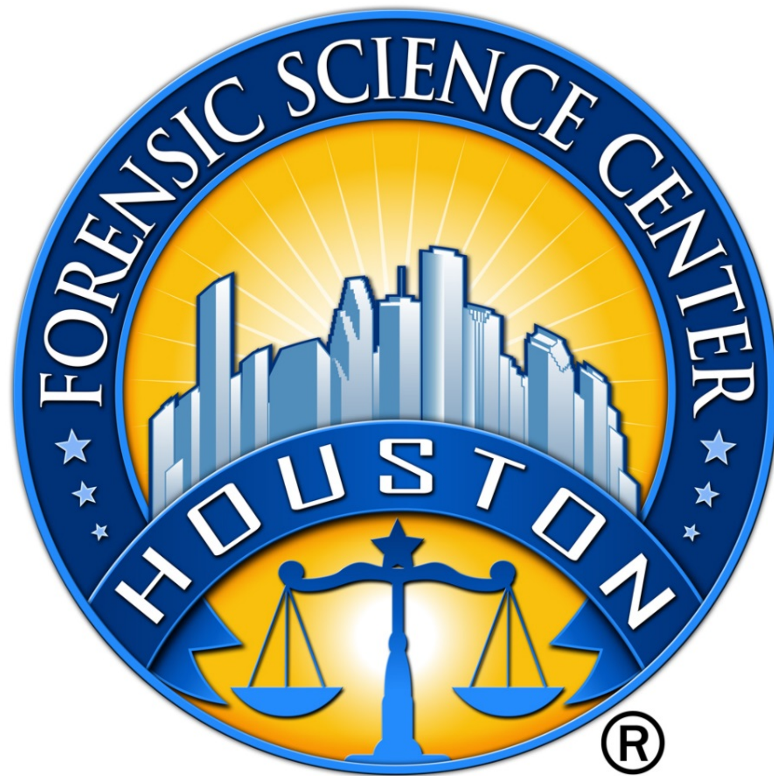

Biology/DNA

DNA General SOP

Biology/DNA Division

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1. Overview

1.1. The DNA Standard Operating Procedure (SOP) manual specifies procedures for DNA analyses of biological evidence for human identification. It is approved for use in the DNA section of the laboratory in conjunction with the Biology SOP. The purpose of this document is to ensure high quality DNA analysis while adhering to FBI Quality Assurance Standards (QAS) requirements and ISO/IEC 17025 requirements.

1.2. This document is a controlled document and only the current version of this SOP or other controlled documents, such as equipment and software manuals that are controlled, shall be used for DNA analysis. Obsolete versions of this document are archived.

1.3. It is not possible to anticipate every situation that may arise. Therefore, staff members must exercise good judgment based on experience. Any divergence from accepted protocol requires approval of the DNA Technical Leader and must be documented in the case record.

1.4. Biology staff members are required to follow the DNA and Biology SOP and all Houston Forensic Science Center (HFSC) policies and procedures. Other documents that must be followed include, but are not limited to, the Quality Manual, Health and Safety Manual, and DNA Training Manual.

1.5. **Technical Leader**

1.5.1. The Technical Leader is a full-time staff member of the laboratory and shall be accessible to the laboratory to provide on–site, telephone, or electronic consultation as needed.

1.5.2. **Minimum educational requirements:** The DNA Technical Leader shall have, at a minimum, a master's degree in a biology or forensic science-related area with successful completion of 12 semester or equivalent credit hours from a combination of undergraduate and graduate coursework covering the following subject areas: biochemistry, genetics, molecular biology, and statistics or population genetics.

1.5.2.1. The 12 semester or equivalent credit hours shall include at least one graduate-level course registering 3 or more semester or equivalent credit hours.

1.5.2.2. The specific subject areas listed above shall constitute an integral component of any coursework used to demonstrate compliance with QAS.

1.5.2.3. Individuals who have completed coursework with titles other than those noted above shall demonstrate compliance through provision of pertinent materials such as a transcript, syllabus, letter from the instructor, or other document that supports the course content.

1.5.3. **Minimum experience requirements:** The DNA Technical Leader shall have at least three years of forensic DNA laboratory experience obtained at a laboratory where forensic DNA testing was conducted for the identification and evaluation of biological evidence in criminal matters. Any newly appointed Technical Leader shall have a minimum of three

years of human DNA experience (current or previous) as a qualified analyst on forensic samples. The DNA Technical Leader shall have previously completed or must successfully complete the FBI-sponsored auditor training within one year of appointment.

1.5.4. **Responsibilities:** The Technical Leader is responsible for overseeing the technical operations of the laboratory. The Technical Leader has the authority to initiate, suspend, and resume DNA analytical operations of the laboratory or an individual. The Technical Leader works with Forensic Biology Manager, Quality Director, and other top management concerning operations of the laboratory. The specific responsibilities of the Technical Leader include:

- To evaluate and document approval of all validations and methods used by the laboratory and propose new or modified analytical procedures to be used by analysts
- To review the academic transcripts and training records for newly qualified analysts and approve their qualifications prior to independent casework analysis
- To approve the technical specifications for outsourcing agreements
- To review internal and external DNA audit documents and, if applicable, approve corrective action(s) and document such review
- To review, on an annual basis, the procedures of the laboratory and document such review
- To review and approve the training, quality assurance, and proficiency testing programs in the laboratory
- To review and approve of all DNA analyses outsource contracts with vendor laboratories before they are awarded. Approval of such a contract includes acceptance of ownership of the DNA data generated in analysis performed under that contract
- To review requests by contract staff members for employment by multiple NDIS participating and/or vendor laboratories, and if no potential conflict of interest exists, may approve such requests

1.5.4.1. In addition, newly appointed Technical Leaders shall be responsible for the documented review of the following:

- Validation studies and methodologies currently used by the laboratory
- Educational qualifications and training records of currently qualified analysts

1.5.5. **Technical Leader Contingency Plan:** In the event that the Technical Leader position is vacated, an Acting Technical Leader will be appointed by top management from current Forensic Biology staff members who meet the requirements outlined above. In the event that the Technical Leader position is vacated and there are no staff members who meet the requirements, the laboratory shall immediately contact the FBI and submit a contingency plan within 14 days to the FBI for its approval. Work in progress by the DNA section may be completed during this 14-day period, but new casework shall not be started until the plan is approved by the FBI.

1.6. **DNA Analyst**

1.6.1. DNA Analysts are HFSC staff members. The laboratory shall retain at least two full-time staff members who are qualified DNA analysts.

1.6.2. **Minimum educational requirements:** The analyst must meet the educational requirements stated in QAS. The Technical Leader shall document approval of compliance.

1.6.3. **Minimum experience requirements:** The analyst shall have six (6) months of forensic human DNA laboratory experience.

1.6.4. **Minimum training requirements:** DNA analyst training includes completion of the following prior to beginning independent casework:

- Analysis of a range of samples routinely encountered in forensic casework prior to independent work using DNA technology
- A competency test

1.6.4.1. Analysts enter into the proficiency test program within 6 months of being deemed competent on any portion of casework analysis.

1.6.4.2. Prior forensic human DNA laboratory experience and/or training may be accepted to fulfill training and experience requirements. However, a competency test must be successfully completed prior to beginning casework. Prior experience or training used to fulfill any portion of this requirement shall be documented and approved by the Technical Leader.

1.6.4.3. Because technical reviewers are also qualified DNA analysts, as outlined above, they satisfy QAS Standard 5.5, and all subcategories of QAS Standard 5.5, through the following:

- Technical reviewers are staff members of the laboratory
- Technical reviewers are currently qualified analysts in methodologies being reviewed
- Technical reviewers have successfully completed a competency test prior to participating in the technical review of DNA data
- Technical reviewers participate in an external proficiency testing program on the same technology, platform, and typing amplification test kit used to generate the DNA data being reviewed

1.7. **DNA Technician**

1.7.1. Staff members who have all academic qualifications for DNA analysts may serve as DNA technicians. Technicians perform specific portions of the DNA analysis procedure after they have been trained and deemed competent in the specific procedure, following Quality Manual and sectional training requirements.

1.7.2. Training in a specific DNA technique consists of completing only the section of the DNA analyst training program relevant to the specific procedure; however, all reading

assignments, practice samples, and competency testing requirements related to that specific procedure must be completed successfully and documented prior to performing the procedure on forensic casework samples. DNA technicians do not interpret DNA data, nor do they issue DNA analytical reports.

1.7.3. DNA technicians participate in proficiency testing to the extent that they perform laboratory analysis following proficiency testing requirements detailed in the Quality Manual.

2. Quality Assurance

2.1. The HFSC Quality Manual establishes requirements in order to maintain an environment of continuous improvement in the management system and in services provided by HFSC. These requirements are based on the ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories and the FBI DNA Quality Assurance Standards for DNA Testing Laboratories (QAS) which the DNA section must adhere to. In order to participate in the National DNA Index System (NDIS) the DNA section must comply with QAS standards, the FBI's *NDIS Standards for Acceptance of DNA Data*, and the FBI's prescribed NDIS procedures.

