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 only in "Pre-Amplification" areas. Blue lab coats are worn in "Amplification" and "Post Amplification" areas when working with opened amplified DNA tubes, such as preparing an injection plate in the 3500 area. For post-amplification labs, placing closed tubes onto a Real-Time PCR or thermalcycler, and/ or running or reinjecting a 3500 plate does not need a blue lab coat, but a blue lab coat may be worn if desired.

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 with bleach and ethanol 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 the FBI Quality Assurance Standards (QAS). 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. The disposition of evidence and extracts will be documented on reports and in LIMS.

Casework knowns/reference samples submitted for processing are considered evidence and are handled as such according to GL4, GL13, and the FBI QAS.

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 considered evidence and treated as such, 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 labeled with a case number and item number. Control extracts have a unique identifier by being labelled with the batch number (initials/date in XXXMMDDYY format). Reagent blanks,

extraction positive controls, 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, "#001-001-01 - DNA Extract of #001-001". When the extract is exhausted; the extract will be virtually transferred to "sample consumed in testing".

For controls (reagent blank/extraction positive control), 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-2021-2 for the second quarter of 2021. In each case, month names are entered as submissions, and control extracts are added as sub-items of the corresponding month. If the controls are exhausted; they will be virtually transferred to "sample consumed in testing". The extracts and controls will be maintained in storage boxes, and virtually in the "DNA Extract- Long Term Freezer" location in JusticeTrax. The storage box number will be noted in the notes field of each extract and control.

While the DNA extracts are in the process ofDNA testing,, they may be virtually placed in the "In Progress – DNA Lab" location and kept where each analyst has a slot for their extract racks or with the analyst during the steps of processing. This is for overnight refrigerator storage and all steps in the DNA process until samples are completed and put into long term storage.

Casework known/reference sample and database extracts are not preserved for future testing, and therefore not tracked. Work products stored in 96-well plates (used for known and database samples) will have a unique identifier to the plate itself, see DNA SOP-12. There will be no separate identifier for controls on these plates.

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 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 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 training (not competency) and, for research purposes past their control date where appropriate and are labelled as such.

1.1.10 When examining evidentiary samples, DNA analysts (qualified in collection of evidence samples) will use Forensic Biology protocols found in Qualtrax. Refer to: FB SOP-01: Evidence Exam, FB SOP-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 JusticeTrax.

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 There are points in the process that a second individual (however titled) will verify sample tubes. Please see DNA SOP-20 for guidance on verifications for using the QIAcube Connect. Both the processor and verifier are responsible for ensuring the samples retrieved for extraction are labelled correctly, according to the extraction QR. Verifications will also ensure the sample tube order corresponds to the extraction worksheet setup. Once verified, the samples can proceed to DNA extraction. Please see DNA SOP-20 for guidance on verifications for using the QIAcube Connect.

The verifier will document the review on the appropriate QR. In the event of a labelling discrepancy, the Technical Leader and DNA Manager(s) will be informed so that corrective action can be taken if necessary.

1.2 Preparation of Discovery Materials (in conjunction with GL-11, Control of Records):

1.2.1 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. 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.

1.2.2 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' making 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's designated folder).

1.2.3 Preparation of GeneMapper (ID and IDX) electronic data sample files and project files for discovery: The project containing samples for discovery is opened, and all files not associated with the case in question are deleted from the project. "Save Project As" should then be selected, and given a name different from original project name. Using the GeneMapper Manager, the newly created project shall be exported to the appropriate folder on the S: Drive in Case Management's 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 (i.e. "technicians") may not make decisions about sample processing, such as; which samples to halt or move forward to amplification, or reinjection. This is in accordance with the FBI DNA QAS. Qualified analysts will review DNA quantitation to create halt at quantitation and amplification QRs, and inform technicians of any samples in need of re-work, including re-injections or re-extractions.

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 GL-18, Case Reviews. 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.
Note: The preferred signature method is certified electronic signatures using the available Adobe software.

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, may be compared and interpreted at overlapping loci.

1.4.2 The results from each tested item and information on not tested items are included in the DNA report. 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, and 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 in 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.

1.4.6.1 After a case is marked "draft complete" in JusticeTrax, 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 or complete the Administrative Review themselves, as appropriate. Upon completion of the administrative review, the request will be released in JusticeTrax. This release will be confirmed by the analyst and documented on the appropriate QR.

