1.1 General Procedures:

Evidence and samples from evidence, must be collected, received, handled, sampled and stored so as to preserve the identity, integrity, condition, and security of the item.

1.1.1 Persons handling physical evidence will wear gloves, lab coats, facemasks, and other appropriate attire for safety considerations and to prevent contamination. White lab coats are worn in "Pre-Amplification" areas and blue lab coats are worn in "Amplification" and "Post Amplification" areas. Only one piece of evidence is examined at a time.

1.1.2 Change bench covering between cases, or between items if needed.

1.1.3 Single use consumable supplies will be used (when appropriate) for each item. Forceps, scissors, scalpels, and other non-consumables will be cleansed appropriately between items.

1.1.4 Polymerase Chain Reaction (PCR) product and genomic DNA are separated by space.

1.1.5 Physical evidence in the DNA Unit will be handled and documented according to standard DESPP Division of Scientific Services procedures (GL-4 and GL-13) and FBI QAS (Standard 7). All case results are documented on the appropriate DNA records. Evidence sampled for DNA extraction will not be consumed unless testing is expected to require the entire sample. Disposition of evidence will be documented on reports and in LIMS.

1.1.6 Genomic DNA (extract tubes or plates), and amplification product (amplification tubes or plates) are considered to be work product-material that is generated as a function of analysis. Work product is not evidence and therefore not sub-itemized in LIMS or given a barcode.

1.1.7 Extracts containing genomic DNA are uniquely identified and are maintained in locked freezers within the DNA Unit. The boxes containing the DNA extracts are sealed with tape and initialled.

1.1.8 Consumption of Evidence (exhaustive testing only)

Per the Connecticut Practice Book (40-9), the defense must be notified in the event of exhaustive testing (if the suspect has been arrested) to determine whether the defense wishes to observe the testing. See Case Management Work Instruction (CM WI-04): Consumption Letters for procedures about notification. If the defense wants a representative to be present during the exhaustive testing, the case management section or designee will notify the TL/supervisor and the analyst assigned to the case (if already assigned). The case management section, designee or analyst assigned to the case will schedule the testing date with the expert. If no arrest has been

made, the analyst/technician may proceed with the testing without a defense expert being present/notified. If there is no documentation within the case jacket, return the case jacket to case management or designee for confirmation. Consult with the TL or your supervisor with any questions or concerns.

If no suspect is listed on the evidence receipt, the analyst/technician may proceed with the testing without notifying the State's Attorney/Police Department. If a suspect is listed on the evidence receipt, check for documentation in the case jacket stating that the case management section or designee contacted the State's Attorney/Police Department and confirmed that the listed "suspect" has not been arrested at this time.

1.1.9 dH_2O is defined as de-ionized water. The source of this water can be from the laboratory's in-house purification system or Molecular Biology Grade Water from an approved vendor. Molecular Biology Grade Water is manufactured and certified to be de-ionized, purified and DNase free. dH_2O used for sample preparation through DNA amplification is lot tracked and quality tested before use in case work.

All casework analysis is conducted using the appropriate lot-tested reagents as defined in DNA SOP-6 Section 4 and DNA SOP-8. Reagents are lot tested before their use in casework. Reagents are not used for casework analysis or validations past their control date. Reagents may be used for research purposes past their control date where appropriate and are labelled as such.

1.1.10 When examining evidentiary samples, DNA analysts will use as guidelines Forensic Biology protocols found in Qualtrax. Refer to: SOP-FB-01: Physical Evidence Examination, SOP-FB-03: Guidelines- Collection & Forwarding, and GL 4. Physical Evidence Worksheets DNA-QR-1 and DNA-QR-1A may be used to document evidence and examination.

1.1.11 A complete list of all applicable DNA Unit abbreviations can be found below in APPENDIX II.

Note: To describe the collection of a sample from evidence, the FB SOPs refer to 1S1, 1S2, etc. When a DNA analyst collects a sample, the items should be noted as 1G1, 1G2, etc. (see GL 4).

Note: The words submission and item are interchangeable unless describing a sub-item taken at the laboratory. (i.e. An evidentiary sample may be called a submission in a forensic biology report but called an item in a DNA report).

1.1.12 DNA analyst can perform screening and confirmatory tests for which they are authorized. Qualified DNA analysts will follow Forensic Biology protocols. These protocols are found in Qualtrax. Refer to FB SOPs 1 through 27 and FB Appendices 1 and 2.

1.2 Preparation of Discovery Materials:

Requests for discovery should be made (in writing) at least 1 month prior to the anticipated court date where practicable. Discovery materials (official laboratory receipts and records) will be made available to authorized individuals according to DESPP policy. The Director of the Division of Scientific Services or designee must approve the discovery request to release the case material. Any sample identifying information not pertaining to the case in question will be obliterated. The requested information will be duplicated, scanned, and maintained on the S Drive. Once on the S Drive, the duplicated hard copy will be shredded. In addition, all requested electronic data will be maintained on the S Drive. The case jacket records and electronic data (if requested) maintained on the S Drive will be the retained copy of the discovery materials at the Laboratory. A designee will send the discovery materials to the requesting agency. Release of case material will be noted in the case file. A fee may be charged to cover administrative costs. Requests for discovery that are non-case specific will be performed on site at the laboratory.