2.2. Quality Control

2.2.1. Staff members keep quality control records as required by the Quality Manual and DNA SOPs.

2.2.2. For the monitoring of analytical procedures throughout DNA analysis, the following controls and standards are used:

2.2.2.1. Extraction reagent blank

- An extraction reagent blank is an analytical control sample with each extraction set that contains all reagents used in that extraction process but no template DNA and is used to monitor contamination from extraction to final fragment analysis. This control is treated the same as, and parallel to, the forensic and or casework reference samples being analyzed.
- Must be extracted concurrently with its associated forensic samples
- Must be subjected to the same concentration conditions as required by its associated forensic sample(s) containing the least amount of DNA
- Must be amplified using the same primers and the same instrument model as its associated forensic samples
- Must be amplified using the same concentration conditions as required by the associated forensic sample(s) containing the least amount of DNA
- Must be amplified concurrently with its associated forensic sample(s) if it is not quantified prior to amplification
- Must be typed using the same instrument model
- Must be typed using the same injection conditions as required by its associated forensic sample(s) containing the least amount of DNA
- If multiple reagent blanks are set up within an extraction set, all reagent blanks must be quantified. At least one of those reagent blanks shall be amplified, if carrying on any of the specimens associated with the extraction set on to amplification. The reagent blank that demonstrates the greatest signal, if any, must be amplified.

2.2.2.2. A hair shaft negative control shall be analyzed for each hair root analyzed.

2.2.2.3. Quantification negative control

- A quantification negative control is an analytical control sample that is used to detect DNA contamination of the quantification reagents

- Must have an IPC value and less than 5 x 10-3 ng/µl of detectable DNA present

2.2.2.4. Amplification controls

2.2.2.4.1 Amplification positive controls

- An amplification positive control is an analytical control sample that is used to determine if the PCR was successful
- Must be amplified and typed concurrently in the same instrument with the associated forensic samples, at all loci using the same primers

2.2.2.4.2. Amplification negative controls

- An amplification negative control is an analytical control sample that is used to detect DNA contamination of the amplification reagents
- Must be amplified and typed concurrently in the same instrument with the associated forensic samples, at all loci using the same primers
- Must be subjected to the same injection conditions as required by its associated forensic sample(s) containing the least amount of DNA

2.2.2.5 Allelic ladders and internal size markers

2.3 Critical reagents

2.3.1. Critical reagents are those that require testing prior to use on evidentiary samples in order to prevent unnecessary loss of sample. These are itemized in Critical Reagents Section (Section 5) and are quality control tested using the appropriate forms.

2.3.2. The critical reagent quality control logs shall contain documentation associated with the quality control testing of critical reagents. The quality control documentation shall show reagent name(s), lot number(s), expiration date(s), quality control test instructions, evaluation criteria, and results. Critical reagents may not be used on casework without acceptable quality control testing results.

2.3.3. Reagents and supplies that have passed their expiration dates may not be used on casework samples. Outdated reagents may be used for training purposes only and must be clearly marked as training samples.

2.4 Validation

2.4.1 The DNA section does not generally develop novel methodology but, if it does, the methodology will undergo full developmental validation in compliance with all relevant standards prior to use in casework. Methodology that has undergone developmental validation elsewhere shall be internally validated in compliance with all relevant standards prior to use in casework. Substantial changes in an existing protocol shall be subjected to an appropriate internal validation, comparing it to the original procedure to demonstrate, at a minimum, no loss of reliability, reproducibility, precision, or sensitivity.

2.4.2 No new or modified method, i.e., any method not already described in the SOP, is to be used without the documented approval of the Section Manager, the Technical Leader, and Quality Director.

2.4.3 New software, or software with significant modifications, must be validated prior to its use in casework.

2.5 Analysis Training

2.5.1 Please refer to the Biology/DNA Training Manual.

2.5.2 Continuing education training

2.5.2.1 Continuing education shall be conducted for enhancement of staff members' skills. As part of their continuing education, each staff member approved to perform DNA analysis shall read at least one current scientific article per quarter and document the completion of this assignment via Qualtrax. Section management maintains records of articles that are discussed at section meetings. In addition, the Technical Leader, CODIS Administrator, and DNA analysts shall complete a minimum of eight hours of continuing education annually.

2.5.2.2 The Technical Leader and the Quality Director may identify areas for which remedial training is necessary based on the results of proficiency or competency test results, laboratory audits, or peer review activities.

2.6 Review of casework

2.6.1 All case records and laboratory reports must be administratively and technically reviewed prior to release from the laboratory. Reviews must follow the appropriate checklist and a copy of the completed checklist must be included in each case record.

2.6.2 Technical/administrative reviews shall be conducted by a second DNA analyst qualified (or previously qualified) and proficient in the DNA platform currently in use in the DNA section. The technical reviewer cannot be the author of any examination documentation within the case record being technically reviewed. All reviews of DNA case folders shall include a review of the following:

2.6.2.1 All case notes, worksheets, and electropherograms

2.6.2.2 Raw data for the samples being used for interpretation (all electropherogram plots in the case record must match the electropherogram plots generated during the reviewer's second read; the reviewer shall indicate agreement with the second read by initialing and dating the GeneMapper project table for each run reviewed in the case folder)

2.6.2.3 DNA types to verify they are supported by the raw or analyzed data. This includes allele calls, edits, and any manual calculations performed by the initial analyst

2.6.2.4 Profiles to verify correct inclusions and exclusions as well as the appropriate use of "inconclusive"

2.6.2.5 All controls, internal lane standards, and allelic ladders to verify that the expected results were obtained

2.6.2.6 Statistical analysis, if applicable

2.6.2.7 The final report to verify that the results/conclusions are supported by the data as well as to verify that each tested item is addressed

2.6.2.8 CODIS profiles are to be reviewed for:

- Eligibility
- Correct type
- Correct specimen category

2.6.3 Technical review of cases shall be completed by a qualified analyst in the methodologies used in the case, as defined by the QAS. A DNA analyst can be competent to perform a methodology, to review a methodology, or to do both. DNA analysts who have obtained performance competency through the testing of samples shall be considered review competent and performance competent. DNA analysts may obtain review competence through training as directed by the DNA Technical Leader. This may be obtained through previous experience. Review competence allows a DNA analyst to review the methodology, but they shall not perform the methodology until performance competence is obtained.

2.6.4 Technical/administrative/quality reviews must include, at a minimum, a review of the following:

- The final report for clerical errors
- The final report for the presence and accuracy of the following elements:
    - Case identifier
    - Description of the evidence examined
    - Description of the technology used
    - Inclusion of loci used for analysis
    - Results and/or conclusions
    - A qualitative or quantitative interpretive statement
    - Date issued
    - Disposition of evidence
    - Signature and title of the person accepting responsibility for the content of the report
- Chain of custody
- Disposition of evidence

2.6.5 All changes, including any additions such as notes, made to the case file after the analyst submits the case file for technical review must be tracked on the case file checklist.

2.6.6 All cross-outs made or notes and checkmarks added to the case file after the analyst submits the case file for technical review must be initialed and dated.

2.6.7 No documents shall be removed from the case file after the analyst submits the case file for technical review. Any newly included or generated documents (eg, different control electropherograms or statistics calculations) shall supplement the case file and not replace the original records. Any records rendered obsolete after the case file is submitted for technical review shall be crossed out, initialed, and dated.

2.6.8 Refer to the Quality Manual for further guidance on Technical and Administrative Reviews.

2.6.9 The analyst and technical reviewer should typically be able to come to agreement on all issues of substance in the laboratory report after sufficient discussion. Disagreements of substance shall be brought to the attention of a Forensic Biology Supervisor or Technical Leader whether or not they are resolved at the analyst level and will be recorded in the case record. If disagreements of substance between the analysts and a reviewer cannot be resolved, the Technical Leader shall make the final determination. Should the analyst not agree with the Technical Leader, the Quality Director will be consulted.

2.7 Proficiency testing

2.7.1 Proficiency testing and review shall follow the requirements of the Quality Manual. In addition, the Quality Division shall maintain a copy of the analysis documentation for each proficiency test. Proficiency tests shall be analyzed and interpreted according to standard operating procedures including technical review. Proficiency test participants shall be notified of their final test results.

2.7.2 Analysts shall enter into a proficiency test program within 6 months of being deemed competent on any portion of casework analysis. Proficiency testing shall include each technology to the full extent to which analysts and technicians participate in casework. It is required that if both manual and automated methods are used, the analyst must be proficiency tested in each at least once per year. It is required that each technology (STRs, Y-STRs) be proficiency tested semi-annually.

2.7.3 Proficiency tests should be treated like casework. In doing so, DNA results reported to CTS (or other approved external proficiency test provider) should not vary from DNA results included within the case record, as established by the DNA SOPs. For example, notations to distinguish major and minor components in a mixture shall be included in results submitted to the test provider, if applicable. If symbols are used in the reporting of data to the proficiency testing agency, they must be defined in the results submission form. It is also advisable to include comments in the comments section of the proficiency results form to explain unusual results.

