Department of Forensic Science

FORENSIC BIOLOGY
TRAINING MANUAL

MITOCHONDRIAL DNA ANALYSIS

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1 OVERVIEW OF TRAINING PROGRAM

The requirements set forth in this manual are intended to apply to the training of an examiner to perform mitochondrial DNA analysis (mtDNA).

The sequence in which the tasks are presented should not necessarily be considered as a mandatory order of instruction. Exposure to legal aspects and testimony will be continuous throughout the training.

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

1.1 Purpose and Scope

The purpose of this document is to provide a uniform training program for the analysis of forensic casework using mitochondrial DNA 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 is designed to develop a person with a solid scientific background ***and*** forensic casework experience into a qualified forensic mtDNA examiner by providing the trainee with the knowledge of and experience with the accepted procedures of forensic mtDNA analysis, as well as their legal significance and evidentiary value.

The program will provide exposure to methods, techniques, and procedures presently used in the field of forensic mtDNA analysis and accepted by the courts. Most of the training will be concentrated on the methods currently used by the Department of Forensic Science, thus allowing the trainee to become proficient in these. 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. A practical examination (i.e., analysis of a validated fabricated case) is used to ascertain the trainee’s technical skills and abilities. 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.

If the individual has no prior testimony experience, a minimum of two mock trials with attendant practical examinations staged throughout the training are required prior to the comprehensive oral competency examination, the analysis of the final validated fabricated case, and the formal comprehensive mock trial.

Satisfactory performance in all areas is required prior to upgrading a trainee to a qualified mtDNA 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 a mtDNA 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 six to eight months, which is to include successful completion of the comprehensive oral competency examination and 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

Training will be conducted at the Central Laboratory.

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, the training coordinator, and (optionally) 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, the training coordinator, and the Laboratory Director or designee (if present) 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 mtDNA 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 panel members 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, 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 a mtDNA 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. 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 mtDNA anaylsis including, but not limited to:
  - Maternal inheritance of mtDNA
  - Role of the mitochondrion in a cell
  - Composition of the mitochondrial genome
  - Primary and secondary structures of DNA
  - Replication, transcription and translation of DNA
  - Heteroplasmy
  - Methods used to recover mtDNA from forensic biological specimens
- Knowledge of the theory and application of instrumentation and specialized techniques used in the mtDNA laboratory including, but not limited to:
  - Types/sizes of samples required for mtDNA analysis
  - Methods used to recover mtDNA from forensic biological specimens
  - Methods for determining quality and quantity of extracted DNA
  - mtDNA amplification procedures (primer set strategy, control region)
  - The polymerase chain reaction (PCR)
  - Gel and capillary electrophoresis of DNA
  - mtDNA sequencing methods
  - Post-amplification assessment using product gels and capillary electrophoresis
  - Contamination minimization during mtDNA analysis procedures
  - Methods of specimen preservation and storage
- 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 A copy of the completed Training Documentation Form 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 technique/test/examination
- Review of pertinent literature

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 mtDNA sequence analysis and quality control procedures can be found in the Mitochondrial DNA Section Procedures Manual and the FB PM QA Program.

1.9.4 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.

1.9.5 The trainee will assist with casework 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.

2 SAFETY

2.1 Bloodborne pathogens and Chemical Hygiene

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 mtDNA 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
- Mechanical/Physical hazards, such as:
  - Use of powered equipment (e.g., Dremel rotary tool, power drill, etc.)
  - Use of chisel/hammer
  - Use of blender cups
  - Use of scalpels

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) 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 RECEIVING AND HANDLING PHYSICAL EVIDENCE

3.1 Purpose and Scope

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

- Gain a working knowledge of factors influencing the deterioration of evidence as these relate to proper vs. improper packaging, handling and storage.
- Develop a thorough understanding of evidence handling procedures, including preservation of chain of custody, use of the laboratory information management system (LIMS) and intra/inter-laboratory transfer of evidence.
- Gain knowledge of court procedures involving identification and introduction of evidence.
- Develop a thorough understanding of the necessity for:
    - Detailed, comprehensive notes.
    - Adequate labeling of evidentiary material.
    - Drawings/photographs.

3.2 Tasks

3.2.1 Read the FB PM QA.

3.2.2 Read the FB PM Documentation and Evidence Handling Requirements.

3.2.3 Read the Mitochondrial DNA Section Procedures Manual.

3.2.4 Observe operations in the Evidence Receiving Section.

3.2.5 Observe and obtain instruction from qualified examiners performing routine examinations on case material.

3.2.6 Participate in an oral question and answer session covering the receipt, transfer, routine examination, and note-taking/documentation of evidence.