1.3 DNA Analysis Workflow:

Upon receipt and documentation of any item for DNA analysis, the first step is to extract the DNA and estimate the quantity of human DNA recovered. The quantity of human DNA is estimated using a human qPCR kit or equivalent for question samples. The Nuclear DNA typing system is fluorescent STR or Y-STR analysis.

Samples for cases with potential Mito testing, such as unidentified person cases, bones, or decomposed tissues are extracted using the mtDNA extraction protocol (mtDNA SOP-2, SOP-3, SOP-4).

The starting date of DNA testing is documented on the DNA Extraction Worksheets. Testing is completed on the date of the report.

1.4 Report Writing:

DNA results are reported according to standard DESPP Division of Scientific Services guidelines and ASCLD/LAB and the FBI QAS. Please refer to General Laboratory Procedures: Case Reviews (GL: 18). Reports are signed by two individuals. The signature on the left is the analyst responsible for the case. The signature on the right is the technical reviewer of the case.

1.4.1 Final reports will include DNA typing results and a qualitative interpretative statement. A quantitative statement is given where appropriate. Please refer to DNA SOP-6 and the DNA Report Template file for general report templates.

Note: Casework forensic samples and knowns, processed using different STR kits such as Fusion 6C and Identifiler Plus (etc.), may be compared and interpreted at overlapping loci.

1.4.2 The results from each item tested (or its probative fraction) are included in the DNA report. With differential extractions, the probative fraction(s) are those with DNA profiles containing information that is relevant to the investigation, e.g., profile(s) detected on a vaginal swab that could not have come from the individual swabbed. Conclusions are required in the DNA report. At a minimum, a qualitative statement will be made for every comparison. A statistical statement will also be made where appropriate. A DNA report is not issued until the work on a set of samples tested is completed and reviewed.

1.4.3 Evidence Examined by a DNA analyst authorized in Forensic Biology methods:

If the evidence was examined and forensic biology tests were performed by a DNA analyst authorized in those testing methods, the results may be incorporated into a DNA report. Refer to FB-SOP-05 for Forensic Biology result statements.

1.4.4 All DNA Reports will clearly communicate all items tested and those not tested, all evidence dispositions, all eliminations (e.g., elimination of a victim or other known source) when comparisons are made, all profiles which were entered into CODIS or which were not suitable for CODIS entry.

1.4.5 Draft reports, while being worked and/or in the review process, should be located on the U drive in the case reports folder and the subfolder for the proper year of the case.

1.4.6 Draft reports that have not been issued, will not have a completed "date of report" filled out until report is issued.

1.4.7 Final Report

1.4.7.1 The Draft report is edited; the date of report filled out and saved as the Final report (without signatures).

1.4.7.2 The Final report (without signatures) will be located on the U drive in the case reports folder and the subfolder for the proper year of the case.

1.4.7.3 The original Final report with signatures will reside in the Case File in evidence receiving.

1.4.7.4 A copy of the Final report with signatures will reside in the case jacket.

1.5 General Considerations for PCR Amplification of Evidentiary Genomic DNA:

1.5.1 All amplification reactions for evidence samples and controls must be prepared in a PCR hood.

1.5.2 Gloves and lab coats must be worn at all times when working in PCR set-up.

1.5.3 All reagents and microfuge tubes must be opened with caution. At no time should open tubes of reagents be removed from the laminar flow hood.

1.5.4 For all mixing and pipetting pertaining to PCR set-up: only use designated pipettes from the PCR set-up hood. At no time should these pipettes be removed from the set-up area, except for calibration checks. Pipettes from other areas of the lab should not be brought into PCR set-up.

1.5.5 Aerosol blocking pipette tips should be used at all times when preparing PCR reactions.

1.5.6 At no time should any amplified product be brought into the PCR set-up area.

1.5.7 All PCR reactions must be prepared using designated "PCR set-up only" microfuge tube racks.

1.5.8 Prior to setting up any PCR amplifications, the working area (laminar flow hood) must be cleaned with bleach solution made in-house (see DNA WI-21) or 10% stabilized bleach followed by ethanol and subjected to UV irradiation for at least 15 minutes. See DNA WI-21 for 20% Bleach formulation.

1.5.9 A PCR master mix is prepared and dispensed to each tube prior to the addition of DNA.

1.5.10 After PCR reactions are set-up; the work area must be cleaned as in 1.5.8.

1.5.11 The standard quantity of template used for PCR amplifications is ~ 0.5ng (F6C) and ~ 0.5ng-2ng (IDP) (note that Y-STR testing may require more than 2 ng of total DNA: male + female). Amplification results from template quantities less than 0.5 ng will be interpreted with caution.

