Department of Forensic Science

FORENSIC BIOLOGY TRAINING MANUAL

DNA ANALYSIS OF FORENSIC CASEWORK

TABLE OF CONTENTS

1 OVERVIEW OF TRAINING PROGRAM

The requirements set forth in this chapter are intended to apply to the training of an examiner to perform DNA PCR-based STR casework using the PowerPlex® Fusion System. It is intended that this training be a continuation of the training received in accordance with the FB TM Case Approach and Identification of Biological Substances. Requirements or tasks set forth in this manual that have already been completed during the completion of the requirements or tasks set forth in the FB TM Case Approach and Identification of Biological Substances need not be repeated.

Trainees with prior applicable experience may follow an abbreviated training plan as deemed appropriate by the Biology Program Manager.

Modifications for Forensic Scientist I (Forensic Scientist – Biologist) trainees will be made on an individual basis as deemed appropriate by the Biology Program Manager.

This manual also addresses the supplemental training of qualified examiners in areas such as Y-STR casework, kinship statistical analysis, TrueAllele® statistical analysis, familial DNA searching, and STRmix™ System statistical analysis. Not all examiners in the Forensic Biology Section will necessarily be trained in these areas. The specific requirements for these supplemental training programs are set forth in the applicable chapters of this manual.

The reestablishing of the technical skills and knowledge of analysts and technical reviewers who reinterpret legacy data for which they were previously qualified and whose external proficiency testing does not include a legacy technology, typing test kit, or platform is conducted through a training in Qualtrax. As a final step, the technical leader shall review the documentation of an analyst's or technical reviewer's maintenance or reestablishment of the technical skills and knowledge and authorize the analyst or technical reviewer to reinterpret legacy data for no more than a two year period. This authorization is documented with an email generated by Qualtrax once the technical leader has completed this final step.

1.1 Purpose and Scope

The purpose of this document is to provide a uniform training program for the analysis of forensic casework using DNA PCR-based STR 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**.**

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. Methods previously employed by the Department should be reviewed in the event the trainee eventually becomes involved in a cold case analysis.

Because each case a forensic examiner analyzes has the potential of involving him/her as an expert witness in courtroom testimony, testimony training is equally important as the analytical training. Therefore, the training will also provide exposure to court procedures and assistance in developing the skills necessary for effective expert witness testimony. 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.

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.

The sequence in which the tasks are presented in the outline should not necessarily be considered as a mandatory order of instruction. If a particular sequence is considered to be mandatory, that sequence will be specified in the task lists below. If a Procedures Manual has been read and the information retained, the trainee is not required to re-read it in its entirety simply because it is listed as a task in a particular area of study. Exposure to legal aspects and testimony will be continuous throughout the training.

Oral and practical examinations and/or mock trials encompassing several topics will be staged periodically. Upon completion of DNA training, the trainee will undergo a comprehensive oral competency examination. The comprehensive 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. A practical examination (i.e., 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 recorded formal comprehensive 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.

Once the trainee has satisfactorily completed all training requirements and given a satisfactory performance on the comprehensive oral competency examination, satisfactory performance on the practical examination, and a satisfactory performance in the final comprehensive mock trial, 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.

1.2 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 and monthly training reports.

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 comprehensive 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, section supervisor (if different) 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. Certain phases of instruction may be scheduled at any of the four laboratories. Such arrangements will be made through the Biology Program Manager and Regional Director(s). Oversight and direction of the training will be provided by the Biology Program Manager.

1.5 Guidelines for Comprehensive Oral Competency Examination

1.5.1 A comprehensive oral competency examination of the trainee will be conducted by the section supervisor, the group supervisor (if different), the Biology Program Manager, and the Laboratory Director or designee to ascertain the technical knowledge of the individual. Questions will be used to ascertain whether the goals, as set forth in each technical portion of the training program, have been achieved. The questions that are asked and the outcome of the oral competency will be documented.

1.5.2 Immediately following the comprehensive oral competency examination, the trainee may be released while the supervisor(s), the Biology Program Manager, and the Laboratory Director or designee evaluate the trainee's performance.

1.5.3 The outcome of the oral competency examination evaluation will be one of the following:

- Satisfactory
- Unsatisfactory

1.5.3.1 If the panel deems the trainee's performance to be unsatisfactory, steps will be taken to effect the appropriate action, as determined by the panel.

1.6 Guidelines for the Final Comprehensive Mock Trial

1.6.1 Refer to the Department Quality Manual in determining who will attend and serve as an evaluator for the final mock trial.

1.6.2 The final mock trial will not exceed four (4) hours. Prior to the 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.

1.6.3 The atmosphere of the trial will be formal (i.e., it will be conducted in the same manner as a real courtroom situation). This includes conduct, protocol, and all other aspects.

1.6.4 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.

1.6.5 There may be two "defense lawyers" at the trial, one of whom must be a qualified examiner in the Forensic Biology Section.

1.6.6 The trial may be stopped at any time upon the request of any of the involved parties.

1.6.7 The trial will be recorded.

1.6.8 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.

1.6.9 The outcome of the final comprehensive mock trial evaluation will be:

- Satisfactory
- Unsatisfactory

1.6.9.1 If the panel deems the trainee's performance to be unsatisfactory, steps will be taken to effect the appropriate action, as determined by the panel.

1.6.10 The evaluation may be followed by a short performance critique.

1.6.11 The training coordinator will review the recording with the trainee as soon as possible. Other comments should be gathered by the trainee from trial participants/observers as soon as possible.

1.7 Transition from Trainee to Examiner

Once the individual has successfully completed all training (case approach, identification of biological substances, and DNA analysis), the supervisor will ensure that the transition from trainee to qualified examiner takes place as smoothly as possible. Guidance will continue to be offered.

1.7.1 For a period of time, all of the newly qualified examiner's reports will be reviewed by the supervisor, or designee, prior to release.

1.7.2 Casework will be monitored closely for at least six (6) months.

1.7.3 The supervisor, or designee, may, depending upon previous testimony experience and the evaluation of the first testimony after qualification, accompany the newly qualified examiner to court for the first few cases.

1.8 Instructions for the Training Coordinator

NOTE: Refer to the Department Quality Manual for the requirements for training documentation and for the retention requirements for those records.

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 PRIOR TO IMPLEMENTATION.

At the culmination of the training, the trainee should be able to demonstrate through the Comprehensive Oral Competency Examination, the final practical examination, and the Final Comprehensive Mock Trial:

- Knowledge of the principles and practices of forensic body fluid identification and DNA, as these relate to the analysis of case material.
- Knowledge of the theory and application of instrumentation and specialized techniques used to examine biological evidence.
- 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.
- The ability to skillfully present and defend analytical findings in a court of law.

1.8.1 The training coordinator, or designated examiner, will document the completion of each required training task by the trainee on the Training Documentation Form.

1.8.1.1 The completed Training Documentation Form will be retained by the trainee in his/her training notebook.

1.8.1.2 One copy of all completed Training Documentation Forms 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.

1.8.2 The training coordinator will continually evaluate the trainee's performance and submit a monthly training report of progress to the Biology Program Manager and Laboratory Director using the Qualtrax workflow in accordance with the requirements set forth in the Department Quality Manual.

1.8.2.1 A monthly training report will be submitted individually for each trainee.

1.8.2.2 Each monthly training report will be maintained and used as documentation of the trainee's progress toward qualification as an examiner.

1.9 Instructions for the Trainee

1.9.1 The trainee will keep a notebook of all work completed, including the Training Documentation Form and the training coordinator's monthly training reports.

1.9.2 The notebook will be organized by subject.

1.9.2.1 Within each subject category the following will be included:

- The types of tests or examinations observed and performed
- Notes and comments on each type of test
- Review of pertinent literature
- Answers to study questions (see 1.9.4)

1.9.2.2 For each procedure performed, comments/notes will include the following, as applicable:

- Principle behind the procedure
- A procedural outline including the purpose of critical reagents
- Sensitivity of the procedure
- Specificity of the procedure
- Contemporaneous results of testing
- Interpretation of results
- Possible interferences/problems
- Other comments including comparisons to other methods or procedures

1.9.3 The DNA PCR-based STR analysis and quality control procedures can be found in the Forensic Biology Procedures Manuals.

1.9.4 A list of study questions is located in Appendix C. Each trainee is expected to write out the answers to the questions after completing the required tasks and readings for each subject area.

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 opening of, screening of, collection for DNA from, and documentation of casework evidence throughout the training only after successful completion of an applicable competency test and under the direct supervision of a qualified examiner. All FS Lab numbers and/or names must be redacted from the training notes/copies maintained in the training notebook.

1.9.6.1 Refer to the FB TM Case Approach and ID of Biological Substances for the requirements for applicable competency testing.

1.9.6.2 A trainee previously qualified to perform these duties under supervision as detailed in the FB TM Case Approach and ID of Biological Substances remains qualified and need not be competency tested again.

2 SAFETY

2.1 Bloodborne pathogen

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

2.2 Hazards

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

- Infectious agents, such as those associated with:
  - Hepatitis
  - HIV/AIDS
  - Sexually transmitted diseases
  - Parasitic infections
  - Bacterial infections
- Hazardous materials, such as:
  - Acids and bases
  - Organic chemicals

2.3 Safety Procedures

2.3.1 All trainees will read and become familiar with the Department of Forensic Science Safety Manual.

2.3.2 All trainees will follow personal protective measures.

2.3.2.1 Gloves, safety glasses and other protective clothing and equipment will be worn.

2.3.2.2 The production of aerosols will be avoided.

2.3.2.3 No mouth pipetting is allowed.

2.3.2.4 Trainees will read and become familiar with the prescribed precautions for the handling of all chemicals used in a particular procedure before performing the procedure.

2.3.2.4.1 This will include a review of any applicable Safety Data Sheets (SDS).

2.3.3 All trainees will follow biosafety practices.

2.3.3.1 Prescribed personal, work space and equipment cleaning procedures will be followed.

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 the procedures outlined in the Department Safety Manual.

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

2.3.3.4 Organic and other hazardous chemicals (e.g., phenol, tetramethylbenzidine) will be retained in appropriately labeled containers in a designated, marked area in the section or building until disposed of following the procedures outlined in the Department Safety Manual (i.e., picked up by a disposal company).

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 AmpliTaq Gold™ (PE Applied Biosystems) 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.

4 DNA ISOLATION

4.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Develop an understanding of the theory and procedures of DNA isolation from blood stains, other biological stains, tissue, bone, teeth, hair and mixed biological samples.
- Become acquainted with the sensitivity of the isolation procedure.
- Become familiar with the limitations of the isolation procedure.
- Become familiar with the use of controls incorporated at this stage of the procedure.
- Become familiar with the reagents used for DNA isolation and the function of each.
- Become familiar with proper documentation of DNA isolation.

4.2 Tasks

NOTES: Ensure that all appropriate controls are isolated with all training samples required below.

4.2.1 Read the Forensic Biology Procedures Manual Extraction of DNA.

4.2.2 Prepare the reagents necessary for DNA isolation.

4.2.3 Observe a project coordinator or robot operator run a minimum of one of the following sets of samples through the entire DNA isolation process, deck setup, and initiation of the Biomek® NX^{P} Software.