2.7.4 Only one proficiency results form shall be completed for proficiency tests to ensure that only the most complete information is submitted to the proficiency testing provider in

order to be included in the provider's published external summary report when submitting by fax or mail. The screening analyst shall complete the proficiency test results form if the test does not proceed to DNA analysis; the DNA analyst shall complete the proficiency test results form if the test does proceed to DNA analysis.

2.7.5 During the case record reviews, the proficiency results form (including the screening data, DNA data, DNA interpretations, and comments sections), along with the case record, shall be reviewed by both the technical/administrative and quality reviewers to ensure proper transcription of results by the author of the results form.

2.7.6 If the only extraction method for which an analyst is competent in is the differential extraction method, he/she must perform this extraction method in both proficiency tests in a given year, regardless of whether or not one of the evidence samples is found to be semen-containing. This permits him/her to be proficiency-tested in the differential extraction method semi-annually. If the analyst is competent in other extraction methods, he/she may perform the appropriate extraction method, given the screening results of the proficiency samples, as long as at least one differential is performed in a given year.

2.8 Audits

2.8.1 The Quality Director shall plan, arrange, and direct audits according to ISO/IEC 17025 and QAS requirements. This audit shall be completed once each calendar year, with the intervals between FBI Quality Assurance Audits being no less than 6 months and no more than 18 months. The auditor(s) shall use both the ISO/IEC 17025 and FBI Quality Assurance Audit Documents as a checklist for compliance.

2.9 Personnel Records

2.9.1 The Quality Division maintains transcripts, approval memos, proficiency records, continuing education records, and testimony monitoring records for each staff member. The laboratory must maintain the competency notebook, original or copies of training records, and proficiency test records for each staff member.

2.10 Chemical and reagent labels

2.10.1 Purchased chemicals and reagents shall be marked on the container with the date received and/or date opened. An expiration date shall be placed on the outer container. In general, the manufacturer's labeling should be followed to determine expiration dates of purchased chemicals and reagents. If no manufacturer information exists for a purchased reagent, it shall be considered expired 5 years from the received date.

2.10.2 The labels of reagents prepared in-house shall include the reagent name, lot number (consisting of preparation date and preparer's initials), and date of expiration. The expiration date of the overall reagent shall be no later than the expiration date of the

individual reagent with the nearest expiration date, unless otherwise indicated. Additional information may be documented in a reagent log.

2.10.3 Reagents that are subjected to quality control testing prior to use must also be marked with the “QC date”.

2.10.4 Analysts are responsible for checking expiration dates and that reagents have been appropriately quality control tested prior to use in casework.

2.11 Equipment

2.11.1 Equipment operation manuals shall be readily available to each staff member approved to use the equipment. Calibration, maintenance, and repair activities shall be recorded in an equipment calibration and maintenance log or in a logbook dedicated to that specific piece of equipment. The equipment calibration and maintenance log shall include at a minimum the date, activity, laboratory personnel performing or overseeing the activity, non-HFSC technician(s) performing or overseeing the activity, and a record of quality control checks performed to verify operation prior to returning a piece of equipment to casework use. See the DNA Maintenance SOP for additional information.

2.12 Contamination documentation

2.12.1 Contamination events, resolved and unresolved, shall be summarized as stated below. Document the details of the contamination event, including the cases involved, the date of detection, the investigative actions taken, the source of the contamination if known, and any corrective actions taken. See 3.3 for additional information on contamination.

2.13 HFSC DNA Profile Policy

2.13.1 Refer to the HFSC DNA Profile Policy.

2.13.2 The DNA of each Biology section staff member is typed. The DNA profile for other staff and visitors may also be typed to allow for the detection of possible contamination. All DNA profiles are stored by the laboratory. For additional information, refer to the Elimination Database Procedure SOP.

2.13.3 Samples collected for testing as part of the HFSC DNA Profile Policy should be clearly labeled with a first and last name, and an organization, should the sample be from a non-HFSC staff member.

2.13.4 Samples should be stored in individual packaging that is sufficient to prevent loss, deterioration, and contamination.

2.13.5 Samples should be stored in the area designated for samples collected as part of the HFSC DNA Profile Policy until testing begins.

2.13.6 At the completion of testing, samples collected as part of the HFSC DNA Profile Policy will be disposed of in biohazard waste receptacles.

2.13.7 DNA profiles generated from these samples will be housed in a database that can be used for comparison purposes only.

2.14 Quality Check of the DNA analysis system through NIST-SRM

2.14.1 A NIST-SRM or a NIST-traceable stain must be extracted, quantified, amplified, and analyzed one time per year, or whenever substantial changes are made to a procedure, to verify the DNA section's system of analysis as required in the QAS. This sample must type correctly by comparison with the known DNA profile. If the correct profile is not obtained from the NIST-SRM or NIST-traceable stain, the DNA Technical Leader shall be informed and the analyst shall work backwards to determine where any deficiencies in the system exist. If a deficiency in the system is identified, then analysis of casework samples in the DNA section shall cease until the NIST-SRM or NIST-traceable stain can be typed correctly.

2.15 References

2.15.1 SWGDAM Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories, approved January 12, 2017.

3. Facilities

3.1. Laboratory facility and security requirements are described in the Quality Manual and HFSC Security Manual. In addition to procedures and policies in the Quality Manual, the following policies apply to the DNA section.

3.2. Work Areas

3.2.1. The DNA section shall have designated space for the following activities. These activities must occur only in these designated areas:

- **DNA extraction** - DNA extraction, purification, and concentration; microscopy may also be performed in this area
- **PCR setup** - setup of real-time PCR quantification and PCR amplification reactions. A laminar flow hood or PCR setup hood dedicated to amplification setup is recommended when manual set-ups are performed
- **Amplified DNA product** - generation, analysis, and storage of amplified DNA product

3.2.2. The extraction of known samples shall be performed at a separate time or location from the extraction of evidentiary samples to prevent known to unknown sample contamination. Decontamination of work areas shall be performed between set-up of the evidentiary samples and set-up of the known samples.

3.2.3. The DNA extraction area and PCR setup area shall be separate from each other. This is accomplished by maintaining separate physical spaces for each task or by conducting these tasks at separate times. If conducted in the same space at separate times, the space shall be decontaminated between tasks.

3.2.4. The amplified DNA product area shall be physically separate from all other areas. Doors to the amplified product area shall remain closed at all times, except for passage. Once amplified, no samples shall leave the amplified DNA product area unless securely packaged. Equipment, reagents, and supplies in the amplified product area are dedicated and shall not be removed unless properly decontaminated through treatment with UV or thorough wiping with a decontaminant.

3.3. Contamination

3.3.1. Contamination is the unintentional introduction of exogenous DNA into a DNA sample or PCR reaction. As human DNA is pervasive throughout the environment, contamination may not be completely avoided. In addition, the improved sensitivity of DNA methodologies and the introduction of new DNA technologies may allow low-level or previously undetected contamination to be detected and potentially cause DNA interpretational difficulties.

3.3.1.1. Contamination can be in negative controls, such as reagent blanks and amplification negative controls, as well as samples. For example, samples that are expected to be

single source may indicate a contamination event by producing a DNA profile from more than one contributor.

3.3.1.2. Sources of contamination include:

3.3.1.2.1. DNA from laboratory personnel to an evidentiary item or DNA sample

3.3.1.2.2. DNA from contaminated reagents or consumables to an evidentiary item or DNA sample

3.3.1.2.3. Laboratory environment, such as surfaces, equipment, ventilation system, to an evidentiary item or DNA sample

3.3.1.2.4. Cross contamination of an evidentiary item or DNA sample to another evidentiary item or DNA sample

3.3.1.3. Contamination can occur directly or indirectly:

3.3.1.3.1. Direct contamination involves the transfer of DNA from the source of the contamination to the evidentiary item or DNA sample. This may occur when laboratory personnel handle an evidentiary item or DNA sample but may also occur without direct physical contact, such as speaking, sneezing or coughing on an evidentiary item or DNA sample.

3.3.1.3.2. Indirect contamination (i.e., secondary transfer) is a result of the transfer of DNA from the source of contamination to the evidentiary item or DNA sample through an intermediary such as gloves, tools, pens, packaging and laboratory surfaces.

3.3.2. Prevention and Decontamination

3.3.2.1. The following policies are designed to prevent contamination of DNA samples:

3.3.2.1.1. To minimize the potential for contamination from staff and/or visitors, unnecessary traffic into each of the work areas shall be avoided.

3.3.2.1.2. Use 10% bleach or DNA Away as a decontaminant. Other commercially available decontaminants may be used if they are shown to completely inactivate DNA for the purposes of amplification. Autoclaving is acceptable for decontamination. Selection of either bleach or DNA Away shall take into consideration the potential caustic effects of the surface being decontaminated. For example, DNA Away should be used to clean rotors of centrifuges and surfaces of the Tecan robots. Bleach is appropriate for decontaminating writing utensils and bench tops. DNA Away and ethanol should be used to clean the EZ1 and Tecan instruments.

