer than the other alleles in the same sample (heterozygote). The overestimation is due to the fact that the stutter peak resides on the shoulder of the peak for the shorter allele. It may be possible to minimize this effect by running longer gels or implementing other gel condition modifications that increase peak sharpness or resolution.

Taq Polymerase has no 3' → 5' exonuclease (proofreading) activity, but does have a 5' → 3' exonuclease activity.

The mechanism known as slipped strand mispairing has been proposed to explain the stutter bands that result from amplification. According to this proposal, the template strand and extending strand can break apart during synthesis through the repeat region, perhaps when/if the DNA polymerase has fallen off during PCR. A single repeat unit can then loop out in the template strand before the two strands re-anneal. The result is that the newly extended strand will have one fewer repeat unit than the template strand when synthesis is complete.

It is also conceivable in slipped strand mispairing that the extending strand could loop out, thus resulting in a newly extended strand having an additional repeat unit relative to the template strand. Even though this is possible, the most prevalent stutter bands are shorter than the main allele.

A speculative explanation for the relative lack of longer stutter bands is that the DNA polymerase may associate with the stretch of DNA at the 3' end of the extending strand when the strands are unpaired. This association may then somehow inhibit loop out formation in the extending strand.

It is possible that the template strand has more of an opportunity and/or tendency to loop out when long stretches of core repeats are present. Also the strand alignment that exists when loop out does occur in an interrupted core repeat stretch is less likely to position the 3' end of the extending strand across from a complementary base. The polymerase then would not complete synthesis of what would otherwise become the shortened, stutter strand.

However in the case of vWA and most other tetranucleotide repeat loci, stutter bands longer than the main allele have not been observed.

When choosing loci to incorporate into a multiplex to reduce the overall amount of stutter, it is recommended to choose loci whose alleles contain overall fewer repeat units and a core repeat that is interrupted by a four base pair sequence substitution.

"Defining Microsatellite Alleles by Genotyping Global Indigenous Human Populations and Non-Human Primates," Li Jinj, Peter Underhill, Martin Buoncristiani, and James M. Robertson, Journal of Forensic Sciences, Vol. 42: 496-499, 1997.

Chimpanzee DNA cannot be amplified at the D7S820 locus. This is either due to a nucleotide substitution(s) at priming regions or an absence of the locus in the Chimp genome.

D5S818 alleles in Chimpanzees differ by increments of two base pairs instead of four, suggesting that there may be a dinucleotide repeat associated with the locus.

"Mixture Interpretation: Guidelines for Defining the Relevant Features for Assessment of Mixed DNA Profiles in Forensic Casework", Bruce Budowle, Anthony Onorato, Thomas Callaghan, et al. Journal of Forensic Sciences, Vol. 54 (3), 2009.

All of the relevant electropherogram information (such as peak height ratios) must be considered when differentiating whether or not an individual can be included as a contributor to a mixture or not. Generally, reproducible patterns are observed, i.e. a donor contributing the majority of DNA to a mixture will generate larger peaks than the minor contributor. Peak height ratios may be used to aid in differentiating which alleles may have come from the same individual when there is a large enough disparity in the peak heights for the alleles in the mixture. Peaks that overlap from contributors will generally display an additive effect on the shared peak height. A peak amplitude threshold (PAT) should be defined for the typing system and detection platform used. A minimum interpretation threshold (MIT) is defined as the threshold above which stochastic effects are no longer observed. A lab may choose to set the PAT and MIT at the same value or choose to define different thresholds for these.

WHAT IS MEANT BY A NON-NUCLEOTIDE ADDITION?

A terminal nucleotide addition occurs when *Taq* DNA polymerase adds a nucleotide, generally adenine, to the end of amplified DNA fragments in a template-independent manner. Thus, an artifact band, one base shorter than expected (i.e. missing the terminal addition), is sometimes seen. This activity often does not occur with 100% efficiency and varies with different primer sequences.

IF YOU WERE DESIGNING PRIMERS FOR PCR AMPLIFICATION, HOW WOULD YOU DESIGN THE REVERSE PRIMER TO REDUCE THE AMOUNT OF NON-TEMPLATE NUCLEOTIDE ADDITIONS? WHY?

As a general rule, it was demonstrated that when the 5' end nucleotide of the reverse primer was replaced with a "T", the result would be a 3' "A" terminal nucleotide on the forward strand. The presence of an adenine on the 3' end of the PCR product is inhibitory to adenine addition by *Taq* DNA polymerase. The removal of "A" as terminal substrate nucleotide markedly enhances the PCR product to proceed to $\geq 70\%$ allele + A.