1.6 Controls for DNA analysis:

Four types of controls are processed with each case:

1.6.1 Reagent Blank (RB). A reagent blank (RB) is processed with each set of extractions. The extract volume of a RB must be at or below the extract volume of all samples for which it controls. During a manual extraction, after measuring the volume with a pipette, if a sample elutes at a lower volume than its RB, dH_2O is added to the sample to raise it to the volume of the RB. Extractions performed on automation instruments where volumes are set, such as the EZ1 or Biomek instrument are an exception to this. For automated extraction instruments, the elution volume is set. After the extraction and elution, a visual inspection of the elution volumes is made. If any visual discrepancies are noted, a manual measurement of the extract is conducted. If it is determined that an adjustment is needed, dH_2O will be added to the sample to bring the final volume to 50µL (as intended by the program setting). Any adjustments made to the samples will be recorded on the corresponding DNA QR sheet. The volume of the RB used for the amplification step must be the same as (or greater than) the maximum volume used to amplify the evidentiary samples. Re-amplification of the RB may be omitted when re-amplifying the same volume (or less) of the evidentiary sample(s). When re-amplifying a greater volume of the evidentiary sample(s), the RB must be re-amplified with that volume. With differential extractions, the RB is subjected to the same processing steps as the evidence.

If multiple amplification test kits are to be used and the reagent blank associated with the extraction set or sample being amplified has been depleted, continuation on to a different amplification test kit shall not be performed. This is specifically for DNA extracted after July 1, 2009. Multiple RBs may be processed in a single extraction set to avoid this situation; if this is done, each RB may control for any sample in the extraction set. RBs for an extraction set should be used for testing in order of highest signal to lowest.

1.6.2 Lab Positive Control. A positive control (EP1) is processed for each set of extractions. This positive control is carried through all STR analysis steps. The EP1 is also considered an amplification positive control that is used to determine if the PCR performed properly. The EP1 may be omitted for re-amplifications if expected results were previously generated for the extraction set.

1.6.3 Negative Control. An amplification blank (NEG) is processed with each set of amplifications. The maximum volume (dH_2O) possible for each kit is used for the amplification step. When practicable the same lot of dH_2O used in extraction/dilution is used for amplification.

1.6.4 Kit Positive Control (POS). A kit amplification positive control is processed with each set of amplifications.

1.6.5 Each RB and EP1 extraction tube will be labelled with a unique identifier. An example could be RB-date-initials. The unique identifier information for the control can be found on the DNA extraction worksheets under the Sample # field in line with the appropriate Control. The unique identifier will be used for any extract tubes that will be stored in a long-term freezer boxes. Work product stored in 96-well plates (used for database samples) do not have the controls labelled as such, but do have a unique identifier to the plate itself, see DNA SOP-12.

1.6.6 Expected results of controls will be verified and documented on DNA QR-4A, QR-4b, or QR4c which is maintained in the case file. For Positive Controls and Extraction Controls verified using the POS and EP1 Concordance Check Excel spreadsheets for the specific test kit (DNA-QR-45A, D, E, F, QR-49A,E for POS& EP1, or QR-37 GeneMarker Concordance Check) the corresponding QR worksheet is maintained in the batch paperwork. Instructions to use these worksheets are within the excel spreadsheet workbooks. When using a positive extraction control besides EP1 (i.e. KJL, RKO,TMP), the control may be checked using other means.

1.7 Contamination:

Contamination is defined as the introduction of a secondary source of DNA (genomic or amplified) into a sample at the DESPP Division of Scientific Services. This is to be distinguished from sample mixtures or contamination at the time of collection, which may require different action.

See below for examples of contamination prevention methods.

The following are two possible techniques to further reduce the potential for contamination during DNA analysis of forensic unknown samples:

1. Kimwipe method: Use a fresh Kimwipe or a portion of a Kimwipe to open and close flip-top tubes.

2. Tube Opener method: Use a plastic tube opener to open tubes. Bleach tube openers between uses.

All Laboratory personnel are to supply a known sample for elimination purposes prior to them working in the lab. If a known sample is not submitted by an individual that person cannot work on evidence involving DNA requests.

If contamination is detected, review the results to determine the source of the contamination. Perform appropriate corrective measures as warranted by the nature/source of the contamination. Each incident of contamination (peaks ≥ 25 rfu for casework items and ≥ 50 rfu for knowns) will be documented and maintained by the Quality Section (i.e. workflow in Qualtrax). The TL,

CODIS Admin, Assistant Director and Deputy Director will be notified of all incidents of contamination. If a QAR is required, it will be approved by the TL. Please see DNA-SOP-5 Section 5.5.5.2 and DNA SOP-31 Section 31.5.3 for more details.

All applicable profile results are checked against the staff index (see DNA SOP 13.8.1)

If contamination is detected:

1.7.1 Discard any reagents that are contaminated.

1.7.2 Thoroughly clean the affected lab area.

1.7.3 On occasion, when objects/equipment are repeatedly identified as contaminated, after a thorough cleaning, set up blank reactions to verify that the contamination has been eliminated. Do not conduct DNA testing of case samples until the situation is rectified.