3.3.2.1.3. In general, clean glassware after each use wearing gloves and an appropriate soap (e.g., Liquinox or Alconox) and water. Rinse with deionized or distilled water and allow to air-dry inverted. DNA reagent bottles require sterilization after cleaning. Autoclave or rinse with sterile water prior to use. For glassware in the amplified product area, rinse thoroughly with water after each use, with a final rinse of distilled or deionized water, and invert to air-dry.

3.3.2.1.4. To help prevent contamination:

3.3.2.1.4.1. Wear appropriate personal protective equipment (PPE) including disposable gloves, face masks (to cover mouth and nose), disposable lab coats, and hair coverings during all testing and reagent preparation (face masks and hair coverings are optional in post-amplification). If examining heavily soiled evidentiary items, coats shall be changed immediately after examination and before continuing to examine other evidentiary items or cases. Soiled coats shall be disposed of. Refer to the HFSC Health and Safety Manual for additional safety-related PPE requirements. Safety eye wear must be used when handling biological material that may pose the threat of splashing, such as liquid blood.

3.3.2.1.4.1.1. Change gloves frequently and whenever gloves may have become contaminated. Gloves shall be changed between examinations of different evidentiary items. Gloves may be wiped with bleach or DNA Away after donning. Discard gloves when leaving a work area, except when transporting samples or reagents.

3.3.2.1.4.1.2. To reduce transfer from outer package(s) to the evidentiary items, gloves shall be changed after handling or opening the outer package and prior to proceeding with examination.

3.3.2.1.4.2. Non-disposable tools (e.g., scissors, forceps, etc.) that come into contact with packaging shall be cleaned before and after use. The same tool shall not be in contact with evidence before cleaning. Whenever possible, use disposable tools and discard after single use.

3.3.2.1.4.3. Centrifuge all liquid to the bottom of closed microcentrifuge tubes before opening. A de-capper or a clean Kimwipe may be used for opening microcentrifuge tubes.

3.3.2.1.4.4. Use sterile, disposable pipet tips and microcentrifuge tubes. Use aerosol-resistant pipet tips while working with any sample that may be subsequently amplified. Change pipet tips between samples.

3.3.2.1.4.5. Set up reagents and tools in work space in such a way that used tips do not cross over or near stock reagents or clean tubes/wells.

3.3.2.1.4.6. In all pre-PCR areas, clean work surfaces thoroughly with decontaminant at least at the beginning and the end of each DNA extraction, quantification set-up or PCR set-up session.

3.3.2.1.4.7. Outer packaging and reagent bottles shall be wiped down with decontaminating agent/bleach before opening at one’s bench.

3.3.2.1.4.8. Limit talking during sample handling.

3.3.2.1.4.9. Use good lab practices to prevent cross-contamination between samples, from analyst to samples, from samples to analyst, and from the environment to samples.

3.3.2.1.4.10. In the PCR setup area, add DNA template last to the PCR setup tubes to minimize inadvertent transfer between setup tubes and stock reagents. Limit talking during sample handling.

3.3.2.1.4.11. In the amplified DNA product area, wear a dedicated disposable lab coat when handling amplified samples. Do not wear the lab coat or gloves outside the amplified DNA product area. These lab coats shall be disposed of when necessary. Clean work surfaces thoroughly with decontaminant after use.

3.3.2.1.4.12. Signs may be posted to designate appropriate personal protective equipment (PPE) in certain areas. PPE shall be worn in these areas as indicated.

3.3.3. Detecting and Responding to Possible Contamination Events

3.3.3.1. Unacceptable activity in a reagent blank or negative control that cannot be readily attributed to an artifact, or unexpected activity in sample, such as a mixture on a sample expected to be single-source, or male DNA on a sample expected to be female, must be investigated to determine if it is reproducible contamination. Unacceptable activity includes a pattern of data that can be differentiated from background. A single activity point may not be evidence of contamination. Forensic Biology Supervisors and the Technical Leader have discretion in determining if a single point is acceptable or if it requires further processing.

3.3.3.2. Investigation and corrective action shall be guided by the nature of the specific event and may include the following:

- Compare the unknown profile to the staff/visitors database
- Compare the unknown profile to profiles from samples worked with the contaminated sample
- Work backwards to determine where the contamination occurred:
  - Re-prepare the amplified product (addition of formamide and internal lane size standard) and re-inject
  - Re-amplify and analyze the DNA extract
  - Re-extract and analyze the sample (if this may be done without consuming the sample)
- Extract, amplify, and/or inject known samples (to test suspected reagents and/or equipment)
- Discard suspected buffers and prepared reagents and clean reagent bottles
- Clean and decontaminate work areas, glassware, pipets, etc.

Generally, the first course of action is to re-inject the sample on the genetic analyzer to determine if the activity is in the amplified DNA product or if it was perhaps introduced during post-amplification sample set-up. To rule out the introduction of exogenous DNA at the set-up of the capillary electrophoresis plate, the sample shall be re-plated to determine if the activity is reproduced upon re-injection. If not reproduced upon re-injection, the data from samples associated with the reagent blank or amplification negative control may be used for interpretation.

If reproduced upon re-injection, the sample or negative control is then re-amplified to determine if the activity is in the DNA extract or if it was introduced during the amplification set-up. If the activity is in the amplification negative control and reproduced upon re-injection, the samples associated with the amplification negative control must be re-amplified. If activity in a reagent blank is not reproduced upon re-amplification, the data from samples associated with the reagent blank may be used for interpretation. If reproduced upon re-amplification, the DNA activity is determined to be in the DNA extract and all samples associated with the contaminated reagent blank must be re-extracted because the data from samples associated with that reagent blank may not be used for interpretation due to unacceptable quality controls.

When data cannot be used for interpretation due to confirmed contamination in the sample or an associated control, the contamination event must be reported.

Report language examples:

Contaminated Regent Blank:
"The reagent blank associated with these items was contaminated; therefore, it did not meet quality assurance standards. No results will be reported for these items. Retesting will require the consumption of these items, which will not be conducted until a court-approved consumption order is received."

Contaminated Sample:
"A mixture of DNA from at least two contributors, at least one of whom is male, was obtained from this item. Major and minor components were identified. The major component is consistent with the CSU Officer who collected the evidence. Suspect #1 cannot be excluded from the minor component."

Activity that is resolved with re-injections and re-amplification shall be tracked with a contamination log. Tracking is intended to detect trends that may need further review. Activity that is reproduced upon re-amplification shall be tracked with an HFSC Incident Report form. The detection of trends or an abundance of incidents may warrant a root cause analysis that would be documented with a Corrective Action Form.

It is recommended that any steps taken to investigate potential contamination are performed by a second technician to enable a transparent exploration.

3.4. Safety

3.4.1. There are biological and chemical hazards in the laboratory. Each staff member is responsible for familiarity with the HFSC Safety Manual. Any incident or condition that occurs in or under the control of the laboratory that threatens the immediate or future health of any individual must be immediately brought to the attention of the section

supervisor and Laboratory Safety Specialist. The Safety Specialist will define corrective action related to health and safety issues.

4. Casework Processing

4.1. Analysis Methods

4.1.1 The DNA section conducts short tandem repeat (STR) analysis. The GlobalFiler™ PCR Amplification Kit is a STR multiplex assay that amplifies 21 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, and D2S1338), one Y-STR (DYS391), one insertion/deletion polymorphic marker on the Y chromosome (Y indel), and the sex determining marker (Amelogenin) in a single reaction. The section may also conduct Y-STR analysis. The Yfiler® Plus PCR Amplification Kit is a STR multiplex assay that amplifies 27 Y-STR loci (DYS576, DYS389I/II, DYS635, DYS627, DYS460, DYS458, DYS19, YGATAH4, DYS448, DYS391, DYS456, DYS390, DYS438, DYS392, DYS518, DYS570, DYS437, DYS385a/b, DYS449, DYS393, DYS439, DYS481, DYF387S1a/b, and DYS533) in a single PCR reaction.

4.1.2 The amount of human DNA must be quantified prior to nuclear DNA amplification. However, when a reference sample is re-extracted for extraction/exclusion confirmation purposes it is not necessary to also re-quantify the sample. Quantification data from the initial extraction may be used for amplification since the resulting profile is not used for interpretation.

4.1.3 Samples suspected of containing semen shall be processed using a differential extraction method.

4.1.4 Unknown or suspect profiles developed from evidence are routinely databased in CODIS for searching against other evidentiary profiles and convicted offender profiles at the state and national levels.

4.1.5 Comparisons that yield a probative match between known and questioned items are evaluated to estimate statistical significance (see the DNA Interpretation SOP).

4.2 Case Acceptance and Evaluation

4.2.1 Before a case is accepted for analysis into the DNA section, the case shall be evaluated. The staff member completing the evaluation should be thoroughly aware of the requested examinations, the reason(s) for the requested analyses, the potential probative value of the evidence, and the quality and quantity of the evidence. Because each case is different, only guidelines can be prescribed. The case evaluation may include consultation with the investigator/prosecutor as necessary to determine what evidentiary items should be analyzed. Fully document conversations related to case evaluation and ask the customer to change analysis requests, as appropriate. A submitting agency offense report may be helpful in assessing the evidentiary material.

