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Department of Forensic Science

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**MITOCHONDRIAL DNA SECTION
TRAINING MANUAL**

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1 INTRODUCTION

1.1 Purpose and Scope

- 1.1.1 The purpose of this document is to provide a uniform training program for the analysis of forensic casework utilizing Mitochondrial DNA analysis (mtDNA), and in so doing, adhere to the “FBI Quality Assurance Standards for Forensic DNA Testing Laboratories” (effective July 1, 2009). It is not designed to be all inclusive. Molecular biology and DNA analysis are ever changing fields, wherein new theories, knowledge, methods, determinations, equipment, and instrumentation are constantly being added, refined and improved.
- 1.1.2 It 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.
- 1.1.3 The Department of Forensic Science requires all mtDNA 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 mtDNA analysis on casework by the Department.
- 1.1.4 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 mtDNA Unit, 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.
- 1.1.5 The sequence in which the tasks are presented in the outline should not necessarily be considered as a mandatory order of instruction. Exposure to legal aspects and testimony will be continuous throughout the training.
- 1.1.6 Oral and practical examinations and/or mock trials encompassing several topics will be staged periodically. The training program will culminate with a formal oral competency examination and satisfactory completion and defense of a mock case. The oral competency examination is used to ascertain the trainee’s technical knowledge. The analysis of the practical examinations and the formal mock case are used to ascertain the trainee’s technical skills and abilities. Finally, the trainee will testify to the examinations performed on the mock case at the final 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 fully qualified examiner in the mtDNA Unit.

1.2 Coordination of the Program

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

1.3 Training Period

- 1.3.1 It is estimated that this training program can be completed in six to eight months, which is to include successful completion of the formal oral examination and final mock. 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 in consultation with the training coordinator.

1.4 Location of the Training

Training will be conducted at the Central laboratory.

1.5 Mock Trials

- 1.5.1 Each case a forensic examiner analyzes has the potential of involving him/her as an expert witness in courtroom testimony. The trainee must never underrate this important aspect of the work. It is the training coordinator's responsibility to ensure that the trainee is thoroughly prepared for legal questioning. This can be done by a combination of mock trials, prearranged as well as impromptu question and answer sessions, pertinent literature review, and observation of courtroom testimony given by experienced examiners.
- 1.5.2 A mock trial may take place after the trainee has completed a block of this training protocol and a practical examination of a case incorporating that block of the training. The case will be fabricated so that the training coordinator knows the correct answers. The fabricated case thus serves as a monitor of the trainee's proficiency in applying techniques and procedures to actual casework examinations.
- 1.5.3 A final mock trial will incorporate all aspects of this training program and will be held subsequent to the final practical examination of a fabricated case. **THE TRAINEE WILL NOT RECEIVE THE FINAL MOCK CASE UNTIL ALL PHASES OF THIS TRAINING PROTOCOL HAVE BEEN SATISFACTORILY COMPLETED.**
- 1.5.4 If the individual has no prior testimony experience, a minimum of 2 mock trials with attendant practical examinations are required prior to the final mock trial.
- 1.5.5 All mock trials will cover both in-depth technical questioning appropriate for a courtroom setting, as well as the typical chain of custody and standard procedural questioning. Each mock trial should serve as a constructive learning process and a good evaluation tool.
- 1.5.6 The scheduling of mock trials is to be done by the training coordinator as frequently as he/she deems necessary. The final comprehensive mock trial will be videotaped for viewing at a later date and can be used to identify weak and strong points of the trainee's testimony. The videotape will be retained for future training purposes.
- 1.5.7 Other related legal training will be integrated into the program on a continual basis.
- 1.5.8 It cannot be overemphasized that testimony training is just as important as the analytical training. The trainee must successfully meet acceptable performance standards in both areas before he/she is deemed to be qualified to conduct forensic examinations on evidential material.

1.6 Guidelines for Comprehensive Oral Examination and Final Comprehensive Mock Trial

- 1.6.1 Approximately two weeks prior to the final mock trial, a formal oral examination of the trainee will be conducted by the Unit Technical Leader and supervisor, training coordinator, and the Biology Program Manager to ascertain the technical knowledge of the individual. This will be limited to two (2) hours. Questions should be confined to technical aspects of the training and should be used to ascertain whether the goals, as set forth in each technical portion of the training program, have been achieved.
- 1.6.2 Immediately following the oral examination the trainee may be released while administrative peers evaluate the trainee's performance.
- 1.6.3 The outcome of the oral examination evaluation will be:
 - 1.6.3.1 Satisfactory.
 - 1.6.3.2 Not satisfactory.