WHAT PORTION OF THE AMPLIFICATION CYCLE IS DESIGNED TO REDUCE NON-TEMPLATE NUCLEOTIDE ADDITIONS?

Addition of a final extension step at 60°C for 30 minutes to the amplification protocol can lead to conditions of essentially full terminal nucleotide addition.

WHAT IS MEANT BY STUTTER?

The PCR amplification of tetranucleotide short tandem repeat (STR) loci produces a minor product band 4 base pairs shorter than the corresponding main allele band. This is also referred to in the literature as shadow bands or DNA polymerase slippage product.

WHAT CAUSES STUTTER?

The mechanism known as slipped strand mispairing has been proposed to explain the stutter bands that result from amplification. According to this proposal, the template strand and extending strand can breath apart during synthesis through the repeat region, perhaps when/if the DNA polymerase has fallen off during PCR. A single repeat unit can then loop out in the template strand before the two strands re-anneal. The result is that when synthesis is complete, the newly extended strand will have one fewer repeat unit than the template strand.

It is also conceivable in slipped strand mispairing that the extending strand could loop out, thus resulting in a newly extended strand having an additional repeat unit relative to the template strand. Even though this is possible, the most prevalent stutter bands are shorter than the main allele.

WHY IS IT MORE COMMON FOR THE TEMPLATE STRAND TO LOOP OUT VERSUS THE EXTENDING STRAND?

A speculative explanation for the relative lack of longer stutter bands is that the DNA polymerase may associate with the stretch of DNA at the 3' end of the extending strand when the strands are unpaired. This association may then somehow inhibit loop out formation in the extending strand.

It is possible that the template strand has more of an opportunity and/or tendency to loop out when long stretches of core repeats are present. Also the strand alignment that exists when loop out does occur in an interrupted core repeat stretch is unlikely to position the 3' end of the extending strand across from a complementary base. The polymerase then would not complete synthesis of what would otherwise become the shortened, stutter strand.

TRUE OR FALSE:

TAQ POLYMERASE HAS A 3' → 5' EXONUCLEASE (PROOFREADING) ACTIVITY. False

TRUE OR FALSE:

THE MOST COMMON REPEAT MOTIF IN THE VARIABLE EXPANSION REGION OF A STR LOCUS IS REFERRED TO AS THE "CORE" REPEAT SEQUENCE. True

TRUE OR FALSE:

THE 5' END OF THE REVERSE PRIMER CARRIES THE FLUORESCENT LABEL, WHICH IS DETECTED BY THE FMBIO. False, the 5' end of the forward primer is labeled.

WHEN CHOOSING LOCI TO INCORPORATE INTO A MULTIPLEX TO REDUCE THE OVERALL AMOUNT OF STUTTER, IT IS RECOMMENDED TO CHOOSE:

- A. Loci whose alleles contain overall fewer repeat units
- B. Loci that contain dinucleotide repeats
- C. Loci that contain a core repeat that is uninterrupted by a four base pair sequence substitution
- D. A and C
- E. All of the above

D. A and C

DEFINE MICROVARIANT AND GIVE AN EXAMPLE OF A MICROVARIANT.

Alleles differing from one another by lengths other than the full repeat length. Examples: TH01 9.3 and 8.3

WHAT ARE SOME OF THE REASONS FOR "FUZZY" BANDS THROUGHOUT THE GEL?

1. Poor quality polyacrylamide gel
2. Electrophoresis temperature is too high

EXPLAIN THE NOMENCLATURE THAT IS USED TO CALL AN ALLELE AT A PARTICULAR LOCUS, FOR EXAMPLE, FGA 22 VERSUS FGA 22.2.

Alleles are assigned based upon their repeat lengths. The FGA 22.2 allele is a microvariant with 22 complete repeats, with an incomplete repeat of an additional 2 bases.

AS A GENERAL RULE, WE WORK WITH STR LOCI WITH MODERATELY HIGH POLYMORPHISM. WHY DO WE NOT WORK WITH HIGHLY POLYMORPHIC LOCI?

There appears to be a correlation between a high degree of polymorphism and a tendency for microvariants and increased mutation rates.