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. The report is considered finalized when the primary analyst makes all necessary edits to include adding a date to the report. This is saved as the final report (without signatures) on the U: Drive.

1.4.7.2 The final report (with signatures) will be scanned or saved as a PDF. The PDF will be saved to the U: Drive in the case reports folder and the subfolder for the proper year of the case. This PDF will also be uploaded to LIMS.

1.4.7.3 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. Pre-Amplification and Post-Amplification areas are separated from one another.

1.5.2 Gloves, masks, and lab coats must always be worn during 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 shall be used at all times when preparing PCR reactions.

1.5.6 No amplified product should 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 Before setting up any PCR amplifications, the working area (laminar flow hood) must be cleaned with an in-house bleach solution 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 before 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 described in 1.5.8.

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 (most stringent 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 elution volumes are set, such as the EZ1 or EZ2 instruments, are an exception. However, a visual inspections of all elution volumes are made after the extraction and elution. If any visual discrepancies are noted, a manual measurement of all the extracts in the set will be 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 will be used for testing in order of highest signal to lowest.

The reagent blank shall be extracted in parallel and concurrently with its respective samples. The reagent blanks will be quantified, and if multiple, the one with the highest signal will be processed.

1.6.2 Lab Positive Control. A positive extraction control (EP1, EP2 or EP-MS) is processed for each set of extractions, as appropriate. EP1 is utilized for non-differential samples. EP-2 is used for differential samples, and EP-MS is used during the male screen process. When an EP2 or EP-MS is utilized, it is processed only through quantification. The EP1 is carried through all STR analysis steps that the evidence samples go through. The EP1 may be 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 for that kit. If all evidence samples are halted in an extraction set at quantification, the EP1 (associated with those samples) does not have to be carried through to CE.

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 Expected results of controls will be verified by the analyst and technical reviewer and documented on DNA QR-4A, QR-4E, or QR-4F, which is maintained in the case file.

EP-MS controls are assessed based on the Y quantitation value. EP2 controls are assessed based on the "B" fraction (sperm-rich fraction) Y quantitation value. EP-MS and EP2 passing values are lot specific determined during the QC process and maintained in the Reagent Lot Log on the Shared Drive. Amplification and EP1 positive controls are acceptable by yielding the correct genotype and are verified using DNA-QR-37.
When using a legacy positive extraction control (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 actions.

See below for examples of contamination prevention methods 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: Open tubes with a plastic tube opener. 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 contamination incident (peaks ≥ AT for casework items and knowns) will be documented and maintained by the Quality Section as workflow instances in Qualtrax. If a control produces one allele peak above the analytical threshold, this may indicate drop-in. Drop-in peaks are typically low-level (at or near the analytical threshold, but below the stochastic threshold), typically homozygote in nature, and are not associated with a pattern indicative of a DNA profile (i.e., contamination). A drop-in event in one negative control may be classified as an artifact and not considered contamination. An example would be having one single peak detected in one negative control. Two peaks detected in a negative control or one peak in each negative control will require a Qualtrax contamination workflow. The TL, CODIS Admin, Assistant Director and/or Deputy Director will be notified of all contamination incidents through the workflow in Qualtrax. If a QAR is required, it will be approved by the TL. Please see DNA-SOP-39 and DNA SOP-40 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 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 and 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, GeneMapper IDX, sample and project files: .sgf, .fsa, .hid, 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 regard 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 and authorized for the process they are reviewing and has had casework experience prior to performing any technical reviews. The Technical Reviewer must be qualified in the method, technology, typing test kit, platform and interpretation software that the review encompasses. For individuals only authorized in Technical Review, they will perform such on the two proficiency tests a year.

Administrative Review: is an evaluation of the report and supporting documentation for consistency with laboratory policies and editorial correctness.

Administrative Reviewer: is an employee who is not an author of the applicable report.

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 reviewer performs the technical review and is documents it on DNA QR-4, QR-4C or QR-D.

Technical reviews are performed on all case files before 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 administratively review DNA cases and have access to LIMS. These reviews are used to ensure that the case demographics are correct, there are no typographical errors, the chain of custody is accurate, and the documentation is consistent with laboratory policies.

A single qualified individual will conduct technical and administrative reviews on a DNA report with no comparisons. The TR will document this combined review on DNA QR-4 by signing in both review spots.

