

The Arkansas State Crime Laboratory

Forensic DNA Section

Quality Assurance Manual

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DNA

Forensic DNA Section

QUALITY ASSURANCE MANUAL

SECTION 1: SECTION OVERVIEW

It is the mission of the Forensic DNA section to analyze biological evidence utilizing PCR technology in order to determine its source. This evidence is used to include or exclude individuals from having deposited the evidence in the commission of a criminal act.

Goals: It is the goal of the Arkansas State Crime Laboratory DNA section to:

- A. Provide the users of laboratory services access to forensic analysis including biological fluid identification and DNA typing of selected biological materials associated with official investigations.
- B. Ensure the quality, integrity and accuracy of the DNA typing data and its presentation through the implementation of a detailed Quality Assurance/Quality Control program.
- C. Provide the criminal justice system with a functional DNA database (CODIS) to help law enforcement agencies solve criminal cases.

Objectives: It is the objective of the Quality Assurance (QA) program to:

- A. Monitor on a routine basis the analytical testing procedure for DNA typing by means of Quality Control (QC) standards, proficiency test and audits.
- B. Verify that the entire DNA typing procedure is operating within the established performance criteria, as stated in the Analytical section of the Quality Manual and that the quality and validity of the analytical data are maintained.
- C. Ensure that problems are noted and that corrective action is taken and documented.
- D. Ensure the overall quality as outlined in the DNA Advisory Board Guidelines.

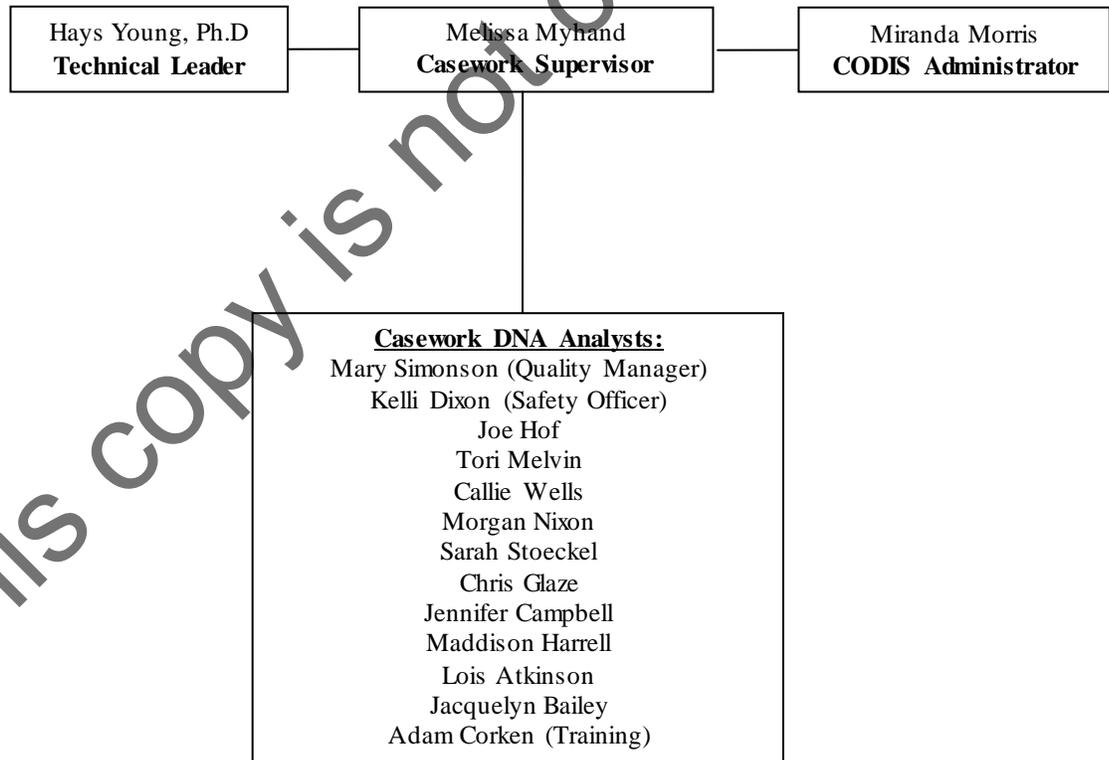
1.1: Organization and Management

1.1.1: Relationship of QA Program, DNA Analysis, Lab Operations and Management:

This QA Manual has been approved by the DNA Casework Supervisor, CODIS Administration, DNA Technical Leader, lab wide Quality Assurance Manager, Scientific Operations Director and Executive Director. and is accepted as routine operating policy of the Forensic DNA Section within the Arkansas State Crime Laboratory. The QA standards prepared by the FBI provided the model for the Arkansas State Crime Laboratory DNA QA program. Any supplements and revisions to the FBI guidelines will be reviewed for possible incorporation into the QA program. To discuss possible revisions, meetings between the Casework Supervisor and the DNA Analysts will be held as needed. Any changes to this QA manual must be approved by the same individuals as stated above, with affected manual pages and files updated. Previous versions of revised documents are maintained in a separate Historical Archive Manual. All DNA Analysts must be notified of the changes and must be given any necessary training.

1.1.2: Relationship of Individuals and Job Responsibilities:

Forensic DNA Section's Organizational Chart



SECTION 2: PERSONNEL QUALIFICATIONS AND JOB DESCRIPTIONS

The following establishes the job function, responsibility and qualifications for each position. This includes specification and description of lines of responsibility for

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developing, implementing, recording and updating the QA program. Job descriptions for personnel are established and located in each employee history binder. Each subordinate is accountable to one supervisor per function.

2.1: Personnel

2.1.1: DNA Technical Leader

2.1.1.1: Responsibility

The technical leader is ultimately responsible for technical operations and the QA program and thus the management of the DNA analysis program including technical troubleshooting, validation and systems management. The technical leader also has the authority to initiate, suspend, and resume the DNA analytical operations for the laboratory or an individual. In the event that the technical leader position is vacated then the contingency plan is detailed in appendix A.

2.1.1.2: Job Function

- a. Monitoring of development, validation, and implementation of the QA program, new methods and new technologies.
- b. Review the academic transcripts and training records for newly qualified analysts and approve their qualifications prior to independent casework analysis and document such review.
- c. Establishing professional liaisons with colleagues engaged in DNA testing and research.
- d. Approve the technical specifications for outsourcing agreements.
- e. Review internal and external DNA audit documents and, if applicable, approve corrective action(s), and document such review.
- f. Monitoring training and proficiency testing programs for DNA Casework Section personnel.
- g. Review, on an annual basis, the procedures of the laboratory and the quality system, then approve and document such review.
- h. Analyzing samples, providing expert testimony, and performing other routine duties of a Casework Analyst.
- i. Review and approve training, quality assurance, and proficiency testing programs in the laboratory.
- j. Review request by contract employees for employment by multiple NDIS participating and/or vendor laboratories and, if no potential conflict of interests exist, may approve such request.
- k. Technical leaders must review validation and methodologies currently used by the laboratory and educational qualifications and training records of currently qualified analysts.

l. Ensure compliance with ASCLD/LAB International requirements.

2.1.1.3: Qualifications

a. Education

The technical leader shall meet the following qualifications:

- Minimum educational requirements: The technical leader of a laboratory shall have, at a minimum, a Master's degree in a biology-, chemistry- or forensic science- related area and successfully completed 12 semester or equivalent credit hours from a combination of undergraduate and graduate course work covering the following subject areas: biochemistry, genetics, molecular biology, and statistics or population genetics.
- The 12 semester or equivalent credit hours shall include at least one graduate level course registering three (3) or more semester or equivalent credit hours.
- The specific subject areas listed above shall constitute an integral component of any course work used to demonstrate compliance with this Standard.
- Individuals who have completed course work with titles other than those listed above shall demonstrate compliance with this Standard through a combination of pertinent materials such as a transcript, syllabus, letter from the instructor or other document that supports the course content.
- If the degree requirements of listed above were waived by the American Society of Crime Laboratory Directors (ASCLD) in accordance with criteria approved by the Director of the Federal Bureau of Investigation (FBI), such a documented waiver is permanent and portable.

b. Training

The technical leader shall have three years of forensic DNA laboratory experience obtained at a laboratory where DNA testing was conducted for identification and evaluation of biological evidence in criminal matters.. As of the effective date of this revision, any newly appointed technical leader shall have a minimum of three years of human DNA (current or previous) experience as a qualified analyst on forensic samples. The technical leader shall have previously completed the FBI sponsored auditor training or successfully complete the FBI sponsored auditor training within one year of appointment.

c. Continuing Education

The technical leader must stay abreast of developments within the field of DNA typing by reading current scientific or DNA applicable literature, attending seminars, courses or professional meetings. Management provides the opportunity to comply with this requirement through travel budget, membership dues and education expense reimbursement.

d. Other

Must additionally meet the requirements specified for a DNA Analyst.

2.1.2: Casework Supervisor

2.1.2.1: Responsibility

The Casework Supervisor is responsible for the daily operation, including the supervision and management of personnel and casework flow through the Forensic DNA Section. The Casework Supervisor is also responsible for the technical operations and provisions of the resources needed to ensure the required quality of the laboratory operations. The Casework Supervisor has the responsibility and authority to receive and take action on Casework employee concerns. If the Casework Supervisor is going to be absent for more than 2 days, a labwide email notification will be sent appointing another casework analyst for any managerial issues and/or emergencies.

2.1.2.2: Job Function

- a. Overseeing day-to-day operation of the Forensic DNA Section, i.e., scheduling workload, supervising analysts, monitoring and reviewing results and case reports. These duties may be distributed among the DNA Analysts to facilitate case flow.
- b. Establishing professional liaisons with colleagues engaged in DNA testing and research.
- c. Conducting informational seminars for the principal users of the laboratory, i.e. judges, prosecutors, police administrators and investigators.
- d. Monitoring training programs for DNA unit personnel
- e. Enforcing safety procedures.
- f. Analyzing casework, providing expert testimony, and performing other routine duties of a DNA Analyst.
- g. Ensure compliance with ASCLD/LAB International requirements.

2.1.2.3: Qualifications

a. Education

The Casework Supervisor shall have at a minimum, a BS/BA degree in a biological, chemical, or forensic science, with undergraduate or graduate coursework in genetics, chemistry, statistics, biochemistry, and molecular biology (molecular genetics or recombinant DNA technology).

b. Training

The Casework Supervisor shall complete the DNA training program with individuals, agencies, or other laboratories that have an established training program and considerable experience in DNA methods and casework.

c. Experience

The Casework Supervisor of the laboratory is recommended to have a minimum of three (3) years of experience as a forensic DNA analyst. The Casework Supervisor is recommended to have successfully completed the FBI sponsored auditor training within one year of appointment.

d. Continuing Education

The Casework Supervisor must stay abreast of developments within the field of DNA typing by reading current scientific or DNA applicable literature, attending seminars, courses or professional meetings. Management provides the opportunity to comply with this requirement through travel budget, membership dues and education expense reimbursement.

e. Other

Must additionally meet the requirements specified for a DNA Analyst.

2.1.3: CODIS Administrator

2.1.3.1: Responsibility

The CODIS administrator is responsible for the administration of the laboratory's local CODIS network. The CODIS Administrator is also responsible for the technical operations and provisions of the resources needed to ensure the required quality of the laboratory operations. The CODIS Administrator has the responsibility and authority to receive and take action on CODIS employee concerns. In the event that the CODIS Administrator position is unoccupied the laboratory shall not upload any DNA profiles to NDIS.