I GENERAL MOLECULAR BIOLOGY QUESTIONS

1. Approximately how many base pairs are found in a single human diploid cell?
2. DNA is found both in the ? and the ? of a typical eukaryotic cell.
3. What is one difference between a prokaryote and eukaryote cell?
4. What is the name of the process by which a diploid parent cell gives rise to two diploid daughter cells?
5. A change in a wild type genetic sequence is commonly referred to as a ? .
6. Who is known as the "Father of Genetics"?
7. If an individual has a genetic profile consisting of the same alleles at a locus (e. g., CSF1PO 12, 12), he is said to be ? at this locus.
8. If an individual has a genetic profile consisting of different alleles at a locus (e. g., TH01 8, 10), he is said to be ? at this locus.

9. Replication of DNA is accomplished using an enzyme called a ?.
10. DNA replication involves the disruption of the double helix at a junction known as the ?.
11. With regard to DNA replication, synthesis occurs in what direction?
12. The enzyme responsible for unwinding the DNA in preparation for replication is called ?.
13. An enzyme which cleaves nucleotides from the end of a DNA chain is called a ?.
14. A triplet of nucleotides in a DNA sequence representing an amino acid is called a ?.
15. Proteins are composed of linked organic molecules called ?.
16. DNA is cloned by inserting sequence fragments into circular DNA vectors called ?.
17. What are the names of the four bases found in DNA?

Define the following terms:

18. Phenotype
19. Genotype
20. Haploid cell
21. Diploid cell
22. Gamete
23. Zygote
24. Allele
25. Locus
26. Chromosome
27. Genome
28. Base Pair
29. T_m
30. Which of the following would have a higher T_m and why?
 - A. CTGTTTTTGCAATGCAATATTAC
 - B. AGCCCCCATTTCGGGCGGCCCG
31. What is the difference between a nucleotide and a nucleoside?
32. What is the basic difference between an intron and an exon?
33. What is the difference between mRNA sequences and their DNA counterpart sequences?

ANSWERS TO GENERAL MOLECULAR BIOLOGY QUESTIONS

1. 6 billion
2. Nucleus and Mitochondria
3. Nucleus
4. Mitosis
5. Mutation
6. Gregory Mendel
7. Homozygote
8. Heterozygote
9. Polymerase
10. Replication fork
11. 5' → 3'
12. Helicase
13. Exonuclease
14. Codon
15. Amino Acids
16. Plasmids
17. Adenine, Thymine, Guanine, and Cytosine
18. Phenotype: The physical characteristic expressed as a result of the genotype
19. Genotype: The pair of alleles at a locus or set of loci
20. Haploid cell: A cell containing a complement of DNA for a given species (Example: one half of a diploid cell)
21. Diploid cell: A cell containing the full complement of DNA from a female and male
22. Gamete: Sex cell (Examples: sperm and eggs)
23. Zygote: Union of a sperm and egg to make a single diploid cell
24. Allele: Alternative form or version of a gene; a sequence difference could account for this form
25. Locus: A specific physical position on a chromosome at which a gene or gene pair reside
26. Chromosome: The package containing a combination of genetic material (DNA) and proteins.
27. Genome: The total genetic complement of an organism defined by one copy of the DNA found in each cell.

28. Base Pair: The bonding of an Adenine (A) to Thymine (T) and Cytosine (C) to Guanine (G)
29. T_m: Melting temperature (i.e., the temperature at which 50% of the strands of a double-helix (DNA) are denatured)
30. "B" since there are more G:C bonds
31. A nucleotide has a phosphate group while a nucleoside does not.
32. Introns are non-transcribed DNA sequences where exons are transcribed into proteins.
33. mRNA contains no introns; it carries the code to make a protein.

II GENETICS PROBLEMS AND QUESTIONS

1. A gene for a particular genetic characteristic for hemoglobin production can be inherited in two forms: "Z" or "z". "Z" is dominant and when expressed, offspring will have normal hemoglobin levels. Recessive "z" always results in high hemoglobin levels. A second genetic characteristic for the trait "Progragy" is inherited as "X" where "X" is dominant and non-lethal. Recessive "x" homozygote is always lethal within the first decade of a child's life.

A mother is known to have the genetic profile "ZZ" and "Xx" and the father has the profile "Zz" and "Xx".

