

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. Only one item is examined at a time.
- 1.1.2 For each case/item, fresh Benchkote/paper will be used.
- 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. Organic extractions are performed in a laminar flow hood.
- 1.1.4 Polymerase Chain Reaction (PCR) product and genomic DNA are separated by space.
- 1.1.5 Physical evidence in the DNA **Unit section** will be handled and documented according to standard DESPP Forensic Laboratory procedures (GL-4 and GL-13) and FBI QAS (Standard 7). All case results are documented on the appropriate DNA QR worksheets. When possible, a portion of the evidence sampled for DNA extraction will be retained at the laboratory. Disposition of evidence will be documented on reports and in LIMS.
- 1.1.6 Genomic DNA (extract tubes or plates), and amplification product (amplification tubes or plates) are considered to be work product--material that is generated as a function of analysis. Work product is not evidence therefore not sub-itemized in LIMS or given a barcode.
- 1.1.7 Extracts containing genomic DNA are uniquely identified and are maintained in locked freezers within the DNA Unit. The boxes containing the DNA extracts are sealed with tape and initialled.
- 1.1.8 Consumption of Evidence (exhaustive testing only)
Per the Connecticut Practice Book (40-9), the defense must be notified in the event of exhaustive testing (if the suspect has been arrested) to determine if the defense wishes to observe the testing. See Case Management Work Instruction (CM WI-04): Consumption Letters for procedures about notification. If the defense wants a representative to be present during the exhaustive testing, the case management section or designee will notify the TL/supervisor and the analyst assigned to the case (if already assigned). The case management section, designee or analyst assigned to the case will schedule the testing date with the expert. If no arrest has been made,

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

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

1.1.9 dH₂O 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. dH₂O used for sample preparation through DNA amplification is lot tracked and quality tested before use in case work.

1.1.10 When examining evidentiary samples, DNA analysts will use as guidelines Forensic Biology protocols found on the S: drive. Refer to: SOP-FB-01: Physical Evidence Examination, SOP-FB-32: Swabbing Guidelines, and GL 4.

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

Note: The words submission and item are interchangeable. (i.e. An evidentiary sample may be called a submission in a forensic biology report but called an item in a DNA report).

1.1.11 DNA analyst can perform screening and confirmatory tests for which they are competent and proficient. Qualified DNA analysts will follow Forensic Biology protocols. These protocols are found on Qualtrax. Refer to the following:

- SOP-FB-01 Evidence Exam
- SOP-FB-02 SAEC Kit Exam
- SOP-FB-03 Flow Charts
- SOP-FB-04 Definitions-Abbreviations
- SOP-FB-05 Case Records and Reports
- SOP-FB-06 Whole Blood
- SOP-FB-07 Screening Tests for Blood
- SOP-FB-08 Confirmatory Test for Blood
- SOP-FB-09 Spp Double Diffusion (ouch)

SOP-FB-10 RIA's for Blood
SOP-FB-11 Screening Test for Semen
SOP-FB-12 Extraction of Samples for Semen
SOP-FB-13 Identification of Spermatozoa
SOP-FB-14 RIA's for Semen
SOP-FB-15 Test to Amylase (Phadebas)
SOP-FB-16 RIA for Urine
SOP-FB-17 Test for Urobilinogen

1.2 Preparation of Discovery Materials:

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

1.3 DNA Analysis Flow Chart:

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 DNA typing system is fluorescent STR analysis.

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

1.4 Report Writing:

DNA results are reported according to standard DESPP Division of Scientific Services guidelines and ASCLD/LAB and the FBI QAS. Please refer to General Laboratory Procedures: Case Reviews (GL: 18). Reports are signed by two individuals. The signature on the left is the analyst responsible for the case. The signature on the right is the technical reviewer of the case. Please refer to DNA SOP-6 and the DNA Report Template file for general report template information.

- 1.4.1 Final reports will include DNA typing results and a qualitative interpretative statement. A quantitative statement is given where appropriate. Please refer to DNA SOP-6 and the DNA Report Template file for general report templates.
- 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. If an allele table is present in the DNA report, profile results from both fractions are included. Conclusions are required in the DNA report. 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 competent and proficient in Forensic Biology

If the evidence was examined and forensic biology tests were performed by a DNA analyst competent and proficient in those testing methods, the results may be incorporated into a DNA report. Refer to FB-SOP-05 for Forensic Biology result statements.
- 1.4.4 All DNA Reports will clearly communicate all items tested, all eliminations (e.g., elimination of a victim or other known source) when comparisons are made, all profiles which were entered into CODIS or which were not suitable for CODIS entry.
- 1.4.5 Draft reports, while being worked and/or in the review process, will be located on the U drive in the case reports folder and the subfolder for the proper year of the case.
- 1.4.6 Draft reports that have not been issued, will not have a completed "date of report" filled out until report is issued.
- 1.4.7 Final Report

- 1.4.7.1 The Draft report is edited; the date of report filled out and saved as the Final report (without signatures).
- 1.4.7.2 The Final report 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 Final report with signatures will reside in the Case File in evidence receiving.
- 1.4.7.4 A copy of the Final report with signatures will reside in the case jacket.