2.1.3.2: Job Function

- a. Scheduling and documentation of the CODIS computer training of casework analysts.
- b. Assurance that the security of data stored in CODIS is in accordance with state and/or federal laws and NDIS operational procedures.
- c. Assurance that the quality of data stored in CODIS is in accordance with state and/or federal laws and NDIS operational procedures.
- d. Assurance that matches are dispositioned in accordance with NDIS operational procedures.
- e. The CODIS administrator has authority to terminate an analyst's or laboratory's participation in CODIS until the reliability and security of the computer data can be assured in the event of an issue with the data identified.
- f. Ensure compliance with ASCLD/LAB International requirements.
- g. Maintain a list of all employees with access to the CODIS database.
- h. Notify the NDIS Custodian, within five business days, of the following:

1. If a CODIS User, CODIS IT User or CODIS WAN User in its laboratory has been arrested for, or convicted or, a criminal offense
2. If the laboratory loses its criminal justice agency status;
3. If the laboratory loses its accreditations, has its accreditation suspended or has its accreditation revoked;
4. if the laboratory losses the capability to perform DNA analysis at its facility;
5. If the laboratory has fewer than two full-time employees who are qualified DNA analyst;
6. If the laboratory has a vacancy in the laboratory's Technical Leader position when there is no one in the laboratory who meet the Quality Assurance Standards' qualifications and is available to serve in that positions; or
7. If the laboratory is not in compliance with the external QAS audit requirement

2.1.3.3: Qualifications

a. Education

The CODIS administrator shall have at a minimum, a BS/BA degree in a biological, chemical, or forensic science, with undergraduate or graduate coursework in genetics, chemistry, statistics, biochemistry, and molecular biology (molecular genetics or recombinant DNA technology).

b. Training

The CODIS administrator shall complete the DNA training program with individuals, agencies, or other laboratories that have an established training program and considerable experience in DNA methods and casework.

c. Experience

The CODIS administrator of the laboratory shall be or have been a qualified DNA analyst with documented training in mixture analysis. The CODIS administrator shall participate in CODIS software training within six (6) months of assuming CODIS administrator duties. The CODIS administrator shall have successfully completed the FBI sponsored auditor training within one year of appointment.

d. Continuing Education

The CODIS administrator must stay abreast of developments within the field of DNA typing by reading current scientific or DNA applicable literature, attending seminars, courses or professional meetings. Management provides the opportunity to comply with this requirement through travel budget, membership dues and education expense reimbursement.

- e. Other

Must additionally meet the requirements specified for a DNA Analyst.

2.1.4: Forensic DNA Analyst

2.1.4.1: Responsibility

The forensic DNA analyst is responsible for performing DNA analysis and specifically delegated QA responsibilities from the Casework Supervisor.

2.1.4.2: Job Function

- a. Implementing the QA program.
- b. Handling reagents.
- c. Establishing liaisons with colleagues in the field.
- d. Analyzing, interpreting and reporting casework.
- e. Providing expert testimony.
- f. Interacting with investigative personnel.
- g. Executing all duties of QA Manager, if so designated.
- h. Assisting in training new employees.
- i. All other duties as assigned.

2.1.4.3: Qualifications

- a. Education

The DNA analyst shall have at a minimum, a BS/BA degree in a biological, chemical, or forensic science, with undergraduate or graduate coursework in genetics, statistics, biochemistry, and molecular biology (molecular genetics or recombinant DNA technology). With a minimum of nine (9) cumulative semester hours, or equivalent that cover the required subject areas.

- b. Training

The DNA analysts shall complete the DNA training program with individuals, agencies, or other laboratories that have an established training program and considerable experience in DNA methods and casework.

- c. Experience

The DNA analyst shall have a minimum of six (6) months of experience of forensic human DNA lab experience. This training entails the analysis of a range of samples routinely encountered in forensic casework prior to independent work using DNA technology. Additionally the analyst shall successfully complete a competency test and proficiency test before beginning independent DNA analysis. A complete list of training

requirements can be located in the DNA Section Training Manual Continuing Education

The DNA analyst must stay abreast of developments within the field of DNA typing by reading current scientific or DNA applicable literature, attending seminars, courses or professional meetings. Management provides the opportunity to comply with this requirement through travel budget, membership dues and education expense reimbursement.

2.1.6: DNA Quality Manager

2.1.6.1: Responsibility

The DNA quality manager is responsible for implementing the quality assurance program for the Forensic DNA section.

2.1.6.2: Job Function

- a. Ensure proper maintenance is being performed according to the quality assurance manual.
- b. Ensure that the quality manual procedures are being followed.
- c. Maintain all logs documenting the quality check of new chemicals.

2.1.7: DNA Safety Officer

2.1.7.1: Responsibility

The DNA safety officer is responsible for all aspects of the safety program for the Forensic DNA section.

2.1.7.2: Job Function

- a. Test safety equipment and complete required documentation.
- b. Maintain chemical inventory within the section as well as maintain MSDS binder.
- c. Responsible for the disposal of any chemical/biological waste.
- d. Complete safety survey on a semi-annual basis.
- e. Insures incident reports are completed and returned when an accident occurs.
- f. Maintain first aid kit.
- g. Provide safety orientation for new employees and manage the overall safety of the section.

2.2: Training

Training will be guided by the DNA Training Manual.

The required six-month training program for forensic laboratory personnel will depend upon previous training and experience. The training period may consist of continuous training or it may consist of a period of training plus time spent in supervised casework. The DNA technical leader will assess and document any adjustments to the established training program. At the completion of the training program each employee shall successfully complete a competency test which includes: an external proficiency test, a qualifying test and a moot court before performing independent casework. See the Training Manual for the complete training program. (The DNA Technical Leader must approve any deviations).

As new technology or methodology is added to the DNA Section each analyst may be required to become qualified in the procedure. For an analyst to become qualified they must complete a qualifying exam. A proficiency test in the technology must be completed within six (6) months of the qualifying exam.

2.2.1: Scientific or DNA Applicable Literature

All Casework employees have access to scientific or DNA applicable literature. Each member of the Section will read articles of scientific interest periodically. An excel sheet, that is located on the S drive will be filled out to document the article read. The analyst can disperse the article to the rest of the Section either by email or via hand carry.

2.3: Actions and Approval

2.3.1: DNA Technical Leader

- a. Can initiate, suspend, and resume DNA analytical operations for the laboratory or an individual.
- b. Must approve DNA quality manager's action.

2.3.2: DNA Quality Manager

- a. Can reject any chemical, reagent, supply or material which fails to meet the specifications set forth in the Quality Manual. The rejection of any such item must be documented in the Reagent Preparation Manual.
- b. Can terminate DNA testing if a technical problem is identified and is not resolved by the Technical Leader. The CODIS Administrator and the rest of the DNA Section must be notified and the specific problem(s) must be documented in the QA manual where the Casework Supervisor, CODIS Administrator and/or Technical Leader will initial to signify approval.

2.3.3: Casework Supervisor

- a. Can reject materials or suspend testing in the same manner as the DNA Quality Manager, following the same unit notification and problem documentation specifications.
- b. Must approve the DNA Quality Manager's actions.

2.3.4: DNA Analysts (Other than DNA Quality Manager)

- a. May recommend rejection of chemicals, reagents, supplies or materials that are found to be inadequate.
- b. May recommend termination of DNA testing if a technical problem is found.

SECTION 3: FACILITIES

3.1: Overall Laboratory Security

The Arkansas State Crime Laboratory building has security monitors that cover the external perimeter of the building and parking lots. Security cameras are also located on the first floor of the Crime Laboratory. Only authorized personnel are allowed access to the 2nd and 3rd floor unless accompanied by authorized personnel. Security fobs and keys are issued to authorized personnel in order to access the certain areas of the laboratory and must be approved by the Executive Director. The ASCL has a security fob access system controlled by a computer placed in the Administrative Section (access reports can be generated from the security fob access system software). Refer to the Arkansas State Crime Laboratory Quality Manual for comprehensive details regarding laboratory wide security.

3.2: Forensic Biology Laboratory Security

(Physical Evidence, CODIS and Casework DNA analysis areas)

The Physical Evidence, CODIS and DNA Casework area of the laboratory is limited in access to other laboratory personnel through the security system. Each analyst is assigned a unique programmed fob that enables entry into the laboratory.

3.3: Forensic DNA Casework Laboratory Set-up

The Forensic DNA Section is designed to minimize contamination during the processing of evidence. The sensitivity of PCR-based analysis, involving the amplification of minute quantities of DNA, makes it necessary to take certain precautions to avoid sample contamination. The best way to prevent PCR contamination is to have a separate lab for pre-PCR work and post-PCR work.

3.3.1: DNA Pre-PCR Laboratory

The DNA Pre-PCR area consists of evidence handling, DNA extraction and isolation, and preparation of samples for quantitation and amplification. The CODIS section shares this space for the processing, extraction, and amplification setup of database samples.

Special Precautions (DNA)

1. Use disposable gloves at all times.
2. Sterilize the bench top before and after you use it with diluted bleach solution.
3. Sterilize those solutions that can be heated in an autoclave without affecting their performance. Steam sterilization under bacterial decontamination conditions degrades DNA to a very low molecular weight, rendering it unamplifiable.
4. Always change pipette tips between handling each sample even when dispensing reagents.
5. Store reagents as small aliquots to minimize the number of times a given tube of reagent is opened. Record the lot numbers of reagents used in each set of samples so that if contamination occurs, it can be traced more readily. It is recommended that the small aliquots are retained until typing of the set of samples for which the aliquots were used is completed.
6. Centrifuge tubes before opening.
7. Include reagent blank controls with each set of DNA extractions to check for the presence of contaminating DNA in the reagents.
8. Never "blow out" the last bit of sample from a pipette. Blowing out may cause aerosols that may contaminate the sample.
9. Use disposable bench paper to prevent the accumulation of human DNA on permanent work surfaces. Bleach should be used to decontaminate exposed work surfaces after each use.
10. Wear a dedicated lab coat for pre-amplification sample handling when working in the pre-PCR DNA extraction work area.
11. Facemasks and/or face shields must be worn when working with evidence and setting up amplifications.
12. Lab coats should be washed on a monthly basis.
13. General housekeeping should be performed as needed (e.g. sweeping, mopping dusting).

3.3.2: DNA Casework Post-PCR Laboratory

The DNA Casework Post-PCR area consists of quantitation, amplification and PCR product typing. It is important that there is a one-way flow from the Pre-PCR lab to the Post-PCR lab. This is to prevent possible contamination between areas.

Special Precautions

Even in the amplified DNA work area, amplified DNA should be handled carefully. Steps should be taken to avoid dispersing it around the room. Reducing the dispersal of amplified DNA within this work area will reduce the potential for transfer of amplified DNA to other work areas.

1. Always remove gloves and lab coat when leaving the Amplified DNA Work Area to avoid the transfer of amplified DNA into other work areas.
2. Sterilize the bench top before and after you use it with diluted bleach solution.
3. Reduce the unnecessary dispersal of DNA around the work area by changing gloves whenever they may have become contaminated with amplified DNA.
4. Use disposable bench paper to cover the work area used to perform the typing steps to prevent the accumulation of amplified DNA on permanent work surfaces.
5. Plates of amplified DNA will be kept in the work area until all reviews are completed.

SECTION 4: EVIDENCE CONTROL

*See Arkansas State Crime Laboratory Quality Manual for lab wide policy regarding Evidence Control and Case Management.

NOTE: Arrestee samples are handled differently than casework evidence; see CODIS Quality Manual for sample processing. When referencing the sample in Casework reports the ASCL CODIS section Arrestee number (YYYY-1-XXXXX) should be used.

4.1: Evidence Handling Procedures

Evidence is submitted to the Arkansas State Crime Laboratory from investigating agencies only. Evidence is submitted to the Evidence Receiving section of the laboratory and assigned a unique identifying case number. These case files are then distributed to the proper sections of the Crime Laboratory.

Evidence that is screened for DNA evidence is processed through the Physical Evidence Section of the lab. Evidence is then packaged, submitted, and a request for DNA examination is made.

Evidence is collected, received, handled, sampled and stored so as to preserve the identity, integrity, condition and security of the item.

Before analysis begins, a second review is conducted by the Casework Supervisor and/or the DNA analyst to determine if there is anything more specific about the request and to determine if the laboratory has the capability and resources to perform the services requested (i.e. adequate standards, controls and approved test methods). Documentation is only noted if significant changes are observed. By starting analysis the analyst agrees to the request.

If the contract needs to be amended after work has begun, all affected personnel shall be notified.

4.2: Chain of Custody

A clear, well-documented chain of custody is maintained from the time the evidence is first received until it is released from the laboratory by the case file management system JusticeTrax.

4.3: Prioritizing

All cases may be prioritized based on a system that allows for a timely response. Priority may be made for the following reasons:

- Investigating Officer request
- Court Official request (including court date and court orders)
- Threat to public safety (homicides, rapes, and violent crimes)

Other cases or types of cases may be prioritized at the request of the DNA Supervisor, Scientific Operations Director, or the Executive Director.

All priority requests will be documented in the LIM systems under the "Request Tab" with a brief description of the prioritization request.

4.4: Packaging

After analysis, the DNA Analyst re-packages the evidence in a manner that will preserve the evidence while in storage and awaiting trial. At times, evidence submitted to the laboratory is not adequately packaged.

In this case, the DNA Analyst may re-package the evidence properly, retaining the inadequate packaging, since this original packaging may bear important markings necessary for identification.

4.5: Seals

During the evidence processing procedure, the DNA Analyst should, if possible, avoid damaging seals on the evidence made by others. For example, a box or a bag should be cut open in an area not sealed with evidence tape. After processing, the DNA Analyst completely seals all openings made in the packaging with tape. The tape is marked with the analyst's initials, and the package is checked to ensure that the State Crime lab number as well as a number to identify the item of evidence is present.

