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 Amplification products (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. They are not preserved for future testing.

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. Evidence extracts have a unique identifier by being labelled with case number and item number. Reagent Blank, EP1, and evidence extracts are tracked in LIMS with a chain of custody when the samples are preserved for future testing. The extracts will be clearly designated in LIMS; for example, #001-001 - DNA Extract. When the extract is exhausted; the extract will be transferred to "sample consumed in testing". For controls (RB/EP1), a "DNA controls" case covering a time period specified in the case name will be made in LIMS where the controls assigned to specific batches will be tracked. For example: CONTROLS-2019-2 for the second quarter of 2019. In each case, month names are entered as submissions, and control extracts are added as sub-items of the corresponding month. When the controls are exhausted; they will be transferred to "sample consumed in testing". The extracts and controls will be maintained in storage boxes in the "DNA Extract- Long Term Freezer" location in Justice Trax. In the notes field of each extract and control, the storage box number will be noted. While the DNA extracts are in

progress and not in the analyst's custody, they may be placed in the "DNA Extract – Temporary Refrigerator" where each analyst has a slot for their extract racks. This is for overnight refrigerator storage if not all steps in the DNA process are completed in one day.

Known reference extracts are not tracked and are not preserved for future testing.

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. Case Management will determine arrest status prior to an analyst/technician receiving a case. There is no need for any further confirmation by the analyst or technician.

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 (expiration 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 Forensic Biology protocols found in Qualtrax. Refer to: SOP-FB-01: Physical Evidence Examination, SOP-FB-03: Guidelines-Collection & Forwarding, and GL 4 LIMS. Physical Evidence Worksheets DNA-QR-1 and DNA-QR-1A may also be used to document evidence and examination. Please see GL-4 LIMS for details on itemizing in Justice Trax.

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

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 analysts can perform Forensic Biology 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.

1.1.13 Prior to beginning an extraction, all samples retrieved for DNA testing will be verified for correct labeling and contents by a second individual (however titled).

The first individual will review the samples retrieved and verify that the labeling and contents agree with the LIMS evidence transfer sheet. They will initial and date the evidence transfer sheet to document their review.

The second individual will review the samples retrieved and verify that the labeling and contents agree with the LIMS evidence transfer sheet.

a. If in agreement, the second individual will initial and date the LIMS evidence transfer sheet.
b. If the second individual discovers a discrepancy, the Technical Leader, Assistant Director and/or Deputy Director will be informed and the root cause will be determined and corrected.

Further appropriate action may be taken by the Technical Leader, Assistant Director and/or Deputy Director.

The initialed and dated LIMS evidence transfer sheet(s) (or copy) will be retained in the appropriate case jacket(s).

Once verified, the samples can proceed to DNA extraction.

1.2 Preparation of Discovery Materials:

It is preferred that discovery requests are made in writing at least 1 month prior to the anticipated court date. 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. No additional information is to be added to the discovery copy of the original case material. The requested information will be duplicated, scanned, and maintained on the dps

domain shared drive (S:). 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. Please refer to CM WI-06 "Discovery and FOIA Requests" for further details.

1.2.1 Preparation of GeneMarker electronic data sample files and project files for discovery. The samples applicable to the discovery request are selected by opening the saved project with GeneMarker, and then selecting the applicable samples, controls, and ladders by double clicking on them. Next, select 'Save Selected Samples' and make sure to save the project for discovery with a name that is different from the original file name (e.g. original project name_case#discovery; save on S-drive in Case Management designated folder).

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.

Personnel not qualified in DNA analysis may not make decisions about sample processing, such as which samples to halt or move forward to amp, or reinject. This is in accordance with the QAS. Qualified analysts will analyze Quantifiler Trio Runs, create halt at quantitation and amplification QRs, and inform technicians of any samples in need of re-work, including re-injections or re-extractions.

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 for a request is documented on the DNA Extraction Worksheets for the particular request. Testing is completed on the date of the draft report for that particular request.

1.4 Report Writing:

DNA results are reported according to standard DESPP Division of Scientific Services guidelines, ANAB ISO 17025:2017, and the current FBI DNA 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 statements.

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 or DNA SOP-6.