If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.

- 1.6.4 The final mock trial will not exceed four (4) hours. Prior to trial, the "prosecutor" and the "defense attorney" may reach an agreement as to selected items to be introduced at trial in order to remain within the set time constraints.
- 1.6.5 The atmosphere of the trial will be formal. That is, it will be conducted in the same manner as a real courtroom situation. This includes conduct, protocol, and all other aspects.
- 1.6.6 Harassment of the expert witness by defense counsel or prosecutor will be kept to the minimum necessary to achieve the desired goal. Questioning by both the prosecutor and defense attorney(s) should be relevant and realistic.
- 1.6.7 There may be two defense lawyers at the trial, one of whom must be a qualified examiner in the mtDNA Unit.
- 1.6.8 The trial may be stopped at any time upon the request of any of the involved parties.
- 1.6.9 Immediately following the trial, the trainee may be released while the Department Director or his designee, the Biology Program Manager, Unit supervisor, and trial participants evaluate the trainee's performance.
- 1.6.10 The outcome of the trial evaluation will be:
 - 1.6.10.1 Satisfactory.
 - 1.6.10.2 Not satisfactory.

If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.

- 1.6.11 This evaluation may be followed by a short performance critique.
- 1.6.12 The training coordinator will review the videotape with the individual as soon as possible. Other comments should be gathered by the individual from trial participants/observers as soon as possible.
- 1.6.13 Satisfactory performance on technical aspects and testimony must be achieved before the individual is qualified to perform the duties of an examiner.

1.7 Transition from Trainee to Examiner

- 1.7.1 After the individual has successfully completed all training there follows a somewhat awkward period of adjustment. The supervisor must ensure that the transition from trainee to qualified examiner takes place as smoothly as possible. A newly qualified examiner cannot function without some guidance.
- 1.7.2 For a period of time, all of the newly qualified examiner's reports must be reviewed prior to release by the supervisor or designee. Casework must be monitored closely for at least six (6) months.
- 1.7.3 The supervisor, or designee, will accompany the newly qualified examiner to court for the first few cases.

1.8 Instructions for the Training Coordinator

- 1.8.1 The intent of the training program is to ensure that each and every trainee is provided with certain basic principles and fundamentals necessary for the complete education of an examiner in the mtDNA Unit. 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 APPROVED BY THE BIOLOGY PROGRAM MANAGER.

- 1.8.2 The trainee will be evaluated on his/her performance during the course of the program. The training coordinator must submit regular written evaluations of the trainee's progress to the Biology Program Manager.
- 1.8.3 The training report must include:
- 1.8.3.1 A summation of the progress made.
 - 1.8.3.2 An evaluation of the trainee's notebook.
 - 1.8.3.3 An evaluation of the progress during the period, to include:
 - 1.8.3.3.1 Problem areas, as applicable, and their solutions or proposed solutions.
 - 1.8.3.3.2 Trainee's strong points.
 - 1.8.3.3.3 Trainee's weak points and suggested remedies.
 - 1.8.3.3.4 Statement concerning trainee's overall performance.
 - 1.8.3.3.5 Plans for the upcoming training.
- 1.8.4 This report will be in memorandum format, one memorandum per trainee. Each memorandum will become a part of the training history of the trainee and will be used to document the trainee's progress toward qualification. The report format is located at the end of this section.
- 1.8.5 A review of the checklists with the trainee will enhance the training coordinator's ability to prepare the written evaluation, and may also give the trainee a greater sense of accomplishment. The coordinator is to discuss this evaluation with the trainee and the trainee's supervisor prior to forwarding it to the Biology Program Manager. Any comments by the trainee, coordinator, or supervisor are to be included with the report.
- 1.8.6 When the trainee has satisfactorily completed all training requirements 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 Unit. If the trainee cannot meet the criteria expected of him/her during the period allowed for training in each of the areas, steps will be taken to effect the appropriate action.
- 1.8.7 The training should culminate so that the trainee has the following:
- 1.8.7.1 Knowledge of the principles and practices of mtDNA analysis.
 - 1.8.7.2 Knowledge of the theory and application of instrumentation and specialized techniques used in the mtDNA laboratory.
 - 1.8.7.3 The ability to perform accurate forensic analyses independently and proficiently, to accurately document the findings of all analyses in accordance with Department and Unit policies and procedures, and to accurately report those findings in a Certificate of Analysis.
 - 1.8.7.4 The ability to skillfully present and defend analytical findings in a court of law.