4.2.2 If the necessary equipment or expertise is not available to comply with a valid, pertinent request, the submitting officer must be advised. If another non-HFSC laboratory is capable of performing the requested analysis, consider coordinating portions of the analysis or referring the investigator/prosecutor directly to the other laboratory.

4.2.3 Both suspect and non-suspect cases are accepted. The section supervisor may evaluate unusual submissions for acceptance on an individual basis.

4.2.4 Unused evidence should be returned to the submitting agency once analysis is complete.

4.3 Evidence Evaluation

4.3.1 **Evidence** is defined as any original item submitted to the laboratory for analysis, related to a specific incident, and/or any cutting or swabbing taken from that item.

4.3.2 **Work product** is defined as any derivative item obtained as a result of the analysis of evidence including but not limited to:

- Microscopy slides
- DNA extracts
- PCR amplification products

4.3.3 Before the case is worked, and in an effort to support an efficient laboratory, an evaluation should be made to determine the quality and quantity of the evidence that is going to be analyzed initially. Emphasis should be placed on items of significant evidentiary value. Additional items/stains may be analyzed at a later date depending on case development and initial DNA analysis results. Decisions have to be made concerning the analytical approach that must be taken to obtain the most useful information. It is often helpful to consult with another qualified analyst, the Technical Leader, and/or the supervisor. Cases must be evaluated to:

- Eliminate the loss of potentially valuable information.
- Maximize the meaningful information obtained from the evidence.
- Determine if the requested examinations can be performed with the submitted evidence and with the available resources.

4.3.4 Some of the considerations in evaluating the evidence and deciding which items should be analyzed for DNA include:

- The age of the evidence, especially when the evidence is biological material.
- The storage conditions of the samples prior to submission.
- Whether wet samples were dried before submission.
- Whether the evidence is moldy and/or putrefied.
- Possible dilution of the samples.
- Whether weapons or other objects require fingerprinting or have been fingerprinted.
- Whether all pertinent evidence has been submitted.
- The availability of suspect, complainant, and/or elimination reference sample.

- The analyses that should be run if sample is limited.
- Possibility of sample remaining after analysis.
- Possibility of cross-contamination.

4.4 Evidence Handling

4.4.1 Please refer to the Quality Manual for the Handling of Evidence (5.8).

4.4.2 Storage of Evidence

4.4.2.1 Biological evidence must be properly stored by the laboratory to preserve biochemicals assayed in body fluid identifications and DNA typing for current and future analyses. Storage conditions for all types of evidence, including both evidence and work product, must be considered so that none are compromised through sample loss or deleterious change. It is not necessary to track the movement of work product while in process using a chain of custody. However, long term storage for DNA extracts, which is defined as post-processing storage, will be maintained using a chain of custody. DNA extracts shall be stored so that additional or future technologies may be employed.

4.4.2.2 During the initial analysis of the case, DNA extracts may be stored refrigerated. After a report has been issued, DNA extracts should be relocated to a freezer for long-term storage. Repeated freezing and thawing of extracts should be minimized. DNA extract tubes must be clearly labeled with the case number and item number and sealed with parafilm prior to long-term storage. DNA extracts may be stored individually with remaining evidence from that case or in “batches” that contain several items from multiple cases that went through the analysis process simultaneously. When stored as “batches”, the storage container must be clearly labeled with a unique batch number. Examination documentation must indicate to which “batch” a sample belongs so that a DNA extract may be easily located at a later time. Documentation of which box(es) the extract(s) are stored in long-term shall be on the extraction worksheet(s).

4.4.2.3 It is not necessary to maintain or store amplified product, amplification controls, or dilutions of DNA extracts.

4.4.3 Consumption of Evidence

4.4.3.1 The evidence quality and quantity should be preserved as much as possible without sacrificing the quality of the analyses. Whenever possible, at least half of the evidence sample shall be preserved for possible re-analysis. When this is not possible, consumption of the evidence may be necessary. Refer to the Quality Manual for policy and procedures on consumption of evidence (5.10.1).

4.4.4 Documentation

4.4.4.1 Refer to the Quality Manual for chain-of-custody policies and procedures, documentation of chain-of-custody, and documentation required in all case records (4.13.2, 5.8).

4.4.4.2 Documentation must be in such a form that another qualified analyst or supervisor, in the absence of the primary analyst, would be able to evaluate what was done and interpret the data. The reviewer of the case must be able to determine from the notes that sufficient testing, relevant testing, and correct methods of testing were used. To this end, all documentation of procedures, standards and controls used, observations made, results of tests performed, charts, graphs, photographs, sketches, electropherograms, etc. that are used to support the analyst's conclusions must be preserved as a record. Observations, data, and calculations shall be recorded at the time they are made. The date of an in-house photograph shall be included on the photograph or associated examination documentation. Examination records shall be of a permanent nature. If a written examination record is created (or if original observations are made) on non-traditional media (for example: sticky notes, paper towels, gloves, etc.), then either the original media or an electronic scan/picture of the original media shall be retained in the case record. Once an electronic scan/picture is created, the original hardcopy may be destroyed. Examination documentation shall reflect the name and/or initials of the individual who performed the work.

4.4.4.3 Appropriately completed SOP worksheets shall be used during the analyses. Examination documents should have notes that help in the identification of the item of evidence. A written description may suffice for some items, whereas others may need a drawing, sketch, or photograph.

4.4.4.4 If an item is submitted for immediate analysis, such as a mobile phone to be swabbed for possible contact DNA and immediately returned to the submitting party, it is not necessary for the evidence to be sealed and it may not be possible to establish a proper seal and/or to be marked with a unique identifier. However, case record documentation must include identifying information that permits a later identification of the item handled. For example, in the absence of analyst markings on the actual item of evidence, a photograph of the mobile phone's serial number, along with a photo of the mobile phone, enables later identification of the actual item handled.

4.4.4.5 Items collected at autopsy do not always include the name of the complainant, as it may be unknown at the time of the autopsy. If morgue evidence is received without the name of the complainant, the evidence should be described using the Medico-Legal number (ML#) and "unknown". Alternatively, if written notice is provided by the investigator, the complainant name provided by the investigator may be used in the evidence descriptions, in conjunction with the ML#. This written notice must be maintained in the case record and may be referred to in the report.

4.4.4.6 Case records must include documentation of long-term storage of DNA extracts and reagent blanks (i.e., storage after the completion of analysis) as well as certain quality control documentation including, but not necessarily limited to:

- a copy of the standard curve quality parameters used
- a copy of amplification results of any reagent blanks associated with the case,
- copies of results of positive and negative amplification controls.

4.5 Naming DNA Extracts

4.5.1 Each DNA extract shall be assigned a unique identifier at the beginning of analysis. This identifier is intended to assist the analyst in tracking the extract through the analysis process. The unique identifier shall be indicated on the DNA Extract Log and any subsequent DNA analysis worksheets. Each analyst shall number his or her extracts sequentially from 1 using this format: #initialslasttwodigitsofyear. Each calendar year, every analyst shall start his or her unique identifier-naming scheme at 1. An example of a unique identifier series that was extracted in 2005 follows: 1VN05, 2VN05, 3VN05, etc. Reagent blanks shall be given a unique identifier in sequence with DNA extracts. Alternatively, a LIMS generated item identifier may also be used. LIMS item identifiers include the case #, the item #, and the portion #.

4.5.2 Samples must be marked in such a way to distinguish them throughout processing. Extract tubes containing the final eluate must include the case # and item # unique to that sample. Labeling can be hand-written or printed (e.g., barcode label).

4.5.3 In the event a sample is re-extracted, efforts should be made to distinguish it from the original extraction. Whether the unique identifier is LIMS-generated or not, the item # shall include the next sequential number for the portion number, so the item number is not the same. Item # 1, portion # 1 would be 1.1 during the first extraction; it would be 1.2 for the second extraction. When combining DNA extracts, case record documentation must be clear about which samples were combined and naming shall appropriately reflect the extract combination.

4.5.4 When not using a LIMS identifier, extraction confirmations may be marked with the same original item name and “EC” to distinguish it from the original extraction if it is being re-extracted by the same individual. It is also acceptable to assign a new unique identifier to the extraction confirmation.

4.5.5 In the event a sample is re-amplified, the sample shall retain its original unique identifier and the designation “RA” shall follow. Thus, a re-amplification of sample 8CDA06 would be identified as 8CDA06RA. Additional re-amplifications of the same sample would be identified using sequential numbers starting from 2. An additional re-amplification of sample 8CDA06RA would be identified as 8CDA06RA2. The sample description in LIMS can be modified to indicate a re-amplification by pressing F9 on the well to add

comments, such as “RA”. Samples re-amplified using Y-STRs shall be designated with a “Y” from amplification forward and in a manner similar to the one described above when multiple Y-STR amplifications are warranted. For example, the initial Y-STR amplification of 8CDA06 would be recorded as 8CDA06Y, while its subsequent amplification would be recorded as 8CDA06Y2, and so on. All re-amplification requests must be accompanied by an “Amplification Request” form that has been reviewed for accuracy.