- A. Prepare a Punnet square diagram to demonstrate the possible patterns of inheritance for these two traits considering the mother's and father's profiles described above.
 - B. Based on the Punnet square data that was generated for the "Progragy" trait, what is the possible ratio of children who may die to children who will live within the first decade of life?
 - C. What would it mean if the literature has reported that these two genetic traits are in linkage disequilibrium?
2. Name at least two types of genetic mutations that can occur in the DNA strand.
 3. What is the difference between a "mutation" and a "polymorphic sequence"?
 4. The complete DNA sequence of a new species of plant has recently been found in the rain forest.
 - A. Using the conversion chart below, what would the protein sequence be if the following RNA sequence was obtained from the plant?

5' AUG UUU GCU UUU CGG GGC CUA CUA AAA UAG 3'

- B. What would the DNA sequence be for the above plant RNA sequence?

1st Position	2nd Position				3 rd Position
	U	C	A	G	
U	PHE	SER	TYR	CYS	U
	PHE	SER	TYR	CYS	C
	LEU	SER	STOP	STOP	A
	LEU	SER	STOP	TRP	G
C	LEU	PRO	HIS	ARG	U
	LEU	PRO	HIS	ARG	C
	LEU	PRO	GLN	ARG	A
	LEU	PRO	GLN	ARG	G

A	ILE	THR	ASN	SER	U
	ILE	THR	ASN	SER	C
	ILE	THR	LYS	ARG	A
	MET	THR	LYS	ARG	G
G	VAL	ALA	ASP	GLY	U
	VAL	ALA	ASP	GLY	C
	VAL	ALA	GLU	GLY	A
	VAL	ALA	GLY	GLY	G

ANSWERS TO GENETICS PROBLEMS AND QUESTIONS

1A.

		FATHER			
		ZX	Zx	zX	zx
MOTHER	ZX	ZZXX	ZZXx	ZzXX	ZzXx
	Zx	ZZXx	ZZxx	ZzXx	Zzxx

1B. 1:3, one child will die for every 3 that live

1C. Inheritance of the hemoglobin-related gene is linked to inheritance of the Progragy gene. The phenotype associated with either of these two traits is predictable.

2. Insertion: An addition of one or several nucleotides into the DNA strand.

Deletion: A chromosomal mutation characterized by the loss of a chromosome segment or a nucleotide

Transversion: A base pair substitution mutation resulting in the replacement of a purine by a pyrimidine, or vice versa.

Transition: A base pair substitution mutation resulting in the replacement of one purine by another purine, or a pyrimidine by another pyrimidine.

Duplication: A chromosomal mutation characterized by the presence of two copies of a chromosome segment in the haploid genome.

Inversion: A chromosomal mutation characterized by the reversal of a chromosome segment.

3. A mutation is any inheritable change in the DNA sequence that occurs during reproduction or cell division. A polymorphism is a genetic locus for which there are may alleles in existence based on the mutation.

4A. 5' MET PHE ALA PHE ARG GLY LEU LEU LYS STOP 3'

4B. 5' ATG TTT GCT TTT CGG GGC CTA CTA AAA TAG 3'
3' TAC AAA CGA AAA GCC CCG GAT GAT TTT ATC 5'

III CAPILLARY ELECTROPHORESIS (CE) QUESTIONS

1. What is the capillary made of? Provide approximate dimensions for the capillary.

2. Describe the mechanism of separation of DNA fragments using CE.
3. Describe the function of the following components of the CE:
 - i. Polymer block
 - ii. Oven
 - iii. Laser
 - iv. Prism/spectrograph
 - v. CCD Camera
4. What is the name of the polymer used to separate STR fragments on the CE? What does it consist of?
5. What buffer is used for separation of STR fragments on the 3130xl for use with PP16?
6. What would the effect of the following be on an electropherogram:
 - i. significant change in temperature during the electrophoresis?
 - ii. decrease in injection voltage?
 - iii. POP-6 was used instead of POP-4?
 - iv. the prepared sample plate sat out for a week at room temperature prior to injection?
 - v. the polymer was left on the instrument for 3 weeks?
7. Briefly define or explain the significance of the following:
 - i. Excitation wavelength
 - ii. Emission wavelength
8. What fluorophores are used for the PowerPlex[®] 16 System?
9. You prepared your samples on Friday and forgot to run them on the CE. You realized your forgetfulness on Tuesday. What would be the best action to take?
10. Why do we use Hi-Di[™] Formamide as opposed to non-Hi-Di[™] varieties available?
11. What happens to the Hi-Di[™] Formamide as a result of storage at room temperature for extended lengths of time? Why is this a problem?