4.2.4 Perform DNA isolation on at least 28 blood stains prepared on a variety of different substrates commonly encountered in casework.

4.2.4.1 Use the organic extraction method for 14 of the blood stains.

4.2.4.2 Use the manual DNA IQ™ extraction method for 7 of the blood stains.

4.2.4.3 Use the automated DNA IQ™ extraction method for the remaining 7 blood stains.

4.2.4.4 Initially use large stains (200 µL) and gradually move to smaller stains (5 µL) to test the ability to obtain DNA from smaller stains.

4.2.4.5 All blood stain extracts will be quantitated using the PowerQuant® human quantitation method.

4.2.4.6 All blood stain extracts will be taken through the entire DNA analysis prior to proceeding to 4.2.5.

4.2.5 Perform DNA isolation on at least 28 other unmixed biological stains including semen, vaginal fluid, saliva, vasectomized seminal fluid, urine, and feces prepared on a variety of different substrates commonly encountered in casework. These stains will vary in size from large stains (200 µL) to smaller stains (5 µL).

4.2.5.1 Use the organic extraction method for 14 of the samples.

4.2.5.2 Use the manual DNA IQ™ extraction method for 14 of the samples.

4.2.5.3 All unmixed biological stain extracts will be quantitated using the PowerQuant® human quantitation method.

4.2.5.4 All unmixed biological stain extracts will be taken through the entire DNA analysis prior to proceeding to 4.2.6.

4.2.6 Perform DNA isolation on at least 20 mixed biological stains, each consisting of a mixture of two biological fluids to include semen, vaginal fluid, blood or saliva prepared on a variety of different substrates commonly encountered in casework. These stains will vary in size and quantity of fluid present.

4.2.6.1 Use the Organic extraction method for mixed body fluid stains (differential procedure) for 10 of the mixed biological stains.

4.2.6.2 Use the manual DNA IQ™ extraction method for mixed body fluid stains (differential procedure) for 5 of the mixed biological stains.

4.2.6.3 Use the automated DNA IQ™ extraction method for mixed body fluid stains (differential procedure) for 5 of the mixed biological stains.

4.2.6.4 All mixed biological stain extracts will be quantitated using the PowerQuant® human quantitation method.

4.2.6.5 All mixed biological stain extracts will be taken through the entire DNA analysis prior to proceeding to 4.2.7.

4.2.7 Perform DNA isolation on the following sample types using either an organic extraction method or DNA IQ™ extraction method (manual or robotic) and carry through quantitation and the entire DNA analysis:

- Two bone samples
- Six tissue samples (preferably multiple types of tissue, if available)
- Five animal samples
- Ten stains contaminated with contaminants such as bacteria, soil, grass, cleaning agents, etc.
- Five hair samples (root ends identified for the trainee)
- A family study (at least 3 people; greater than 3 people, if available)
- Aged stains – at least 5 years old (10 samples total)
- Two teeth

4.2.8 Perform the Casework Direct (CD) extraction method on the following sample types:

- Two unmixed biological stains prepared on swabs – one from a male and one from a female
- Two mixed biological stains prepared on swabs – semen or seminal fluid mixed with another body fluid on each

NOTE: These extracts are to be quantitated on the same day on which they are extracted.

4.2.9 Read applicable literature, become familiar with applicable glossary terms and complete the applicable study questions.

4.3 Evaluation

4.3.1 Because the DNA profiles to be obtained from each of the training samples are unknown in advance to the trainee, the satisfactory completion of DNA analysis on the above listed non-CD training samples

(which cover the spectrum of anticipated work to be performed) will serve as the competency set for DNA analysis.

4.3.2 Knowledge of the trainee will be evaluated through:

- Review of notes and worksheets in the training notebook by the training coordinator.
- Mini-mock trials/oral and practical exams.

4.3.3 The trainee should, through performing DNA isolation on the variety and number of samples detailed in this chapter, 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.

5 DNA QUANTITATION

5.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Develop an understanding and working knowledge of the PowerQuant® quantitation method, including limitations and interpretation of results and proper documentation.
- Become familiar with the controls used with the PowerQuant® System.
- Develop an understanding and working knowledge of the amplification reaction used in the PowerQuant® System.

5.2 Tasks

5.2.1 Read the Forensic Biology Procedures Manual Quantitation of DNA.

5.2.2 Quantitate the non-CD DNA extracts from the blood stains, unmixed biological stains, mixed biological stains and validation sample sets addressed in Chapter 4, DNA Isolation.

NOTE: In accordance with the instructions provided in Chapter 4, DNA Isolation, the extracts from the blood stains, unmixed biological stains, mixed stains and validation study sets will have been isolated at different times and will be quantitated at different times.

5.2.3 Observe a project coordinator or robot operator run a minimum of one set of samples through the entire PowerQuant® quantitation process, deck setup, and initiation of the Biomek® NX^{P} Software and the QuantStudio™ Design & Analysis Software for running an experiment and data analysis.

5.2.4 Interpret the results from the PowerQuant® Analysis Tool.

5.2.5 Interpret the results of the quantitation of the CD extracts using the appropriate flowchart in the Forensic Biology Procedures Manual Quantitation of DNA and document whether each sample is potentially suitable for autosomal STR analysis, Y-STR analysis, or discontinuation. Additionally, document what extraction method would be most suitable, if applicable.

NOTE: Successful extraction and interpretation of this set of extracts serves as the trainee's competency for the use of the CD extraction method for screening for male DNA.

5.2.6 Look at results of the quantitation of degraded samples using the PowerQuant® quantitation process in order to become familiar with the appearance of human DNA and potential bacterial DNA.

NOTE: Data from previously run training samples may be used for this purpose. If necessary, other degraded samples or previously isolated samples may also be obtained and extracted and/or quantitated.

5.2.7 Read applicable literature, become familiar with applicable glossary terms and complete the applicable study questions.

5.3 Evaluation

5.3.1 Knowledge of the trainee will be evaluated through:

- Review of notes and worksheets in the training notebook by the training coordinator.
- Mini-mock trials/oral and practical exams.

5.3.2 The trainee should develop and exhibit an unquestionably sound technique for quantitating DNA and interpreting the associated results. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

6 NORMALIZATION WIZARD AND AMPLIFICATION PROCESS

6.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Develop an understanding and working knowledge of the amplification process, including proper documentation.
- Become familiar with the controls used during the amplification process.
- Become familiar with the problems associated with amplification.
- Develop an understanding of the importance of an amplification environment being free of contamination.
- Develop an understanding of the importance of quality control associated with the amplification process.

6.2 Tasks

6.2.1 Read the Forensic Biology Procedures Manual PowerPlex® Fusion Amplification and Long Term Storage.

6.2.2 Work in an environment free of contamination and follow proper guidelines to prevent contamination.

6.2.3 Program a thermal cycler and perform the quality control test on the thermal cycler, completing all appropriate documentation.

6.2.4 Perform manual amplification set up on half of the training samples addressed in Chapter 4, DNA Isolation, using the PowerPlex® Fusion System. Refer to the Forensic Biology Procedures Manual PowerPlex® Fusion Amplification and Long Term Storage.

6.2.5 Observe a project coordinator or robot operator run a minimum of one set of samples through the entire Normalization Wizard, amplification set up and deck set up using the Biomek® NXP Automation Workstation.

6.2.6 Read applicable literature, become familiar with applicable glossary terms and complete the applicable study questions.

6.3 Evaluation

6.3.1 Knowledge of the trainee will be evaluated through:

- Review of notes and worksheets in the training notebook by the training coordinator.
- Mini-mock trials/oral and practical exams.

6.3.2 The trainee should develop and exhibit 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.

7 CAPILLARY ELECTROPHORESIS

7.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Become familiar with the theories of electrophoresis as they apply to capillary electrophoresis used in STR typing analysis.
- Learn the parameters used for electrophoresis of DNA amplified with the PowerPlex® Fusion System.
- Develop an understanding and working knowledge of the use of capillary electrophoresis instrumentation, including the limitations and proper documentation.
- Become familiar with the controls associated with capillary electrophoresis.

7.2 Tasks

7.2.1 Read the Forensic Biology Procedures Manual CE for PowerPlex® Fusion.

7.2.2 Observe the bubble remove wizard and common maintenance tasks associated with the CE instrument such as changing the polymer, changing the buffer, running the water wash wizard and flushing the water trap.

7.2.3 Prepare the reagents necessary to perform capillary electrophoresis.

7.2.4 Run the training samples addressed in Chapter 4, DNA Isolation, on the capillary electrophoresis instrument.

7.2.5 Read applicable literature, become familiar with applicable glossary terms and complete the applicable study questions.

7.3 Evaluation

7.3.1 Knowledge of the trainee will be evaluated through:

- Review of notes and worksheets in the training notebook by the training coordinator.
- Mini-mock trials/oral and practical exams.

7.3.2 The trainee should develop and exhibit an unquestionably sound technique for running consistently interpretable PowerPlex® Fusion 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.

8 CAPILLARY ELECTROPHORESIS DATA ANALYSIS AND INTERPRETATION

8.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Develop a working knowledge of the GeneMapper® ID-X (GMID-X) Software used for the analysis of the electropherogram data.
- Become familiar with the interpretation of electropherograms.
- Become familiar with the base pair size range of different PowerPlex® Fusion loci.
- Develop an understanding of the use of controls, ladders and the internal lane standard.
- Develop an understanding of the problems that may be encountered with regard to interpretation.
- Become skilled in the deconvolution of mixture profiles.
- Become familiar with the proper documentation of the results.

8.2 Tasks

8.2.1 Read the Forensic Biology Procedures Manual Analysis of CE Results Using GeneMapper® ID-X.

8.2.2 Read the Forensic Biology Procedures Manual Interpretation of PowerPlex® Fusion CE Data.

8.2.3 Analyze the CE data generated from the training samples using GMID-X.

8.2.4 Interpret all results successfully and properly document the results.

8.2.5 Assess and deconvolve training mixture profiles, as appropriate.

8.2.6 Read applicable literature, become familiar with applicable glossary terms and complete the applicable study questions.

8.3 Evaluation

8.3.1 Knowledge of the trainee will be evaluated through:

- Review of notes, copies of all electropherograms (including the landscape printout), deconvolutions, and other data related to the analytical process in the training notebook by the training coordinator.
- Mini-mock trials/oral and practical exams.

8.3.2 The trainee should develop and demonstrate a thorough understanding of all aspects of interpretation of capillary electrophoresis data by accurately interpreting PowerPlex® Fusion 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.

9 STATISTICS, REPORT WRITING AND CODIS

9.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Become familiar with the format and report wording presently used by DFS Forensic Biology examiners.
- Become skilled in expressing the results of the PowerPlex® Fusion typing of a sample in a clear, concise and technically correct fashion.
- Become skilled in the computation and explanation of statistical calculations associated with PowerPlex® Fusion typing results including random match probabilities, likelihood ratios, and CPE calculations.
- Become familiar with the Combined DNA Index System applications, policies and procedures, and associated documentation required.

9.2 Tasks

9.2.1 Read the Forensic Biology Procedures Manual Interpretation of PowerPlex® Fusion CE Data.

9.2.2 Read the Forensic Biology Procedures Manual Report Writing.

9.2.3 Read the Forensic Biology Procedures Manual CODIS Operating Policies and Procedures.

9.2.4 Review Certificates of Analysis prepared by examiners and compare to the corresponding electrophoretic data, landscape printouts, and applicable deconvolution documentation.