1.7.4 Review the data and the analytical techniques of the analyst(s) in question and take remedial action as required.

1.8 Monitoring, cleaning, and decontaminating facilities and equipment:

1.8.1 DNA testing facilities and equipment are monitored, cleaned, and decontaminated (when applicable) per FBI Quality Assurance Standards (Standard 6) and laboratory SOPs.

1.8.2 For each set of amplifications, document the batch number, the results of the controls and cleaning performed on worksheet DNA QR-8.

1.9 Case documentation:

Only official case receipts and records shall be maintained in the DNA case file. Where appropriate case results/records shall be documented on controlled worksheets. See SOP-23 Case Documentation and Review for specific procedures. In addition, phone calls and email correspondence can be maintained in the DNA case file. For STR analysis, the electropherograms are included in the case folder with the exception of convicted offender samples. All electronic files regarding STR analysis (Complete run folder, GeneMarker HID, GeneMapper ID sample and project files: .sgf, .fsa and .ser files) are archived on optical disks. In addition, the U drive is backed up on a regular basis. See APPENDIX I below for more detail in regards to Archiving Data.

1.10 Case review:

Definitions as per the 2011 FBI QAS:

Technical Review: is an evaluation of reports, notes, data, and other documents to ensure that there is appropriate and sufficient basis for the scientific conclusions.

Technical Reviewer: is an employee who is a current or previously qualified analyst in the methodology being reviewed and is not an author of the applicable report. The Technical Reviewer is authorized to conduct technical reviews and has had casework experience prior to performing any technical reviews. A methodology is used to describe the analytical processes and procedures used to support a DNA typing technology: for example, extraction methods (manual vs. automated), quantification methods (real-time); typing test kit, and platform (capillary electrophoresis).

Administrative Review: is an evaluation of the report and supporting documentation for consistency with laboratory policies and editorial correctness. Administrative review includes a review of the chain of custody that is documented on the "DNA QA/QC Casework Checklist Review Worksheet" (DNA QR-4, QR-4b, or QR-4c). For batch paperwork, the Administrative Review is documented on DNA QR-4A.

See DNA SOP-23: General Procedures for Case Documentation and Review

1.10.1

All DNA cases/reports are technically (100%) and administratively (100%) reviewed by 2 laboratory employees in addition to the primary examiner. The technical review function is performed by the technical reviewer and documented on DNA QR-4, QR-4b, or QR-4c. For batch paperwork, the Technical Review is documented on DNA QR-4A.
Administrative reviews are performed on all case files prior to reports being issued. In cases where no DNA profile interpretations are reported, administrative reviews are performed by any laboratory personnel who have been trained to review DNA cases and have access to LIMS. These reviews are used to ensure that the case demographics are correct, there are not typographical errors, and the documentation is consistent with laboratory policies.
In cases where DNA profile interpretations are reported, administrative reviews are performed by any qualified DNA analyst or DNA analyst trainee who has been approved to be an administrative reviewer. In addition to the criteria listed above, the administrative reviewer will verify that the conclusions made in the report are supported through the documentation in the case file. For outsourced cases where there will be no ownership in the data, QR-4d is used. Please see DNA SOP-21 for further outsourcing guidance.

Note: No analyst may technically review their own work.

1.10.2 In the event of discrepant conclusions, the examiners will discuss/review the results. Should a discrepancy persist, the data will be reviewed and conclusions approved by the Technical Leader (TL).

1.10.3 Where practicable, one case per analyst will be reviewed quarterly by the TL.

1.11 Corrective Action:

Where warranted, corrective action will be taken as outlined in the Quality Manual GL-9. Any corrective action in the DNA Unit shall be approved by the TL (documented on GL-9:1 or in Qualtrax) prior to implementation. The TL has the authority to initiate, suspend, and resume DNA analytical operations for the DNA Unit or an individual.

1.12 Laboratory Safety protocols:

Laboratory safety protocols are outlined in the Division of Scientific Services Quality Manual GL-2. The DNA health and safety program is reviewed annually by the TL and documented on QR-258. The program includes (1) a blood borne pathogen and chemical hygiene plan and (2) documented training on the blood borne pathogen and chemical hygiene plan.

1.13 Quality Manual:

General DESPP, Division of Scientific Services protocols are outlined in the Quality Manual (GL 1-21). The DNA Unit follows accordingly to these SOPs.

GL-1 (Quality Manual)
GL-2 (Safety)
GL-3 (Security)
GL-4 (LIMS)
GL-5 (Ethics)
GL-6 (Purchasing)
GL-7 (Audits)
GL-8 (Management System)
GL-9 (Quality Action Requests)
GL-10 (Customer Inquiries)
GL-11 (Records Control)
GL-12 (Evidence Receiving)
GL-13 (General Evidence Handling)
GL-14 (General Training)

GL-15 (Professional Development)
GL-16 (Proficiency Tests)
GL-17 (Court Monitoring)
GL-18 (Case Reviews)
GL-19 (Document Control)
GL-20 (Review of Requests and Tenders)
GL-21 (General Lab Equipment)

1.14 DNA Analyst Training and Continuing Education:

1.14.1 Transcripts and educational qualifications of all analysts are documented and maintained in each analyst's professional development file by the Quality section which are approved by the Technical Leader and documented by memo.