4.6: Release of Evidence

No evidence will be released from the laboratory, unless to the submitting agency, a police property custodian, or to a person with a court order or search warrant. (See Arkansas State Crime Laboratory Quality Manual)

4.7: Release of Information

See the Arkansas State Crime Laboratory Quality Manual for the policy on the release of information. . Statute 12-12-312 Records confidential and privilege.

4.8: Disposition

All appropriate DNA evidence will be retained by the Forensic DNA Section.

4.9: Purging

The Arkansas State Crime Laboratory is currently using the JusticeTrax LIMS-plus software program. All case documentation will be stored electronically. Once reviewed, this electronic version is considered the official case record.

Since 2008, the case file is stored electronically within JusticeTrax. For any case submitted prior to 2008, the paper case files are stored in a secure location. These files are stored either on site or in the Arkansas State Crime Laboratory annex.

4.10: Destruction of Evidence

The Forensic DNA Section destroys no evidence, except in the case of fetal tissue samples as described in 4.11.10.

4.11: Sample Handling and Storage

The following written policy ensures that evidence samples will be handled, processed and preserved so as to protect against loss, contamination or deleterious change. Testing of evidence and evidence samples is conducted to provide the maximum information with the least consumption of the sample. Whenever possible, a portion of the original sample is retained by the Forensic DNA Section. However, the Forensic DNA section reserves the right to consume the entire sample to maximize the information from a sample.

**See NRC1996 recommendations at <http://www.nap.edu/openbook.php?isbn=0309053951>*

4.11.1: Acceptance for DNA

Any felony, criminal case that has biological fluids identified will be a candidate for DNA testing. Misdemeanor or drug cases may be excluded from being processed by the DNA Section with the approval of the Casework Supervisor.

4.11.2: Identification of Evidence and Work Product

DNA extracts, excluding known samples, are considered as evidence and will be dried, sealed, and stored in DNA storage after the completion of the case. Work products are all other materials produced during the DNA analysis procedure and can be discarded after the product has been used.

4.11.3: Additional Samples

In cases where the source of the DNA has been identified through evidence that has already been processed, the Casework Supervisor should approve any requests for additional testing.

4.11.4: Evidence Marking

The Evidence Receiving Section of the Arkansas State Crime Laboratory generates a unique case file number. This number is designated by the year and numerical order of cases submitted to the laboratory (ex: YYYY- 000000). Each item of evidence will receive a specific identifier. Agency evidence numbers will be used whenever practical. Other identifiers may be assigned by the Forensic Serologists, and in such case, the DNA Analyst will use that same identifier. Other identifiers may be utilized if appropriate for the specific case. All evidence tubes are labeled with case number, analyst initials, and item number.

4.11.5: Evidence Collection and Examination

Requests for DNA examination are designated on an Arkansas State Crime Laboratory Submission Sheet. Evidence may be submitted and checked out for examination through the Evidence Receiving Section or received from another laboratory analyst via secure laboratory storage. Detailed procedures required for obtaining evidence can be found in the Evidence Receiving Section's Quality Manual.

Evidence is brought directly to the section where it is properly secured in a drawer, cabinet, refrigerator or freezer.

Preparation for the examination:

1. Review all the information provided to determine what questions an investigator needs to have answered. A discussion with a supervisor or other colleague may be helpful.

2. Items which are submitted and are transferred directly for DNA testing, should be assigned the same identifier in which it was submitted under, so not to assign redundant item numbers.
3. Prepare the work area. The bench space must be clean and free of clutter. The work area should be covered with white paper to prevent loss of small evidence and to prevent the cross transfer of trace evidence from one item to another. The necessary tools and reagents for examination should be conveniently placed. Adequate lighting should be provided to allow close visual inspection of evidence. Lab work sheets should be at hand to note observations.
4. A lab coat must be worn to protect ones clothing from contamination. Gloves must be worn to protect one from infectious diseases that could be present in biological material or for protection from toxic chemicals. Mask must be worn over nose and mouth to prevent contamination of evidence.

4.11.6: Examination

1. Examine one item at a time, making sure the work area and tools are cleaned between examinations.
2. Mark evidence for future identification with your initials. The package should be checked to ensure that the lab case number and item number is present.
3. If items are known to be disease contaminated, handle cautiously yet expeditiously. It is always good laboratory practice to handle all evidence with universal precautions as though the evidence was disease contaminated. Clean hood or work area and instruments with bleach solution.

4.11.7: Evidence Control System

The DNA Casework section will utilize an excel spreadsheet (DNA-FORM-1 or 39 depending on the chemistry used) and the labeling of tubes to ensure the integrity of each sample throughout processing. The DNA-FORM-1 will generate labels for each tube. The label will contain at least the case number, unique sample ID, and analyst's initials. The DNA-FORM-1 will document the location of each sample during the quantitation, amplification, and electrophoretic processes.

4.11.8: Long Term Storage

Upon completion of the testing, the DNA Analyst has the ultimate responsibility for long-term storage of the following case samples.

Items are placed in coin envelopes and placed in storage envelopes to be stored at room temperature when appropriate. Samples requiring sub-zero temperatures may be stored appropriately. All retained samples, including reference samples must be sealed and stored with contents identified. Outer container must contain at a minimum, the laboratory case number, item number, and initials of analyst.

Additional Storage Information:

1. Upon completion of the case, all question sample extracts are placed in a spin-vac and dried. The dried extracts are placed into the appropriate storage container with the original samples/cuttings when possible.
2. All liquid blood samples must be dried down for long term storage.
3. It is not required to retain extracts from reference blood samples or oral samples unless the entire reference sample was consumed during extraction. In that situation, the reference sample extract will be dried down and retained in the appropriate container.
4. Any unused reference samples collected with Q-tip swabs are retained.
5. Blood sample(s) collected by the Medical Examiner's Office, spotted on FTA paper, can be stored at room temperature in long term evidence storage.

4.11.9: Reconstitution of Dried Samples

When a dried sample needs to be used for further testing it may be reconstituted with deionized water. The amount of water to be added should be approximated to the amount of liquid that was removed during dry down. Sample may be placed on the heat blocks to assist the reconstitution. The sample RB must be reconstituted alongside the sample unless the RB has been previously run with the technology requested. If the RB has already been run then a reconstitution RB must be run consisting of the deionized water used for reconstitution.

4.11.10: Retention/Destruction Policy for Fetal Tissue Samples submitted per ACT 725

The fetal sample will be examined, documented and worked in order to obtain a DNA profile, per laboratory policy. This profile will be entered into the "QC" category of CODIS.

The sample extract will be dried down and retained as per normal laboratory policy. Once the report has been released to the investigating agency, the agency will be contacted to request a letter / email stating that the remaining fetal tissue may be destroyed. The letter / email will be scanned into the case file and the remaining fetal tissue will then be destroyed.

SECTION 5: VALIDATION

The laboratory shall only use validated methodologies for DNA analyses. There are two types of validation: developmental and internal.

5.1: Developmental Validation

Developmental validation is required on any novel methodology for forensic DNA analysis. The developmental validation shall include the following studies, where applicable:

1. Characterization of genetic markers.
2. Species specificity.
3. Sensitivity.
4. Stability.
5. Reproducibility.
6. Case-type samples.
7. Population.
8. Mixture.
9. Precision.
10. Accuracy.
11. PCR-based studies.
 - a. Reaction conditions.
 - b. Assessment of differential amplification.
 - c. Assessment of preferential amplification.
 - d. Effects of multiplexing.
 - e. Assessment of appropriate controls.
 - f. Product detection.

5.2: Internal Validation

Internal validation is required on any methodologies that are utilized for forensic DNA analysis in the laboratory. A developmentally validated methodology cannot be utilized in the laboratory until it has been internally validated, reviewed and approved by the technical leader. The internal validation procedure will be tested using known and non-probative evidence samples or database type samples, and contain the following studies where applicable:

1. Accuracy.

2. Precision.
3. Reproducibility.
4. Sensitivity and Stochastic.
5. Mixture.
6. Contamination assessment

Internal validation shall define quality assurance parameters and interpretation guidelines.

Before an analyst can begin using an internally validated procedure for DNA casework, the analyst must successfully complete training and a qualifying test. A proficiency test must be completed within (6) months of qualification of the new technology or methodology. See the Arkansas State Crime Laboratory Quality Manual for specific requirement of validation.

Material modifications made to validation procedures shall be documented and approved by the technical leader

SECTION 6: ANALYTICAL PROCEDURES

6.1: Generic Guidelines

6.1.1: Reagents

The following is a list of critical reagents used in the Forensic DNA Section:

Commercial Kits:

DNA Investigator Kits	Qiagen
Quantifiler-Duo Kits	Applied Biosystems
PowerPlex 16 HS	Promega
PowerPlex Y23	Promega

Miscellaneous Items:

Taq Gold Polymerase	Applied Biosystems
9947A	Promega
2800M	Promega

6.1.1.1: Sources of Materials, Reagents, Chemicals and Supplies

A listing of commercial sources for all materials, reagents, chemicals, and supplies will be maintained in the Reagent Log. All commercial reagents will be labeled with the identity of the reagent, open date and

the expiration date if applicable. All information relevant to material or services that must meet certain specifications for testing will be provided in the External Supply Request to the purchasing department.

6.1.1.2: Supply and Materials Inventory

Upon receipt of all materials, reagents, chemicals and supplies, the packing slip will be checked for agreement with the items received when available. The analysts are responsible for initialing and dating chemical and reagent containers with an "Open Date". Reagents and supplies, which have passed their expiration date, will not be used in casework unless a performance check has been conducted and the technical leader has approved and documented the deviation to extend the expiration date.

6.1.1.3: Material Safety Data Sheets (MSDS)

The MSDS received from the manufacturer for each chemical used in the laboratory can be found in the designated MSDS book or electronically. These data sheets are readily available to all laboratory personnel. Master copies of all MSDS sheets for the laboratory are kept by the Laboratory Health and Safety Manager.

6.1.1.4: Laboratory Prepared Reagents and Solutions

A log will be maintained for each laboratory prepared reagent and solution except dilutions of laboratory concentrates. Each reagent/solution prepared will have the following recorded in the DNA Reagent Log or stored electronically on the DNA drive:

- Identity
- Date of preparation
- Date of expiration
- Instructions on preparation of reagent
- Lot numbers of solvents and/or chemicals used in preparation of reagent
- A method to verify the reagent's reliability (if applicable)
- Initials of the person preparing reagent
- Initials of the person verifying reagent (if applicable)

6.1.1.5: Labeling Requirements

All laboratory prepared reagents and solutions will be clearly labeled. Labels will include identity, date of preparation, identity of preparing analyst, expiration date and, as appropriate, storage requirements.

6.1.1.6: Storage and Disposal

All chemicals must be stored, used, and disposed of in a manner conforming to established safety requirements.

6.1.1.7: Critical reagents and supplies

Critical consumables, supplies, and services which affect the quality of testing will be obtained from reliable suppliers (see ASCL-DOC-01). All critical reagents and supplies must be quality control tested for accurate, reliable performance prior to use in the Forensic DNA Section. Quality control test results will be recorded in the Quality Control of Critical Reagents Log.

6.1.1.7.1: DNA Investigator Kits

DNA investigator kits will be marked with the receive date and initials of the individual who receives the kit. A known blood sample will be processed through the extraction kit to check the quality of the reagents. The DNA extract will be amplified with a QC'd PowerPlex 16 HS kit, and analyzed to ensure the correct profile was produced. Once the lot has been verified the QC date will be placed on all received kits. If the kit does not produce the expected profile, the known blood samples will be re-extracted and re-analyzed. If the kit fails the QC a second time the Technical Leader, or designee will be informed. The Technical Leader, or designee, will examine the problem and contact the manufacturer if necessary.

6.1.1.7.2: Quantifiler-Duo Kits

The quantitation kits will be marked with the receive date and initials of the individual who receives the kit. A dilution of standards, as described in the SOP for each of the quantitation kits, will be run and analyzed to ensure the quality of the newly received kits. Using the guidelines in the appropriate SOP, a R^2 of ≥ 0.98 will be considered passing. Once the lot has been verified the QC date will be placed on all received kits. If the standard curve does not have a R^2 of ≥ 0.98 , the standard will be re-run and re-analyzed. If the standard fails the QC a second time the Technical Leader, or designee will be informed. The Technical Leader, or designee, will examine the problem and contact the manufacturer if necessary.