1.4.4 All DNA Reports will clearly communicate all items tested and those not tested, all evidence and extract 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. Any updates to the level of a CODIS entry (e.g. SDIS, NDIS, deleted from NDIS and/or SDIS) will be communicated, please refer to DNA SOP-13 CODIS Profile Entry and Data Bank.

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 have a date (may be handwritten) to reflect the "end of testing" until the final report is released.

1.4.7 Final Report

1.4.7.1 The draft report is edited, and then changed from a draft report to the final report to include the report date. This is saved as the final report (without signatures) on the U-drive.

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.

1.4.7.5 After a case is "draft complete" in Justice Trax, the analyst will assign a technical review request to a Technical Reviewer. When the Technical Reviewer has completed their technical review of the case, they will assign an administrative review request to the Administrative Reviewer. Upon completion of the administrative review, the request will be released in Justice Trax. This release will be confirmed by the analyst and documented on the appropriate QR.

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, masks, 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 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. Limit the time PCR reagents are out of storage for long exposure of light and incorrect storage temperatures could negatively affect the reagents.

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 for F6C. Please note that Y-STR testing has a target of 1-2 ng of male DNA, but 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. For samples that are both high molecular weight and assumed to be single-source (such as blood stains or non-probative A-fractions), a target of 0.25ng/µl may be amplified at analyst discretion.

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 all the extracts in the set 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-Batch Number or EP1-Batch Number. 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 (as stated above for a control) will be used for any control extract tubes that will be stored in a long-term freezer boxes. Justice Trax is used to track these samples with the respective casework samples. 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. Work product used for database samples and knowns are not kept/preserved for future testing, therefore are not tracked in LIMS.

1.6.6 Expected results of controls (correct genotype) will be verified by the analyst and technical reviewer 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 files 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 workbooks. When using a positive extraction control besides EP1 (i.e. KJL, RKO, TMP), the control may be checked against the known genotype of those controls and documented in the batch paperwork and on the review worksheets in the case file.

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 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 knowns) will be documented and maintained by the Quality Section as workflow instances in Qualtrax. The TL, CODIS Admin, Assistant Director and Deputy Director will be notified of all incidents of contamination through the workflow in Qualtrax. If a QAR is required, it will be approved by the TL. Please see DNA-SOP-5 and DNA SOP-31 for more details. A quarterly review of contamination will be performed and shared with the FB/DNA Section.

All applicable profile results are checked against the staff index (see DNA SOP-13)

If systemic 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 The EZ1extraction robots have maintenance worksheets with each instrument. Cleaning before and after use is required and is documented on the respective worksheets.

1.8.3 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, technical and administrative records shall be maintained in the DNA case file. Where appropriate, case results and 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 and notes are made in LIMS. 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 current 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/competent analyst in the methodology being reviewed and is not an author of the applicable report. The Technical Reviewer is required to have been competency tested for the process they are reviewing. 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 and reports are technically (100%) and administratively (100%) reviewed. 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 or QR-4E.

Technical reviews are performed on all case files prior to reports being issued. Technical reviews are performed by any qualified analyst to verify that the conclusions and data are scientifically supported through the documentation in the case file.

Administrative reviews are performed on all case files prior to reports being issued.

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 addition, a DNA analyst trainee who has been approved to be an administrative reviewer may AR cases where interpretations are reported. 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. 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 For male-screen batch paperwork, the technical review and administrative review are completed simultaneously by one qualified analyst.

1.10.3 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).

1.10.4 For intra-laboratory reviews, 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 in Qualtrax through a DNA- Corrective Action Workflow) 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) GL-22 (Policy on Validation and Performance Checks)

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. Such documentation may also be found in Qualtrax in the analyst's electronic professional development file.

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 and necessary, 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 qualified commercial vendor with ISO 17043 accreditation. 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 that covers the spectrum of anticipated activities related to the work the analyst will perform. Per the Quality Manual, competency tests include the following:

1. The examination of sufficient practical 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.

Competency memos recommending authorization for individuals in a specific methodology or platform shall be approved by the TL. Authorizations are approved by the Director through a Qualtrax workflow. Personnel who review and authorize results, an opinion or an interpretation or technical review of results or testimony shall be competency tested as above.

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 DNA 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 program in the DNA Unit. A Qualtrax workflow "DNA Proficiency Testing" is used to capture the proficiency results, TL notification of results, and analysts' notification of their results.