1.5 General Considerations for PCR Amplification of Evidentiary Genomic DNA:

- 1.5.1 All amplification reactions for evidence samples and controls must be prepared in a PCR hood.
- 1.5.2 Gloves and lab coats must be worn at all times when working in PCR set-up.
- 1.5.3 All reagents and microfuge tubes must be opened with caution. At no time should open tubes of reagents be removed from the laminar flow hood.
- 1.5.4 For all mixing and pipetting pertaining to PCR set-up: only use designated pipettes from the PCR set-up hood. At no time should these pipettes be removed from the set-up area, except for calibration checks. Pipettes from other areas of the lab should not be brought into PCR set-up.
- 1.5.5 Aerosol blocking pipette tips should be used at all times when preparing PCR reactions.
- 1.5.6 At no time should any amplified product be brought into the PCR set-up area.
- 1.5.7 All PCR reactions must be prepared using designated "PCR set-up only" microfuge tube racks.
- 1.5.8 Prior to setting up any PCR amplifications, the working area (laminar flow hood) must be cleaned with 20% (in-house) bleach or 10% stabilized bleach followed by ethanol then subjected to UV irradiation for at least 15 minutes. See DNA WI-21 for 20% Bleach formulation.
- 1.5.9 A PCR master mix is prepared and dispensed to each tube prior to the addition of DNA.
- 1.5.10 After PCR reactions are set-up; the work area must be cleaned as in 1.5.8.

- 1.5.11 The standard quantity of template used for PCR amplifications is 0.5 ng – 2 ng (note that Y STR testing may require more than 2 ng of total DNA-male + female). Amplification results from template concentrations less than 0.5 ng/reaction will be interpreted with caution.
- 1.5.12 All casework analysis is conducted using the appropriate lot-tested reagents as defined in DNA SOP-6 Section 4 and DNA SOP-8. Reagents are lot tested before their use in casework. Reagents are not used for casework analysis or validations past their control date. Reagents may be used for research purposes past their control date where appropriate and are labelled as such.

1.6 Controls for DNA analysis:

Four controls are processed with each case:

- 1.6.1 Reagent Blank (RB). A reagent blank (RB) is processed with each set of extractions. 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. When processing non-differential samples and differential samples in the same set, the RB-A will also serve as the reagent blank for non-differential samples as well as for the epithelial-rich samples (organic extractions only). RB-B will be the reagent blank for the sperm-rich fraction of the differential samples.

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.

- 1.6.2 Lab Positive Control. A positive control (RKO) is processed for each set of extractions. The positive control is carried through all STR analysis steps. The RKO is also considered an amplification positive control that is used to determine if the PCR performed properly. The RKO 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 (dH₂O) possible for each kit is used for the amplification step.

- 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 RKO extraction tube will be labelled with a unique identifier. An example could be RB-date-initials. The unique identifier information for the control can be found on DNA QR-2 "DNA Extraction Worksheet" or any of the DNA extraction worksheets under the Sample # field in line with the appropriate Control. The unique identifier will be used for any extract tubes that will be stored in a long-term freezer boxes. Work product stored in 96-well plates (used for database samples) do not have the controls labelled as such, but do have a unique identifier to the plate itself, see DNA SOP-12.
- 1.6.6 Expected results of controls will be verified and documented on DNA QR-4, QR-4b, or QR4c which is maintained in the case file. For Positive Controls and Extraction Controls verified using the POS/RKO Concordance Check Excel spreadsheets for the specific test kit (DNA-QR-45A, C D, E, F), The corresponding QR worksheet is maintained in the batch paperwork. Instructions to use these worksheets are within the excel spreadsheet workbooks. When using a positive extraction control besides RKO (i.e. KJL or TMP), the control may be checked using other means.

1.7 Contamination:

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

See below for examples of contamination prevention methods.

The following are two 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 tubes containing genomic DNA.
2. Tube Opener method: Use a plastic tube opener to open tubes containing genomic DNA. Bleach tube openers between uses.

All Laboratory personnel must supply a known sample for elimination purposes prior to them working in the lab.