1.9 Instructions for the Trainee

- 1.9.1 The trainee is expected to keep a loose-leaf notebook on all work completed. The completed checklist for each training topic and the training coordinator's report will also be included in the notebook.
- 1.9.2 The notebook should be organized by subject. Within each subject category, the techniques/methods or examinations observed and performed, notes and comments on each technique/method, and the review of pertinent literature should be included. For each procedure performed, comments/notes should include the following, as appropriate: principle, procedural outline (to include the purpose of critical reagents), sensitivity, specificity, interpretation of results, possible interferences/problems, and comments, including comparison to other methods.
- 1.9.3 The mtDNA sequence analysis and quality control procedures can be found in the Department of Forensic Science mtDNA Unit Section Procedure Manual.
- 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 under the direct supervision of a qualified examiner.

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TRAINING REPORT FORMAT
(on letterhead stationary)

MEMORANDUM

TO: (Name), Biology Program Manager

FROM: (Name), Training Coordinator

DATE:

SUBJECT: Training Report: (Name)

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

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

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

2 SAFETY**2.1 Bloodborne Pathogen**

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

2.2 Hazards

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

2.2.1 Infectious agents, such as those associated with:

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

2.2.2 Hazardous materials, such as:

- Acids and bases
- Organic chemicals

2.3 Safety Procedures

2.3.1 Reference: Department of Forensic Science Safety Manual

All trainees are required to read and be familiar with the Department of Forensic Science Safety Manual. The trainee must make every effort to ensure safety within the Unit and will demonstrate an understanding of the following general safety aspects:

2.3.1.1 Fire escape routes and procedures

2.3.1.2 Location and use of safety equipment including, but not limited to fire extinguishers, blankets, eye wash stations, etc.

2.3.1.3 Handling and proper disposal including the decontamination/neutralization of hazardous substances

2.3.1.4 First aid procedures (cuts, burns, sprains, etc.)

2.3.1.5 Proper handling of biological hazardous evidence

2.3.1.6 Marking of biohazard evidence

2.3.2 Personal protection

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

2.3.2.2 Avoid production of aerosols.

2.3.2.3 No mouth pipetting.

2.3.2.4 Material Safety Data Sheets (MSDS)

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

2.3.3 Biosafety Practices

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

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

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

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

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3 EVIDENCE HANDLING

3.1 Goals

- 3.1.1 To obtain a working knowledge of factors influencing the deterioration of evidence as these relate to proper vs. improper packaging, handling, and storage.
- 3.1.2 To develop a thorough understanding of evidence handling procedures, including preservation of chain of custody, use of the laboratory information management system (F.A.C.E.), and intra/interlaboratory transfer of evidence.
- 3.1.3 To develop a knowledge of court procedures involving identification and introduction of evidence.
- 3.1.4 To develop a thorough understanding of the necessity for:
 - 3.1.4.1 Detailed, comprehensive notes.
 - 3.1.4.2 Adequate labeling of evidentiary material.
 - 3.1.4.3 Drawings/photographs.

3.2 Tasks

- 3.2.1 Receive, transfer, and return evidence.
- 3.2.2 Assist in preservation and storage of evidence.
- 3.2.3 Observe and obtain instruction from qualified examiners performing routine examinations on case material.
- 3.2.4 Practical applications: examine, describe, and take notes on case material. **THIS MUST BE DONE UNDER THE DIRECT SUPERVISION OF A QUALIFIED EXAMINER.** It should be noted that this task will continue throughout the training period.

3.3 Training Evaluation

3.3.1 Knowledge

- 3.3.1.1 Evaluation of case notes by training coordinator or designee.
- 3.3.1.2 Review of notes in training notebook by training coordinator.
- 3.3.1.3 Mini-mock trials/oral and practical examinations.
- 3.3.1.4 Completion of trainee checklist by training coordinator.

3.3.2 Skills

- 3.3.2.1 The trainee should handle a sufficient number of cases 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.
- 3.3.2.2 Review of notes in training notebook by training coordinator.
- 3.3.2.3 Mini-mock trails/oral and practical examinations.