ANSWERS TO CAPILLARY ELECTROPHORESIS QUESTIONS

1. What is the capillary made of? Provide approximate dimensions for the capillary.
Fused silica, approx. 50-100µm inner diameter, approx 36 cm length
2. Describe the mechanism of separation of DNA fragments using CE.
In capillary electrophoresis of the STR fragments, a viscous polymer solution acts as a sieving medium. Larger DNA molecules are retarded more by the linear, flexible polymer chains than smaller DNA fragments, which leads to a size-based separation of the DNA. The capillary is placed in two buffer vials, across which an electrical voltage is applied. The fragments of DNA migrate past a detector
3. Describe the function of the following components of the CE:
 - i. Polymer block
This serves to fill (and re-fill between runs) the capillary with polymer for separation.
 - ii. Oven
This heats the capillary/polymer to 60°C, the temperature at which DNA separation occurs using the 3130xl.
 - iii. Laser
This excites the fluorophore attached to the forward primer. The collection of the emitted light is then detected via a CCD camera, which constitutes the raw data collected by the instrument and eventually leads to the electropherogram.
 - iv. Prism/spectrograph

The prism serves to spread out the emitted light from the fluorophores into its various wavelengths, which is then directed to the CCD camera for detection.

v. CCD Camera

The CCD camera detects the emitted light from the fluorophore.

4. What is the name of the polymer used to separate STR fragments on the CE? What does it consist of?

POP-4, which stands for Performance Optimized Polymer. It is a 4% concentration of linear, uncross-linked dimethyl polyacrylamide. It is contained in a solution of high urea concentration (8M), 5% 2-pyrrolidinone, and 100mM TAPS, with the pH adjusted to 8.0 with NaOH.
5. What buffer is used for separation of STR fragments on the 3130xl for use with PP16?

“Genetic Analyzer Buffer” which is 100mM TAPS, 1mM EDTA, pH 8..
6. What would the effect of the following be on an electropherogram:
 - i. significant change in temperature during the electrophoresis?

The resolution of the electropherogram would likely be affected, resulting in either broad peaks or sharp peaks.
 - ii. decrease in injection voltage?

Less DNA would enter the capillary, resulting in decreased peak heights
 - iii. POP-6 was used instead of POP-4?

POP-6 is more viscous, it results in greater resolution, but longer run times. So, you’d likely not detect all of the STR fragments unless the run time had been increased before the run.
 - iv. the prepared sample plate sat out for a week at room temperature prior to injection?

The formamide in the sample will have likely broken down, resulting in a higher concentration of ions. If you injected the samples anyway, your peaks heights would be diminished because the STR fragments would be competing with the higher concentration of ions for injection into the capillary.
 - v. the polymer was left on the instrument for 3 weeks?

The fragments move more slowly through polymer that has been left out for an extended period of time. Thus, you’d likely not detect all of the STR fragments unless the run time had been increased before the run began. In addition, the longer run time would result in broader peaks.
7. Briefly define or explain the significance of the following:
 - i. Excitation wavelength

Optimum wavelength at which a fluorophore is excited
 - ii. Emission wavelength

Wavelength of light given off by a fluorophore
8. What fluorophores are used for the PowerPlex® 16 System?

Fluorescein, JOE (6-carboxy-2’7’-dimethoxy-4’5’ dichlorofluorescein), TMR (tetramethyl rhodamine), and CXR (carboxy-X-rhodamine)
9. You prepared your samples on Friday and forgot to run them on the CE. You realized your forgetfulness on Tuesday. What would be the best action to take?

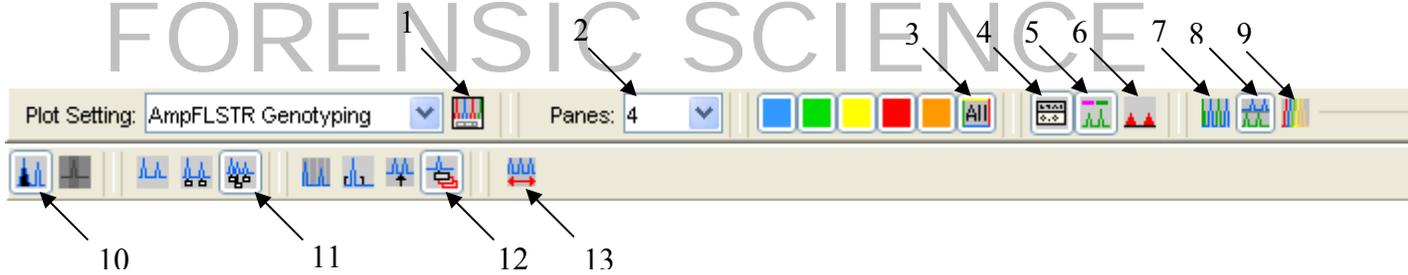
Re-prepare your samples for running on the CE. The formamide is not stable at room temperature for long periods of time (more than approx. 48 hours). Therefore, to get optimal injection of your sample, re-preparing them is likely the best option.
10. Why do we use Hi-Di™ Formamide as opposed to non-Hi-Di™ varieties available?