9.2.5 Prepare Certificates of Analysis based upon completed case files.

9.2.5.1 Compare the Certificates to those released by the examiner.

9.2.6 Compute statistical calculations on actual casework samples typed at the PowerPlex® Fusion System loci.

9.2.6.1 Compare the results to those reported by the examiner.

9.2.7 Perform CODIS searches under the direct supervision of qualified examiners and generate the proper associated documentation.

9.2.8 Document the practice technical review of a minimum of ten (10) case files prepared by a qualified Forensic Biology Examiner which will then be officially technically reviewed by another qualified Forensic Biology Examiner.

9.2.9 Read applicable literature, become familiar with applicable glossary terms and complete the applicable study questions.

9.3 Evaluation

9.3.1 Knowledge of the trainee will be evaluated through:

- Review of notes, computations, and Certificates of Analysis prepared by the trainee in the training notebook by the training coordinator.
- Mini-mock trials/oral and practical exams.

9.3.2 The trainee should develop and demonstrate the ability to:

- clearly, accurately, and concisely set forth STR analytical results, including statistical information, using random match probabilities, likelihood ratios, and combined probabilities of exclusion in a report.

- use CODIS.

This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

10 TESTIMONY AND EXPERT WITNESS QUALIFICATION

10.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Become familiar with the legal aspects of DNA typing, including common challenges, controversial cases, and case histories.
- Successfully demonstrate technical knowledge by satisfactorily completing a comprehensive oral competency examination.
- Successfully demonstrate skill in analysis and testimony by satisfactorily completing the analysis of a practical exam (mock case) and satisfactorily testifying to that analysis during a comprehensive final mock trial.

10.2 Tasks

10.2.1 Become familiar with the DNA testing guidelines/standards set forth by the following:

- Scientific Working Group on DNA Analysis Methods (SWGDAM)
- American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB®)
- ANAB ISO/IEC 17025 Forensic Science Testing Laboratories Accreditation Requirements
- FBI Quality Assurance Standards

10.2.2 Observe examiners testify, when possible.

10.2.3 Observe at least one pretrial conference with a qualified examiner, when possible.

10.2.4 Provide lecture(s) on DNA analysis to law enforcement personnel and lay groups to practice explanations and public speaking skills.

10.2.5 Participate in a comprehensive oral competency examination.

10.2.6 Conduct analysis on a practical exam (mock case), prepare a Certificate of Analysis, and testify to the results of the analysis in a comprehensive final mock trial.

10.2.7 Read applicable literature, become familiar with applicable glossary terms and complete the applicable study questions.

10.3 Evaluation

10.3.1 Knowledge of the trainee will be evaluated through mini-mock trials and/or question and answer sessions.

10.3.2 The trainee should demonstrate the ability to clearly and accurately testify to STR analytical results and conclusions, including associated statistical calculations.

11 COORDINATION OF THE BIOMEK® NXP AUTOMATION WORKSTATION

The requirements set forth in this chapter are intended to apply to the training of a qualified Forensic Biology examiner to become a project coordinator for the Biomek® NXP Automation Workstation and encompasses the training required to become an operator. For the requirements to train a qualified Forensic Biology examiner to be only an operator of the Biomek® NXP Automation Workstation, refer to the FB TM Forensic Laboratory Specialist. Not all Forensic Biology examiners will be trained as a project coordinator or operator.

The trainee will prepare and maintain an organized notebook which will serve as a ready-reference during the examiner's tenure at the Department.

The training will be monitored by a currently qualified project coordinator/training coordinator designated by the Biology Program Manager.

Documentation of this supplemental training will be maintained on the appropriate Forensic Biology Training Documentation Form rather than in monthly Qualtrax workflow format. This training form will be kept up to date and made available to the Biology Program Manager for review upon request.

11.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Learn how to initiate the Biomek® NXP Software.
- Learn how to set up the deck for isolation of DNA, quantitation of DNA, amplification set up, and the 1.5 mL transfer.
- Learn how to trouble-shoot any problems that arise during the operation of the Biomek® NXP Automation Workstation.
- Prepare to oversee the Forensic Laboratory Specialist or examiner who will serve as an operator.
- Become familiar with the QC procedures for the Biomek® NXP Automation Workstation and QuantStudio™ 5 instrument.

11.2 Tasks

11.2.1 Read the following Forensic Biology Procedures Manuals:

- Quality Assurance
- Extraction of DNA
- Quantitation of DNA
- PowerPlex® Fusion Amplification and Long Term Storage

11.2.2 Observe a currently qualified operator perform isolation, quantitation, dilution, amplification setup, and the 1.5 mL transfer on at least one plate of casework samples.

11.2.3 Observe a currently qualified project coordinator perform isolation, quantitation, dilution, and amplification setup of at least one plate of casework samples.

11.2.4 Run a checkerboard training set of 48 samples (24 blood and/or buccal samples and 24 blanks).

11.2.5 Run a zebra stripe training set of 48 samples (24 blood and/or buccal samples and 24 blanks).

11.2.6 Prepare and perform a sensitivity series using the Biomek® NXP Automation Workstation and the PowerQuant® System.

11.2.7 Observe the Biomek® NXP Automation Workstation calibration programs (e.g., deck framing) and QuantStudio™ 5 instrument QC.

11.2.8 Observe the entire DNA isolation, quantitation, dilution, and amplification setup, deck setup, and program design.

11.2.9 Observe a currently qualified project coordinator abort a 16 sample water run and perform the Recovery Dispense Only and Recovery methods on the run.

11.2.10 Perform the Biomek® NXP Automation Workstation calibration programs (e.g., deck framing) and QuantStudio™ 5 instrument QC.

11.2.11 Run a minor validation study as follows:

11.2.11.1 Two checkerboard sets will be run independently of one another and carried through DNA typing.

11.2.11.1.1 One set will consist of at least 32 samples (16 blood and/or buccal samples and 16 blanks).

11.2.11.1.2 One set will consist of at least 40 samples (20 blood and/or buccal samples and 20 blanks).

NOTE: One of these two checkerboard runs will be aborted and the Recovery Dispense Only and Recovery methods will be performed.

11.2.12 Run a set of at least 10 samples that are typically encountered in casework analysis (e.g., blood, buccals, cigarette butts) and at least two mixture samples requiring a differential extraction and carry through DNA typing.

11.2.13 Perform a minimum of three (3) casework runs under the supervision of a qualified coordinator or designee.

11.2.14 Run a set of competency samples and carry through DNA typing.

11.3 Evaluation

11.3.1 Knowledge of the trainee will be evaluated through participation in a semi-formal technical question and answer session covering robot operation, QuantStudio™ 5 and PowerQuant® procedures, QC and trouble-shooting by the training coordinator or a currently qualified project coordinator.

11.3.1.1 The trainee's supervisor or designee will also be present.

11.3.2 The trainee should demonstrate a thorough understanding of and be able to independently operate the Biomek® NXP Automation Workstation and QuantStudio™ 5 instrument. This will be monitored throughout the training by the training coordinator or a currently qualified project coordinator.

11.3.3 At the successful completion of the training requirements detailed in this Chapter, including a satisfactory performance during the semi-formal technical question and answer session described in 12.3.1, a Memorandum for Record (MFR) detailing the recommendation to qualify the trainee as a project coordinator will be submitted to the Biology Program Manager. Once this MFR is accepted and signed by the Biology Program Manager, the examiner will be deemed qualified to perform the duties of a project coordinator.

12 Y-STR ANALYSIS

The requirements set forth in this chapter are intended to apply to the training of a qualified Forensic Biology examiner to perform Y-STR analysis for the Department using the AmpFℓSTR™ Yfiler™ System Kit, to issue associated Certificates of Analysis and to technically review Y-STR casework. Not all Forensic Biology examiners will necessarily be trained as a Y-STR examiner.

The trainee will prepare and maintain an organized notebook which will serve as a ready-reference during the examiner's tenure at the Department.

The training program will be monitored by a training coordinator designated by the Biology Program Manager.

Documentation of this supplemental training will be maintained on the appropriate Forensic Biology Training Documentation Form rather than in monthly Qualtrax workflow format. This training form will be kept up to date and made available to the Biology Program Manager for review upon request.

If a trainee new to the Department is incorporating Y-STR training into the overall training to become a fully qualified Forensic Biology examiner, the training requirements detailed in this Chapter may be combined with those detailed in the FB TM Case Approach and Body Fluid Identification and the previous Chapters of this manual. In that case, the comprehensive oral competency exam, final practical exam (mock case) and comprehensive final mock trial may include all aspects of the training, including Y-STR analysis. In addition, the documentation will adhere to the monthly Qualtrax workflow requirements as a part of the overall examiner training program.

12.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Become familiar with the potential uses and limitations of Y-STR analysis as they relate to forensic casework and other fields.
- Become familiar with assessing the quantity and quality of male DNA using quantitation data.
- Become familiar with the nature of the polymorphic regions examined by the Department on the Y-chromosome.
- Become familiar with the Y-STR amplification kit and internal lane standard used by the Department.
- Become familiar with the methods for the reconstitution of dried DNA extracts, if not already familiar.
- Become familiar with the base pair size range of different AmpFℓSTR™ Yfiler™ System Kit loci.
- Develop an understanding of the problems that may be encountered with regard to interpretation of Y-STR data.
- Become familiar with the proper documentation of the results.
- Become familiar with the format and report wording presently used by DFS Forensic Biology Y-STR examiners.
- Become skilled in expressing the results of the AmpFℓSTR™ Yfiler™ typing of a sample in a clear, concise and technically correct fashion.
- Become skilled in the computation and explanation of statistical calculations associated with Y-STR analysis (i.e., the counting method and application of a 95% upper bounds confidence interval).

12.2 Tasks

12.2.1 Read the appropriate AmpFℓSTR™ Yfiler™ validation summaries.

12.2.2 Read the appropriate sections of the FB PM Quality Assurance Program.

12.2.3 Read the FB PM AmpFℓSTR™ Yfiler™ Amplification and Long Term Storage.

12.2.4 Read the FB PM CE for AmpFℓSTR™ Yfiler™.

12.2.5 Read the applicable sections of the FB PM Analysis of CE Results Using GeneMapper® ID-X.

12.2.6 Read the FB PM Interpretation of AmpFℓSTR™ Yfiler™ data.

12.2.7 Read the applicable chapters of the FB PM Report Writing.

12.2.8 Read the Y-STR references and required readings listed in Appendix A of this manual.

12.2.9 Amplify and type the following sample sets following the procedures outlined in the FB PM manuals for AmpFℓSTR™ Yfiler™.

NOTES: These sample sets must be amplified and typed in a total of at least two (2) separate amplifications and two (2) separate CE runs so as to demonstrate the skills necessary in conducting each of these portions of the analysis.

If extracts and associated quantitation data are available, the trainee may use them. It may be necessary, in the absence of available extracts, to extract and quantitate the samples. If extracts of at least 7 µL are available in the absence of quantitation data, they may be quantitated and used.