1.14.2 All DNA analysts are trained prior to assuming casework duties as outlined in the Quality Manual, the DNA Training Manual, Mitochondrial DNA Training Manual, and FBI QAS (Standard 5). If appropriate the training period may be extended, or an analyst may undergo retraining following guidance in GL-14 and DNA SOP-7.

The TL will assess and document any adjustments to the established training program with aid of the Training Coordinator. DNA analysts will successfully complete a qualifying test prior to independent casework analysis. The TL is responsible for the oversight and approval of training in the DNA Unit and will document the approval by memo. The current DNA Training Manual and Mitochondrial DNA Training Manual is found in DNA SOP 7.

1.14.3 Proficiency Tests are samples submitted to the laboratory by an external source, usually a commercial vendor. Individuals taking the test do not know the results.

Competency Tests are samples that have either been prepared internally or received from an outside source. The Training Coordinator or designee knows the results for the samples but the analyst conducting the test does not. Each analyst must successfully complete a competency test prior to being assigned casework. Per the Quality Manual, competency cases include the following:

1. The examination of sufficient unknown samples to cover the anticipated spectrum of assigned duties.
2. A written report (if applicable) to demonstrate the individual's ability to convey results and the significance of the results.
3. A written or oral examination, which assess the individual's knowledge of the discipline.

4. Court Testimony (if applicable)

Competency memos recommending authorization for individuals in a specific methodology or platform shall be approved by the TL.

1.14.4 Proficiency testing is used to demonstrate the quality of the scientific service offered by the laboratory and it serves as a mechanism for critical self-evaluation. The test due date is the date used to indicate when a DNA proficiency test is performed.

All DNA analysts will undergo external proficiency tests semi-annually (twice a year) as outlined in the Quality Manual (GL-16) and FBI QAS (Standard 13). A report will be written for the proficiency test and kept in the case file.

The TL is responsible for the oversight and approval of proficiency testing in the DNA Unit. Proficiency test results will be documented on the DNA Proficiency Testing Review Sheet GL-16:3. Documentation will be kept in the "Proficiency Test Record" Binder by the Quality Section.

If an analyst is qualified in both manual and automated methods for DNA extraction, then the analyst must be proficiency-tested in each method at least once per year to the full extent in which he or she participates in casework. If multiple manual and/or automated methods are available, the analyst must be proficiency tested on at least one of the manual and one of the automated methods per year.

1.14.5 DNA analysts will complete at least 8hrs of continuing education each year as outlined in the FBI QAS Standard 5. These records are documented using DNA QR-31 and kept in a binder by the Training Coordinator.

Analysts will maintain familiarity with the scientific literature of their field by reading and/or reviewing pertinent articles. In general, analysts should try to read 4-6 articles a year (minimum is 1 article per year by FBI QAS).
Records of this process will be reviewed and approved by the TL, in association with the Training Coordinator, and documented using DNA QR-30, kept in a binder by the Training Coordinator.

1.14.6 The Training Coordinator will work with Quality Section, the Technical Leader and CODIS State Administrator to ensure appropriate levels of training and competence are maintained for DNA Unit personnel. The Training Coordinator will maintain appropriate documentation of education and training processes in the laboratory.

1.15 Method Validation:

The DNA Unit shall only use validated methodologies for DNA analyses.

Before any new/updated method is implemented for casework, the DNA Unit must perform an internal validation to demonstrate the reliability of the procedure in-house.

Validation is the process used by the scientific community to acquire information to assess the ability of a procedure to obtain a desired result, determine the conditions under which such results can be obtained and to determine the limitations of the procedure. The validation process identifies the critical aspects of a procedure and must address the quality assurance parameters and interpretation guidelines for the procedure.

Prior to the start of a validation, a validation plan must be written and approved by the Technical Leader. Internal validations (according to FBI QAS (standard 8) and the Quality Manual), if applicable, must include the following criteria:

1. Reproducibility/Concordance
2. Precision
3. Sensitivity and stochastic studies
4. Mixture studies
5. Knowns and Non-probative casework or Mock Evidence.
6. Contamination assessment

During the validation process, additional testing may be necessary which can alter the validation plan.

Validation Summaries should capture what was tested and the results of the testing. The validation results will be reviewed and approved by the TL. In addition, all methods, work instructions, and SOPs (for nuclear and mtDNA) are approved by the TL. The Assistant Director and/or Deputy Director will also review validations and SOP changes.

1.16 References: DNA Unit:

All DNA protocols are based on standard techniques of molecular biology and forensic science, and have been extensively peer reviewed and conform to the general practices of forensic and molecular biology.

ABI 7500 Getting Started Guide, User's Manuals, ABI, 2006.

ABI 7500 Installation and Maintenance Guide, User's Manuals, ABI, 2006.

ABI 7500 Real-Time PCR System, User Bulletin, ABI, 2007.

ABI Prism 3130xl Genetic Analyzer, Data Collection v3.0 Manual, ABI, 2005.