4.6 Naming Controls

4.6.1 Controls are named using this format: identifierdateinitials (RBQ032818RDG). Identifiers for controls are as follows:

- RBQ (for questioned stains)
- RBK (for known stains)
- RBS (for sperm cell fractions)
- RBE (for epithelial cell fractions)
- RBR (for hair roots)
- RBSH (for hair shafts)
- POS (for PCR positive controls)
- NEG (for PCR negative controls)
- NTC (for qPCR negative controls)*

*Given the NTC does not continue to amplification, it is not necessary to also include date and initials in the naming of this control.

4.6.2 For example, the naming of controls for a differential extraction that was extracted on May 31, 2006 and amplified on June 1, 2006 is as follows: RBS053106VN, RBE053106VN, POS060106VN, and NEG060106VN.

4.6.3 In the event that the analyst performs two extractions or amplifications on the same day, the two events shall be distinguished (e.g., RBQ010101RDG1, RBQ010101RDG2, POS010101RDG1, POS010101RDG2, etc.).

4.6.4 Alternatively, a LIMS-generated identifier that uses a unique ID # assigned to a particular worksheet may be used for all controls.

4.7 Analytical Approaches

4.7.1 Once the case has been evaluated, the staff member decides on an analytical approach. The staff member must choose a scheme of analysis using recognized, accepted, and internally validated scientific procedures designed to develop the information in a logical sequence. In general, the analysis should enable the staff member to make conclusions regarding the source of the evidence.

4.7.2 Once an approach is chosen, the analyst shall evaluate the results at each step in light of previous results. A repeat analysis may be indicated when the first analysis has produced

inconclusive results. Internal inconsistencies shall be investigated. The opinion of a second qualified staff member or the Technical Leader can be helpful when results are unclear.

4.7.3 Hair comparisons can be made using DNA characteristics or microscopic characteristics. With any attempt to DNA type a hair root, a result is not assured and, for a hair root in the telogen phase, not expected. DNA STR hair root analysis consumes the sample but may not yield results. Therefore, the evidentiary value of the hair must be carefully evaluated and the potential loss of information weighed before proceeding with DNA analysis. Typically, an evidentiary hair should be analyzed only after a microscopic examination of the hair by a qualified trace analyst and after consultation with the investigator/prosecutor to determine:

- What is the significance of the particular hair, e.g., collected by pubic combing vs. car vacuum?
- Is it permissible to destroy part of the evidence?
- Are there additional details of the case that may explain the hair?
- What is the condition of the hair, e.g., fragment, telogen root, etc.? What is the likelihood of a DNA typing result?
- Is it desirable to postpone DNA typing at this time?
- Would mitochondrial DNA analysis by another laboratory be possible?

4.8 Casework Outsourcing

4.8.1 All outsourcing activities shall comply with Quality Manual policies, the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories, and therefore, the following:

4.8.1.1 Vendor laboratories shall demonstrate compliance with the most current version of the Quality Assurance Standards for Forensic DNA Testing Laboratories and accreditation requirements of federal and state law.

4.8.1.2 The Technical Leader shall approve technical specifications of outsourcing agreements with a vendor laboratory before contracts are awarded.

4.8.1.3 For any work that may be uploaded into or searched in CODIS, vendor laboratories shall not begin analysis of casework before the HFSC DNA section Technical Leader has accepted ownership of the DNA data.

4.8.1.4 Per the QAS document, ownership occurs when any of the following criteria are applicable:

- The originating laboratory will use any samples, extracts, or materials from the vendor laboratory for the purposes of forensic testing (i.e., a vendor laboratory prepares an extract that will be analyzed by the originating laboratory);
- The originating laboratory will interpret the data generated by the vendor laboratory;
- The originating laboratory will issue a report on the results of the analysis; or
- The originating laboratory will enter or search a DNA profile in CODIS from data generated by the vendor laboratory.

4.8.1.5 An ownership review is a review of DNA records generated by a vendor laboratory in accordance with QAS Standard 17 by the NDIS participating laboratory that accepts responsibility for and enters the DNA records into CODIS or uses the data for interpretation or comparison.

4.8.1.6 To determine whether an ownership review is required, DNA reports received from vendor laboratories shall be evaluated for the following:

4.8.1.6.1 CODIS eligibility of any DNA profiles generated

- Including profile eligibility and evidence eligibility

4.8.1.6.2 Interpretational value of non-CODIS eligible profiles (i.e. profiles not suitable for CODIS but suitable for further comparison such as data foreign to the complainant)

4.8.1.7 Vendor laboratory reports containing CODIS-eligible profiles or profiles with interpretational value shall undergo an ownership review. The ownership review shall be performed by a staff member who is qualified in the technology, platform, and typing amplification test kit used to generate the data. The analyst must participate in the laboratory's proficiency testing program to the full extent in which he or she participates in casework and/or the review of the outsourced data, including the technology, platform and/or amplification test kit used by the outsourcing laboratory. This ownership review shall include, at a minimum:

4.8.1.7.1 Review of all DNA types to verify that they are supported by the raw and/or analyzed data (electropherograms or images).

4.8.1.7.2 A review of all associated controls, internal lane size standards, and allelic ladders to verify that the expected results were obtained.

4.8.1.7.3 A review of the final report to verify that the results/conclusions are supported by the data. The report shall address each tested item (or its probative fractions) submitted to the vendor laboratory.

4.8.1.7.4 Verification of the DNA types, eligibility, and the correct specimen category for entry into CODIS.

4.8.1.8 A report shall be issued at the completion of the ownership review indicating which profile(s) has/have been entered into CODIS or that no items are suitable for CODIS entry.

4.8.1.9 Vendor laboratory reports containing no CODIS-eligible profiles or profiles with no interpretational value beyond comparisons already made (e.g. existing comparison to complainant) do not require an ownership review because no data is entered into CODIS. A second qualified analyst must verify that the ownership review is not required. This verification is captured on the "Outsourced DNA Case-Ownership Review Not Required" form. Vendor laboratory reports not requiring an ownership review shall be forwarded to the investigating officer or submitting agency with a cover letter. Examples of a profile having no interpretational value include samples with no DNA results and samples for which DNA foreign to the complainant is not obtained.

4.8.1.10 On-site visits of vendor laboratories shall be performed as follows:

4.8.1.10.1 An initial on-site visit shall be conducted prior to the vendor laboratory's beginning of casework analysis.

4.8.1.10.2 The Houston Forensic Science Center (HFSC) DNA section Technical Leader, a delegate of the Technical Leader who is a qualified or previously qualified DNA analyst in the technology, platform, and typing amplification test kit used to generate the DNA data, or another NDIS participating laboratory using the same technology, platform, and typing amplification test kit may perform the on-site visit. Alternatively, a designated FBI employee may perform the on-site visit.

4.8.1.10.3 If the outsource agreement extends beyond one year, annual on-site visits will be conducted every calendar year, at least 6 months and not more than 18 months apart. The HFSC Laboratory may accept subsequent visits by another NDIS participating laboratory using the same technology, platform, and typing amplification test kit, or a designated FBI employee.

4.8.1.10.4 The Technical Leader must document review and acceptance of on-site visits not conducted by the Technical Leader.

4.8.1.10.5 Site visits and site visit preparation may include, but not be limited to, a review or direct observation of the following:

- 4.8.1.10.5.1 Accreditation certificates
- 4.8.1.10.5.2 Recent external and internal audit reports
- 4.8.1.10.5.3 SOPs
- 4.8.1.10.5.4 Quality Manual
- 4.8.1.10.5.5 Facilities
  - 4.8.1.10.5.5.1 Security and controlled access
  - 4.8.1.10.5.5.2 Contamination minimized
- 4.8.1.10.5.6 Validation records
- 4.8.1.10.5.7 Proficiency records and educational qualifications of analysts
- 4.8.1.10.5.8 Evidence
  - 4.8.1.10.5.8.1 Unique identifier markings
  - 4.8.1.10.5.8.2 Proper storage
  - 4.8.1.10.5.8.3 Chain of custody
- 4.8.1.10.5.9 Case records
  - 4.8.1.10.5.9.1 Maintenance of case notes and examination documentation that support conclusions
  - 4.8.1.10.5.9.2 Documentation of technical and administrative reviews
- 4.8.1.10.5.10 Contamination or corrective action reports

4.8.1.10.6 Should the outsource lab be an NDIS-participating laboratory that routinely submits profiles for upload into CODIS as part of their analysis process, ownership and administrative reviews are not required by HFSC personnel upon receipt of the DNA report.

4.9 **Reinterpretation of Data Typed with Legacy Amplification Kits (**according to the SWGDAM publication “Clarification on the Reinterpretation of Data Typed with Legacy Amplification Kits”, approved June 6, 2016.)

4.9.1 If an analyst is sufficiently trained according to laboratory requirements, has reviewed the validation and standard operating procedures for the appropriate application of analytical parameters (e.g., thresholds, peak height ratios), the analyst is able to review and reinterpret legacy data in accordance with the following:

4.9.1.1 If an analyst(s) is currently qualified (trained and proficiency tested) in the amplification test kit, the analyst can reinterpret the data.