- At least five (5) dilutions of male DNA
- At least five (5) environmentally exposed samples
- At least five (5) female DNA samples
- At least five (5) male:female mixtures with an excess of female DNA and varying concentrations of male DNA
- At least five (5) non-probative casework samples from normalization wizard plates
- At least five (5) male:male mixtures of DNA of varying concentrations and ratios

12.2.10 Using the Y-Chromosome Haplotype Reference Database (YHRD), generate the haplotype frequency using the 95% confidence interval for a minimum of three (3) different Y-STR profiles provided by the training coordinator and perform the calculation manually on a minimum of one (1) profile for which there are no observations in the database.

12.2.11 Review a minimum of five (5) case files previously generated by a qualified Y-STR examiner.

NOTE: Cases that contain low-level samples, samples with extreme male to female ratios, and/or challenging interpretations should be chosen for this purpose.

12.2.12 Document the practice technical review of a minimum of three (3) case files prepared by a qualified Y-STR examiner which will then be officially technically reviewed by another qualified Y-STR examiner.

12.3 Evaluation

At the culmination of the training, the trainee should:

- exhibit an unquestionably sound technique for DNA amplification by consistently achieving uncontaminated results in the Y-STR typing data.
- exhibit an unquestionably sound technique for running consistently interpretable AmpFℓSTR™ Yfiler™ samples on the capillary electrophoresis instrument using proper documentation.
- exhibit a thorough understanding of all aspects of interpretation of capillary electrophoresis data by accurately interpreting AmpFℓSTR™ Yfiler™ results on all training samples and properly recording results.
- demonstrate the ability to clearly, accurately, and concisely set forth Y-STR analytical results, including statistical information using the counting method and application of a 95% upper bounds confidence interval in a report.
- demonstrate the ability to clearly and accurately testify to Y-STR analytical results and conclusions, including associated statistical calculations.

12.3.1 The training coordinator, or designated examiner, will document the completion of each required training task by the trainee on the checklist.

12.3.1.1 The completed checklist will be retained by the trainee in the training notebook.

12.3.1.2 A copy of the completed checklist 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.

12.4 Examiner Qualification

12.4.1 The final qualification of a trainee to perform Y-STR analysis and issue associated Certificates of Analysis will be based upon the following:

12.4.1.1 Successful completion of the required training samples and tasks

12.4.1.2 Satisfactory completion of a practical exam (mock case extracts/stains) and preparation of an associated Certificate of Analysis

12.4.1.2.1 This practical exam will be worked as if it were an actual case submission and will serve as a means by which the trainee's note taking skills, accuracy in performing Y-STR analysis, interpretation skills, casework documentation and reporting can be evaluated.

12.4.1.2.2 This practical exam will be graded by the training coordinator.

12.4.1.3 Satisfactory performance in an oral competency examination

12.4.1.3.1 Attendees will include, at a minimum, the training coordinator and Biology Program Manager.

12.4.1.3.2 This examination will include both technical questions and court-style questions. Although all aspects of Forensic Biology examination may be covered, the questions will be heavily focused on aspects related specifically to Y-STR analysis and casework.

12.4.2 Upon successful completion of the training requirements detailed in this Chapter, including a satisfactory completion of the practical exam and a satisfactory performance in the oral competency exam, a Memorandum for Record (MFR) detailing the recommendation to qualify the trainee as a Y-STR examiner will be submitted to the Biology Program Manager. Once this MFR is accepted and signed by the Biology Program Manager, the examiner will be deemed qualified to perform Y-STR analysis, issue associated Certificates of Analysis, and to technically review Y-STR casework.

13 KINSHIP STATISTICAL ANALYSIS

The requirements set forth in this chapter are intended to apply to the training of a qualified Forensic Biology examiner to perform kinship statistical analyses on criminal paternity and body identification cases, to issue associated Certificates of Analysis, and to technically review criminal paternity and body identification cases. Not all Forensic Biology examiners will necessarily be trained to perform these functions.

The trainee will prepare and maintain an organized notebook which will serve as a ready-reference during the examiner's tenure at the Department.

The training program will be monitored by a training coordinator designated by the Biology Program Manager.

Documentation of this supplemental training will be maintained on the appropriate Forensic Biology Training Documentation Form rather than in monthly Qualtrax workflow format. This training form will be kept up to date and made available to the Biology Program Manager for review upon request.

13.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Become familiar with the use of the software program currently used by the Department to perform paternity and relationship kinship statistical analyses.
- Learn to choose the appropriate relationship statistical test to perform given the casework question being asked.
- Become proficient at the interpretation of the results obtained.
- Become skilled at expressing the meaning of the statistical results.

13.2 Tasks

13.2.1 Read the applicable chapters of the FB PM Interpretation of PowerPlex® 16 CE Data.

13.2.2 Read the applicable chapters of the FB PM Interpretation of PowerPlex® Fusion CE Data.

13.2.3 Read the applicable chapters of the FB PM Report Writing.

13.2.4 Participate in either internal or external training on the theory and practice of paternity and kinship statistical analysis.

13.2.5 Read the kinship statistical analysis references and required readings listed in Appendix A of this manual and become familiar with paternity and kinship testing terms.

13.2.6 Review at least five (5) released paternity and kinship Certificates and the associated supporting case file documentation.

13.2.7 Perform statistical analysis on at least ten (10) previously analyzed paternity and kinship cases using the software used by the Department and draft associated Certificates of Analysis or draft wording that would appear in associated Certificates of Analysis.

13.2.7.1 These cases will be provided by the training coordinator and will include at least one of each of the following:

- Standard paternity trio case
- Case including a mutation
- Reverse paternity case (missing child)
- Single parent case

- Sibling case
- Paternity case with Y-STR results

13.2.8 Perform manual formula derivations/calculations on ten (10) previously analyzed cases provided by the training coordinator.

13.2.9 Document the practice technical review of a minimum of three (3) case files prepared by a qualified Kinship Statistical Analysis examiner which will then be officially technically reviewed by another qualified examiner.

13.3 Evaluation

13.3.1 Knowledge of the trainee will be evaluated through:

- Review of notes, calculations and Certificates of Analysis/drafted report wording in the training notebook by the training coordinator.
- Mini-mock trial(s) and/or question and answer session(s) as deemed necessary by the training coordinator.

13.3.2 The trainee should demonstrate the ability to clearly, accurately and concisely set forth paternity and kinship testing statistical results for all types of kinships tests performed by the Department.

13.4 Examiner Qualification

13.4.1 The final qualification of a trainee to perform kinship statistical analyses and issue associated body identification and paternity case Certificates of Analysis will be based upon the following:

13.4.1.1 Successful completion of the required training tasks

13.4.1.2 Satisfactory performance in an oral competency examination

13.4.1.2.1 Attendees will include, at a minimum, the training coordinator and Biology Program Manager.

13.4.1.2.2 This examination will include both technical questions and court-style questions focused on aspects related specifically to kinship statistical analysis and casework.

13.4.1.3 Satisfactory completion of a practical exam

13.4.1.3.1 This practical exam will be worked as if it were an actual case and will serve as a means by which the trainee's ability to choose the appropriate relationship statistical test, interpretation skills, casework documentation and reporting can be evaluated.

13.4.1.3.2 This practical exam will be graded by the training coordinator.

13.4.2 Upon successful completion of the training requirements detailed in this Chapter, including a satisfactory performance in the oral competency exam and satisfactory completion of the practical exam, a Memorandum for Record (MFR) detailing the recommendation to qualify the trainee as a kinship statistical analysis examiner will be submitted to the Biology Program Manager. Once this MFR is accepted and signed by the Biology Program Manager, the examiner will be deemed qualified to perform kinship statistical analysis on casework, issue associated Certificates of Analysis, and to technically review kinship statistical analysis casework.

14 TRUEALLELE® STATISTICAL ANALYSIS

The requirements set forth in this chapter are intended to apply to the training of a qualified Forensic Biology examiner to become a qualified TrueAllele® operator, issue associated Certificates of Analysis and technically review TrueAllele® cases. Not all Forensic Biology examiners will necessarily be trained to perform these functions.

The trainee will prepare and maintain an organized notebook which will serve as a ready-reference during the examiner's tenure at the Department.

The training program will be monitored by a training coordinator designated by the Biology Program Manager.

Documentation of this supplemental training will be maintained on the appropriate Forensic Biology Training Documentation Form rather than in monthly Qualtrax workflow format. This training form will be kept up to date and made available to the Biology Program Manager for review upon request.

14.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Develop an understanding of the theory behind the likelihood ratio and the different ways in which it can properly be expressed.
- Become familiar with the TrueAllele® Casework System components and VUIer® Software.
- Become proficient at operating the software for both Operator I (basic upload) and Operator II (advanced analysis) functions.
- Become proficient at determining when variables (e.g., contributor number, cycle time, differential degradation) are to be considered and when they are to be applied.
- Become familiar with the criteria used to evaluate a TrueAllele® run to determine if it is usable/reportable.
- Become familiar with the proper documentation, electronic record retention, and peer review required for each step of the analysis process.
- Become proficient at presenting the results of TrueAllele® statistical analyses to attorneys, juries, and/or other scientists.

14.2 Tasks

14.2.1 Read the FB PM TrueAllele® Casework System.

14.2.2 Read the TrueAllele® statistical analysis references and required readings listed in Appendix A of this manual.

14.2.3 Complete the VUIer® orientation class given by vendor or training coordinator.

14.2.4 Complete the TrueAllele® Operator I (basic upload) assignments and questions in succession.

NOTE: Trainee is to receive feedback on each assignment prior to beginning the next assignment.

14.2.5 Successfully complete the TrueAllele® Operator I exam.

14.2.5.1 The exam is to be completed independently.

14.2.5.2 Successful completion requires a graded score of 90% or higher.

14.2.6 Complete the TrueAllele® Operator II (advanced analysis) assignments and questions in succession.

NOTE: Trainee is to receive feedback on each assignment prior to beginning the next assignment.

14.2.7 Complete the TrueAllele® Operator II exam.

14.2.7.1 The exam is to be completed independently.

14.2.7.2 Successful completion requires a graded score of 90% or higher.

14.2.8 Complete the TrueAllele® Reporter assignments and questions in succession.

NOTE: Trainee is to receive feedback on each assignment prior to beginning the next assignment.

14.2.9 Complete the TrueAllele® Reporter exam.

14.2.9.1 The exam is to be completed independently.

14.2.9.2 Successful completion requires a graded score of 90% or higher.

14.2.10 Document a review of a minimum of fifteen (15) previously released TrueAllele® cases including an interpretation of the analysis of the data previously run.

14.2.11 Perform TrueAllele® statistical analysis on at least seven (7) previously analyzed TrueAllele® cases to demonstrate the ability to achieve concordant results and document those results using the worksheets/supporting documentation used by the Department for real cases.

14.2.12 Document the practice technical review of a minimum of five (5) TrueAllele® cases prepared by a qualified TrueAllele® examiner which will then be officially technically reviewed by another qualified examiner.

14.3 Evaluation

14.3.1 Knowledge of the trainee will be evaluated through:

- Review of notes, training documentation and the training notebook by the training coordinator.
- Mini-mock trial(s) and/or question and answer session(s), as deemed necessary by the training coordinator.

14.3.2 The trainee should demonstrate the ability to clearly, accurately and concisely set forth TrueAllele® statistical results.