3.2.6.1 This oral question and answer session serves as a competency. Successful completion qualifies the trainee to perform the tasks described in 3.2.7-3.2.9 as well as general receipt, transfer, opening/inventorying and documentation of evidence.

3.2.7 Receive, transfer and return evidence, including reconciliation of items of evidence and containers with the associated RFLE(s).

3.2.8 Assist in the preservation and storage of evidence.

3.2.9 Examine, describe and take notes on case material under the direct supervision of a qualified examiner.

NOTES: This examination of the evidence by the trainee does not include chemical testing unless the trainee has successfully completed an applicable competency specific to the type of chemical testing required as detailed in the appropriate chapter(s) of the FB TM Case Approach and ID of Biological Substances. The trainee may, however, handle, examine, describe and take notes on the evidence and observe the chemical testing by the supervising examiner.

This task will continue throughout the training process.

3.3 Evaluation

3.3.1 Knowledge of the trainee will be evaluated through:

- Review of notes in the training notebook by the training coordinator.
- Mini-mock trial(s)/oral and/or question and answer session(s).

3.3.2 The trainee should handle a sufficient number of cases and items of evidence to develop and exhibit an unquestionably sound technique for handling physical evidence with a wide variety of evidentiary material. This will be monitored by continual observation by the training coordinator or designee.

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 for mtDNA analysis from blood stains, buccal swabs, hairs, bones, tissue, and alternate known 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

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

4.2.1 Read/refer to Chapter 2 of the Mitochondrial DNA Section Procedures Manual.

4.2.2 Prepare the reagents necessary for DNA isolation.

4.2.3 Observe a qualified mtDNA examiner perform a Chelex® extraction on at least one reference sample.

4.2.4 Observe a qualified mtDNA examiner perform an organic extraction on at least one hair and at least one bone.

4.2.5 Perform DNA isolation on at least 16 samples.

NOTE: Each sample extracted will be carried through the entire mtDNA analysis process as the trainee works through the tasks required in this manual. The number of samples processed at one time and whether or not a sample is processed completely prior to extraction of another sample will be determined by the training coordinator.

4.2.5.1 Use the organic extraction method for at least 5 hairs.

4.2.5.2 Use the organic extraction method for at least 3 bones.

4.2.5.3 Use the Chelex® extraction method on at least two blood and two saliva samples.

4.2.5.4 Use either the organic extraction method or the Chelex® extraction method on at least two more blood and two more saliva samples.

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

4.3 Evaluation

4.3.1 Because the mtDNA sequencing results to be obtained from each of the training samples are unknown in advance to the trainee, the satisfactory completion of mtDNA analysis on the above listed 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 MITOCHONDRIAL DNA AMPLIFICATION

5.1 Purpose and Scope

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

- Develop an understanding of the amplification scheme to be followed (i.e., which primer set(s) to use and when).
- 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.

5.2 Tasks

5.2.1 Read/refer to Chapter 3 of the Mitochondrial DNA Section Procedures Manual.

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

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

5.2.4 Observe a qualified mtDNA examiner set up at least one primer set amplification.

5.2.5 Observe a qualified mtDNA examiner set up at least one control region amplification.

5.2.6 Amplify, at a minimum, the extracts obtained previously in following Chapter 4 of this manual.

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

6 MITOCHONDRIAL DNA AMPLFICATION PRODUCT EVALUATION

6.1 Purpose and Scope

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

- Develop an understanding and working knowledge of the evaluation of mtDNA amplification products using a product gel, including proper documentation.
- Become familiar with the controls used and evaluated using a product gel.
- Develop an understanding of the importance of a post-amplification environment being free of contamination.
- Develop an understanding of the importance of quality control associated with the amplification and evaluation process.

6.2 Tasks

6.2.1 Read/refer to Chapter 4 of the Mitochondrial DNA Section Procedures Manual.

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

6.2.3 Observe a qualified mtDNA examiner prepare a product gel and 1X TAE buffer.

6.2.4 Observe a qualified mtDNA examiner run and evaluate the results obtained using a product gel.

6.2.5 Prepare the product gel(s) and 1X TAE buffer, as needed, for the training samples previously amplified in following Chapter 5 of this manual.

6.2.6 Run and evaluate the results obtained using the product gel(s) prepared for the training samples previously amplified in following Chapter 5 of this manual.