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 ≥ 50 rfu) will be documented and maintained by the Quality Section with access by the TL. The TL will be notified of all incidents of contamination. If a QAR is required, it will be approved by the TL. Please see DNA-SOP-5 Section 5.5.5.2 for more details.

All forensic unknown DNA profiles (profiles not unambiguously assigned to a known) are checked against the staff index using Searcher in CODIS (for STRs), or using the staff index spreadsheet (for Y-STRs) according to SOP-13.7.1. This staff search is documented on DNA-QR-20 and maintained in the case file.

If contamination is detected:

- 1.7.1 Discard any reagents that are contaminated.
 - 1.7.2 Thoroughly clean the PCR set-up area or the DNA extraction area (depending on which process was affected).
 - 1.7.3 On occasion, when objects/equipment are repeatedly identified as contaminated, after a thorough cleaning, set up blank reactions to verify that the contamination has been eliminated. Do not conduct DNA testing of case samples until the situation is rectified.
 - 1.7.4 Review the data and the analytical techniques of the analyst(s) in question and take remedial action as required.
- 1.8 Monitoring, cleaning, and decontaminating facilities and equipment:**
- 1.8.1 DNA testing facilities and equipment are monitored, cleaned, and decontaminated (when applicable) per FBI Quality Assurance Standards (Standard 6) and laboratory SOPs.
 - 1.8.2 For each set of amplifications, note the condition/results of the negative control, the reagent blank, and the positive controls in DNA QR-8.
 - 1.8.3 Document that laboratory cleaning procedures (section 1.5) were followed for each set of amplifications using worksheet DNA QR-8.

1.9 Case documentation:

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

1.10 Case review:

Definitions as per the 2011 FBI QAS:

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

Technical Reviewer: is an employee who is a current or previously qualified analyst in the methodology being reviewed and is not an author of the applicable report. 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)

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

1.10.1

All DNA cases/reports are technically (100%) and administratively (100%) reviewed by 2 laboratory employees in addition to the primary examiner. The technical review function is performed by the technical reviewer (2nd analyst) and documented on DNA QR-4, QR-4b, or QR-4c.

Approved by Director: Dr. Guy Vallaro

Administrative reviews are performed on all case files prior to reports being issued. In cases where no DNA profile interpretations are reported, administrative reviews are performed by any laboratory personnel who have been trained to review DNA cases and have access to LIMS. These reviews are used to assure that the case demographics are correct, there are not typographical errors, and the documentation is consistent with laboratory policies. In cases where DNA profile interpretations are reported, administrative reviews are performed by any qualified DNA analyst or DNA analyst trainee who has been approved to be an administrative reviewer. These reviews are used to assure that the case demographics are correct, there are not typographical errors, and the documentation is consistent with laboratory policies. In addition, the administrative reviewer will verify that the conclusions made in the report are supported through the documentation in the case file.

Note: All STR projects must be independently second analyzed by at least one qualified DNA examiner. No analyst may technically review their own work.

- 1.10.2 In the event of discrepant conclusions, the two examiners will discuss/review the results. Should a discrepancy persist, the data will be reviewed and conclusions approved by the Technical Leader (TL).
- 1.10.3 Where practicable, one case per analyst will be reviewed quarterly by the TL.

1.11 Corrective Action:

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

1.12 Laboratory Safety protocols:

Laboratory safety protocols are outlined in the Division of Scientific Services Quality Manual GL-2. ~~The TL is responsible for the oversight and approval of the safety program in the DNA section.~~ 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 1-20). The DNA **Unit Section** 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)

1.14 DNA Analyst Training and Continuing Education:

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

1.14.2 All DNA analysts are trained prior to assuming casework duties as outlined in the Quality Manual, the DNA Training Manual, Mitochondrial DNA Training Manual, and FBI QAS (Standard 5). Training may consist of continual training beyond the time schedule in the DNA Training Manual or it may consist of a period of training plus time spent under direct supervision.

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 section~~ and will document the approval by memo. The current DNA Training Manual and Mitochondrial DNA Training Manual is found in DNA SOP 7.

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

Competency Tests are samples that have either been prepared internally or received from an outside source. The Training Coordinator, or designee usually knows the results of the samples but the analyst conducting the test does not. Each analyst must successfully complete a competency test prior to being assigned casework. Competency memos authorizing an individual in a specific methodology or platform shall be approved by the TL by initialing and dating the memo.

- 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 two external proficiency tests semi-annually (twice a year) as outlined in the Quality Manual (GL-16) and FBI QAS (Standard 13). Select statistical calculations for each proficiency test will be performed by hand.