14.4 Examiner Qualification

14.4.1 The final qualification of a trainee to perform TrueAllele® statistical analyses, issue associated Certificates of Analysis and conduct associated technical reviews will be based upon the following:

14.4.1.1 Successful completion of the required training tasks

14.4.1.2 Satisfactory performance in an oral competency examination

14.4.1.2.1 Attendees will include, at a minimum, the training coordinator and Biology Program Manager.

14.4.1.2.2 This examination will include both technical questions and court-style questions focused on aspects related specifically to TrueAllele® statistical analysis and casework.

14.4.1.3 Satisfactory completion of a practical exam

14.4.1.3.1 This practical exam will be worked as if it were an actual case and will serve as a means by which the trainee's proficiency in performing TrueAllele® analysis, interpretation skills, casework documentation and reporting can be evaluated.

14.4.1.3.2 This practical exam will be graded by the training coordinator.

14.4.2 Upon successful completion of the training requirements detailed in this Chapter, including a satisfactory performance in the oral competency exam and satisfactory completion of the practical exam, a Memorandum for Record (MFR) detailing the recommendation to qualify the trainee as a TrueAllele® statistical analysis examiner will be submitted to the Biology Program Manager. Once this MFR is accepted and signed by the Biology Program Manager, the examiner will be deemed qualified to perform TrueAllele® statistical analysis on casework, issue associated Certificates of Analysis, and to technically review TrueAllele® cases.

15 FAMILIAL DNA SEARCHING

The requirements set forth in this chapter are intended to apply to the training of a qualified Forensic Biology examiner to perform familial DNA searching, issue associated Certificates of Analysis, and to technically review familial DNA searching cases. Not all Forensic Biology examiners will necessarily be trained to perform these functions.

The trainee is required to be either currently qualified or concurrently in training in Y-STR analysis (or other current genetic identity testing systems in use for familial DNA searching) and kinship statistical analysis.

Because it is presumed that the trainee is either previously qualified in or concurrently training in Y-STR analysis and kinship statistical analysis, the training requirements for each of those areas of study are not addressed in this chapter. Rather, they are addressed in the applicable chapters in this manual.

The trainee will prepare and maintain an organized notebook, including electronic files, which will serve as a ready-reference during the examiner's tenure at the Department.

The training program will be monitored by a training coordinator designated by the Biology Program Manager.

Documentation of this supplemental training will be maintained on the appropriate Forensic Biology Training Documentation Form rather than in monthly Qualtrax workflow format. This training form will be kept up to date and made available to the Biology Program Manager for review upon request.

15.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Become familiar with the function and use of Microsoft Excel® (e.g., spreadsheets, macros, setting and changing cell functions).
- Become familiar with the function and use of the Denver Familial Search Program (DFSP).
- Develop an understanding of the statistical analysis performed by the DFSP at each step of the analysis and sorting process.
- Become familiar with the theory and practice of the Department's candidate sorting thresholds and determination of the Modified Estimated Kinship Ratio (mEKR) used for setting sorting thresholds.
- Become familiar with the quality control measures used for familial DNA searches.
- Become proficient at interpreting results and communicating conclusions and any limitations.
- Become proficient at properly documenting primary and repeated searches, archiving data and writing Certificates of Analysis in accordance with the Department's policies and procedures.

15.2 Tasks

15.2.1 Read the FB PM Familial DNA Testing.

15.2.2 Read the Department's validation summary pertaining to familial DNA testing.

15.2.3 Read pertinent literature provided by the training coordinator – at minimum, all references cited within the FB PM Familial DNA Testing, which are included in Appendix A.

15.2.4 Shadow an examiner qualified in familial DNA searching to observe and review a minimum of one familial search process from start to finish.

15.2.4.1 If no new search is available, a previous case search may be used and the administrative/agency communication aspects reviewed in detail.

15.2.5 Simulate and run a small (≤10 synthetic profiles) validation-like familial search.

15.2.5.1 Select and verify several of the search likelihood ratio calculations – including both parent/offspring and sibling kinship by hand calculation or using PopStats.

15.2.5.2 Test the software's programmed functions by selecting at least one each for which the following is demonstrated:

- A mutation calculation
- A minimum allele frequency calculation
- A tri-allele locus pattern calculation
- A calculation between profiles with results at a different number of loci

15.2.5.3 Conduct a minimum of one simulated familial search with the assistance of an examiner qualified in familial DNA searching to develop hands-on skills and knowledge of the software and documentation process.

15.2.5.4 Independently perform a minimum of three (3) familial searches on previously analyzed cases and create associated Certificates of Analysis.

15.2.5.4.1 These cases will include at least one of each of the following:

- Positive familial association case
- Negative result case
- Repeated search case

15.2.6 Document the practice technical review of a minimum of two (2) familial DNA search cases prepared by a qualified examiner which will then be officially technically reviewed by another qualified examiner.

15.3 Evaluation

15.3.1 Knowledge of the trainee will be evaluated through:

- Review of notes, calculations, documentation and Certificates of Analysis in the training notebook by the training coordinator.
- Mini-mock trial(s) and/or question and answer session(s), as deemed necessary by the training coordinator.

15.3.2 The trainee should demonstrate the ability to clearly, accurately and concisely set forth familial DNA searching results.

15.4 Examiner Qualification

15.4.1 The final qualification of a trainee to perform familial DNA searching and issue associated Certificates of Analysis will be based upon the following:

15.4.1.1 Successful completion of the required training tasks

15.4.1.2 Satisfactory performance in an oral competency examination

15.4.1.2.1 Attendees will include, at a minimum, the training coordinator and Biology Program Manager.

15.4.1.2.2 This examination will include both technical questions and court-style questions focused on aspects related specifically to familial DNA searching and casework.

15.4.1.3 Satisfactory completion of a practical exam

15.4.1.3.1 This practical exam will be worked as if it were an actual case and will serve as a means by which the trainee's ability to properly conduct familial DNA searches, interpret familial search results, document the familial search results

according to Department protocol and produce a Certificate of Analysis can be evaluated.

15.4.1.3.2 This practical exam will be graded by the training coordinator.

15.4.1.4 Upon successful completion of the training requirements detailed in this Chapter, including a satisfactory performance in the oral competency exam, a satisfactory completion of the practical exam, qualification as a Y-STR examiner, and qualification as a kinship statistical analysis examiner, a Memorandum for Record (MFR) detailing the recommendation to qualify the trainee as a familial DNA search examiner will be submitted to the Biology Program Manager. Once this MFR is accepted and signed by the Biology Program Manager, the examiner will be deemed qualified to perform familial DNA searching on casework, issue associated Certificates of Analysis, and to technically review familial DNA search cases.

16 STRMIX™ SYSTEM STATISTICAL ANALYSIS

The requirements set forth in this chapter are intended to apply to the training of a qualified Forensic Biology examiner to perform STRmix™ System analyses, issue associated Certificates of Analysis, and to technically review STRmix™ cases. Not all Forensic Biology examiners will necessarily be trained to perform these functions.

The trainee will prepare and maintain an organized notebook, including electronic files, which will serve as a ready-reference during the examiner's tenure at the Department.

The training program will be monitored by a training coordinator designated by the Biology Program Manager.

Documentation of this supplemental training will be maintained on the appropriate Forensic Biology Training Documentation Form rather than in monthly Qualtrax workflow format. This training form will be kept up to date and made available to the Biology Program Manager for review upon request.

16.1 Purpose and Scope

During the completion of this area of study the trainee will:

- Develop an understanding of the theory behind the likelihood ratio and the different ways in which it can properly be expressed.
- Become familiar with the STRmix™ System Software.
- Become proficient at operating the software.
- Become familiar with the criteria used to evaluate a STRmix™ deconvolution to determine if it is useable/reportable.
- Become familiar with the critera used to evaluate a STRmix™ Likelihood Ratio analysis to determine if it is useable/reportable.
- Become familiar with the proper documentation, electronic record retention, and peer review required for each step of the analysis process.
- Become proficient at presenting the results of STRmix™ statistical analyses to attorneys, juries, and/or other scientists.

16.2 Tasks

16.2.1 Read the FB PM STRmix™ System.

16.2.2 Read the Department's validation summary pertaining to STRmix™.

16.2.3 Read pertinent literature provided by the training coordinator – at a minimum, all references cited within the FB PM STRmix™ System, which are included in Appendix A.

16.2.4 Attend the STRmix™ Training Workshop offered by the Institute of Environmental Science and Research Limited (ESR).

16.2.5 Attend lectures provided by the training coordinator or designee.

16.2.6 Conduct STRmix™ System analyses on the following training sets of data:

- 2 contributor set
- 3 contributor set
- 4 contributor set

16.2.7 Document a review of a minimum of five (5) previously released STRmix™ cases including an interpretation of the analysis of the data previously run.

16.2.8 Perform STRmix™ statistical analysis on at least seven (7) previously analyzed STRmix™ cases to demonstrate the ability to achieve concordant results and document those results using the worksheets/supporting documentation used by the Department for casework.

16.2.9 Document the practice technical review of a minimum of five (5) STRmix™ cases prepared by a qualified STRmix™ examiner which will then be officially technically reviewed by another qualified examiner.

16.3 Evaluation

16.3.1 Knowledge of the trainee will be evaluated through:

- Review of notes, training documentation and the training notebook by the training coordinator.
- Mini-mock trial(s) and/or question and answer session(s), as deemed necessary by the training coordinator.

16.3.2 The trainee should demonstrate the ability to clearly, accurately and concisely set forth STRmix™ statistical results.

16.4 Examiner Qualification

16.4.1 The final qualification of a trainee to perform STRmix™ statistical analyses, issue associated Certificates of Analysis and conduct associated technical reviews will be based upon the following:

16.4.1.1 Successful completion of the required training tasks

16.4.1.2 Satisfactory performance in an oral competency examination

16.4.1.2.1 Attendees will include, at a minimum, the training coordinator and Biology Program Manager.

16.4.1.2.2 This examination will include both technical questions and court-style questions focused on aspects related specifically to STRmix™ statistical analysis and casework.

16.4.1.3 Satisfactory completion of a practical exam

16.4.1.3.1 This practical exam will be worked as if it were an actual case and will serve as a means by which the trainee's proficiency in performing STRmix™ analysis, interpretation skills, casework documentation and reporting can be evaluated.

16.4.1.3.2 This practical exam will be graded by the training coordinator.

16.4.1.4 Upon successful completion of the training requirements detailed in this Chapter, including a satisfactory performance in the oral competency exam and satisfactory completion of the practical exam, a Memorandum for Record (MFR) detailing the recommendation to qualify the trainee as a STRmix™ statistical analysis examiner will be submitted to the Biology Program Manager. Once this MFR is accepted and signed by the Biology Program Manager, the examiner will be deemed qualified to perform STRmix™ statistical analysis on casework, issue associated Certificates of Analysis, and to technically review STRmix™ cases.

APPENDIX A – REFERENCES AND REQUIRED READINGS

Isolation/Purification

Akane, A., Matsubara, K., Nakamura, H., Takahashi, S., and Kimura, K., "Identification of Heme Compound Copurified with Deoxyribonucleic Acid (DNA) from Bloodstains, a Major Inhibitor of Polymerase Chain Reaction (PCR) Amplification," Journal of Forensic Sciences, Vol. 39: 362-372, 1994.