6.2.7 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 evaluating mitochondrial DNA amplification results using product gels. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

7 PURIFICATION/SEQUENCING AND CE OF MITOCHONDRIAL DNA

7.1 Purpose and Scope

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

- Develop an understanding and working knowledge of the process for enzymatic cleanup of PCR amplification product.
- Develop an understanding and working knowledge of the process of sequencing mtDNA PCR product.
- Develop an understanding and working knowledge of the process of purifying mtDNA sequencing product.
- Become familiar with the theories of electrophoresis as they apply to capillary electrophoresis used in mtDNA analysis.
- 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/refer to Chapters 5 and 6 of the Mitochondrial DNA Section Procedures Manual.

7.2.2 Observe the weekly and monthly maintenance of the CE instrument.

7.2.3 Perform the weekly and monthly maintenance of the CE instrument.

7.2.4 Observe a qualified mtDNA examiner perform enzymatic cleanup, sequencing, purification, and capillary electrophoresis of the sequencing product.

7.2.5 Prepare the reagents necessary to perform enzymatic cleanup of mtDNA amplification product through capillary electrophoresis.

7.2.6 Perform the enzymatic cleanup, sequencing, purification, and capillary electrophoresis on the training samples amplified in following the previous chapters of this manual.

7.2.7 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 the enzymatic cleanup of amplified mtDNA, sequencing, purification of the sequencing product, and capillary electrophoresis using proper documentation. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

8 SEQUENCE ASSEMBLY/ANALYSIS, INTERPRETATION AND CODIS

8.1 Purpose and Scope

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

- Become familiar with mtDNA sequence electropherogram data.
- Develop a working knowledge of the Sequencher™ Software used to edit and assemble sequences to produce a contiguous consensus sequence (contig).
- Develop a working knowledge of the use of the Revised Cambridge Reference Sequence (rCRS) and nomenclature rules in producing a reportable result for mtDNA analysis.
- Develop an understanding of the problems that may be encountered with regard to interpretation (e.g., heterplasmy, ambiguous base calls, etc.) and become skilled in applying appropriate nomenclature, as applicable.
- Become familiar with the Combined DNA Index System applications, policies and procedures, and associated documentation required.
- Become skilled in the export of data for CODIS and the subsequent import of that data into CODIS.
- Become skilled in the creation of proper pedigree trees and their use in CODIS.
- Develop a working knowledge and become skilled in sequence confirmation, evaluation of controls, and the interpretation of comparisons between sample sequences.

8.2 Tasks

8.2.1 Read/refer to Chapters 7 and 8 of the Mitochondrial DNA Section Procedures Manual.

8.2.2 Observe the assembly and analysis of mtDNA contig(s) and the export of data for CODIS and subsequent import of that data into CODIS.

8.2.3 Analyze the CE data generated from the training samples using Sequencher™.

8.2.4 Interpret all results successfully and properly document the results.

8.2.5 Perform CODIS searches and pedigree tree uploads under the direct supervision of qualified examiners and generate the proper associated documentation.

8.2.6 Create pedigree trees and designate the appropriate DNA analysis required for CODIS upload for the following scenarios:

- Biological mother and biological brother searching for son/brother
- Biological mother searching for daughter
- Biological son searching for biological mother
- Biological children (son and daughter) searching for biological mother
- Biological brother searching for biological sister

8.2.7 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 assembling and editing a contig 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 AND REPORT WRITING

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 mtDNA examiners.
- Become skilled in expressing the results of the mtDNA analysis in a clear, concise and technically correct fashion.
- Become skilled in the use of Popstats for searching mtDNA haplotypes against the database and obtaining frequency estimates.
- Become skilled in the explanation of statistical calculations associated with mtDNA analysis results.
- Become familiar with the Combined DNA Index System applications, policies and procedures, and associated documentation required.

9.2 Tasks

9.2.1 Read/refer to Chapters 9 and 10 of the Mitochondrial DNA Section Procedures Manual.

9.2.2 Review Certificates of Analysis prepared by examiners and compare to the corresponding supporting documentation.

9.2.3 Prepare Certificates of Analysis based upon completed case files.

9.2.3.1 Compare the Certificates to those released by the examiner.

9.2.4 Conduct database searches in Popstats under the direct supervision of a qualified examiner on actual casework samples.

9.2.4.1 Compare the results to those reported by the examiner.

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

NOTE: If ten (10) unreleased mitochondrial DNA case files are not available during the appropriate training period, the number of reviews of unreleased casework available may be supplemented with post-release reviews of previous casework.