ABI Prism 3130xl Genetic Analyzer, User's Manuals, ABI, 2004.

AmpF*l*STR Identifiler Plus PCR Amplification Kit, User's Guide, ABI, April 2011.

AmpF*l*STR Minifiler PCR Amplification Kit, User's Guide, ABI, August 2012.

AmpF*l*STR Yfiler PCR Amplification Kit. User's Guide, ABI, September 2012.

Biomek 3000 Laboratory Automation Workstation, User's Manual, Revision AD, Beckman Coulter, December 2009.

Biomek Software, Version 3.3 User's Manual, Revision AE, Beckman Coulter, December 2009.

BSD600 Duet Operator Guidelines, Version 6, BSD Robotics, 2005.

CODIS User's Guides, FBI, CODIS Home Page.

Current Protocols in Molecular Biology, Edited by Ausubel et al., 1988. Breene Publishing Associates and Wiley-Interscience.

DNA Technology in Forensic Science, National Research Council, 1992. National Academy Press. (NRC I)

EZ1 Advance XL, User Manual, Qiagen, May 2009.

EZ1 DNA Investigator Handbook, 4th edition, Qiagen, April 2009.

FBI Quality Assurance Standards for DNA Databasing Laboratories, Effective September 1, 2011.

FBI Quality Assurance Standards, Audit for DNA Databasing Laboratories, Effective July 1, 2009.

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories, Effective September 1, 2011.

FBI Quality Assurance Standards, Audit for Forensic DNA Testing Laboratories, Effective July 1, 2009.

Forensic Biology, DNA Unit Reference Library.

GeneMapper ID Software v3.2, User's Manuals, ABI, 2004.

High-performance DNA from forensic samples using the EZ1 DNA Tissue Kit together with the EZ1 DNA Forensic Card protocols. Application Note, Qiagen, January 2004.

Molecular Cloning: A Laboratory Manual 2nd Edition, Sambrook et al., 1989. Cold Spring Harbor Laboratory Press.

NDIS Procedures Manual, CODIS Home Page.

PCR Protocols, Methods in Molecular Biology, Edited by White, 1993. Humana Press Inc.

PCR Protocols, A Guide to Methods and Applications, Edited by Innis et al., 1990. Academic Press, Inc.

The Evaluation of Forensic DNA Evidence, National Research Council, 1996. National Academy Press. (NRC II).

Quantifiler® HP and Trio DNA Quantification Kit User Guide, Pub# 4485354 Rev B, 2014.

HID Real-Time PCR Analysis Software, Version 1.2 Pub #MAN0009819 Rev. B.0, 2014.

SWGDAM Interpretation Guidelines for Autosomal STR Typing 01/12/2017

GeneMarker HID User Manual v 2.9

Promega Fusion 6C System Protocol rev 6/16

STRmix v2.4 User's Manual rev 7/16

STRmix v2.4 Operation Manual rev 7/16

NOTE: Various other scientific articles and references are kept in a binder by the Training Coordinator.

1.17 Equipment Maintenance and Calibration:

All equipment used for casework analysis is maintained in proper working condition. Any equipment in need of repair or out of calibration is tagged and will not be used for casework until repairs/calibrations are completed. If necessary and depending on the situation, an incident report may be needed.

Performance checks are performed on critical equipment annually. A performance check is typically done following the annual (service contract) preventative maintenance (if applicable) or after a repair of an instrument to ensure the instrument is operating within normal parameters and gives the expected results, prior to the instrument being put back online for casework analysis. (Refer to DNA SOP-9).
Instrument Performance Checks will be documented on the respective DNA Quality Record referenced in SOP-9 and kept in its respective "Performance Check" Binder by the DNA Unit.

1.18 Audits:

Internal audits will be conducted by the CT Division of Scientific Services DNA audit team using the current FBI QAS audit document in accordance with guidelines established in the Laboratory Quality Manual (GL-7).
The DNA audit team (see QR-257A and B) will consist of at least one person that is, or has previously been, a qualified DNA analyst for each specific DNA technology performed, including mtDNA, and at least one person who is a qualified auditor who has successfully completed the FBI QAS auditor training course. The qualifications of each audit team member are documented on Appendix C of the QAS. The audit documents (internal and external) are maintained for a minimum of ten years by the Quality Section. The internal audit review will be documented on DNA QR-257A and B.

In accordance with the FBI QAS standards, the DNA Unit of the laboratory will be audited using the most current standards annually. Every other year, the audit must be performed by personnel external to the Division of Scientific Services. Per the FBI QAS standard 15; "the required annual audit shall, at a minimum, occur once every calendar year and shall be at least 6 months but no more than 18 months apart."

If any difficulty arises in scheduling an external audit during the required year, the laboratory will immediately notify the NDIS Custodian and the NIJ Grants Program Manager of the nature of the problem scheduling the external audit.

The TL will annually review the DNA Quality System (independent of the annual audit) and document the approval on DNA QR-258. This document will be kept indefinitely by the Quality Section.