6.1.1.7.3: PowerPlex 16 HS and Y23 DNA Kits

The genetic typing kits will be marked with the receive date and initials of the individual who receives the kit. The appropriate positive control as described in the corresponding SOP will be amplified in duplicate along with an AMP- sample. The samples will then be analyzed to ensure the appropriate DNA profile is obtained. Once the lot has been verified the QC date will be placed on all received kits. If the kit does not produce the expected profile, the samples should be re-injected or re-amplified. If the positive or negative controls still do not produce the expected result, the Technical Leader, or designee, will be informed. The Technical Leader, or designee, will examine the problem and contact the manufacturer if necessary.

6.1.1.7.4: Taq Gold Polymerase

The Taq gold polymerase will be marked with the receive date and initials of the individual who receives the Taq. The appropriate positive control as described in the corresponding SOP will be amplified in duplicate along with an amplification blank (AMP-) sample. The samples will then be analyzed to ensure the appropriate DNA profile is obtained. Once the lot has been verified the QC date will be placed on all received kits. If the Taq does not produce the expected profile, the samples should be re-injected or re-amplified.. If the Taq fails the QC a second time the Technical Leader, or designee will be informed. The Technical Leader or designee will examine the problem and contact the manufacturer if necessary.

6.1.2: Controls and Standards

It is essential that proper control samples are included when samples are extracted, amplified and typed. The typing results obtained from these controls are important for the interpretation of the profiles obtained. All employees and supervisory personnel must be vigilant for any indication of nonconforming tests and work.

6.1.2.1: Reagent Blank (RB)

The reagent blank consists of all reagents used in the test process minus any sample and is processed through all steps alongside the question or known samples on the same extraction instrument. A reagent blank must be included with each extraction set (up to 7 forensic evidence samples and up to 13 forensic known samples). The reagent blank will be amplified at full strength.

The reagent blank is used to test for possible contamination of the sample preparation, reagents, and/or supplies by an external DNA source. If the reagent blank exhibits any typing results above the analytical threshold (**175 RFU for PP16HS and Y23**), the reagent blank can be re-amplified. If the typing results remain above threshold after re-amplification, then all DNA samples that were associated with reagent blank should be considered inconclusive for analysis and re-extracted. If the DNA sample has been consumed and re-extraction is not possible, then the DNA Technical Leader, Casework Supervisor and/or Laboratory Director will be consulted to analyze the samples and reagent blank. If after analysis the source of the contaminating DNA does not appear to be in the samples, then the contamination will be noted in the report. If the extraneous DNA is present in both the reagent blank and associated sample, then the sample will be reported as inconclusive.

6.1.2.2: Positive Control

The positive control contains DNA from a known source with a known DNA profile. The positive control will be amplified and analyzed with each sample set.

The positive control tests to insure the proper performance of the amplification and typing procedure. 2800M is the positive control for PP16 HS and Y23. If the positive control does not exhibit the appropriate results, then samples associated with that positive control are considered inconclusive for analysis and must be re-amplified. Positive controls may be setup in duplicate to compensate for poor injections, spikes, or other artifacts. Only one of the positive controls is required to produce the expected results. If a positive control is lacking expected allele(s) at a locus, then the control can be used, but that locus will be marked as inconclusive in all samples associated with the positive control. If there are more than two loci that lack the expected allele(s) then all samples associated with the positive control must be re-injected or re-amplified.

6.1.2.3: Negative Control (AMP-)

The negative control (amplification blank) contains all the reagents for the amplification mix but no DNA.

The negative control tests for contamination of samples during the setup of the amplification reactions. If the negative control exhibits unexplainable peaks above the analytical threshold (175 RFU for PP16HS and Y23), that are not eliminated after re-injection, then all

samples associated with the negative control are considered inconclusive for analysis and must be re-amplified.

6.1.2.4: Quantification Standards

Quantification standard consists of 8 dilutions made from the standard stock provided with each quantification kit.

The quantification standard is used to create a standard curve to allow for the quantification of the samples amplified concurrently. The standard curve must consist of at least 6 of the 8 dilutions.

6.1.2.5: Internal Size Marker and Allelic Ladder

Internal size marker is added to each sample and ladder prior to electrophoresis. The internal size marker allows the genetic analysis software to determine the size (in base pairs) of the peaks in the samples and ladders.

The allelic ladder is supplied with each of the amplification kits and is run with each set of samples. The allelic ladder allows GeneMapper ID-X to assign an allele call to any peaks observed based on their size.

6.1.2.6: NIST Standard

DNA procedures will be checked using the NIST Standard Reference Material (SRM; 2391b for autosomal STRs and 2395 for Y-STRs or an internal NIST traceable sample) annually or whenever substantial changes are made to the procedures.

6.1.2.7.1: Internal NIST Standards

Internal NIST Traceable Standards are created by running NIST Standard Reference Material alongside the internal standard. The internal standard will be viable until a new lot is taken or until an internal expiration date (if applicable).

6.1.2.7.2: NIST Standards Handling, Storage, and Prevention of Deterioration

NIST SRM samples will be maintained in as the manufacturer recommends. All internal NIST traceable standards will be maintained at room temperature. All NIST samples will be transported, handled, and used as all casework samples to prevent contamination and deterioration and to protect the integrity of the sample.

6.1.3: Detection and Control of Contamination

The Arkansas State Crime Laboratory employs several safeguards to detect any contamination that might occur. The reagent blank detects contamination during extraction, the amplification blank detects contamination during the setup of amplification, and the monthly swipe test detects contamination of the laboratory spaces. In order to reduce the possibility of contamination, the Arkansas State Crime Laboratory has devised procedures listed in the section on evidence handling and processing.

If contamination has been discovered, the laboratory will try to discover the source of the contamination. The incident will be documented on a Non-Conformance Report (CODIS-FORM-17). If a DNA analyst is found to be the source of the contamination, the Casework Supervisor will be notified and take the necessary corrective actions. If the contamination is from outside the DNA section, the appropriate supervisor will be notified to address the contamination source. If the contamination is systemic issue, the lab wide Quality Manager will be notified and a Corrective Action Request (CAR) may be necessary.

6.2: Standard Operating Procedures

6.2.1: Intake of Arrestee Samples with Submitted Cases

If arrestee samples are submitted to the CODIS Section with case numbers referencing specific cases in which the arrest was made, the sample can be processed for both the database and for the DNA Casework Section. The sample can also be processed if documentation from the submitting agency or the prosecutor requesting the Arrestee sample be referenced to the specific case the individual was arrested. In order for the sample to be used for both sections the qualifying violation the individual was sampled for must also be the case submitted to the DNA Section.

- A. Prior to use in Casework an 'Arrestee Confirmation Sheet' (CODIS-Form-43) must be completed. Once the 'Arrestee Confirmation Sheet' is completed it should be scanned in JusticeTrax along with biographical information.
- B. An 'Arrestee' Request in JusticeTrax must be created and canceled to inform an analyst that a sample related to his/her case is in the CODIS Section.
- C. A duplicate sample is not re-run in the CODIS Section. DNA Casework can work the sample if necessary and retain it with the appropriate evidence. It is noted that this can be changed on a case-by-case basis upon approval of the CODIS Administrator and/or the Casework Supervisor.

- D. If an arrestee sample that is referenced to an ASCL case number is given to the CODIS Section, and it is deemed to have a non-qualifying violation, the sample can be stored for the DNA Casework Section.
- E. All completed 'Arrestee Confirmation Sheets' are stored along the completed CODIS Hit information. Any additional hits from the arrestee sample will need to have the DNA profile confirmed.
- F. All arrestee profiles (autosomal and Y-STR) should be developed and entered into Specimen Manager by a CODIS Analyst for the Casework Analyst to obtain. It is noted that this can be changed on a case-by-case basis upon approval of the CODIS Administrator and/or Casework Supervisor.

6.2.2: Extraction Protocols

Note: All samples not extracted with EZ1 must be concentrated prior to any quantitation, with the exception of reference samples. The quantitation step may be omitted for reference samples.

Extracts from oral samples collected as reference items must be dried down only if the entire sample was used during extraction.

Extraction tubes are stored in the Pre-PCR laboratory refrigerator until cases are completed and samples are stored long term.

All extraction steps must be performed in the Pre-PCR Laboratory.

Extract known samples at a different time and/or space than questioned samples. Use reagents and pipettes dedicated only to this area.

6.2.2.1 Organic and Tissue Extraction

1. Activate Stain Extraction Buffer by dissolving 60.14mg DTT into 10mL of stain extraction buffer (or equivalent ratio) Record activation on tube and store in refrigerator for up to 2 weeks.
2. Using a new, sterile scalpel blade, cut a piece of tissue approximately 3 mm squared.
3. With a scalpel blade, mince tissue into small pieces and transfer the minced tissue to a 1.5ml microcentrifuge tube.
4. To the sample add 300 ul of activated stain extraction buffer and 7.5 ul proteinase K solution. Vortex on low speed (be alert for fluid leakage from the tube cap) for 1 second and spin in a

microcentrifuge for 2 seconds to force the cutting into the extraction fluid.

5. Incubate the tube at 56 °C overnight (minimum of eight (8) hours).
* Blood samples may be incubated for a minimum of 30 minutes.
6. Spin in a microcentrifuge for 2 seconds to force condensate into the bottom of the tube.
7. In a fume hood, add 300 ul phenol/chloroform/isoamyl alcohol to the stain extract. Vortex (low speed) the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge for 3 minutes.
8. Label appropriate 2.0 ml EZ1 sample tube.
9. Transfer the aqueous phase from the tube in step 7 to the labeled sterile 2.0 ml EZ1 sample tube. Avoid pipetting solvent (bottom layer) from the tube into the concentrator.
10. Process on EZ1 with Trace protocol.
 - a. Ensure EZ1 workstation is on.
 - b. Press “Start” to start protocol setup.
 - c. Press “Esc” (for no report)
 - d. Press “1” or “2” (for Trace or Trace TD protocol)
 - e. Press “2” (for elution in TE buffer)
 - f. Choose elution volume, “2” for 50 µl, “3” for 100 µl, “4” for 200 µl. (most applications will be 50 µl, but highly concentrated forensic known samples can be diluted in larger volumes).
 - g. Press any key to continue.
 - h. Follow steps on screen to setup workstation/
 - i. Load cartridges into the rack.
 - ii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - iii. Load tip holders and tips in Row 2 of tip rack.
 - iv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
 - v. Close workstation door.
 - vi. Press “Start” to start protocol.
 - vii. Once display show “Protocol finished”, remove elution tubes. Discard waste appropriately.
11. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kit SOP)

12. The samples are now ready for PCR amplification.

6.2.2.2: EZ1 Robot Extractions

6.2.2.2.1: Whole Blood

When liquid blood samples are submitted, samples should be dried and retained on appropriate paper for storage. Stains should be air dried and stored in individual envelopes at room temperature.

2. Label appropriate 2.0 ml EZ1 sample tube.
3. Add 200 μ l of whole blood to the labeled sterile 2.0 ml EZ1 sample tube.
3. Add 190 μ l of **DILUTED** G2 buffer. (Diluted G2 buffer is a 1:1 dilution with diH₂O) Additional **DILUTED** G2 buffer may be added to absorbent samples to ensure ~190 μ l of liquid in the tube.
4. Add 10 μ l of Proteinase K, mix by vortexing. (**DO NOT** add additional Proteinase K, even if you increase the volume in step 3.)
5. Incubate at 56 °C for a minimum of 15 minutes.
6. If necessary, centrifuge briefly.

4. Process on EZ1 with Trace protocol.
 - a. Ensure EZ1 workstation is on.
 - b. Press "Start" to start protocol setup.
 - c. Press "Esc" (for no report)
 - d. Press "1" or "2" (for Trace or Trace TD protocol)
 - e. Press "2" (for elution in TE buffer)
 - f. Choose elution volume, 50 μ l, 100 μ l, or 200 μ l. (most applications will be 50 μ l, but highly concentrated forensic known samples can be diluted in larger volumes).
 - g. Press any key to continue.
 - h. Follow steps on screen to setup workstation/
 - i. Load cartridges into the rack.
 - ii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - iii. Load tip holders and tips in Row 2 of tip rack.
 - iv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
 - v. Close workstation door.

- vi. Press "Start" to start protocol.
 - vii. Once display show "Protocol finished", remove elution tubes. Discard waste appropriately.
5. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kit SOP)
 6. The samples are now ready for PCR amplification.

6.2.2.2.2: Dried Blood Stains (Swabs, Filter Paper, & FTA), Forensic Surface Samples, Cigarette Butts, Stamps / Envelope Flaps, Tape Lift Swabs.

1. Label appropriate 2.0 ml EZ1 sample tube.

2. ***Blood stains**

Cut the stain, approximately 3mm x 3mm in size and place into the labeled sterile 2.0 ml EZ1 sample tube.