Butler JM. Advanced topics in Forensic DNA typing: methodology. Elsevier Academic Press, Waltham. 2012. Chapter 2 pp 29-48.

Connon, Catherine Cupples. Forensic DNA Analysis: Methods and Protocols. Humana Press, New York. 2023. Chapter 2 pp 23-24 and 30-32, Chapter 3 pp 35-39 and 45-50, Chapter 6 pp. 93-102, and Chapter 7 pp.103-116.

DNA IQ™ System – Frequently Asked Questions (https://www.promega.com/-/media/files/resources/profiles-in-dna/501/dna-iq-system-frequently-asked-questions.pdf?rev=d20abf9fb6b34dedade70d22901d1c51&sc_lang=en).

DNA IQ™ System – Small Sample Casework Technical Manual

Greenspoon, S. and Ban, J., "Robotic Extraction of Mock Sexual Assault Samples Using the BioMek® 2000 and the DNA IQ™ System", Profiles in DNA, Volume 5, No.1, February 2002.

Inman, K. M. and Rudin, N., An Introduction to Forensic DNA Analysis, CRC Press, New York, 2002, Chapters 7, 9, and 11.

Mandrekar, P. V., Krenke, B. E., and Tereba, A., "DNA IQ™: The Intelligent Way to Purify DNA", Profiles in DNA, Volume 4, No.3, July 2001.

Microcon User Guide. Available from:
https://www.sigmaaldrich.cn/deepweb/assets/sigmaaldrich/product/documents/338/456/pr04305-rev0618.pdf

Wallace, D. M., "Large and Small-Scale Phenol Extractions," Methods in Enzymology, Vol. 152: 33-40, 1987.

Wallace, D. M., "Precipitation of Nucleic Acids," Methods in Enzymology, Vol. 152: 41-48, 1987.

Quantitation

Hakim HM, Khan HO, Ismail SA, Ayob S, Lalung J, Kofi EA, Chambers GK, Edinur HA. Assessment of autosomal and male DNA extracted from casework samples using Casework Direct Kit, Custom and Maxwell 16 System DNA IQ Casework Pro Kit for autosomal-STR and Y-STR profiling. Sci Rep. 2019 Oct 10;9(1):14558. doi: 10.1038/s41598-019-51154-4. PMID: 31601905; PMCID: PMC6787247.

Margaret M Ewing 1, Jonelle M Thompson 2, Robert S McLaren 3, Vincent M Purpero 4, Kelli J Thomas 5, Patricia A Dobrowski 6, Gretchen A DeGroot 7, Erica L Romsos 8, Douglas R Storts. Human DNA quantification and sample quality assessment: Developmental validation of the PowerQuant(®) system, Forensic Sci Int Genetics. 2016 Jul:23:166-177. doi: 10.1016/j.fsigen.2016.04.007. Epub 2016 Apr 16.

Krenke, BE Nassif N, Sprecher CJ, Knox C, Schwandt M, Storts DR.Developmental validation of a real-time PCR assay for the simultaneous quantification of total human and male DNA. Forensic Sci Int Genet. 2008 Dec;3(1):14-21.

Poetsch M, Konrad H, Helmus J, Bajanowski T, von Wurmb-Schwark N. Does zero really mean nothing?-first experiences with the new PowerQuant(TM) system in comparison to established real-time quantification kits. Int J Legal Med. 2016 Jul;130(4):935-940. doi: 10.1007/s00414-016-1352-1. Epub 2016 Mar 14. PMID: 26972802.

Amplification

Andersen, J. F., Greenhalgh, M. J., Butler, H. R., Kilpatrick, S. R., Piercy, R. C., Way, K. A., Myhill, H. S., Wright, J. C., Hallett, R., and Parkin B. H., "Further validation of a multiplex STR system for use in routine forensic identity testing," Forensic Science International, Vol. 78: 47-64, 1996.

Bacher, J., Schumm, J.W., "Development of Highly Polymorphic Pentanucleotide Tandem Repeat Loci with Low Stutter", Profiles in DNA, September 1998.

Butler, JM (2012) *Advanced Topics in Forensic DNA Typing: Methodology.* Chapter 5 – Short Tandem Repeat (STR) Loci and Kits.", Elsevier. New York.

Crouse, C. A. and Schumm, J., "Investigation of Species Specificity Using Nine PCR-Based Human STR Systems," Journal of Forensic Sciences, Vol. 40: 952-956, 1995.

Dembinski GM, Sobieralski C, Picard CJ. Estimation of the number of contributors of theoretical mixture profiles based on allele counting: Does increasing the number of loci increase success rate of estimates? Forensic Sci Int Genet. 2018 Mar;33:24-32. doi: 10.1016/j.fsigen.2017.11.007. Epub 2017 Nov 20. PMID: 29175725.

Developmental Validation of the PowerPlex® Fusion System for Analysis of Casework and Reference Samples: A 24-locus Multiplex for New Database Standards. Kathryn Oostdik et al. FSI: Genetics, 12 (2014) 69-76.

Duncan Taylora,b,*, Jo-Anne Brightc, Catherine McGovenc, Christopher Hefforda,Tim Kalafutd, John Buckleton, Validating multiplexes for use in conjunction with modern interpretation strategies, https://doi.org/10.1016/j.fsigen.2015.09.011

Edwards, A., Civetello, A., Hammond, H. A., and Caskey, C. T., "DNA Typing and Genetic Mapping with Trimeric and Tetrameric Tandem Repeats," Am. J. Hum. Genet., Vol. 49: 746-756, 1991.

Hammond, H. A., Jin, L., Zhong, Y., Caskey, C. T., and Chakraborty, R., "Evaluation of 13 Short Tandem Repeat Loci for Use in Personal Identification Applications," Am. J. Hum. Genet., Vol. 55: 175-189, 1994.

Hill, Becky., 2012 STR Loci and Multiplex Kits – NIST PowerPoint

Levinson, G. and Gutman, G. A., "Slipped-Strand Mispairing: A Major Mechanism for DNA Sequence Evolution," Mol. Biol. Evol., Vol. 4: 203-221, 1987.

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

Micka, K. A., Sprecher, C. J., Lins, A. M., Comey, C. T., Koons, B. W., Crouse, C., Endean, D., Pirelli, K., Lee, S. B., Duda, N., Ma, M., and Schumm, J. W., "Validation of Multiplex Polymorphic STR Amplification Sets Developed for Personal Identification Applications," Journal of Forensic Sciences, Vol. 41: 582-590, 1996.

Molecular Biology: Principles and Practice by Michael M. Cox, Jennifer Doudna, Michael O'Donnel© 2015 W. H. Freeman 2nd edition, Chapters 6, 10, 11, 12, 13, and 17, ISBN 1464126143Textbook ISBN: 9781464126147

Mullis, K. B., "The Unusual Origin of the Polymerase Chain Reaction," Scientific American, pp. 56-65, April 1990.

Nakahori, Y., Takenaka, O. and Nakagome, Y., "A Human X-Y Homologous Region Encodes Amelogenin", Genomics 9, 264-269, 1991.

PowerPlex® Fusion System Technical Manual

Puers, C., Linns, A. M., Sprecher, C. J., Brinkmann, B., and Schumm, J. W., "Analysis of Polymorphic Short Tandem Repeat Loci Using Well-Characterized Allelic Ladders," Proceedings from the 4th International Symposium on Human Identification, pp. 161-172, 1993.

Reynolds, R. and Sensabaugh, G., "Analysis of Genetic Markers in Forensic DNA Samples Using the Polymerase Chain Reaction," reprinted from Analytical Chemistry, Vol. 63, 1991.

Ruano, Gualberto, Brash, Douglas E., and Kidd, Kenneth K., "PCR: The First Few Cycles," Perkin Elmer Cetus Amplifications - A Forum for PCR Users, Issue 7, pp. 1-4, October 1991.

Santos, F.R., Pandya, A., and Tyler-Smith, C., "Reliability of DNA Based Sex Tests", Nature Genetics Vol. 18, p 103, February 1998.

Sardelli, A.D., "Plateau Effect - Understanding PCR Limitations," Perkin Elmer Cetus Amplifications - A Forum for PCR Users, Issue 9, pp. 1 and 3-5, March 1993.

Schlötterer, C. and Tautz, D., "Slippage synthesis of simple sequence DNA," Nucleic Acids Research, Vol 20: 211-215, 1992.

Sprecher, C. J., Puers, C., Lins, A. M., and Schumm, J. W., "General Approach to Analysis of Polymorphic Short Tandem Repeat Loci," BioTechniques, Vol. 20: 266-276, 1996.

Sullivan, K.M., Mannucci, A. Kimpton, C.P., and Gill, P., "A Rapid and Quantitative DNA Sex Test: Fluorescence-Based PCR Analysis of X-Y Homologous Gene Amelogenin, BioTechniques, Vol. 15, No. 4, 636-640, 1993.

Walsh, P. S., Fildes, N. J., and Reynolds, R., "Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA, "Nucleic Acids Research, Vol 24: 2807-2812, 1996.

Young, D.R., Tun, Z., Honda, K., and Matoba, R., "Identifying Sex Chromosome Abnormalities in Forensic DNA Testing Using Amelogenin and Sex Chromosome Short Tandem Repeats", Journal of Forensic Sciences, Vol. 46: 346-348, 2001.

Capillary Electrophoresis

Connon, C.C. (2023) *Forensic DNA Analysis Methods and Protocols*. "Chapter 18 – Capillary Electrophoresis with Applied Biosystems' 3500 Genetic Analyzer." Humana Press. New York. pp. 285-305

Butler, JM (2012) *Advanced Topics in Forensic DNA Typing: Methodology.* "Chapter 5 – Short Tandem Repeat (STR) Loci and Kits." "Chapter 6 – Capillary Electrophoresis: Principles and Instrumentation." Elsevier. New York. pp. 99-139, 141-162.

McCord, B.R., Buel, E., "Capillary Electrophoresis in Forensic Genetics", Encyclopedia of Forensic Sciences (Second Edition), pp. 394-401, Elsevier. New York. 2013.

Schumm, J. W., "Why Use a Size Marker and Allelic Ladder in STR Analysis," Profiles in DNA, Vol. 1, No. 2, pp. 11-13, August 1997.

Wenz, H. M.; Robertson, J. M.; Menchen, S.; Oaks, F.; Demorest, D. M.; Scheibler, D.; Rosenblum, B. B.; Wike, C.; Gilbert, D. A.; Efcavitch, J. W. (1998) High-precision genotyping by denaturing capillary electrophoresis. Genome Res. 8: 69-80.

Statistics and Other Topics

Corrigendum to "Linkage disequilibrium analysis of D12S391 and vWA in U.S. population and paternity samples" FSI: Genetics (in press), doi:10.1016/j.fsigen.2010.09.003.

Dalsgaard, S. et al., "Non-uniform phenotyping of D12S391 resolved by second generation sequencing" FSI:Genetics 8 (2014) 195-199.

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories, July 2020.

Gill, Peter et al., "An evaluation of potential allelic association between the STRs vWA and D12S391: Implications in criminal casework and applications to short pedigrees." FSI: Genetics 6 (2012) 477-486.