Hi-Di refers to ‘highly deionized’. Because the formamide is highly deionized, there are fewer ions in the formamide to compete with the DNA fragments for injection into the capillary.

11. What happens to the Hi-Di™ Formamide as a result of storage at room temperature for extended lengths of time? Why is this a problem?
 It breaks down into ionic byproducts (incl. formic acid) that will compete with the DNA for injection, thus, decreasing the peak heights.

IV DATA ANALYSIS AND INTERPRETATION QUESTIONS

1. What is meant by the allele calling thresholds? Why are they different for each dye? How were the thresholds determined?
2. Is a positive control required in each injection? In each run?
3. How can you ensure that the sizing is correct in any injection that does not also have a positive control included in that injection?
4. How does the GeneMapper™ ID software utilize data from multiple allelic ladders included in a single run?
5. What sizing algorithm is used to size the PowerPlex® 16 data? How does that work?
6. Extra peaks were observed in the analysis of a single-source sample. What may they arise from?
7. The following diagram represents commonly used icons in the GeneMapper™ ID software. Match the function to the appropriate icon.



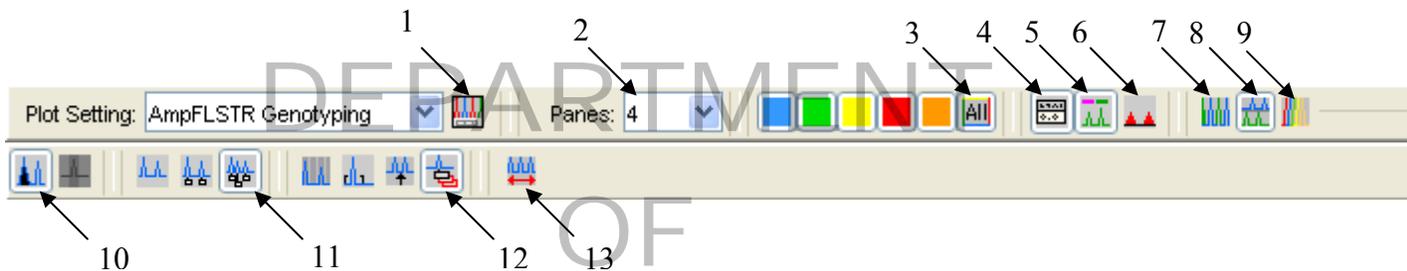
- ___ a. Selects the “combine dyes” plot display
- ___ b. Selects the “separate dyes” plot display
- ___ c. Icon to select the vertical label display
- ___ d. Menu that controls the number of electropherogram panes shown in the view
- ___ e. Show/Hide allele history
- ___ f. Selects the “peak selection” mode
- ___ g. Selects the “overlay all” plot display
- ___ h. Show/Hide the plot header
- ___ i. Plot settings Editor icon to edit the selected profile
- ___ j. Switches view to full x-axis and Y-axis scales
- ___ k. Show/Hide the marker range
- ___ l. Shows all dye colors in the electropherogram(s)

- ___ m. Show/hide the marker indicators
8. What is meant by each of the following flags in the GeneMapper ID software:
- AE
 - OS
 - SQ
 - SQO
9. A colleague asks you to review his STR data. Although the greater majority of the allele calls in your colleague's analysis of his data has resulted in accurate allele calls, you notice there are several "off ladder" calls for some peaks. Which of the following could be causing this? (Check all that apply.)
- ___ Internal size standard peaks were incorrectly identified (325 bp band was identified as 350 bp, etc.).
- ___ Some bands in the allelic ladder lanes were incorrectly identified.
- ___ Overloaded injection resulted in inaccurate migration distances.
- ___ No spectral had been performed on the instrument.
- ___ He has discovered new microvariants which are not present in the allelic ladder.
- ___ The wrong analysis file was used to evaluate an electropherogram.
- ___ One or more allelic ladders was not injected properly.
10. What is meant by the term "capillary cross-talk"? Have you observed this? Describe. How do we know your evidentiary results are not false-positive and simply a result of capillary-cross talk?