4.9.1.2 If an analyst is currently qualified in an amplification test kit (trained and proficiency tested), and the analyst was previously qualified (trained and proficiency tested) in the legacy amplification test kit, the analyst can reinterpret the legacy data if the analyst has been proficiency tested on the legacy test kit within the last two calendar years. If an analyst has not been proficiency tested on a legacy test kit within the last two calendar years, then the Technical Leader must document and approve the completion of the analyst’s review of the validation data, where available, and standard operating procedures of the legacy test kit. If an analyst, who has completed the requirements to interpret legacy amplification test kit data, has not reviewed the required documents within the last two calendar years, the Technical Leader must document and approve the completion of the analyst’s additional review.

4.9.1.3 If an analyst is currently qualified in an amplification test kit (trained and proficiency tested), but has never been previously qualified in the legacy amplification test kit, the analyst can reinterpret the legacy amplification test kit data if the analyst is trained in the legacy test kit interpretation protocols by a previously qualified analyst. The review of the validation data and standard operating procedures of the legacy test kit, training by the previously qualified analyst, and interpretation competency test must be completed by the analyst, and documented and approved by the Technical Leader. Documentation shall contain sufficient information for an auditor to confirm the analyst is qualified to reinterpret legacy amplification test kit data.

4.9.1.4 If a previously qualified analyst is unavailable to train a currently qualified analyst on a legacy amplification test kit, it is highly recommended that the current analyst not reinterpret legacy data. However, if the current analyst must perform legacy interpretation without training from a previously qualified analyst, then it is recommended that the currently qualified analyst and Technical Leader train in the legacy test kit interpretation protocols, review validation data and standard operating procedures. The training and interpretation competency test must be completed by the analyst, and documented and approved by the Technical Leader. Documentation shall contain sufficient information for an auditor to confirm the analyst is qualified to reinterpret legacy amplification test kit data.

4.9.1.5 If a laboratory generates new interpretation protocols from legacy validation data (e.g., developing a stochastic threshold when none previously existed), the laboratory shall document the basis for the new interpretation protocols. These new protocols shall be documented in the laboratory’s standard operating procedures and

memorialized in the Quality Assurance Standards Audit Document. The training and interpretation competency test must also be completed by the analyst, and documented and approved by the Technical Leader. Documentation shall contain sufficient information for an auditor to confirm the analyst is qualified to reinterpret legacy amplification test kit data.

4.9.1.6 Technical reviewers of reinterpretations of the legacy data are held to the same training requirements as the analyst reinterpreting the data.

4.10 Abbreviations

4.10.1 Abbreviations common to the Forensic Biology field or found in an American English dictionary may be used in case records without definition. Additional abbreviations defined in Biology SOP or defined below are also permissible. Any other abbreviation used must be defined on the page on which it is used.

*	Possible alleles below threshold (50-99RFU)	NR	no result	**STR Loci:***	
**	May be attributable to elevated stutter	NSA	Non-specific amplification	Amel	Amelogenin
( )	Minor allele	NTC	No testing conducted, or Non-template control, in relation to qPCR	CSF	CSF1PO
[]	Trace allele	NRC II	National Research Council, *An Update: The Evaluation of Forensic DNA Evidence,* 1996	D1	D1S1656
^	Allele below stochastic threshold	OL	Off-ladder	D3	D3S1358
~	Approximately	OMR	Outside Marker Range	D5	D5S818
AF	alleged father	PCI	phenol:chloroform:isoamyl alcohol	D7	D7S820
AMP	amplification	PCR	polymerase chain reaction	D8	D8S1179
ASCLD/ LAB	American Society of Crime Laboratory Directors/Laboratory Accreditation Board	PI	paternity index	D10	D10S1248
BLK	Black or African American	POS	amplification positive control	D12	D12S391
bp	base pair	PRO	Profiler Plus	D13	D13S317
C	child	PK/ ProK	proteinase K	D16	D16S539
CAU	Caucasian	PU	Pull-up	D18	D18S51
cLLD	Capacitive Liquid Level Detection	QAS	The FBI Quality Assurance Standards Audit for Forensic DNA Testing Laboratories	D19	D19S433
CO	COfiler	QF	Quantifiler	D21	D21S11
CODIS	Combined DNA Index System	qPCR	Quantitative PCR	D22	D22S1045
CPE	combined probability of exclusion	QUANT	quantification	Indel	Y indel
CPI	combined paternity index or combined probability of inclusion	RA	Re-amplification	**Y-STR Loci:**	
CPU	Complex pull-up	RB	reagent blank	19	DYS19
DAB	DNA Advisory Board	RCF	relative centrifugal force	385	DYS385
DB	digest buffer	RFU	relative fluorescence unit	389-I	DYS389-I
DTT	dithiothreitol	RQ	Re-quantification	389-II	DYS389-II

EB	Elevated baseline	SDIS	State DNA Index System	390	DYS390
EC	Exclusion/Extraction confirmation	SDS	sodium dodecyl sulfate	391	DYS391
EDTA	ethylenediaminetetraacetic acid	SEB	stain extraction buffer	392	DYS392
EF	Epithelial Fraction	SEH	Southeast Hispanic	393	DYS393
ES	Elevated stutter	SF	Sperm Fraction	437	DYS437
GF	GlobalFiler	SPEC	specimen	438	DYS438
HB	Heat block	STD	standard	439	DYS439
IDF/ID	Identifiler® Amplification Kit	STR	short tandem repeat	448	DYS448
IDP/ID+	Identifiler® Plus Amplification Kit	SWH	Southwest Hispanic	456	DYS456
INJ	Injection	TE	Tris-HCL and EDTA buffer	458	DYS458
INS	Insufficient	TM	Thermomixer	635	DYS635
KBS	Known buccal swabs	TECH	technical	GATA or GATA H4	Y GATA H4
KSS	Known saliva swabs	TNE	Tris/NaCl/EDTA solution		
LDIS	Local DNA Index System	UNDET	Undetermined		
M	Mother	UV	Ultraviolet Light		
MCON	Microcon	YF/YFP	Yfiler™/Yfiler ™ Plus Amplification Kit		
NDIS	National DNA Index System				

*There is no abbreviation for D2S441 or D2S1338.

5. Critical Reagents

5.1. Extraction Reagents

5.1.1. Each lot and shipment of DNA extraction reagent or DNA extraction kit must be quality control tested prior to use on casework to establish both functionality and a lack of contamination. Digestion components not received with or included in a kit but used in conjunction with a kit, such as QIAGEN MTL buffer or additional QIAGEN proteinase K, must also be quality control tested. At least one appropriate sample of known origin and one reagent blank must be incorporated into the quality control test. A sample is considered appropriate if it is similar to samples generally encountered through the use of a particular reagent (i.e., when testing DTT, a semen-containing sample must be used). All documentation generated through the quality control testing shall accompany the reagent quality control form (e.g., extraction, quantification, and amplification worksheets, electropherograms, etc.) and be maintained in the laboratory. It is acceptable to quality control test multiple reagents simultaneously but unacceptable results may require individual retesting to troubleshoot appropriately. If multiple reagents are tested simultaneously, it is acceptable to include the testing documentation with one of the reagents, as long as the other reagents' quality control form(s) indicates where the documentation may be found. Acceptable results include results for the known sample that are concordant with previously obtained data and a reagent blank free of contaminating DNA (no activity that cannot readily be attributed to an amplification or electrophoretic artifact). Reagent blank samples must be subjected to the most sensitive volume and injection conditions possible. Questionable results must be brought to the attention of a Forensic Biology Supervisor or the Technical Leader immediately.

5.1.2. The following extraction reagents are considered critical reagents and must be quality control tested prior to use on casework samples:

- Sterile di water
- Buffer G2
- Buffer ATL
- DTT, 1M
- Ethanol, denatured
- EZ1 DNA Investigator Kit (QIAGEN)
  - Buffer G2
  - Proteinase K
  - Carrier RNA
- MTL Buffer
- Proteinase K, 10 mg/mL
- TE Buffer
- PrepFiler Automated Forensic DNA Extraction Kit
  - Includes:
    - PrepFiler Lysis Buffer (One 500 ml bottle)
    - PrepFiler Magnetic Particles (Thirteen 1 ml tubes)

        - PrepFiler Wash Buffer A Concentrate (One 500 ml bottle)
        - PrepFiler Wash Buffer B Concentrate (One 250 ml bottle)
        - Elution Buffer (One 200 ml bottle)
- PrepFiler BTA Forensic DNA Extraction Kit
    - Includes:
        - PrepFiler Lysis Buffer (One 35 ml bottle)
        - PrepFiler Magnetic Particles (One 1.5 ml tube)
        - PrepFiler BTA Lysis Buffer (One 25 ml bottle)
        - PrepFiler Wash Buffer A Concentrate (Two 125 ml bottles)
        - PrepFiler Wash Buffer B Concentrate (Two 30 ml bottles)
        - PrepFiler Elution Buffer (One 12.5 ml bottle)
        - Proteinase K (One 0.85 ml tube)
- Ethanol (95%)
- Isopropanol (100%)

5.2. Quantification Kits

5.2.1. Each lot of a DNA quantification kit must be quality control tested prior to use on casework to establish both functionality and a lack of contamination. All documentation generated through the quality control testing shall accompany the reagent quality control form (e.g., quantification worksheets) and be maintained in the laboratory. Questionable results must be brought to the attention of a Forensic Biology Supervisor or the Technical Leader immediately.