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

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 attend at least one continuing education unit (8hrs) each year as outlined in the FBI QAS Standard 5. This record will be approved by the TL and documented using DNA QR-31, kept in a binder by the Training Coordinator.

Analysts will maintain familiarity with the scientific literature of their field by reading and/or reviewing pertinent articles. In general, analysts should try to read 4-6 articles a year.

Records of this process will be reviewed and approved by the TL, in association with the Training Coordinator, and documented using DNA QR-30, kept in a binder by the Training Coordinator.

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

1.15 Method Validation:

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

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

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

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

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

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

Validation Summaries should capture what was tested and the results of the testing. The validation results will be reviewed and approved by the TL. In addition, all methods, work instructions, and

SOPs (for nuclear and mtDNA) are approved by the TL. Other procedures approved by the TL may be used on a case-by-case basis.

1.16 References: DNA **Unit Section:**

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

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

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

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

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

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ABI Quantifiler Duo DNA Quantification Kit, User's Manual, ABI, 2008.

ABI Quantifiler Kits, User's Manuals, ABI, 2006.

AmpF/STR Identifiler PCR Amplification Kit, User's Bulletin, ABI, 2001.

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

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

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

Biomek 2000 Electronic Manuals, Release 3.1a, Beckman Coulter, 1999.

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

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

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

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

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

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

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

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

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

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

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

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

Forensic Biology, DNA ~~Unit Section~~ Reference Library.

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

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

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

NDIS Procedures Manual, CODIS Home Page.

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

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

PowerPlex Y System, Technical Manual, Promega, 2007.

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

1.17 Equipment Maintenance and Calibration:

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

A "performance check" is done following the annual service contracted preventative maintenance or repair of an instrument to ensure the instrument is operating within normal parameters and gives the expected results. A "performance check" is completed 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. ~~Section.~~

1.18 Abbreviations:

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

1.19 Audits:

Internal audits will be conducted annually by the CT Forensic Lab DNA audit team using the current FBI QAS audit document ~~approximately 6 months following the external audit~~ in accordance with guidelines established in the Laboratory Quality Manual (GL-7). The DNA audit team (see QR-257A and B) will consist of at least one person that is, or has previously been, a qualified DNA analyst for each specific DNA technology performed, including mtDNA, and at least one person who is a qualified auditor. 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 indefinitely~~ by the DNA ~~Section~~ Quality Manager. The

internal audit review will be documented on DNA QR-257A and B.

In accordance with the FBI QAS standards, the DNA ~~Unit Section~~ 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. 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 and document the approval on DNA QR-258. This document will be kept indefinitely by the Quality Section.

1.20 Outsourcing:

The Connecticut Department of Emergency Services and Public Protection Division of Scientific Services (CTDSS) may contract or subcontract forensic DNA casework samples to a Vendor Laboratory (e.g. Bode, Cellmark). If samples are outsourced to a Vendor Laboratory, this Vendor Laboratory and CTDSS 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 SOP 21 and GL-1).

1.20.1 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:

1.20.1.1 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 will be printed out and completed by the analyst performing the visit. Any discrepancies or issues found will be documented and reviewed.

1.20.1.2 Instead of a CTDSS employee performing the initial onsite visit as stated in 1.20.1.1, the CTDSS Technical Leader may accept an on-site visit conducted by another NDIS participating

laboratory using the same technology, platform and typing amplification test kit, for the generation of the DNA data and shall document the review and approval of such an on-site visit. If the on-site visit is performed by another NDIS laboratory, the procedure of the laboratory performing the on-site visit will be followed and a copy maintained by the CTDSS.

- 1.20.2 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 1.20.1.1 or 1.20.1.2 may be followed. All reviews and approvals by the DNA TL will be documented.

1.21 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 much more detailed than a SOP and is only created if more detailed instructions are necessary. Work Instructions will be referenced to in specific SOPs or may be appended to the end of an SOP. Work Instructions will be treated as a controlled document.

1.22 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". Access to the Division of Scientific Services is controlled via a secure key card system.

1.23 Examination Record:

The starting date of DNA testing is documented on the DNA Extraction Worksheets. ~~Physical Evidence worksheet (DNA QR-1 or designated FB or DNA exam worksheet)~~. Testing is completed on the date of the report, following the technical and administrative review of the results and report conclusion(s), documented on DNA QR-4, QR-4b, or QR-4c.

APPENDIX I

Data Archived

All 3130 data files (.fsa) and GeneMapper projects (.ser) containing case data are periodically removed from the DNA network and archived on permanent storage disks. Other data may also be archived.

Security of Archived Data

Archived data is stored on non-rewriteable disks. At least 2 copies are made of each disk of archived data before the data is removed from the network. These are stored in separate locations, each of which may only be accessed by authorized personnel.