1.19 Outsourcing:

The Connecticut Department of Emergency Services and Public Protection Division of Scientific Services (CT DSS) may contract or subcontract forensic DNA casework samples to a Vendor Laboratory (e.g. Bode). If samples are outsourced to a Vendor Laboratory, this Vendor Laboratory and CT DSS shall comply with the *FBI Quality Assurance Standards for Forensic DNA Testing Laboratories* and the *FBI Quality Assurance Standards for DNA Databasing Laboratories (QAS)* and the accreditation requirements of federal law. The Vendor Laboratory shall provide documentation of this compliance to the CTDSS. Documentation of this compliance will be maintained by the Quality Section (Refer to DNA SOP-21 and GL-1).

1.19.1 DNA laboratories outsourcing DNA sample(s) to a vendor laboratory or accepting ownership of DNA data from a vendor laboratory shall have and follow a procedure to perform an onsite visit(s).
The CTDSS procedure includes, at a minimum, one of the following elements:

1.19.1.1 A documented initial on-site visit prior to the vendor laboratory's beginning of casework analysis for the CTDSS. This on-site visit is performed by the DNA TL or a designated employee of the CTDSS who is a qualified or previously qualified DNA analyst in the technology, platform and typing amplification test kit, used to generate the DNA data. If the on-site visit is performed by the DNA TL or designated employee of the CTDSS, at a minimum, standard 17 of the FBI QAS will be printed out and completed by the analyst performing the visit. Any discrepancies or issues found will be documented and reviewed.

1.19.1.2 Instead of a CTDSS employee performing the initial on-site visit as stated in 1.19.1.1, the CTDSS Technical Leader may accept an on-site visit conducted by another NDIS participating laboratory (within 12 months) using the same technology, platform and typing amplification test kit, for the generation of the DNA data. If the on-site visit is performed by another NDIS laboratory, the DNA TL will document the review and approval of the on-site visit. The date the onsite visit was performed, a summary of the visit and the personnel who performed the on-site visit will all be maintained.

1.19.2 If the outsourcing agreement extends beyond one year, an annual on-site visit shall be required. Each annual on-site visit shall occur every calendar year and shall be at least 6 months and no more than 18 months apart. Either element described 1.19.1.1 or 1.19.1.2 may be followed. All reviews and approvals by the DNA TL will be documented.

1.20 Work Instructions:

Work Instructions are defined as documents containing detailed instructions that specify exactly what steps to follow to carry out an activity. A work instruction is more detailed than a SOP and is only created if more detailed instructions are necessary. Work Instructions may be referenced in specific SOPs or may be appended to the end of an SOP. Work Instructions will be treated as a controlled document.

1.21 Facility/Security:

The laboratory Security protocol is outlined in the Quality Manual GL-3 "Security". The Laboratory's approach for maintaining the integrity of evidence is outlined in GL-13 "General Evidence Handling".

APPENDIX I

Data Archived

All 3130 data files (.fsa) and GeneMapper projects (.ser)), GeneMarker projects (.sgf), and STRmix data containing case data are periodically (2x a year) archived on permanent storage disks. Other data may also be archived. For mitochondrial DNA archiving of data, please see mtDNA SOP-1.

Security of Archived Data

Archived data is stored on non-rewriteable disks. These are stored in a location which may only be accessed by authorized personnel. Only designated analysts should have write-access to the network folder containing archived data, to prevent accidental changes.

Considerations When Generating Data that will be Archived

1. Semicolons should not be used in Collection Sample File Names, as they prevent their file from burning properly to DVDs.

2. Run file names generated by 3130s should not be altered, except from the run number onward. This includes changing underscores to dashes and vice-versa. Nor should a casework or QC run folder be placed into a folder of a different name (except from the run number onward). These changes affect the order in which data sorts, which makes it more difficult to determine which data to burn at a given time.

3. Run folders and GeneMapper projects should be moved into the "Completed" folder as soon as they are done being analyzed.

4. Never place anything into a subfolder containing the word "Archived" in its name, which may be present within the "Completed" folder.

Procedure for Archiving Data

1. Leave at least one month between the latest data archived and the date of archiving. The data from this month(s) should remain on the network in the "Completed" folder until the next data burn. Use the injection date in the 3130 run folder name as the "date" of everything in that folder.

2. Convicted Offender data is archived separately from case data. Follow the same procedure, using the Convicted Offender plate number for sorting.

3. Create a new folder within the "Completed" folder. Call this folder "Archived [date]".

4. Move all data to be archived into the "Archived [date]" folder. Check carefully to be sure that only data from the proper date range has been moved, and that no data from the date range to be burned remains in the "Completed" folder.

5. Follow the instructions in a data-burning program to burn the "Archived [date]" folder to a disk. Indicate the type of data (Nuclear/Mt/CO 3130 Data) and the date of burning in the disk title.

6. When the burn is finished, insert the disk into a computer, open it, and right-click the "Archived [date]" folder and choose "Properties". Do the same for the "Archived [date]" folder which is still on the network. Compare the Size and the Contains fields. The Size of each folder and the numbers of Files and Folders Contained should be identical. If not, find what is causing the difference and fix it before continuing. Note: the Size-on-disk may be slightly different between the two folders; this is ok.