***Forensic Surface Samples (Shirt collars, transfer evidence etc...)**

Cut approximately 3 mm x 3mm portion of the gauze or filter paper, or an appropriate portion of a swab and place into the labeled sterile 2.0 ml EZ1 sample tube..

***Cigarette Butts**

Cut an approximately 5 mm wide strip from the cigarette butt (including filter) in the area which would have been in contact with the mouth and place into the labeled sterile 2.0 ml EZ1 sample tube.

***Stamps / Envelope Flaps**

Carefully open envelope flap or remove stamp using steam and clean tweezers. Using a sterile cotton swab moistened in sterile, distilled, deionized water, swab gummed envelope flap or stamp. Cuttings may also be used for this extraction. Cut cotton swab from stick and place into the labeled sterile 2.0 ml EZ1 sample tube.

***Tape Lift Swabs**

Using a sterile cotton swab moistened in sterile, distilled, deionized water, swab the entire tape lift to remove any epithelial cells which may be present. Cut cotton swab from stick and place into the labeled sterile 2.0 ml EZ1 sample tube.

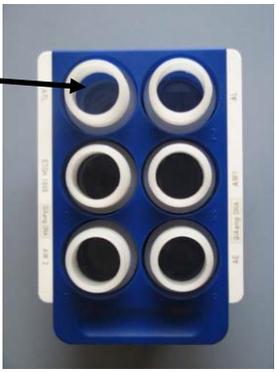
7. Add 190 μ l of **DILUTED** G2 buffer. (Diluted G2 buffer is a 1:1 dilution with diH₂O) Additional **DILUTED** G2 buffer may be added to absorbent samples to ensure ~190 μ l of liquid in the tube.
8. Add 10 μ l of Proteinase K, mix by vortexing. (**DO NOT** add additional Proteinase K, even if you increase the volume in step 3.)
9. Incubate at 56 °C for a minimum of 15 minutes.
10. If necessary, centrifuge briefly.
11. Process on EZ1 with Trace TD protocol.
 - a. Ensure EZ1 workstation is on.
 - b. Press “Start” to start protocol setup.
 - c. Press “Esc” (for no report)
 - d. Press “1” or “2” (for Trace or Trace TD protocol)
 - e. Press “2” (for elution in TE buffer)
 - f. Choose elution volume, 50 μ l, 100 μ l, or 200 μ l. (most applications will be 50 μ l, but highly concentrated forensic known samples can be diluted in larger volumes).
 - g. Press any key to continue.
 - h. Follow steps on screen to setup workstation
 - i. Load cartridges into the rack.
 - ii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - iii. Load tip holders and tips in Row 2 of tip rack.
 - iv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
 - v. Close workstation door.
 - vi. Press “Start” to start protocol.
 - vii. Once display show “Protocol finished”, remove elution tubes. Discard waste appropriately.
12. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kit SOP)
13. The samples are now ready for PCR amplification.

6.2.2.2.3: Semen-containing Stains (Qiacube) (2-6 samples)

1. Label appropriate 1.5 ml Qiacube sample tube as Semen Fraction (SF) and label appropriate 2.0 ml EZ-1 sample tube as Epithelial Fraction (EF).

2. Cut an appropriately size sample and place into the labeled sterile 1.5 ml Qiacube sample tube.
3. Add 480 μ l G2 buffer.
4. Add 20 μ l of Proteinase K, mix by vortexing.
5. Incubate at 56 °C for a minimum of 15 minutes.
6. Centrifuge tube briefly to remove drop from lid.
7. Remove any solid material from tube. Use a toothpick to remove cloth or swab from tube. Twist sample on side of tube to remove excess fluid. Discard solid material in appropriate waste.
8. Sample is now ready for Qiacube separation
 - a. Ensure Qiacube is on.
 - b. Press “DNA”.
 - c. Select “Pipetting” and Press “Select”.
 - d. Select “Epithelial and Sperm Cell” and Press “Select”.
 - e. Select “Separation and Lysis 6” and Press “Select”.
 - f. Follow steps on screen to setup workstation
 - i. Press “Start”.
 - ii. Empty waste drawer and Fill tips racks with 1000ul wide-bore tips.
 - iii. Fill reagent bottle to fill line with G2 Buffer and place in position 1 in the reagent bottle rack.

Postion 1



- iv. Mix Sperm Lysis Buffer according to chart below, then place it in a 2 ml screw top tube and place in position A on tip rack.

# Samples	ul Sperm Lysis	G2 (ul)	Pro K (ul)	DTT (ul)
-----------	----------------	---------	------------	----------



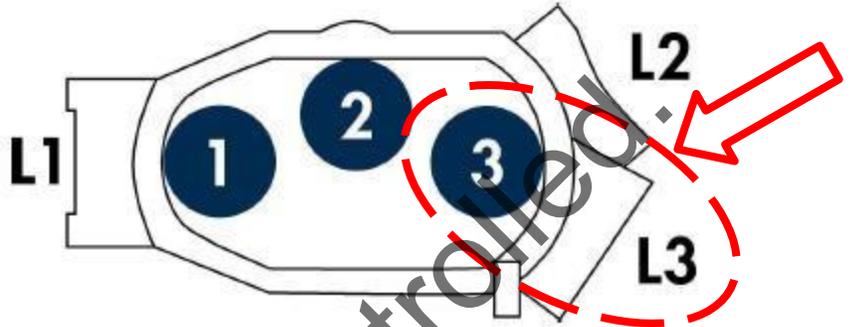
Document ID: DNA-DOC-01

Approved By: Morris, Miranda, Myhand, Melissa, Young, Hays, Cha Ryan

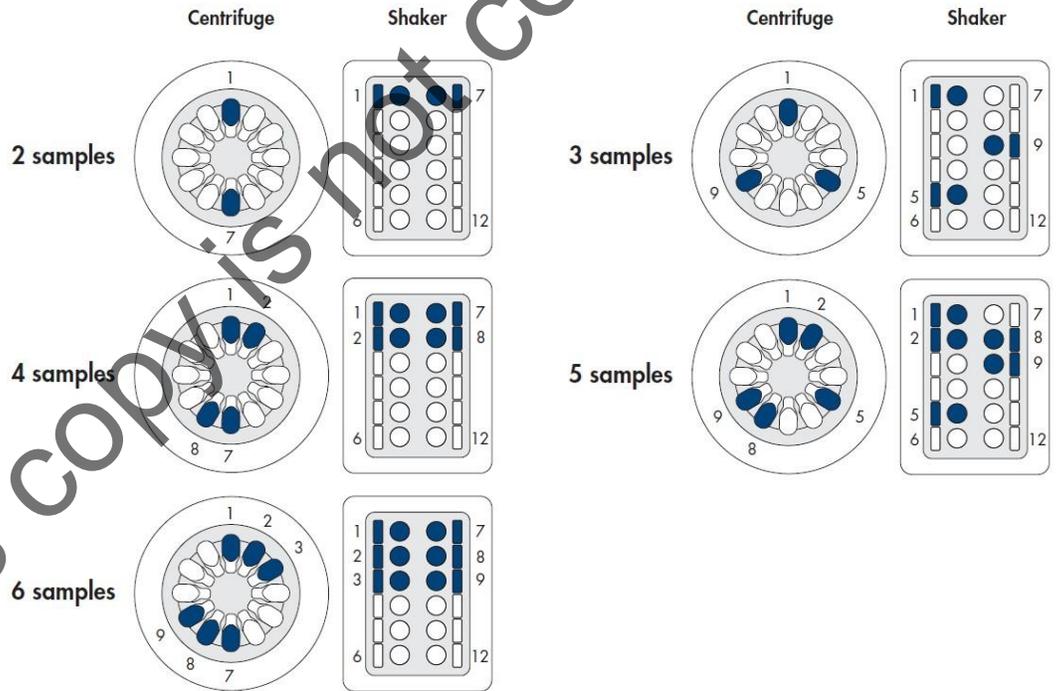
015 k,

2	500	374	26	100
3	750	561	39	150
4	1000	748	52	200
5	1250	935	65	250
6	1500	1122	78	300

v. Load rotor adapters with 1.5 ml Qiacube tubes containing semen and epithelial mixture from step 7.



vi. Place rotor adaptors in rotor according to chart below (or on side of Qiacube)



vii. Place empty 2 ml EZ1 sample tubes in the shaker following the setup above. Ensure the plastic nubs are in the holes next to each tube.

viii. Press "Start".

9. Process Epithelial Fraction on EZ1 with Trace protocol.

- a. Ensure EZ1 workstation is on.
- b. Press “Start” to start protocol setup.
- c. Press “Esc” (for no report)
- d. Press “1” (for Trace protocol)
- e. Press “2” (for elution in TE buffer)
- f. Choose elution volume, 50 μ l, 100 μ l, or 200 μ l. (most applications will be 50 μ l, but highly concentrated samples can be diluted in larger volumes).
- g. Press any key to continue.
- h. Follow steps on screen to setup workstation
 - i. Load cartridges into the rack.
 - ii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - iii. Load tip holders and tips in Row 2 of tip rack.
 - iv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
 - v. Close workstation door.
 - vi. Press “Start” to start protocol.
 - vii. Once display show “Protocol finished”, remove elution tubes. Discard waste appropriately.
 - viii. Save samples for step 15.

10. Remove 1.5 ml tube from the rotor adapter and close tube.

11. Incubate at 56 °C for a minimum of 10 minutes.

12. Centrifuge tube briefly to remove drops from lid.

13. Transfer sample from the 1.5 ml tube to a labeled 2.0 ml EZ1 sample tube.

14. Process Sperm Fraction on EZ1 with Trace protocol.

- a. Ensure EZ1 workstation is on.
- b. Press “Start” to start protocol setup.
- c. Press “Esc” (for no report)
- d. Press “1” or “2” (for Trace or Trace TD protocol)
- e. Press “2” (for elution in TE buffer)
- f. Choose elution volume, 50 μ l, 100 μ l, or 200 μ l. (most applications will be 50 μ l, but highly concentrated samples can be diluted in larger volumes).
- g. Press any key to continue.
- h. Follow steps on screen to setup workstation
 - ix. Load cartridges into the rack.
 - x. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - xi. Load tip holders and tips in Row 2 of tip rack.

- xii. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
- xiii. Close workstation door.
- xiv. Press “Start” to start protocol.
- xv. Once display show “Protocol finished”, remove elution tubes. Discard waste appropriately.

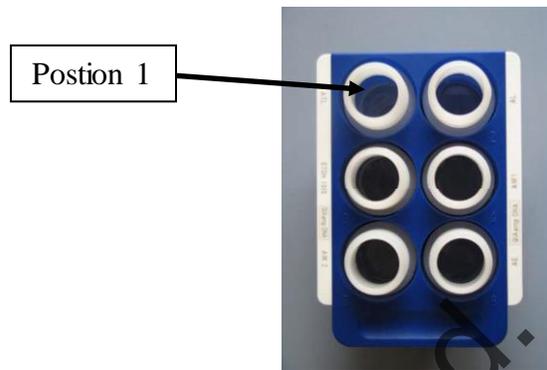
15. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kit SOP)

16. The samples are now ready for PCR amplification

6.2.2.2.4: Semen-containing Stains (Qiacube) (7-12 samples)

1. Label appropriate 1.5 ml Qiacube sample tube as Semen Fraction (SF) and label appropriate 2.0 ml EZ-1 sample tube as Epithelial Fraction (EF).
2. Cut an appropriately size sample and place into the labeled sterile 1.5 ml Qiacube sample tube.
3. Add 480 µl G2 buffer.
4. Add 20 µl of Proteinase K mix by vortexing.
5. Incubate at 56 °C for a minimum of 15 minutes.
6. Centrifuge tube briefly to remove drop from lid.
7. Remove any solid material from tube. Use a toothpick to remove cloth or swab from tube. Twist sample on side of tube to remove excess fluid. Discard solid material in appropriate waste.
8. Sample is now ready for Qiacube separation.
 - a. Ensure Qiacube is on.
 - b. Press “DNA”.
 - c. Select “Pipetting” and Press “Select”.
 - d. Select “Epithelial and Sperm Cell” and Press “Select”.
 - e. Select “Separation and Lysis 12 A” and Press “Select”.
 - f. Follow steps on screen to setup workstation
 - i. Press “Start”.
 - ii. Empty waste drawer and Fill tips racks with 1000ul wide-bore tips.

- iii. Fill reagent bottle to fill line with G2 Buffer and place in position 1 in the reagent bottle rack.