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), the range of dates substantially covered, and “Copy #1”.
8. Burn a second copy onto a second disk. Repeat steps 7 with the second disk, labeling it “Copy #2”.
9. Import at least 2 projects from each disk, preferably all generated by different 3130s and different analysts, into GeneMapper. 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. Make a copy of the printout of the disk contents. Store one printout with Copy #1 of the disk and the other printout with Copy #2. Currently Copy #1 is stored in the Technical Leader’s office and Copy #2 is stored in the DNA ~~Unit-Section~~ storage room (206).
12. Do one final check immediately before deleting to be sure that the Size and numbers of Files and Folders Contained in the “Archived [date]” folder on the network have not changed,

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then delete this folder. Do this by comparing to the copy of the disk that was not used in step 6. (If any of this information *has* changed, the folder has been altered since it was burned – resolve the discrepancy before deleting and be sure that what you are deleting is exactly what was burned to the disks.)

13. Any disk generated that contains an error and is therefore not being used for the final archive should be disposed of. It must be rendered unreadable first (ie. by breaking in pieces or shredding).
14. In addition to disk archival, all data residing on the U drive is archived on a weekly basis.

ARCHIVED

APPENDIX II

List of Abbreviations

1. a = allele
2. @ = at or about
3. -A = minus A peak
4. ABI = Applied Biosystems
5. AP = Acid Phosphatase
6. * Peak = peak ≥ 50 RFU & < 75 RFU (potential allele below threshold)
7. BLS = Blood-like stain
8. bp = base pair
9. Č = contained
10. CDMCS = Central district major crime squad
11. CIDI = case, item, date, initials
12. CO = Click Off
13. CODIS = Combined DNA Index System
14. da = dye artifact
15. dH₂O = de-ionized water
16. DOI = date of incident
17. DNA = deoxyribonucleic acid
18. DNR = Data Not Reported
19. DTT = Dithiothreitol
20. EDMCS = Eastern district major crime squad
21. EF = epithelial rich fraction
22. GMID = GeneMapper ID
23. HBAP = High Background Artifact Peak
24. HY = High Yield
25. ID = Identifiler
26. ID + = Identifiler Plus

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- 27. IDP = Identifiler Plus
- 28. IFC = Insufficient For Comparison
- 29. KM = Kastle-Meyer
- 30. LY = Low Yield
- 31. MF = Minifiler
- 32. MM = master mix
- 33. mt = mitochondrial
- 34. mito = mitochondrial
- 35. N = No
- 36. NE = No Edits
- 37. NEATT = not examined at this time
- 38. NEG = Negative amplification control
- 39. NR = No Results
- 40. nt = nucleotides
- 41. NTATT = not tested at this time
- 42. OCME = Office of the Chief Medical Examiner
- 43. OLa = Off Ladder allele
- 44. OL = Off Ladder
- 45. pd = Pull down peak
- 46. PD = Police Department
- 47. Pks = peaks
- 48. PHR = Peak Height Ratio
- 49. POI = person of interest
- 50. POS = Positive amplification control
- 51. PPY = PowerPlex Y
- 52. Prot K = Proteinase K
- 53. pu = pull up peak
- 54. Q samples = question samples

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- 55. QC samples = quality control samples
- 56. rb = raised baseline
- 57. RAL = retained at the laboratory
- 58. RB = extraction negative control
- 59. R/B = Reddish Brown
- 60. RBS = Reddish Brown Stain
- 61. Re-amp = re-amplification
- 62. re-inj. = re-inject
- 63. RET = red evidence tape
- 64. RKO = laboratory positive control
- 65. rxns = reactions
- 66. SA = sexual assault
- 67. SAK = sex assault kit
- 68. SAO = State's Attorney's Office
- 69. SF = sperm rich fraction
- 70. Sld. = sealed
- 71. sm. amt. = small amount
- 72. sp = spike
- 73. ss = single source
- 74. st = stutter peak
- 75. std = standard
- 76. sus = suspect
- 77. vic = victim
- 78. WDMCS = Western district major crime squad
- 79. Y = yes
- 80. YF = Y-Filer
- 81. D8 = D8S1179
- 82. D21 = D21S11

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- 83. D7 = D7S820
- 84. CSF = CSF1PO
- 85. D3 = D3S1358
- 86. TH0 = TH01
- 87. D13 = D13S317
- 88. D16 = D16S539
- 89. D2 = D2S1338
- 90. D19 = D19S433
- 91. D18 = D18S51
- 92. Amel = Amelogenin
- 93. D5 = D5S818

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