In addition, a DNA analyst trainee who has been approved/authorized 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 no typographical errors, and the documentation is consistent with laboratory policies. For outsourced cases where there will be ownership, DNA QR-4C will be used, and where there will be no data ownership, 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 The technical review is documented on DNA QR-4A, QR-4E, or QR-4F for batch paperwork. Batch and case corrections will be documented on DNA QR-347.

For male-screen batch paperwork and Known batch paperwork, one qualified analyst completes the technical and administrative reviews simultaneously.

1.10.3 If there are discrepant conclusions, the examiners will discuss/review the results. Should a discrepancy persist, the data will be reviewed, and conclusions will be approved by the Technical Leader (TL).

1.10.4 The TL will conduct a DNA case file/batch/Database review at least twice a year; but preferably every quarter. The scope of each review will be defined and approved by the TL. The scope may change based on factors such as corrective actions or interpretational issues as needed. Where practicable, the TL will review one case per analyst. In general, a representative sampling of cases will be selected for TL review that covers the types of cases and range of tasks that are typically encountered by the DNA Unit. Review will be documented.

1.10.5 If you are taking ownership of a case that had a previous report in which you were not the signatory or the Technical Reviewer, a review must be completed of the relevant examination and supplemental records, as well as previous conclusions, drawn that are necessary to review in order to write the additional and/or associated reports for that case. In addition to appropriate initials on supporting paperwork, this additional review will be documented on DNA QR-315 and maintained in the case jacket.

1.10.6 If you are to testify to a report that references known samples not processed/analyzed by you, a review of the case record(s) pertaining to those known/reference samples processed, including electropherograms and data projects, must be reviewed. Your agreement with the results reported for the known/reference sample will be documented on DNA-QR-316 and maintained in the case jacket. In addition, initials will be added to all paperwork reviewed to support this, if not already added during the initial case review.

1.10.7 In the circumstance where the original analyst and Technical Reviewer cannot testify to their report, the analyst to testify must complete a full technical review of the case record. This includes all electropherograms, data projects, and conclusions reported.

1.11 Corrective Action:

Corrective action will be taken where warranted, 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) before 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 1-22 and subcomponents). 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 (Control of Records)
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)
Gl 23 (Teleworking)

1.14 DNA Analyst Training and Continuing Education:

1.14.1 Transcripts and educational qualifications of all analysts are approved by the Technical Leader and documented in Qualtrax through a workflow. Such documentation may also be found in Qualtrax in the analyst's electronic professional development file in Qualtrax.

1.14.2 All DNA analysts are trained prior to assuming casework duties as outlined in the Quality Manual, the DNA Training Manual, and the FBI QAS. 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/competency 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 is found in DNA SOP-7.

1.14.3 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 (where/when applicable).

Competency memos recommending authorization for individuals to conduct independent forensic DNA testing/analysis or databasing using the applicable methods, technology, typing test kits and platforms shall be approved by the TL. Personnel who review and authorize results, an opinion or an interpretation or technical review of results or testimony shall be competency tested as above. The authorization for technical review may be concurrent with authorization as an analyst or as a separate authorization but will be clearly addressed. Authorizations are approved by the Director through a Qualtrax workflow.

1.14.4 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.

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.

The TL is responsible for overseeing and approving the proficiency testing program in the DNA Unit. A Qualtrax workflow, "DNA Proficiency Testing," captures the proficiency results, TL notification of results, and analysts' notification of their results.

All DNA analysts will undergo external proficiency tests semi-annually (twice a year) as outlined in the Quality Manual (GL-16) and the FBI DNA QAS. A report will be written for the proficiency test and kept in the case file. Semi-annual requires testing to take place two times during one calendar year, with the first event taking place in the first six months of that year and the second event taking place in the second six months of that year, and where the interval between events is at least four months and not more than eight months.

If the analyst is qualified in only one technology, then the analyst will take both semi-annual tests in that technology. All applicable samples in a single proficiency test shall be worked for each technology. It is permissible for multiple technologies to be reported on a single proficiency test. Alternatively, and typically, an analyst qualified in multiple technologies may be separately tested in each technology. For example, the laboratory may administer one test in the year's first half with their Y-STR technology and one test in the year's second half with their autosomal STR technology. Proficiency tests are done according to the Lab's DNA SOPs. If only doing Y-STR, there is no need to amplify the female with STRs. A reference sample may be assumed on a proficiency test for STRs and Y-STRs, especially if the evidence is taken from the "person". If statistics are not needed per scenario or results generated, they will not be calculated as we would per the normal course of business.