ANSWERS TO DATA ANALYSIS AND INTERPRETATION QUESTIONS

- What is meant by the allele calling thresholds? Why are they different for each dye? How were the thresholds determined?
The allele calling thresholds are an RFU cutoff value below which alleles are not reported. The thresholds were determined by calculating the limit of detection for each dye using many samples. Any signal that was not explainable as having originated from a true allele, pull-up, stutter, etc. was considered noise. The LOD (a.k.a. threshold) was calculated as three times the largest observed noise peak in the given electropherogram. Thus, since the noise level is inherent to each dye, it follows that the threshold would be different for each dye.
- Is a positive control required in each injection? In each run?
No, a positive control is not required in each injection. At least one positive control is required in each run (plate) on the CE.
- How can you ensure that the sizing is correct in any injection that does not also have a positive control included in that injection?
An internal lane standard (ILS 600) and an allelic ladder are included in each injection – both of which ensure accurate sizing.
- How does the GeneMapper™ ID software utilize data from multiple allelic ladders in included in a single run?
Data from multiple allelic ladders, if imported into GeneMapper ID at the same time, are averaged by GeneMapper ID.

5. What sizing algorithm is used to size the PowerPlex® 16 data? How does that work?
Local Southern. This sizing algorithm uses the size of two peaks on either side of the peak being measured in order to calculate the size of the peak being measured.
6. Extra peaks were observed in the analysis of a single-source sample. What may they arise from?
 - Biology-related: Stutter, -A, extra chromosomal fragments
 - Instrument/Technology related: pull-up, spike (bubbles, urea crystals, voltage spike), dye blobs
 - Sample contaminants
7. The following diagram represents commonly used icons in the GeneMapper™ ID software. Match the function to the appropriate icon.



- 7 a. Selects the “combine dyes” plot display
 - 8 b. Selects the “separate dyes” plot display
 - 11 c. Icon to select the vertical label display
 - 2 d. Menu that controls the number of electropherogram panes shown in the view
 - 12 e. Show/Hide allele history
 - 10 f. Selects the “peak selection” mode
 - 9 g. Selects the “overlay all” plot display
 - 4 h. Show/Hide the plot header
 - 1 i. Plot settings Editor icon to edit the selected profile
 - 13 j. Switches view to full x-axis and Y-axis scales
 - 5 k. Show/Hide the marker range
 - 3 l. Shows all dye colors in the electropherogram(s)
 - 6 m. Show/Hide the marker indicators
8. What is meant by each of the following flags in the GeneMapper ID software:
 - a. AE: Allele edit = This box is checked when the allele calls have been edited by the analyst for that sample.
 - b. OS: Offscale = A flag is displayed when there are off-scale peaks present within the size range.
 - c. SQ: Sizing Quality = A red octagon is shown when the sizing for a sample is of low quality. A yellow triangle indicates that the size standard curve should be checked. A green square indicates that the size standard curve is of good quality.

d. SQO: Sizing Quality Override = This box is checked when the sizing quality for a sample was flagged but the size standard curve has been edited and/or accepted by the analyst.

9. A colleague asks you to review his STR data. Although the greater majority of the allele calls in your colleague's analysis of his data has resulted in accurate allele calls, you notice there are several "off ladder" calls for some peaks. Which of the following could be causing this? (Check all that apply.)

Internal size standard peaks were incorrectly identified (325 bp band was identified as 350 bp, etc.).

Some bands in the allelic ladder lanes were incorrectly identified.

Overloaded injection resulted in inaccurate migration distances.

No spectral had been performed on the instrument.

He has discovered new microvariants which are not present in the allelic ladder.

The wrong analysis file was used to evaluate an electropherogram.

One or more allelic ladders was not injected properly.

10. What is meant by the term "capillary cross-talk"? Have you observed this? Describe. How do we know your evidentiary results are not false-positive and simply a result of capillary-cross talk?

Capillary cross-talk is the phenomenon in which signal from one capillary is detected in another capillary. Typically this would be the result of off-scale signal in the originating capillary. No capillary cross-talk has been observed at DFS.

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