5.2.1.1. Automation enhancer is considered a critical reagent and shall be tracked as any other critical reagent and quality control tested prior to use on casework as any other critical reagent.

5.2.2. Acceptable results include:

- The control DNA must be diluted to make a standard curve according to the procedure outlined in the sectional SOP. This DNA must then be run in duplicate on a plate. IPC values must be obtained from all points on the curve to ensure the reagents are working correctly and the standard curve must be within acceptable ranges for slope, y-intercept, and r^2 values, without having to remove more than 3 points.
- A negative control consisting of master mix and TE buffer or Dilution Buffer (whichever reagent was used to create the standards) must be run on the same plate. This negative control must have an IPC value and detect less than 5×10^{-3} ng/µl of DNA.

5.3. Amplification Kits

5.3.1. Each lot of a DNA amplification kit must be quality control tested prior to use on casework to establish both functionality and a lack of contamination. All documentation generated through the quality control testing should accompany this form (e.g., amplification worksheets, electropherograms, etc.). Negative samples must be subjected to the most sensitive volume and injection conditions possible. Questionable results must be brought to the attention of a Forensic Biology Supervisor or the Technical Leader immediately.

5.3.2. Acceptable results include:

- The positive control DNA must be run and the correct profile (as reported by the manufacturer) must be obtained.
- An amplification blank must be run and shown to have no detectable alleles (no activity that cannot readily be attributed to an amplification or electrophoretic artifact).
- All reagents in the kit must be used.
- The allelic ladder must be run to determine that all of the appropriate alleles are detected.

5.4. Post-Amplification Reagents

5.4.1. Post-amplification reagents, with the exception of allelic ladders and deionized water, are not considered critical and are tested concurrently with samples. Reagents not performing as expected must be brought to the attention of a Forensic Biology Supervisor or the Technical Leader. A plan for troubleshooting may be devised or a reagent may be discarded and replaced with a properly functioning equivalent. No post-amplification reagents are prepared in-house; genetic analyzer Buffer 10X w/ EDTA is, however, diluted with water prior to use. The allelic ladder lot # must be recorded in a user defined column of the capillary electrophoresis plate record.

5.4.2. These reagents include the following:

- GeneScan 500/600 Liz size standard
- HiDi formamide
- POP-4 Polymer
- Buffer 10X w/ EDTA

6. **Appendix A: Glossary of Terms included in the SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories, Approved January 12, 2017**

Allele	form of a gene that is located at a specific location on a specific chromosome. Alleles targeted in STR analysis vary in length.
Analytical threshold	the minimum height requirement at and above which detected peaks can be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles.
Artifact	a non-allelic product of the amplification process (e.g., stutter, non-templated nucleotide addition, or other non-specific product), an anomaly of the detection process (e.g., pull-up or spike), or a by-product of primer synthesis (e.g., "dye blob")
Assumed contributor	an individual whose DNA on an item of evidence is reasonably expected
Binary model	an interpretation scheme in which there are only two values (possible or not possible) for each decision (e.g., a peak is either "an allele" or "not an allele"; a genotype is "included" or "not included")
Composite profile	a DNA profile generated by combining typing results from different loci obtained from multiple injections of the same amplified sample and/or multiple amplifications of the same DNA extract. When separate extracts from different locations on a given evidentiary item are combined prior to amplification, the resultant DNA profile is not considered a composite profile.
Conditional	an interpretation category that incorporates assumption(s) as to the number of contributors and/or the presence of specific contributor(s)
CPE	combined probability of exclusion; produced by multiplying the probabilities of inclusion from each locus and subtracting the product from 1; (i.e., 1-CPI)
CPI	combined probability of inclusion; produced by multiplying the probabilities of inclusion from each locus
Deconvolution	separation of contributors to a mixed DNA profile based on quantitative peak height information and any underlying assumptions
Deduced profile	inference of an unknown contributor's DNA profile after taking into consideration the contribution of a known/assumed contributor's DNA profile based on quantitative peak height information
Differential Degradation	a DNA typing result in which contributors to a DNA mixture are subject to different levels of degradation (e.g., due to time of deposition), thereby impacting the mixture ratios across the entire profile
Distinguishable Mixture	a DNA mixture in which relative peak height ratios allow deconvolution of the profiles of major/minor contributor(s)
Dropout	when one or more alleles present in a sample are not observed above the analytical threshold

Evidence sample	also known as Questioned sample
Exclusion	a conclusion that eliminates an individual as a potential contributor of DNA obtained from an evidentiary item based on the comparison of known and questioned DNA profiles (or multiple questioned DNA profiles to each other)
Genotype	results of autosomal STR analysis of an individual at one or more genetic loci
Guidelines	a set of general principles used to provide directions and parameters for decision making
Heterozygote	an individual having different alleles at a particular locus; usually manifested as two distinct peaks for a locus in an electropherogram
Homozygote	an individual having the same (or indistinguishable) alleles at a particular locus; manifested as a single peak for a locus in an electropherogram
Inclusion	a conclusion for which an individual cannot be excluded as a potential contributor of DNA obtained from an evidentiary item based on the comparison of known and questioned DNA profiles (or multiple questioned DNA profiles to each other)
Inconclusive	a determination that no conclusion (i.e., inclusion/exclusion) can be drawn from the comparison of a reference sample to suitable data. This could also result from statistical analyses that fail to provide sufficient support for an inclusion or exclusion.
Indistinguishable mixture	DNA mixture in which relative peak height ratios are insufficient to attribute alleles to individual contributor(s)
Intimate sample	a biological sample from an evidence item that is obtained directly from an individual's body; it is not unexpected to detect that individual's allele(s) in the DNA typing results
Known sample	biological material for which the identity of the donor is established and used for comparison purposes (referred to as a "K")
Locus	the specific physical location of a gene on a chromosome. In forensic DNA analysis, it refers to the specific sites being tested (e.g., D3S1358, vWA or D5S818)
Major contributor(s)	an individual(s) who can account for the predominance of the DNA in a mixed profile
Masked allele	an allele of the minor contributor that may not be readily distinguishable from the alleles of the major contributor or an artifact
Minor contributor(s)	an individual(s) who can account for the lesser portion of the DNA in a mixed profile
Mixture	a DNA typing result originating from two or more individuals
Mixture ratio	the relative proportion of the DNA contributions of multiple individuals to a mixed DNA typing result, as determined by the use of quantitative

	peak height information; when expressed as a percentage it is termed a mixture proportion
Noise	background signal detected by a data collection instrument
Obligate allele	an allele in a mixed DNA typing result that is (a) foreign to an assumed contributor, or (b) based on quantitative peak height information, determined to be shared with the assumed contributor
Partial profile	a DNA profile for which complete typing results are not obtained at all tested loci due, for example, to DNA degradation, inhibition of amplification and/or low- quantity template
Peak height ratio (PHR)	the relative proportion of two alleles at a given locus, as determined by dividing the peak height of an allele with a lower relative fluorescence unit (RFU) value by the peak height of an allele with a higher RFU value, and then multiplying this value by 100 to express the PHR as a percentage; used as an indication of which alleles may be heterozygous pairs and also in mixture deconvolution
Probability of exclusion (PE)	the percentage of the population that can be excluded as potential contributors to a DNA mixture at a given locus
Probability of inclusion (PI)	the percentage of the population that can be included as potential contributors to a DNA mixture at a given locus; also known as Random Man Not Excluded
Questioned sample	biological sample recovered from a crime scene or collected from persons or objects associated with a crime (referred to as a “Q”)
Random Match Probability (RMP)	the probability of randomly selecting an unrelated individual from the population who could be a potential contributor to an evidentiary profile
Reference sample	also referred to as known sample or reference standard
Single-source profile	DNA typing results determined to originate from one individual based on peak height ratio assessments and the number of alleles at given loci
Stochastic effects	the observation of intra-locus peak imbalance and/or allele drop-out resulting from random, disproportionate amplification of alleles in low-quantity template samples
Stochastic threshold	the peak height value below which it is reasonable to assume that, at a given locus, allelic dropout of a sister allele in a heterozygous pair may have occurred
Stutter	a minor peak typically observed one repeat unit smaller than a primary STR allele resulting from strand slippage during amplification
Theta (θ)	a value used to adjust statistical calculations that rely on population databases to correct for substructure within populations
Uninterpretable	the determination that DNA results at one or more loci cannot be interpreted due to poor or limited data quality

Unsuitable (for comparison)	uninterpretable results or those that fail to meet quality assurance requirements as defined by the laboratory and as a result are not usable for comparisons