1.14.5 DNA analysts will complete at least 8 hours of continuing education each year as outlined in the FBI DNA QAS. 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 their lead. Any 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, some information may still be 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.

Continuing education in topics relevant to the field of forensic or databasing DNA analysis may include seminars on new methods and techniques for obtaining DNA profiles, lectures on troubleshooting current methods or techniques, courses on providing testimony on DNA results and conclusions, as well as the QAS auditor training or relevant CODIS training.

Reading of scientific literature and subsequent lab-sponsored discussions (e.g., journal club, article presentation) do not count toward the continuing education hours. Activities required as part of the laboratory's training program and/or that are required for establishing an individual's competency do not count toward the continuing education hours.

Regional, national, or international conferences related to forensic or biological sciences that include presentations relevant to forensic or databasing DNA typically provide sufficient content to satisfy the continuing education requirement. The program agenda, record of presentations, or curriculum vitae of presenters is not required for regional, national, or international conferences.

Completion is documented through Qualtrax to include the time required to complete the program. For multimedia training that is internally generated (e.g., video recording of an internal lecture), technical leader approval and the time needed to complete the training may be documented prior to or with the dissemination of such training.

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 the FBI QAS 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 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.

Please refer to DNA SOP-9 (Section 9.19) and Appendix 1 and 2 for software and macro performance checks and validation requirements.

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 DNA SOP-9 and kept in its respective "Performance Check" binder by the DNA Unit.

1.18 Audits:

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 in accordance with Standard 15 of the FBI QAS documents. Per the FBI QAS, "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 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 will consist of at least one person that is, or has previously been, a qualified DNA analyst for each specific DNA technology performed (STR and Y-STR) and platform used 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 internal audit review/team will be documented on DNA QR-257A and B. Internal Audits shall include direct observation of DNA personnel performing a sampling of the spectrum of services provided.

The audit documents (internal and external) are maintained for a minimum of ten years by the Quality Section.

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. The Quality Section or DNA Technical leader will maintain this compliance documentation (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).

1.19.1.1 The CTDSS procedure includes, at a minimum, one of the following elements:
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 above, 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 above 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 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 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 GL11 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.

1.23 Reinterpretation of Legacy Data

1.23.1 Reinterpretation of legacy data is defined as re-evaluating allele/base calls, genotype calls (to include potential allelic drop-out), changing the assumptions used, or removing alleles (or

entire loci) from statistics estimates. Generating a report for the comparison of two profiles as a result of a CODIS high stringency match is not considered reinterpretation of legacy data. Likewise, if the interpretation of the DNA profile has been previously documented regarding the genotypes of possible contributors, that interpretation is not considered reinterpretation.

1.23.2 DNA analysts qualified (or previously qualified) in STR, Y-STR and/or mtDNA technology, may be authorized to reinterpret data from a legacy STR, Y-STR and/or mtDNA amplification kit(s). The elements of the analyst's training (listed below) for reinterpretation of legacy data are documented on DNA QR-305. TL approval is documented on DNA QR-305, which is emailed to the Quality Manager and Assistant Director/Deputy Director. The approval to reinterpret legacy data is good for a period of two years.

1. The analyst will review the applicable portions of DNA SOP-33 (Interpretation of STR profiles from Legacy Protocols) and/or DNA SOP-43 (mtDNA Legacy) and the applicable portions of DNA SOP-29 (Stutters and Controls for Legacy STR kits).

2. Legacy kit validation data covering non-probative casework, mixture interpretation, kit sensitivity, reproducibility, thresholds, and stutter will be reviewed by the analyst.

APPENDIX I

Data Archived

All 3500 data files (.hid) and GeneMapper projects (.ser), GeneMarker projects (.sgf), GeneMapper IDX projects (.ser), and STRmix data containing case data are periodically (2x a year) archived on permanent storage disks. Other data may also be archived.

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 3500s should not be altered, except by adding to the end if necessary.

3. Run folders, GeneMapper, GeneMapper IDX, and GeneMarker 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 3500 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/CO 3500 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 1 project from the disk into GeneMarker.

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

10. 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.)

12. 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).

13. 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)

ABC = Anode Buffer Container

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

CBC = Cathode Buffer Container

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 or A-fraction = epithelial-rich fraction

EP1 = Extraction Positive Control

EP2 = Differential Extraction Positive Control

EP-MS = Extraction Positive – Male Screen

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

mtDNA = mitochondrial DNA

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 or Penta D

PE = Penta E

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 or B-Fraction = 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