7. Label the disk with the disk title (as in step 5) and the range of dates substantially covered.

8. Import at least 2 projects from the disk, preferably all generated by different 3130s and different analysts, into GeneMapper.

9. Assure that they open properly and that all data appears to display correctly.

10. Paste screenshots depicting a list of all of the folders and files within the outermost folder on the disk into a file. Print this file and save it on the DNA network.

11. Store the printout with the currently archived data which is stored in the DNA Unit Section storage room (206).

12. Do one final check immediately before moving data to be sure that the Size and numbers of Files and Folders Contained in the "Archived [date]" folder on the network have not changed, then move this folder to the designated Archived Data folder on the DNA network. (If any of this information *has* changed, the folder has been altered since it was burned – resolve the discrepancy before moving and be sure that what you are moving is exactly what was burned to the disks.)

13. Any disk generated that contains an error and is therefore not being used for the final archive should be disposed of. It must be rendered unreadable first (ie. by breaking in pieces or shredding).

14. In addition to disk archival, all data residing on the U drive is archived on a weekly basis.

APPENDIX II

List of Abbreviations

a = allele

@ = at or about

-A = minus A peak

ABI = Life Technologies (formerly Applied Biosystems Incorporated)

A(L)L = Average log likelihood

AP = Acid Phosphatase

* Peak = peak ≥ 50 RFU & <75 RFU (potential allele below threshold)

AT = analytical threshold

AV = Allele variance

BLS = Blood-like stain

bp = base pair

Č = contained

CBE = cannot be eliminated

CDMCS = Central district major crime squad

CIDI = case, item, date, initials

CO = Click Off

CODIS = Combined DNA Index System

da = dye artifact

dH_20 = de-ionized water

DOI = date of incident

DNA = deoxyribonucleic acid

DNR = Data Not Reported

DTT = Dithiothreitol

E = evidence

EDMCS = Eastern district major crime squad

EF = epithelial rich fraction

EP1 = Extraction Positive Control

ESS = effective sample size

F6C = PowerPlex Fusion 6C System

GM-HID = GeneMarker HID Software

GMID = GeneMapper ID Software

GR = Gelman-Rubin Convergence

H_1 = Hypothesis consistent with inclusion of POI

H_2 = Hypothesis consistent with a random person matching

HBAP = High Background Artifact Peak

H_p = Prosecutor's Hypothesis

H_d = Defense's Hypothesis

HPB = Heterozygote peak balance

HPD = Highest Posterior Density

ID = Identifiler

ID + = Identifiler Plus

IDP = Identifiler Plus

IFC = Insufficient For Comparison

ILS = Internal Size Standard

KM = Kastle-Meyer

KJL = Laboratory Positive Control (used previously)

LR = Likelihood ratio

MajorMix = major mixture

MCMC = Markov Chain Monte Carlo

MF = Minifiler

MM = master mix

mt = mitochondrial

mito = mitochondrial

N = No

n = additional allele potentially dropped out

NAA = no alleles assigned

N/A = not applicable

ND = not deduced

NE = No Edits

NEATT = not examined at this time

NEG = Negative amplification control

NR = No Results

nt = nucleotides

NTATT = not tested at this time

OB = out of bin

OCME = Office of the Chief Medical Examiner

OLa = Off Ladder allele

OL = Off Ladder

ORI = Originating Agency Identifier

pd = Pull down peak

PD = Police Department

Pks = peaks

PHR = Peak Height Ratio

POI = person of interest

POS = Positive amplification control

PPY = PowerPlex Y

Pr = Probability

Prot K = Proteinase K

pu = pull up peak

Q samples = question samples

QC samples = quality control samples

rb = raised baseline

RAL = retained at the laboratory

RB = extraction negative control (reagent blank)

R/B = Reddish Brown

RBS = Reddish Brown Stain

R-CPI = restricted CPI

Re-amp = re-amplification

re-inj. = re-inject

RET = red evidence tape

RFU = relative fluorescent units

RKO = Laboratory Positive Control (used previously)

RMP = random match probability

rxns = reactions

SA = sexual assault

SAK = sex assault kit

SAO = State's Attorney's Office

SF = sperm rich fraction

Sld. = sealed

sm. amt. = small amount

sp = spike

SR = stutter ratio

ss = single source

ST = stochastic threshold

st = stutter peak

std = standard

sus = suspect

SV = Stutter variance

TL = Technical Leader

TMP = Laboratory Positive Control (used previously)

U-CPI = unrestricted CPI

vic = victim

WDMCS = Western district major crime squad

Y = yes

YF = Y-Filer

CSF = CSF1PO

D1 = D1S1656

D2B = D2S441

D2G = D2S1338

D3 = D3S1358

D5 = D5S818

D7 = D7S820

D8 = D8S1179

D10 = D10S1248

D12 = D12S391

D13 = D13S317

D16 = D16S539

D18 = D18S51

D19 = D19S433

D21 = D21S11

D22 = D22S1045

TH0 = TH01

Amel = Amelogenin

| = given