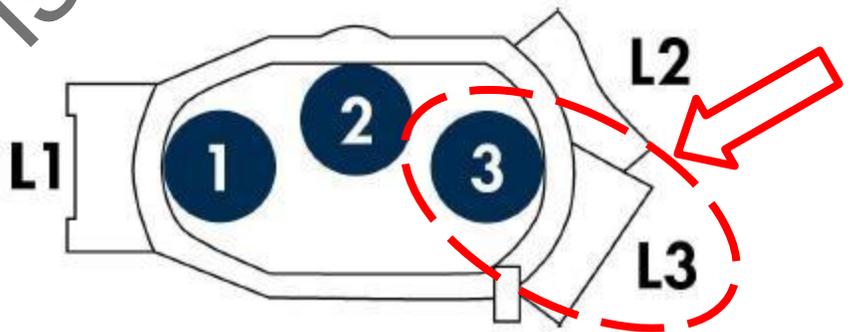


- iv. Mix Sperm Lysis Buffer according to chart below, then place it in a 2 ml screw top tube and place in position A on tip rack.

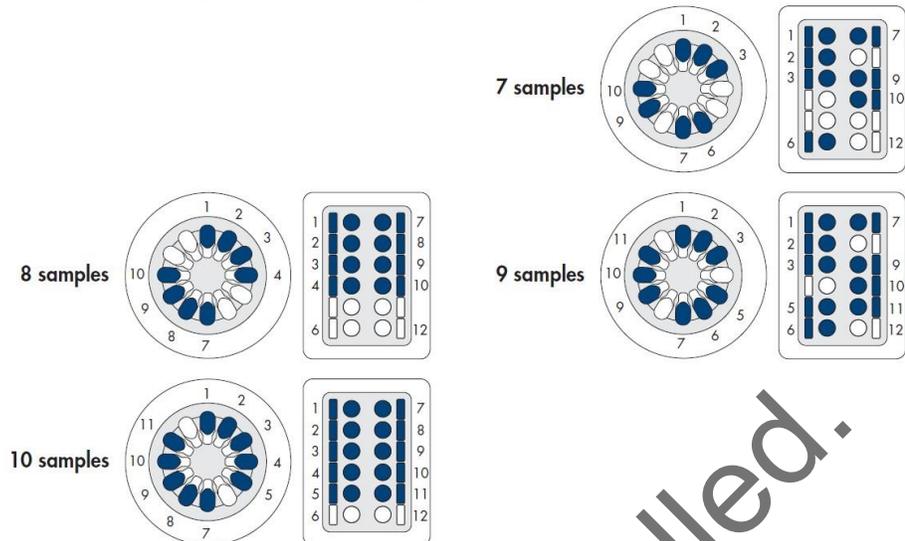
# Samples	ul Sperm Lysis	G2 (ul)	Pro K (ul)	DTT (ul)
7	1150	862	58	230
8	1300	975	65	260
9	1475	1106	74	295
10	1625	1219	81	325
12	1960	1470	98	392



- v. Load rotor adapters with 1.5 ml Qiacube tubes containing semen and epithelial mixture from step 7.



- vi. Place rotor adaptors in rotor according to chart below (or on side of Qiacube)



- vii. Place empty 2 ml EZ1 sample tubes in the shaker following the setup above. Ensure the plastic nubs are in the holes next to each tube.
- viii. Press “Start”.

9. Process Epithelial Fraction on EZ1 with Trace protocol. (This step can be done alongside step 15)

- a. Ensure EZ1 workstation is on.
- b. Press “Start” to start protocol setup.
- c. Press “Esc” (for no report)
- d. Press “1” (for Trace protocol)
- e. Press “2” (for elution in TE buffer)
- f. Choose elution volume, 50 μ l, 100 μ l, or 200 μ l. (most applications will be 50 μ l, but highly concentrated samples can be diluted in larger volumes).
- g. Press any key to continue.
- h. Follow steps on screen to setup workstation
 - i. Load cartridges into the rack.
 - ii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - iii. Load tip holders and tips in Row 2 of tip rack.
 - iv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
 - v. Close workstation door.
 - vi. Press “Start” to start protocol.
 - vii. Once display show “Protocol finished”, remove elution tubes. Discard waste appropriately.
 - viii. Save samples for step 16.

10. Sample is now ready to continue the Qiacube separation.

- a. Press “DNA”.

- b. Select "Pipetting" and Press "Select".
 - c. Select "Epithelial and Sperm Cell" and Press "Select".
 - d. Select "Separation and Lysis 12 B" and Press "Select".
 - e. Follow steps on screen to setup workstation
 - i. Press "Start".
 - ii. Fill tips racks with 1000ul wide-bore tips.
 - iii. Press "Start".
11. Remove 1.5 ml tube from the rotor adapter and close tube.
12. Incubate at 56 °C for a minimum of 10 minutes.
13. Centrifuge tube briefly to remove drops from lid.
14. Transfer sample from the 1.5 ml tube to a labeled 2.0 ml EZ1 sample tube.
15. Process Sperm Fraction on EZ1 with Trace protocol.
- a. Ensure EZ1 workstation is on.
 - b. Press "Start" to start protocol setup.
 - c. Press "Esc" (for no report)
 - d. Press "1" or "2" (for Trace or Trace TD protocol)
 - e. Press "2" (for elution in TE buffer)
 - f. Choose elution volume, 50 µl, 100 µl, or 200 µl. (most applications will be 50 µl, but highly concentrated samples can be diluted in larger volumes).
 - g. Press any key to continue.
 - h. Follow steps on screen to setup workstation
 - i. Load cartridges into the rack.
 - ii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - iii. Load tip holders and tips in Row 2 of tip rack.
 - iv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
 - v. Close workstation door.
 - vi. Press "Start" to start protocol.
 - vii. Once display show "Protocol finished", remove elution tubes. Discard waste appropriately.
16. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kit SOP)
17. The samples are now ready for PCR amplification

6.2.2.2.5: Semen-containing Stains (Manual)

1. Label appropriate 2.0 ml EZ1 sample tube.

2. Cut an appropriately size sample and place into the labeled sterile 2.0 ml EZ1 sample tube.
3. Add 190 μ l G2 buffer. Additional G2 buffer may be added to absorbent samples to ensure \sim 190 μ l of liquid in the tube.
4. Add 10 μ l of Proteinase K, mix by vortexing. (**DO NOT** add additional Proteinase K, even if you increase the volume in step 3.)
5. Incubate at 56 $^{\circ}$ C for a minimum of 15 minutes.
6. Centrifuge tube briefly to remove drop from lid.
7. Remove any solid material from tube. Use a toothpick to remove cloth or swab from tube. Twist sample on side of tube to remove excess fluid. Discard solid material in appropriate waste.
8. Centrifuge tube at approximately 15000 x g for 5 minutes. Carefully transfer the supernatant (epithelial fraction) to a new tube without disturbing the sperm cell pellet. (NOTE: sperm cell pellet may not be visible)
9. Process Epithelial Fraction on EZ1 with Trace protocol.
 - i. Ensure EZ1 workstation is on.
 - j. Press "Start" to start protocol setup.
 - k. Press "Esc" (for no report)
 - l. Press "1" or "2" (for Trace or Trace TD protocol)
 - m. Press "2" (for elution in TE buffer)
 - n. Choose elution volume, 50 μ l, 100 μ l, or 200 μ l. (most applications will be 50 μ l, but highly concentrated samples can be diluted in larger volumes).
 - o. Press any key to continue.
 - p. Follow steps on screen to setup workstation/
 - viii. Load cartridges into the rack.
 - ix. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - x. Load tip holders and tips in Row 2 of tip rack.
 - xi. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
 - xii. Close workstation door.
 - xiii. Press "Start" to start protocol.

- xiv. Once display show "Protocol finished", remove elution tubes. Discard waste appropriately.
 - q. Save samples for step
10. Wash sperm cell pellet by adding 500 μ l of G2 buffer. Vortex and centrifuge tube at approximately 15000 x g for 5 minutes. Discard supernatant.
 11. Repeat step 10 two (2) times for a total of three (3) washes.
 12. Add 180 μ l G2 buffer to the sample tube.
 13. Add 10 μ l Proteinase K and 10 μ l 1 M DTT.
 14. Vortex for 10 seconds.
 15. Incubate at 56 °C for a minimum of 10 minutes.
 16. Centrifuge tube briefly to remove drops from lid.
 17. Process Sperm Fraction on EZ1 with Trace protocol.
 - i. Ensure EZ1 workstation is on.
 - j. Press "Start" to start protocol setup.
 - k. Press "Esc" (for no report)
 - l. Press "1" or "2" (for Trace or Trace TD protocol)
 - m. Press "2" (for elution in TE buffer)
 - n. Choose elution volume, 50 μ l, 100 μ l, or 200 μ l. (most applications will be 50 μ l, but highly concentrated samples can be diluted in larger volumes).
 - o. Press any key to continue.
 - p. Follow steps on screen to setup workstation/
 - xv. Load cartridges into the rack.
 - xvi. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - xvii. Load tip holders and tips in Row 2 of tip rack.
 - xviii. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
 - xix. Close workstation door.
 - xx. Press "Start" to start protocol.
 - xxi. Once display show "Protocol finished", remove elution tubes. Discard waste appropriately.
18. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kit SOP)
 19. The samples are now ready for PCR amplification.

6.2.2.2.5: Hair Extractions

1. Label appropriate 2.0 ml EZ1 sample tube. One tube is for the hair root and the other is for the hair shaft.
2. Cut an appropriately size sample and place into the labeled sterile 2.0 ml EZ1 sample tube.
3. Add 180 µl G2 buffer to the sample tube.
4. Add 10 µl Proteinase K and 10 µl 1 M DTT.
5. Incubate at 56 °C for a minimum of 30 minutes.
6. Centrifuge tube briefly to remove drops from lid.
7. Process on EZ1 with Trace TD protocol.
 - a. Ensure EZ1 workstation is on.
 - b. Press “Start” to start protocol setup.
 - c. Press “Esc” (for no report)
 - d. Press “1” or “2” (for Trace or Trace TD protocol)
 - e. Press “2” (for elution in TE buffer)
 - f. Choose elution volume, 50 µl, 100 µl, or 200 µl. (most applications will be 50 µl, but highly concentrated samples can be diluted in larger volumes). Press any key to continue.
 - g. Follow steps on screen to setup workstation/
 - i. Load cartridges into the rack.
 - ii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - iii. Load tip holders and tips in Row 2 of tip rack.
 - iv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
 - v. Close workstation door.
 - vi. Press “Start” to start protocol.
 - vii. Once display show “Protocol finished”, remove elution tubes. Discard waste appropriately.
8. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kits SOP)
9. The sample is now ready PCR amplification.

6.2.2.2.6: Tissue Extraction

1. Label appropriate 2.0 ml EZ1 sample tube.
2. Cut an appropriately size sample and place into the labeled sterile 2.0 ml EZ1 sample tube.
3. Add 190 μ l G2 buffer to the sample tube.
4. Add 10 μ l Proteinase K.
5. Incubate at 56 °C for a minimum of 3 hour.
6. Centrifuge tube briefly to remove drops from lid.
7. Process on EZ1 with Trace TD protocol.
 - a. Ensure EZ1 workstation is on.
 - b. Press “Start” to start protocol setup.
 - c. Press “Esc” (for no report)
 - d. Press “1” or “2” (for Trace or Trace TD protocol)
 - e. Press “2” (for elution in TE buffer)
 - f. Choose elution volume, 50 μ l, 100 μ l, or 200 μ l. (most applications will be 50 μ l but highly concentrated samples can be diluted in larger volumes).
 - g. Press any key to continue.
 - h. Follow steps on screen to setup workstation/
 - viii. Load cartridges into the rack.
 - ix. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - x. Load tip holders and tips in Row 2 of tip rack.
 - xi. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
 - xii. Close workstation door.
 - xiii. Press “Start” to start protocol.
 - xiv. Once display show “Protocol finished”, remove elution tubes. Discard waste appropriately.
8. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kits SOP)
9. The sample in now ready for PCR amplification.

6.2.2.2.7: Nail Clippings and Gum Extraction

1. Label appropriate 2.0 ml EZ1 sample tube.

2. Cut an appropriately size sample and place into the labeled sterile 2.0 ml EZ1 sample tube.
3. Add 190 µl G2 buffer to the sample tube.
4. Add 10 µl Proteinase K.
5. Incubate at 56 °C for a minimum of 15 minutes.
6. Centrifuge tube briefly to remove drops from lid.
7. Remove any solid material from tube with tweezers or a toothpick. Try to remove as much liquid from the material as possible.
8. Process on EZ1 with Trace protocol.
 - a. Ensure EZ1 workstation is on.
 - b. Press “Start” to start protocol setup.
 - c. Press “Esc” (for no report)
 - d. Press “1” or “2” (for Trace or Trace TD protocol)
 - e. Press “2” (for elution in TE buffer)
 - f. Choose elution volume, 50 µl, 100 µl, or 200 µl. (most applications will be 50 µl, but highly concentrated samples can be diluted in larger volumes).
 - g. Press any key to continue.
 - h. Follow steps on screen to setup workstation/
 - xv. Load cartridges into the rack.
 - xvi. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - xvii. Load tip holders and tips in Row 2 of tip rack.
 - xviii. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
 - xix. Close workstation door.
 - xx. Press “Start” to start protocol.
 - xxi. Once display show “Protocol finished”, remove elution tubes. Discard waste appropriately.
9. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kits SOP)
10. The sample is now ready for PCR amplification.