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 8 hours of continuing education each year as outlined in

the FBI DNA QAS Standard 5. Continuing education documentation and necessary approvals per the FBI DNA QAS are maintained in Qualtrax through the "DNA Continuing Education" workflow. The analysts may self-enter their CE and the workflow will automatically send the information to the DNA TL and Lead for that individual. Pre-approval for webinars and other various items (i.e. sign in sheets for internal training) are kept in a binder in the Training Coordinator's office. CV's of outside lecturers are kept on the U: drive in a specific folder.

Please note: As electronic record keeping is phased in and paper format phased out, there may be some information still maintained in paper format. Eventually, all Professional Development files and future Continuing Education documentation are to be kept electronically.

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). This program 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 program found in DNA SOP-7, to the extent necessary based on job function, shall include criteria for acceptable performance. Retraining will occur if acceptable performance is not found. The Training Coordinator will maintain documentation of education and training processes in the laboratory.

1.15 Method Validation:

The DNA Unit shall only use validated methodologies for DNA analyses and computer software programs that affect the results of testing. Any software program developed in the laboratory will also have a planned validation.

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
7. Associated data interpretation
8. Data required reporting a result, opinion or interpretation
9. Identify the limitations of a method, reported results, opinions and interpretations

During the validation process, additional testing may be necessary which can alter the validation plan. Please refer to GL-22 "Policy on Validation and Performance Checks" for more details and other necessary requirements for validations.

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. The Director will be the final approver of the validation of a new method as described in GL-22.

DNA validations will support that all DNA test methods that involve the comparison of an unknown to a known shall require the evaluation of the unknown to identify characteristics suitable for comparison and, if applicable, characteristics suitable for statistics prior to comparison to one or more known items.

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. Please refer to the controlled DNA Manual list found in Qualtrax.

NOTE: Various other scientific articles and references are kept by the Training Coordinator. Developmental Validations of methods used in the DNA unit are kept on the dps-dnasrv01 (DNA Server).

1.17 Equipment Maintenance and Calibration Checks:

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 (STR, Y-STR, and 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." Internal Audits shall include direct observation of DNA analysts performing a sampling of the spectrum of services provided.

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, DNA Labs International). 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".

1.22 **Technical Records:** Please see GL-11 Control of Records for more details on technical records. Below are some of the specific requirements found in GL-11.

1.22.1 All records of observations, data or calculations made while performing specific tests shall be documented at the time they are made, within the constraints of reasonable and accepted scientific practice.

1.22.2 If an observation, data, or a test result is rejected, the reason, the identity of the individual(s) taking the action and the date shall be recorded in the technical record.

1.22.3 Testing dates shall be clearly document in the case records. Testing dates may be reflected as a range of dates or the date of individual test performance.

1.22.4 All pertinent records generated during the course of laboratory analysis shall be maintained in the case file or within a batch file.

1.22.5 It is incumbent upon case analysts to enable the test to be reproduced under conditions as close as possible to the original and to enable a competent analyst to come to the same conclusion and/or results. In addition, all personnel responsible for the sampling, performance, and review processes of these tests shall be readily identifiable in case documentation within the casefile. For units that perform batch analyses, casefiles will have documentation that shows where records can be located when not based in casefiles. Case documentation shall be clear as to which samples are associated with which batches. The batch documentation will be maintained per unit guidance and in a manner similar to case files.

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 (i.e. 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

CM or Conv Match = Conviction Match

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

FH or For Hit = Forensic Hit

GF = GlobalFiler

GFE = GlobalFiler Express

GM-HID = GeneMarker HID Software

GMID = GeneMapper ID Software

GR = Gelman-Rubin Convergence

H (circled) = high stringency match

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

II or Inv Info = Investigative Information

Inc. = Inconclusive

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

NFT = No further testing

NR = No Results

nt = nucleotides

NTATT = not tested at this time

OB = out of bin

OCME = Office of the Chief Medical Examiner

OH or Off hit = Offender Hit

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

V (circled) or vic = victim

WDMCS = Western district major crime squad

Y = yes

YF = Y-Filer

YFP = Y-Filer Plus

YHRD = Y-Chromosome STR Haplotype Reference Database

Y-Mix = Y-Mix Database Filter

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