4 FORENSIC DNA ANALYSIS

4.1 Goals

4.1.1 The National Quality Assurance Standards for Forensic DNA testing require that forensic DNA examiners successfully complete college course work (graduate and/or undergraduate level) covering the subject areas of biochemistry, genetics, and molecular biology or other subjects that provide a basic understanding of the foundation of forensic DNA analysis. The trainee will demonstrate a thorough knowledge and understanding of the following topics:

- 4.1.1.1 Primary and Secondary structures of DNA
 - 4.1.1.2 Methods for determining quality and quantity of extracted DNA
 - 4.1.1.3 Replication, transcription and translation of DNA
 - 4.1.1.4 The polymerase chain reaction (PCR)
 - 4.1.1.5 DNA sequencing
 - 4.1.1.6 Forensic detection and analysis (RFLP, PM/DQA1, D1S80, STRs, mtDNA, Y-STRs, SNPs)
 - 4.1.1.7 Population genetics
 - 4.1.1.8 Gel electrophoresis of DNA
 - 4.1.1.9 Capillary electrophoresis of DNA
 - 4.1.1.10 Enzymes: kinetics and inhibition
 - 4.1.1.11 Theories, methods and techniques used in the study and analysis of gene structure, organization and function
 - 4.1.1.12 Principles of Mendelian genetics
 - 4.1.1.13 Genomic organization
- 4.1.2 To demonstrate a thorough knowledge and understanding of forensic mtDNA analysis and the underlying principles supporting the use of the technique. Including, but not limited to:
- 4.1.2.1 Maternal inheritance of mtDNA
 - 4.1.2.2 Role of the mitochondrion in a cell
 - 4.1.2.3 Composition of the mitochondrial genome
 - 4.1.2.4 Heteroplasmy
 - 4.1.2.5 Derivation of mtDNA numbering system (Cambridge Reference Sequence – CRS)
 - 4.1.2.6 Methods used to recover mtDNA from forensic biological specimens
 - 4.1.2.7 Type/size of specimens required
 - 4.1.2.8 mtDNA amplification procedures (linear array, primer set strategy, control region)
 - 4.1.2.9 Basis for controls in mtDNA analysis

- 4.1.2.10 Various DNA sequencing methods, including dye-terminator cycle sequencing and linear arrays
- 4.1.2.11 Contamination minimization during mtDNA procedures
- 4.1.2.12 Post amplification assessment using product gels and capillary electrophoresis
- 4.1.2.13 Methods of specimen preservation and storage
- 4.1.3 To become familiar with the Combined DNA Index System and its application to unidentified human remains and mtDNA analysis.
- 4.1.4 Study the analytical procedures and theoretical principles of operation for the Applied Biosystems 3130xl Genetic Analyzer used for sequencing mtDNA.
- 4.1.5 Documentation required for case file.

4.2 Tasks

- 4.2.1 Work with qualified examiners while performing casework and analyze training samples.
- 4.2.2 Read applicable literature.

4.3 Training Evaluation

- 4.3.1 Knowledge
 - 4.3.1.1 Review of notes in training notebook by training coordinator.
 - 4.3.1.2 Mini-mock trials/oral and practical examinations.
- 4.3.2 Skills
 - Observation by training coordinator or designee.

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5 TRAINING SAMPLES

5.1 Goals

The trainee will successfully extract, amplify and sequence at least 16 samples to include 5 hairs, 3 bones, 4 bloods, and 4 saliva training samples using the appropriate VDFS mtDNA protocols. The number of samples analyzed should ensure a minimum of 50 successful amplifications of mtDNA hypervariable regions. Sample analysis will include Linear Array Hybridization Analysis.

5.2 Tasks

The trainee will familiarize him/herself with all aspects of the mtDNA sequencing protocol including:

5.2.1 Evidence handling procedures to minimize contamination

5.2.2 Prepare reagents necessary for mtDNA analysis

5.2.3 Control samples

5.2.4 Equipment maintenance and calibration

5.2.5 Steps in laboratory analysis

5.2.6 Interpretation of results

5.3 Training Evaluation

5.3.1 Knowledge

5.3.1.1 Review of notes, worksheets and results in training notebook by training coordinator.

5.3.1.2 Mini-mock trials/oral and practical examination.

5.3.2 Skills

The trainee should perform mtDNA analysis on a sufficient variety and number of samples to develop and exhibit an unquestionably sound technique for successfully obtaining quality mtDNA sequence results utilizing both the linear array hybridization and capillary electrophoresis technique. The trainee must accurately interpret the associated results. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

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