6.2.2.2.8: Modified Semen-Containing Stains Extraction

1. Label appropriate 2.0 ml EZ1 sample tube.

2. Cut an appropriately size sample and place into the labeled sterile 2.0 ml EZ1 sample tube.
3. Add 190 μ l G2 buffer. Additional G2 buffer may be added to absorbent samples to ensure \sim 190 μ l of liquid in the tube.
4. Add 10 μ l Proteinase K and 10 μ l 1M DTT, mix by vortexing. (**DO NOT** add additional Proteinase K, even if you increase the volume in Step 3.)
5. Incubate at 56 $^{\circ}$ C for a minimum of 10 minutes.
6. Centrifuge tube briefly to remove drops from lid.
7. Remove any solid material from tube with tweezers or a toothpick. Try to remove as much liquid from the material as possible.
8. Process on EZ1 with Trace protocol.
 - i. Ensure EZ1 workstation is on.
 - j. Press "Start" to start protocol setup.
 - k. Press "Esc" (for no report)
 - l. Press "1" or "2" (for Trace or Trace TD protocol)
 - m. Press "2" (for elution in TE buffer)
 - n. Choose elution volume, 50 μ l, 100 μ l, or 200 μ l. (most applications will be 50 μ l, but highly concentrated samples can be diluted in larger volumes).
 - o. Press any key to continue.
 - p. Follow steps on screen to setup workstation/
 - xxii. Load cartridges into the rack.
 - xxiii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
 - xxiv. Load tip holders and tips in Row 2 of tip rack.
 - xxv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
 - xxvi. Close workstation door.
 - xxvii. Press "Start" to start protocol.
 - xxviii. Once display show "Protocol finished", remove elution tubes. Discard waste appropriately.
9. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kits SOP)
10. The sample is now ready for PCR amplification.

6.2.3: Excel Sample Management Form

6.2.3.1 DNA-FORM-01 Excel file for DNA

1. Open form from S:\Excel Form\
2. Enable macros
3. Select the type of run.
4. Select location to save and then save the file with a unique identifier (AA_MMDDYYYY)(AA = initials)
5. Enter Analyst's First Name, Initials, Last Name, and the Run name in Samples Tab.
6. Enter Case number and Samples Name on the Samples tab.
7. Press the Continue to Quant duo setup button.
8. Select location to save and then save the text file for the labels.
 - a. On the computer in the Clean Rooms select DNA labels on the desktop
 - b. Select Menu item TOOLS, then IMPORT DATA, then ADVANCED
 - c. Data Source is TEXT then Press NEXT
 - d. Press BROWSE and select the file you saved at the beginning of Step 8
 - e. Select Delimiter TAB, then Press NEXT
 - f. Select USE EXISTING OBJECTS located in the middle of the screen, then Press Next
 - g. Press Next
 - h. Press Next
 - i. Press Next
 - j. Press Next
 - k. Select Copies to 2
 - l. Press Finish
 - m. Select Menu item FILE, then PRINT
 - n. Press PRINT
 - o. Complete Extraction and return to the Excel Sheet
9. Enter the Date.
10. Press Print page button and select the printer to print to.
11. Press the Save Txt for 7500 button.
12. Select location to save and then save the file with the run name.
13. Write in Lot Numbers and Standard prep. info on printed sheet.

14. Setup Quant. in clean room
15. Go to Post room, and start the 7500 program, open a new run select DUO as template.
16. Click File Import Sample Setup, select text file you saved on your flash drive.
17. Save the file then Start Quant.
18. While Quant is running, enter lot numbers and standard prep info into Excel file.
19. After Quant is complete Click file, export, results. Then select your flash drive and click save.
20. Go to Quantifiler Duo Setup in Excel sheet and click Import Quantifiler Duo Data button.
21. Input your target DNA amount.
22. Select the file you save from the 7500 (It will be a .csv file) click OK.
23. Now go to Total DNA Quantity Tab and examine your data.
24. Adjust any dilutions you would like to make and then Press the Continue to STR AMP Page.
25. Type in your Thermocycler used, add your controls to the end of the list. Changing the amount of DNA to the appropriate amount.
26. Press Print Sheets for Amplification button.
27. Select injections needed from list.
28. Select location and then save file as File name to XXXX(Run Name),
29. Take flash drive to /3500xl
30. On the 3500xl in the Plate manager click the import plate button.
31. Select the txt file that you saved on the flash drive and click either ok or
32. 3500xl should say that it imported ok. If gives an error of invalid symbol or something about the name you probably had a space in your sample name on the first page.
33. Start run.
34. Press Print Worksheets to Justice Trax Imaging and Select Justice Trax imaging.
35. Once the 3500xl run is completed copy your files and analyze with GeneMapper ID-X.

36. Once all files have been analyzed go back to the main GeneMapper windows and change your table settings to the export selection.
37. Next click FILE, Export Combined Table.
38. *MAKE SURE TO SELECT ONE LINE PER SAMPLE* on the right
39. Place the file anywhere you can find it later.
40. Click export combined table.
41. In the Excel file on the Final tab select the samples for each injection time.
42. Click Generate Call Sheets.
43. Press the injection time that needs to have data imported to.
44. Select the file and click OK.
45. Correct all calls on the call sheets
46. Press the injection time that needs to be printed to JusticeTrax imaging and select JusticeTrax imaging

6.2.4: DNA Quantification Protocols

6.2.4.1: Quantifiler Duo Kit

Quantification using the ABI 7500 and Quantifiler DUO DNA Quantification Kit.

The DNA quantification assay combines a target-specific human DNA assay, target-specific human male DNA assay, and an internal PCR control (IPC) assay. The Quantifiler Duo DNA quantification kit quantifies the amount of amplifiable total human and human male DNA in a sample.

**Note* Thaw the reagents completely before using*

6.2.4.1.1: Preparing the DNA quantification standard

6.2.4.1.1.1: Qiagility Preparation

- a. Label 0.2ml tubes A-H and A-H
- b. Mix 300ul Diluent and 100ul Standard into the 0.5ml Tube
- c. Place 200ul from the 0.5ml tube into the each of the tubes labeled A
- d. Place the rest of the Diluent into one of the 2.0ml Tubes (You can pour. It doesn't have to be measured and doesn't have to be all of it)

- e. Make Master Mix: 250ul Reaction Mix and 210 Primer Mix into the other 2.0ml Tube.
- f. Turn on the Qiagility
- g. Start the program called Standards
- h. Replace the 50ul tips (top left) with the 200ul tips from the cabinet.
- i. Put the Master Mix in the center well of the 5 tube holder block
- j. Put the Diluent in the bottom left well of the 5 tube holder block
- k. Place the Tubes labeled A-H in the location for Standards
- l. Put a labeled plate on the machine.
- m. Start the machine, then place it on the 7500. You can just run the template for DUO. It has the standard as part of the layout.
- n. After running you need to export the results (just like casework)
- o. Log off of my account and back into yours
- p. Open the Duo QC Check. Go to Sheet 2
- q. Delete all the data on the page if anything is there.
- r. Go to DATA (On Menu Bar), Import External Data, Import Data
- s. Select your thumb drive and the file you exported
- t. Select Delimited and click next
- u. Select Tab and Comma then Finish
- v. Go to Sheet 1 and see if you have two passes on the first screen.

6.2.4.1.1.2: Manual Preparation

- a. Label eight microcentrifuge tubes: Std. A, Std. B, Std. C, etc. Vortex the Quantifiler Duo DNA Standard 3 to 5 sec.
- b. In tube labeled Std. A, dispense 150ul Quantifiler Duo dilution buffer
- c. To all tubes labeled Std. B thru Std. H, add 100ul Quantifiler Duo dilution buffer
- d. Add 50ul of standard into tube labeled Std. A. mix well.
- e. Add 50ul Std. A into tube labeled Std. B. Mix well
- f. Add 50ul Std. B into tube labeled Std. C. Mix well
- g. Add 50ul Std. C into tube labeled Std. D. Mix well
- h. Add 50ul Std. D into tube labeled Std. E. Mix well
- i. Add 50ul Std. E into tube labeled Std. F. Mix well
- j. Add 50ul Std. F into tube labeled Std. G. Mix well
- k. Add 50ul Std. G into tube labeled Std. H. Mix well

6.2.4.1.2: Preparing the Reactions

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Revision Date: 08/03/2015

Approved By: Morris, Miranda, Myhand, Melissa, Young, Hays, Channell, Kermit, Moran, Cindy, Black, Ryan

6.2.4.1.2.1: Qiagility Preparation

- a. Prepare Quantifiler master mix:
 - # wells (+ 3-5 extra) x 12.5ul PCR Reaction Mix
 - # wells (+ 3-5 extra) x 10.5ul Primer Mix
- b. Vortex and dispense 23ul of the master mix into each well a 96-well reaction plate.
- c. Add a total of 2ul of sample, standard or control to the appropriate well
- d. Seal the reaction plate with the optical adhesive cover
- e. Centrifuge the plate at 3000rpm for about 20 seconds

6.2.4.1.2.2: Manual Preparation

- a. Prepare Quantifiler master mix:
 - # wells (+ 3-5 extra) x 12.5ul PCR Reaction Mix
 - # wells (+ 3-5 extra) x 10.5ul Primer Mix
- b. Turn on the Qiagility
- c. Start the program called 7500
- d. Put the Master Mix in the center well of the 5 tube holder block
- e. Place the Tubes labeled A-H in the location for Standards
- f. Put a labeled plate on the machine
- g. Place sample tubes in the sample blocks according to the Qiagility Setup sheet that is printed from the excel sheet
- h. Press the **Green Triangle** Button to start run.
- i. Seal the reaction plate with the optical adhesive cover
- j. Centrifuge the plate at 3000rpm for about 20 seconds

6.2.4.1.3: Running the Reactions

- a. Turn on the computer and then turn on the instrument
- b. Position the plate in the instrument thermal block so that well A1 is in the upper-left corner
- c. Initialize the ABI software
- d. Select "New" from the File menu
- e. From the drop-down menu under **Template** select **DUO**
- f. Select "Import Samples Setup" from File menu
- g. **Save As-** with your file name (i.e. Init_MMDDYY.sds)
- h. On **Instrument** tab, press **start** when you are ready to run

6.2.4.1.4: Data Analysis

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- a. Press the **green triangle icon** to automatically analyze your run. All analyzed data is viewed under the **Results** tab
- b. In the **Results** tab, select the **Standard Curve** tab. Examine the standard curve to see if $R^2 \geq 0.98$. If not, outlying standard results can be eliminated to a minimum of one set of 6 standard measurements. If it still does not have a $R^2 \geq 0.98$, then the DNA quantities should be used with caution.

6.2.4.1.5: Results of analysis

- a. If samples results are negative, amplify using the maximum volume of extract allowed for the amplification kit.
- b. Overblown samples (<10ng/ul) can be diluted appropriately and amplified.