Kelly, Hannah, Bright, Jo-Anne, Buckleton, John S., Curran, James M., "A comparison of statistical models for the analysis of complex forensic DNA profiles" https://doi.org/10.1016/j.scijus.2013.07.003

O'Connor, Kristen L, Tillmar, Andreas O., "Effect of linkage between vWA and D12S391 in kinship analysis." FSI: Genetics 6 (2012) 840-844.

O'Connor, Kristen L, Butts, Erica, Hill, Carolyn R., Butler, John M., and Vallone, Peter M. "Evaluating the Effect of Additional Forensic Loci on Likelihood Ratio Values for Complex Kinship Analysis." Proceedings of the 21st International Symposium on Human Identification (October 12, 2010).

Steffen C.R., Coble M.D., Gettings K.B., Vallone P.M. (2017) Corrigendum to 'U.S. Population Data for 29 Autosomal STR Loci' [Forensic Sci. Int. Genet. 7 (2013) e82-e83]. Forensic Sci Int Genet. 31:e36-e40.

U.S. Department of Justice, Office of Justice Programs, *National Institute of Justice*. National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach, 2018.

U.S. Population Data for 29 Autosomal STR Loci. Hill et al. FSI-genetics 2013 (7) e82-e83.

Variability of New STR Loci and Kits in US Population Groups. Butler et al. Profiles in DNA

Trace and Transfer DNA

Bowman, Z.E., Mosse, K.S.A, Sungaila, A.M., van Oorschot, R.A.H., Hartman, D., "Detection of offender DNA following skin-to-skin contact with a victim," Forensic Science International: Genetics, Vol. 37, pp. 252-259, 2018.

Cale, Cynthia M., Earll, Madison E., Latham, Krista E., and Bush, Gay L., "Could Secondary DNA Transfer Falsely Place Someone at the Scene of a Crime?" JFS 2016 Jan;61(1):196-203. doi: 10.1111/1556-4029.12894. Epub 2015 Sep 1.

Magee, A.M., Breathnach, M., Doak, S., Thornton, F., Noone, C., McKenna, L.G., "Wearer and non-wearer DNA on the collars and cuffs of upper garments of worn clothing," Forensic Science International: Genetics, Vol. 34, pp. 152-161, 2018.

Murphy, C., Kenna, J., Flanagan, L., Gorman, M.L., Boland, C., Ryan, J., "A study of the background levels of male DNA on underpants worn by females," Journal of Forensic Science, Vol. 65, pp. 399-405, 2020.

Taylor, D., Biedermann, A., Samie, L., Pun, K., Hicks, T., Champod, C., "Helping to distinguish primary from secondary transfer events for trace DNA," Forensic Science International: Genetics, Vol. 28, pp. 155-177, 2017.

Van Oorschot RAH, Meakin GE, Kokshoorn B, Goray M, Szkuta B. DNA Transfer in Forensic Science: Recent Progress towards Meeting Challenges. Genes (Basel). 2021 Nov 7;12(11):1766. doi: 10.3390/genes12111766. PMID: 34828372; PMCID: PMC8618004.

Y-STR REFERENCES AND REQUIRED READINGS

AmpFlSTR® Yfiler® PCR Amplification Kit User Guide 2014

Bosch, E., Calafell, F., Santos, F. R., Perez-Lezaun, A., Comas, D., Benchemsi, N., Tyler-Smith, C., and Bertranpetit, J. (1999) Variation in short tandem repeats is deeply structured by genetic background on the human Y chromosome. *Am.J.Hum.Genet.* 65(6): 1623-1638.

Butler, J.M. (2012) Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Waltham, MA, U.S.A. Chapter 13 Y-Chromosome DNA Testing.

Corach, D., Risso, L.F., Marino, M., Penacino, G., and Sala, A. (2001) routine Y-STR typing in forensic casework. *Forensic Sci. Int.* 118(2-3): 131-5.

Delfin, F.C., Madrid, B.J., Tan, M.P., and De Ungria, M.C. (2005) Y-STR analysis for detection and objective confirmation of child sexual abuse. *Int J Legal Med.* 119(3): 158-63.

Diederiche, M., Martin, P., Amorim, A., Corte-Real, F., and Gusmao, L. (2005) A case of double alleles at three Y-STR loci: forensic implications. *Int. J. Legal Med.* 119(4):223-5.

Gross, A.M., Liberty, A.A., Ulland, M.M., and Juriger, J.K. (2008) Internal validation of the AmpFlSTR® Yfiler™ Amplification Kit for use in forensic casework. *J Forensic Sci*. 53(1): 125-34.

Honda, K., Roewer, L., and de Knijff, P. (1999) Male DNA typing from 25-year-old vaginal swabs using Y-chromosomal STR polymorphisms in a retrial request case. *J.Forensic Sci.* 44(4): 868-872.

Honda, K., Tun, Z., Young, D., and Terao, T. (2001) Examination of Y-STR mutations in sex chromosomal abnormality in forensic cases. *Forensic Sci. Int*. 118(2-3): 136-140.

Kayser, M. and Sajantila, A. (2001) Mutations at Y-STR loci: implications for paternity testing and forensic analysis. *Forensic Sci. Int.* 118(2-3): 116-21.

Kayser, M., Brauer, S., Willuweit S., Schadlich H., Batzer, M.A., Zawacki, J., Prinz, M., Roewer, L., and Stoneking, M. (2002) Online Y-chromosomal short tandem repeat haplotype reference deatabase (YHRD) for U.S. populations. *J Forensic Sci.* 47(3): 513-9.

Jobling, M. A., Pandya, A., and Tyler-Smith, C. (1997) The Y chromosome in forensic analysis and paternity testing. *Int.J.Legal Med.* 110(3): 118-124.

Mayntz-Press, K.A., et al. (2008) Y-STR Profiling in Extended Interval (≥3 days) Postcoital Cervicovaginal Samples *J Forensic Sci* 53 (2), 342-348.

Mayntz-Press, K.A., Ballantyne, J. (2007) Performance Characteristics of Commercial Y-STR Multiplex Systems *J Forensic Sci* 52 (5), 1025–1034.

Mitchell, R. J. and Hammer, M. F. (1996) Human evolution and the Y chromosome. *Curr.Opin.Genet.Dev.* 6(6): 737-742.

Mulero, J.J., Chang, C.C., Calandro, L.M., Green, R.L., Li Y., Johnson, C.L., and Hennessy, L.K. Development and validation of the AmpFlSTR® Yfiler™ PCR amplification kit: a male specific, single amplification 17 Y-STR multiplex system. *J Forensic Sci* 2006;51(1):64–75.

Sibille, I., Duverneuil, C., Lorin de la Grandmaison, G., Guerrouache, K., Teissiere, F., Durigon, M., and deMazancourt, P. (2002) Y-STR DNA amplification as biological evidence in sexually assaulted female victims with no cytological detection of spermatozoa. *Forensic Sci. Int.* 125(2-3): 212-6.

KINSHIP STATISTICAL ANALYSIS REFERENCES AND REQUIRED READINGS

Allen, Robert W. et al., 2007. Considerations for the interpretation of STR results in cases of questioned half-sibship. Transfusion (March 2007). 47:515-519.

Baur, M. P. et al., No Fallacies in the Formulation of the Paternity Index. American Journal of Human Genetics (1986) 39:528-536.

Chang En Pu MS and Adrian Linacre PhD. Increasing the confidence in half-sibship determination based upon 15 STR loci. Journal of Forensic and Legal Medicine 15 (2008) 373-377.

Chang En Pu MS and Adrian Linacre PhD. Systematic evaluation of sensitivity and specificity of sibship determination by using 15 STR loci. Journal of Forensic and Legal Medicine 15 (2008) 329-334.

Gjertson, D., Appendix 8. AABB Guidelines document. 12 ed.

Ge et al., Developing criteria and data to determine best options for expanding the core CODIS loci. Investigative Genetics 2012, 3:1

Gjertson, David W. et al., ISFG: Recommendations on biostatistics in paternity testing. FSI: Genetics 1 (2007) 223-231.

Mickey, M. R., Gjertson, D. W., and Terasaki, P. I., Empirical Validation of the Essen-Möller Probability of Paternity. American Journal of Human Genetics (1986) 39:123-132.

Poetsch, Micaela, et al., The problem of single parent/child paternity analysis – Practical results involving 336 children and 348 unrelated men. FSI (2005) – Article in Press.

Reid, Thomas M. et al., Specificity of Sibship Determination Using the ABI Identifiler Multiplex System. Journal of Forensic Science. November 2004. Vol. 49. No. 6.

Silver, H. and Schoppmann, A., Limitations of paternity testing calculations. Transfusion 1987. 27:288-290.

Sulzer, A., Dion, D., and Kratzer, A., Reliability of Results in Sibship Cases. FSI: Genetics Supplement Series 3 (2011) e95-e96.

Valentin, Jack. Chapter 8: Positive Evidence of Paternity Calculated According to Essen-Möller: The Bayesian Approach. Inclusion Probabilities in Parentage Testing. American Association of Blood Banks. 1983.

Wenk, R.E., Traver, M., and Chiafari, F.A., 1996. Determination of sibship in any two persons. Transfusion 1996. 36:259-262.

TRUEALLELE® STATISTICAL ANALYSIS REQUIRED REFERENCES AND READINGS

Lindley, Dennis V. (2006) Understanding Uncertainty. Wiley-Interscience. Chapters 1-6.

1. Uncertainty
2. Stylistic Questions
3. Probability
4. Two Events
5. The Rules of Probability
6. Bayes Rule

TrueAllele® Casework: VUIer® Tutorial Manual

Lectures:

A. Introduction
   1. Casework Validation of Genetic Calculator Mixture Interpretation
      http://www.cybgen.com/information/presentations/2010/AAFS/Perlin_Casework_validation_of_genetic_calculator_mixture_interpretation/page.shtml
   2. Three Match Statistics, One Verdict
      http://www.cybgen.com/information/presentations/2010/AAFS/Perlin_Three_match_statistics_one_verdict/page.shtml
   3. Computer Interpretation of Uncertain DNA Evidence
      http://www.cybgen.com/information/presentations/2011/NIJ/Perlin_Computer_interpretation_of_uncertain_DNA_evidence/page.shtml

B. Basics
   4. Biology and Information
      http://www.cybgen.com/information/courses/2010/TAVC/Perlin_DNA_Identification_for_Scientists_Basics/page.shtml
   5. Bayesian Belief Update
      http://www.cybgen.com/information/courses/2010/TAVC/Perlin_DNA_Identification_for_Scientists_Basics/page.shtml
   6. Inclusion Genotype and LR
      http://www.cybgen.com/information/courses/2010/TAVC/Perlin_DNA_Identification_for_Scientists_Basics/page.shtml

C. Methods
   7. Stochastic Effects
      http://www.cybgen.com/information/courses/2010/TAVC/Perlin_DNA_Identification_for_Scientists_Methods/page.shtml
   8. Quantitative Data Modeling
      http://www.cybgen.com/information/courses/2010/TAVC/Perlin_DNA_Identification_for_Scientists_Methods/page.shtml
   9. Mixture Weight and Inference
      http://www.cybgen.com/information/courses/2010/TAVC/Perlin_DNA_Identification_for_Scientists_Methods/page.shtml