9.2.6 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 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 mtDNA analytical results, including statistical information 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 mtDNA analysis, 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 mtDNA testing guidelines/standards set forth by the following:

- Scientific Working Group on DNA Analysis Methods (SWGDAM)
- ISO/IEC 17025 Accreditation Requirements
- ANAB 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 (validated fabricated 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 mtDNA analytical results and conclusions, including associated statistical calculations.

11 HAIR EVALUATION

11.1 Purpose and Scope

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

- Become familiar with the microscopic structures of hair.
- Become acquainted with the basic differences between human and animal hairs.
- Develop an understanding of the fundamentals of hair biology as it relates to hair growth, regression and rest.
- Learn to recognize the microscopic characteristics of human hair roots in the growth, regression and rest stages.
- Learn to recognize the hair root forms which may be suitable for STR typing based upon their microscopic morphology.
- Learn to prepare temporary microscope slides containing hairs for examination for STR typing suitability.
- Become familiar with photomicroscopic documentation of hairs.

11.2 Tasks

11.2.1 Read the following Reference Materials:

- Microscopy of Hair, A Practical Guide and Manual. Hicks, JW FBI Publication Issue 2, Jan. 1977, U.S. Government Printing Office 1977-226-1201.

- Human hair histogenesis for the mitochondrial DNA forensic scientist. Linch CA, Whiting DA, Holland MM. *J For Sci* 2001; 46(4); 844-853 (only pages 844-848).

- Evaluation of the human hair root for DNA typing subsequent to microscopic comparison. Linch CA, Smith SL, Prahlow JA. *J For Sci* 1998; 43(2): 305-314.

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11.2.2 Attend the following four lectures:

- Hair Biology; Hair Microscopic Examination and Hair Root Evaluation for Nuclear DNA Typing
- Stereomicroscopic Views of Human Hair Roots
- Hair Evaluation for Nuclear DNA Typing (Examples)
    - Part I: Animal versus Human versus Textile Fiber
    - Part II: Root Suitability for STR Typing
- Use of the Digital Camera with the Compound Microscope

11.2.3 Examine and sketch from reference slides the microscopic appearance of:

- At least 2 dog hair telogen roots absent tissue.
- At least 2 cat hair telogen roots absent tissue.

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

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

- Telogen pubic hair root club with attached follicular tissue
- Telogen pubic hair root club absent follicular tissue

11.2.5 Using several head hairs ***gently*** removed by trainee from trainee's own scalp:

- Mount them on temporary microscopic slide(s).
- Sketch and identify the root forms.
- Capture a microscopic root image with digital photography using a 20x objective; 10x ocular.

11.2.6 Using several head hairs ***forcibly*** removed by trainee from trainee's own scalp:

- Mount them on temporary microscopic slide(s).
- Sketch and identify the root forms.
- Capture a microscopic root image with digital photography using a 20x objective; 10x ocular.

11.2.7 Complete a practical exercise determining hair root suitability for STR typing using the compound microscope to visualize hairs mounted in Permount on microscope slides.

11.2.8 Complete a practical exercise determining hair root suitability for STR typing using the stereomicroscope to visualize hairs placed on post-it style notes.

11.3 Evaluation

11.3.1 Knowledge of the trainee will be evaluated through question and answer session(s) and discussion of the practical exercises.

11.3.2 The trainee should demonstrate a thorough understanding of determination of the suitability of hairs for STR typing. This will be monitored by review of the trainee's sketches, notes, and photographs in the training manual as well as observation, and a review and discussion of the practical exercises by the training coordinator.

APPENDIX A – REFERENCES AND REQUIRED READINGS

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APPENDIX B – GLOSSARY

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

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

ALLELE:
One of two or more alternative forms of a gene.

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

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

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

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

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

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

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

BASE SEQUENCE:
The order of nucleotide bases in a DNA molecule.

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

1. Describe the mitochondrial genome.
2. How is mitochondrial DNA inherited?
3. Compare the mutation rate of mtDNA to nuclear DNA.
4. What is the control region?
5. What is the revised Cambridge reference sequence?
6. What is the function of the demineralization buffer in the isolation procedure?
7. What is the purpose of the ExoSAP-IT step?
8. What is dGTP added to the sequencing reaction separately?
9. What is the purpose of the Performa DTR clean up step?
10. What is heteroplasmy?
11. Why are stricter contamination prevention measures taken in the mtDNA laboratory?
12. What is a pedigree?
13. Explain Sanger Sequencing.
14. What is a “C-stretch”?
15. Why is it challenging to sequence through a “C-stretch”?
16. Can we exclude individuals due to length differences at the “C-stretch”? Why or why not?