D. Information
   10. Sherlock Holmes and the DNA Likelihood Ratio
       http://www.cybgen.com/information/presentations/2011/AAFS/Perlin_Sherlock_Holmes_and_the_DNA_likelihood_ratio/page.shtml
   11. Explaining the Likelihood Ratio in DNA Mixture Interpretation
       http://www.cybgen.com/information/presentations/2010/ISHI/Perlin_Explaining_the_likelihood_ratio_in_DNA_mixture_interpretation/page.shtml
   12. The Science of Quantitative DNA Mixture Interpretation
       http://www.cybgen.com/information/presentations/2011/SWGDAM/Perlin_The_science_of_quantitative_DNA_mixture_interpretation/page.shtml

Validation Studies:

1. Perlin, M.W., et.al. Virginia TrueAllele® Validation Study: Casework Comparison. 2013.

2. VA DFS Validation of TrueAllele®
3. VA DFS Fusion Validation

2017 SWGDAM DNA Interpretation Guidelines

Perlin, MW, Legler MM, Spencer CE, Smith JL, Allan WP, Belrose JL, Duceman BW. Validating TrueAllele® DNA mixture interpretation. Journal of Forensic Sciences. 2011; 56(6): 1430-47. (Manuscript available at: http://www.cybgen.com/information/publication/2011/JFS/Perlin-Duceman-Validating-TrueAllele-DNA-mixture-interpretation/page.shtml)

Perlin, M.W., Explaining the likelihood ratio in DNA mixture interpretation in the proceedings of Promega's Twenty-First International Symposium on Human Identification. San Antonio, TX. 2010. (Manuscript available at: http://www.cybgen.com/information/publication/2010/ISHI/Perlin-Explaining-the-likelihood-ratio-in-DNA-mixture-interpretation/page.shtml)

Perlin, M.W., Belrose, J.L., and Duceman, B.W. New York State TrueAllele® Casework Validation Study. Journal of Forensic Sciences. 58(6), 2013.

Thompson, William C., Uncertainty in probabilistic genotyping of low template DNA: A case study comparing STRmix and TrueAllele. *Journal of Forensic Sciences*. 2023; 00:1-15.

Greenspoon, Susan A., et al., Establishing the Limits of TrueAllele® Casework: A Validation Studay. Journal of Forensic Sciences. 60 (5), 2015.

FAMILIAL DNA SEARCHING REFERENCES AND REQUIRED READINGS

Scientific Working Group on DNA Analysis Methods Ad Hoc Committee on Partial Matches. SWGDAM recommendations to the FBI Director on the "interim plan for the release of information in the event of a 'partial match' at NDIS". Forensic Sci. Commun. 11 (2009).

Myers SP, Timken MD, Piucci ML, et. al. Searching for first-degree familial relationships in California's offender DNA database: Validation of a likelihood ratio-based approach. Forensic Sci. Int. Genet. (2010)j.fisgen.2010.10.010

Jobling, M.A. and Tyler-Smith, C. (2003) "The Human Y Chromosome: An Evolutionary Marker Comes of Age," Nature Reviews: Genetics, 4: 598-612.

Butler, J.M. (2005) Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers, 2nd Ed., Elsevier: Burlington, MA, USA.

STRMIX SYSTEM STATISTICAL ANALYSIS REFERENCES AND REQUIRED READINGS

STRmix™ Operation Manual, v2.6.

STRmix™ Users Manual, v 2.6.

A Guide to STRmix™ - March 2017 (Client Guide).

Bright, J., *et al.* (2013) Developing allelic and stutter peak height models for a continuous method of DNA Interpretation, *Forensic Science International: Genetics*, Volume 7, pages e296-e304.

Bright, J. *et al*. (2016) Developmental validation of STRmix™, expert software for the interpretation of forensic DNA profiles, *Forensic Science International: Genetics*, Volume 23, pages e226-e239.

Bright J.. *et al*. (2018) Internal validation of STRmix™ – A multi laboratory response to PCAST, *Forensic Science International: Genetics,* https://doi.org/10.1016/j.fsigen.2018.01.003.

Curran, J., *et.al.* (2002) Assessing uncertainty in DNA evidence caused by sampling effects, *Science and Justice,* Volume 42, No.1, pages 29-37.

Kalafut, T. *et al*. (2018) Implementation and validation of an improved allele specific stutter filtering method for electropherogram interpretation. *Genetics*, Volume 35, pages e50-e56.

Perlin, M.W., Explaining the likelihood ratio in DNA mixture interpretation in the proceedings of Promega's Twenty-First International Symposium on Human Identification. San Antonio, TX. 2010. (Manuscript available at:

http://www.cybgen.com/information/publication/2010/ISHI/Perlin-Explaining-the-likelihood-ratio-in-DNA-mixture-interpretation/page.shtml)

Richey, M. (2010) The Evolution of Markov Chain Monte Carlo Methods. The Mathematical Association of America, Monthly 117, pages 383-386.

Taylor, D. *et al*. (2017) A fully continuous system of DNA profile evidence evaluation that can utilise STR profile data produced under different conditions within a single analysis, *Forensic Science International: Genetics,* Volume 31, pages e149-e154.

Taylor, D., *et al*. (2014) Considering relatives when assessing the evidential strength of mixed DNA profiles, *Forensic Science International: Genetics,* Volume 13, pages e259-e263.

Thompson, William C., Uncertainty in probabilistic genotyping of low template DNA: A case study comparing STRmix and TrueAllele. *Journal of Forensic Sciences*. 2023; 00:1-15.

Hansson, O., *et al*. (2017) Characterization of degradation and heterozygote balance by simulation of the forensic DNA analysis process, *International Journal of Legal Medicine,* Volume 131, pages e303-e317.

Tvedebrink, T., *et al*. (2012) Statistical model for degraded DNA samples and adjusted probabilities for allelic drop-out, *Forensic Science International: Genetics,* Volume 6, pages e97-e101.

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.

CAPILLARY:
A tube that has an internal diameter of hairlike thinness.

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 HETERODUPLEX:
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

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 peaks 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 peak 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.

NUCLEASE:
An enzyme that cleaves the chains of nucleotides in nucleic acids into smaller units.

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.

POLYMER:
A substance that has a molecular structure consisting chiefly or entirely of a large number of similar units bonded together.

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.

APPENDIX C – STUDY QUESTIONS

General DNA:

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?

DNA Isolation:

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 for nuclear DNA analysis 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?

DNA Quantitation:

1. What DNA standards are used for the PowerQuant® quantitation method?

2. What primers are used in the PowerQuant® quantitation method and why?

3. What dye is utilized to detect the human male (Y-chromosomal) DNA concentration? The human autosomal DNA concentration? The degradation target? The IPC? The background?

4. For what is the degradation target useful?

5. For what is the IPC useful?

6. Explain how the PowerQuant® Quantitation System works.

7. Can the PowerQuant® Quantitation System quantitate highly degraded DNA samples? Why or why not?

8. Can the PowerQuant® Quantitation System quantitate DNA with inhibitors present? Why or why not?

Normalization Wizard and Amplification Process:

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® Fusion 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® Fusion System kit?

21. How many primers are used in the PowerPlex® Fusion 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?

Capillary Electrophoresis:

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?

Capillary Electrophoresis Data Analysis and Interpretation

1. What are the PowerPlex® Fusion loci and on which chromosomes are they located?
2. The allelic ladder used to interpret the results at the PowerPlex® Fusion 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 positive control 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® Fusion loci are considered to have a complex repeat unit?
14. Why does Fusion include locus DYS391?

Statistics, Report Writing, and CODIS

1. What is linkage disequilibrium and how does this affect PowerPlex® Fusion analyses? What does independence mean?
2. What is Hardy Weinberg Equilibrium? Is your database in Hardy Weinberg Equilibrium?
3. How do allele frequencies differ from genotypic frequencies? How are allele frequencies calculated? How are genotype frequencies calculated?
4. What are substructures or subpopulations and how does the existence of these affect your reporting of statistics?
5. Why do you report Caucasian, African American, and Hispanic frequencies versus reporting only one general frequency? If my client were Asian, how would you determine the frequency?
6. What does the Power of Discrimination mean (Pd)? How is this computed?
7. What is a random match probability? How is it calculated? When is it used?
8. What is a likelihood ratio? How is it calculated? When is it used? Why is it used instead of a random match probability?

9. 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?

10. Why is D12S391 not used in the statistical calculations?

11. How many loci are considered "core" for CODIS and which are they?

12. What is the difference between LDIS, SDIS and NDIS?

13. How does a search or entry into CODIS differ between a regional lab and the Central lab?

Testimony and Expert Witness Qualification:

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 does DAB stand for and what is its function? How was it created and why? Is it still in existence?

17. Explain the purpose of the FBI Quality Assurance Standards.

18. What does ASCLD/LAB® stand for and what is its function?

19. Who serves as the Department's Quality Assurance Officer?

20. Who serves as the Department's Safety Officer?

21. What is the Department's Mission Statement?

Coordination of the Biomek® NX^{P} Automation Workstation 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 PowerQuant® procedure and Normalization Wizard and amplification setup steps?

6. What instrumentation is used to detect the PowerQuant® System data?

7. What happens to the fluorescent signal during the real-time PCR process when a sample contains DNA?

8. Can the PowerQuant® System detect PCR inhibitors? If so, what effect do PCR inhibitors have on the Cq (Ct)?

9. Please explain the 96 deep well plate setup when more than one DNA examiner's evidence samples are isolated using the Biomek® Automation Workstation.

10. How does the 96 deep well setup differ when evidence and known samples are isolated at the same time using the Biomek® Automation Workstation?

11. 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?

12. If a signal is detected in a reagent blank, how does this affect the rest of the samples isolated during that particular run?

APPENDIX D – 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 peaks, 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 Stephen, 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 4^0C or room temperature is enough to allow the PCR to proceed further towards the allele + A (5%-25% after 2 days at 4^0C).

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 peak base pairs shorter than the corresponding main allele peak. This is also referred to in the literature as shadow peaks, DNA polymerase slippage product, or n-4 peaks.

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

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 altering the injection parameters.

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 peaks 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 peaks are shorter than the main allele.

A speculative explanation for the relative lack of longer stutter peaks 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 peaks 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.

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. Tm

30. Which of the following would have a higher Tm and why?

A. CTGTTTTTGCAATGCAATATTAC
B. AGCCCCCATTTCGGGCGGCCCCG

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?

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				3rd Position
	U	C	A	G	
U	PHE PHE	SER SER SER SER	TYR TYR STOP STOP	CYS CYS STOP TRP	U C A G

	LEU LEU				
C	LEU LEU LEU	PRO PRO PRO PRO	HIS HIS GLN GLN	ARG ARG ARG ARG	U C A G

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

	VAL				
	VAL				
	VAL				

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 3500*xl* for use with PPFusion?

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® Fusion System?
9. What dye is used for the internal lane standard?

10. 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?

11. Why do we use Hi-Di™ Formamide as opposed to non-Hi-Di™ varieties available?

12. 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?

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-X software utilize data from multiple allelic ladders in included in a single run?

5. What sizing algorithm is used to size the PowerPlex® Fusion data? How does that work?

6. Extra peaks were observed in the analysis of a single-source sample. What may they arise from?

7. 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 peak was identified as 350 bp, etc.).

   ____ Some peaks 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.

8. 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?