6.2.5: Amplification Protocols

6.2.5.1: Background

6.2.5.1.1: PowerPlex 16 HS

The PowerPlex® 16 HS System(a-e) allows co-amplification and three-color detection of sixteen loci (fifteen STR loci and Amelogenin), including Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818. One primer for each of the Penta E, D18S51, D21S11, TH01 and D3S1358 loci is labeled with fluorescein (FL); one primer for each of the FGA, TPOX, D8S1179, vWA and Amelogenin loci is labeled with carboxytetramethylrhodamine (TMR); and one primer for each of the Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818 loci is labeled with 6-carboxy-4',5'-dichloro-2',7'-dimethoxy-fluorescein (JOE). All sixteen loci are amplified simultaneously in a single tube and analyzed in a single injection or gel lane. (Table 1)

Table 1. The PowerPlex 16 HS PCR Amplification System

STR Locus	Label	Chromosomal Location	Alleles Included in PowerPlex 16 HS Allelic Ladder	Control 9947a	Control 2800M
Penta E	FL	15q	5 - 24	12, 13	7,14
D18S51	FL	18q21.3	8-10, 10.2, 11-13, 13.2, 14-27	15, 19	16,18
D21S11	FL	21q11-21q21	24, 24.2, 25, 25.2, 26-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33,	30, 30	29,31.2

			33.2, 34, 34.2, 35, 35.2, 36-38		
TH01	FL	11p15.5	4-9, 9.3, 10-11, 13.3	8, 9.3	6,9.3
D3S1358	FL	3p	12-20	14, 15	17,18
FGA	TMR	4q28	16-18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26-30, 31.2, 43.2, 44.2, 45.2, 46.2	23, 24	20,23
TPOX	TMR	2p24-2pter	6-13	8, 8	11,11
D8S1179	TMR	8q	7-18	13, 13	14,15
vWA	TMR	12p12-pter	10-22	17, 18	16,19
Amelogenin	TMR	Xp22.1-22.3 and Y	X, Y	X, X	X,Y
Penta D	JOE	21q	2.2, 3.2, 5, 7-17	12, 12	12,13
CSF1PO	JOE	5q33.3-34	6-15	10, 12	12,12
D16S539	JOE	16q24-qter	5, 8-15	11, 12	9,13
D7S820	JOE	7q11.21-22	6-14	10, 11	8,11
D13S317	JOE	13q22-q31	7-15	11, 11	9,11
D5S818	JOE	5q23.3-32	7-16	11, 11	12,12

6.2.5.1.2: PowerPlex Y23

The PowerPlex® Y23 PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies 23 Y-STR loci in a single PCR reaction. The kit amplified the following loci
The following table shows the loci amplified by the Y23 kit and the corresponding dyes used. The Y23 Kit Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder and the genotype of the Control DNA 2800M are listed in the table. (Table 3)

Table 3. The PowerPlex® Y23 PCR Amplification System

STR Locus	Label	Alleles Included in Y23 Allelic Ladder	Control 2800M
DYS576	Fluorescein	11-23	18
DYS389I	Fluorescein	9-17	14
DYS448	Fluorescein	14-24	19
DYS389II	Fluorescein	24-35	31
DYS19	Fluorescein	9-19	14
DYS391	JOE	5-16	10
DYS481	JOE	17-32	22
DYS549	JOE	7-17	13
DYS533	JOE	7-17	12
DYS438	JOE	6-16	9
DYS437	JOE	11-18	14
DYS570	TMR-ET	10-25	17
DYS635	TMR-ET	15-28	21
DYS390	TMR-ET	17-29	24
DYS439	TMR-ET	6-17	12

DYS392	TMR-ET	4-20	13
DYS643	TMR-ET	6-17	10
DYS393	CXR-ET	7-18	13
DYS458	CXR-ET	10-24	17
DYS385 a/b	CXR-ET	7-28	13, 16
DYS456	CXR-ET	11-23	17
Y GATA H4	CXR-ET	8-18	11

6.2.5.1.3: Yfiler

The AmpF ℓ STR[®] Yfiler[™] PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies 17 Y-STR loci in a single PCR reaction. The kit amplified the loci in the

- “European minimal haplotype” (DYS19, ~~DYS385a/b~~, ~~DYS389I/II~~, ~~DYS390~~, ~~DYS391~~, ~~DYS392~~, ~~DYS393~~)
- Scientific Working Group-DNA Analysis Methods(SWGDAM)-recommended Y-STR panel (European minimal haplotype plus ~~DYS438~~, and ~~DYS439~~)
- Additional highly polymorphic loci: ~~DYS437~~, ~~DYS448~~, ~~DYS456~~, ~~DYS458~~, ~~DYA635 (Y GATA C4)~~ AND Y GATA H4

The following table shows the loci amplified by the Yfiler kit and the corresponding dyes used. The AmpF ℓ STR Yfiler Kit Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder and the genotype of the Control DNA 007 are listed in the table. (Table 4)

Table 4. The AmpF ℓ STR[®] Yfiler[™] PCR Amplification System

STR Locus	Label	Alleles Included in Yfiler Allelic Ladder	Control 007
DYS456	6-FAM	13-18	15
DYS389I	6-FAM	10-15	13
DYS390	6-FAM	18-27	24
DYS389II	6-FAM	24-34	29
DYS458	VIC	14-20	17
DYS19	VIC	10-19	15
DYS385 a/b	VIC	7-25	11,14
DYS393	NED	8-16	13
DYS391	NED	7-13	11
DYS439	NED	8-15	12
DYS635	NED	20-26	24
DYS392	NED	7-18	13
Y GATA H4	PET	8-13	13
DYS437	PET	13-17	15
DYS438	PET	8-13	12
DYS448	PET	17-24	19

6.2.5.2: Amplification Setup

6.2.5.2.1: PowerPlex 16 HS

For samples amplified using PowerPlex 16 HS chemistries.

Create a master mix of PCR reagents by combining the reagents following ratios:

PowerPlex 16 HS PCR Reaction Mix	5.0 μ L
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PowerPlex 16 HS Primer Set	2.5 μ L
----------------------------	-------------

Dispense 7.5 μ L of master mix and 17.5 μ L of sample into a 96-opti well plate, cover the plate with PCR septa. Briefly spin the plate in the centrifuge and place into the thermocycler. To start the run, follow these steps:

1. Turn on the power to the thermocycler
2. Select the appropriate program
3. Ensure the proper volume is entered
4. Start the program

The following are the thermocycler parameters that are used during amplification of PowerPlex 16 HS:

96°C	2min	
ramp 100% to 94°C		30sec
ramp 29% to 60°C		30sec
ramp 23% to 70°C		45sec
10cycles		
ramp 100% to 90°C		30sec
ramp 29% to 60°C		30sec
ramp 23% to 70°C		45sec
22cycles		
60°C	30min	
4°C	forever	

6.2.5.2.2: PowerPlex Y23

For samples amplified using PowerPlex Y23 chemistries.

Create a master mix of PCR reagents by combining the reagents following ratios:

Y23 PCR 5x Master Mix 5.0 μ L

Y23 10x Primer Pair Mix 2.5 μ L

Dispense 7.5 μ L of master mix and 17.5 μ L of sample into a 96-opti well plate, cover the plate with PCR septa. Briefly spin the plate in the centrifuge and place into the thermocycler. To start the run, follow these steps:

1. Turn on the power to the thermocycler
2. Select the appropriate program
3. Ensure the proper volume is entered
4. Start the program

The following are the thermocycler parameters that are used during amplification of PowerPlex Y23:

96°C 2min

94°C 10sec

61°C 1min

72°C 30sec

29cycles (ramp speed should be set at Max mode)

60°C 20min

4°C forever

6.2.5.3: Sample Setup for the 3500xl Instrument

6.2.5.3.1: PowerPlex 16 HS

After amplification is complete, samples are set up for the 3500xl. A 96 opti-well plate is used. Create a master mix solution in the following ratios:

1.0 μl of Internal Lane Standard (ILS-600)

9.0 μl of HiDi Formamide

1. Pipette 10 μl of mix into each well used.
2. Ensure that all the wells of an injection contain master mix. The 3500xl should never inject sample from a dry well.
3. Add 1 μl of sample to each well (a multi-channel pipette is beneficial).
4. Add 1 μl of ladder to each ladder sample. At minimum, 1 ladder per plate must be present.
5. Briefly spin the plate in the centrifuge.
6. Heat the plate for approximately 3 minutes.
7. Chill the plate for approximately 3 minutes.
8. Place the plate into the 3500xl instrument. The plate only fits into the instrument in one direction.

6.2.5.3.2: Y23

After amplification is complete, samples are set up for the 3500xl. A 96 opti-well plate is used. Create a master mix solution in the following ratios:

1.0 μl of Internal Lane Standard (ILS-500)

10.0 μl of HiDi Formamide

1. Pipette 11 μl of mix into each well used.
2. Ensure that all the wells of an injection contain master mix. The 3500xl should never inject sample from a dry well.
3. Add 1 μl of sample to each well (a multi-channel pipette is beneficial).
4. Add 1 μl of ladder to each ladder sample. At minimum, 1 ladder per plate must be present.
5. Briefly spin the plate in the centrifuge.
6. Heat the plate for approximately 3 minutes.

7. Chill the plate for approximately 3 minutes.
8. Place the plate into the 3500xl instrument. The plate only fits into the instrument in one direction.

6.2.5.3.3: Yfiler

After amplification is complete, samples are set up for the 3500xl. A 96 opti-well plate is used. Create a master mix solution in the following ratios:

0.5 μ l of GS 600
8.5 μ l of HiDi Formamide

1. Pipette 9 μ l of master mix into each well used.
2. Ensure that all the wells of an injection contain master mix. The 3500xl should never inject sample from a dry well.
3. Add 1 μ l of sample to each well (a multi-channel pipette is beneficial).
4. Add 1 μ l of ladder to each ladder sample. At minimum, 1 ladder per plate must be present.
5. Briefly spin the plate in the centrifuge.
6. Heat the plate for approximately 3 minutes.
7. Chill the plate for approximately 3 minutes.
8. Place the plate into the 3500xl instrument. The plate only fits into the instrument in one direction.

6.2.5.4: 3500xl Instrument Setup

PowerPlex 16HS, Y23, and Yfiler

1. Go to Library
2. Click on Import
3. Select the txt file to import.
4. Click 'OK'

5. It is best to start the oven approximately 15 minutes before the run starts.
 - a. Go to the Dashboard
 - b. Press the Pre-Heat Button
6. Link the appropriate plate to the plate map under the 'Load Plates for Run'.
7. To start the run Click on the Start Run Button

6.2.5.5: Analysis of Raw Data / GeneMapper ID-X

6.2.5.5.1: PowerPlex 16 HS

GeneMapper ID-X analysis software is used to analyze the raw data collected by the 3500xl Genetic Analyzer.

- A matrix file is applied to the raw data to create a single baseline as well as to correct for spectral overlap and produce peaks of the five individual colors.
 - A size curve is created using co-injected DNA fragments of known size and the unknown peaks are assigned a size by interpolation.
1. Open the GeneMapper ID-X program with a blank project window or from the GeneMapper ID-X program select **File>Add Samples to Project**.
 2. Select the appropriate run folder saved on the DNA drive and click **Add to List**. Once all samples have been added to the list, click **Add** to import the files.
 3. In the Sample Type column, assign the correct sample type to each sample (i.e. sample, ladder, control)
 4. Select Analysis Method.
 5. Select **PowerPlex_16_IDX_alpha** as the Panel.
 6. Select **ILS 600** as the Size Standard.
 7. Click the green arrow to analyze the project.

8. View the raw data to examine the ILS. Verify that the analysis range is between 60bp and 600bp and the peaks are correctly labeled.
9. Review controls
 - Display each control (including positive and negative amplification controls, and blank controls).
 - If peaks above 175 RFU are observed in the negative controls, the sample can be re-injected.
 - Examine the Positive control and verify the correct calls of the alleles.
10. Examine the allelic ladders.
 - Verify that the allelic ladder is called correctly for each marker.
11. Analyzed samples can be viewed as a group or individually by highlighting the samples to view. After selecting the sample click the **Display Plots** button. There are several options available to view the electropherogram.
12. Edit any labels as appropriate e.g. spike, background, -A
13. Review the remaining sample files. Evaluate the following parameters:
 - Peak shape and height (optimal values between 1000-6000 RFU, although acceptable and type able signals may occur outside of this range).
 - Matrix quality (baselines should be relatively flat and there should not be a pattern of pronounced peaks or dips below true DNA peaks in the other four colors).
 - Peak profile (examine for artifactual peaks e.g. spikes).

6.2.5.5.2: Y23

GeneMapper ID-X analysis software is used to analyze the raw data collected by the 3500xl Genetic Analyzer.

- A matrix file is applied to the raw data to create a single baseline as well as to correct for spectral overlap and produce peaks of the five individual colors.
- A size curve is created using co-injected DNA fragments of known size and the unknown peaks are assigned a size by interpolation.

1. Open the GeneMapper ID-X program with a blank project window or from the GeneMapper ID-X program select **File>Add Samples to Project**.

2. Select the appropriate run folder saved on the DNA drive and click **Add to List**. Once all samples have been added to the list, click **Add** to import the files.
3. In the Sample Type column, assign the correct sample type to each sample (i.e. sample, ladder, control)
4. Select Analysis Method.
5. Select **PowerPlexY23_IDX_v1.0** as the Panel.
6. Select **CC5_ILS_500_IDX** as the Size Standard.
7. Click the green arrow to analyze the project.
8. View the raw data to examine the ILS. Verify that the analysis range is between 60bp and 500bp and the peaks are correctly labeled.
9. Review controls
 - Display each control (including positive and negative amplification controls, and blank controls).
 - If peaks above 175 RFU are observed in the negative controls, the sample can be re-injected.
 - Examine the Positive control and verify the correct calls of the alleles.
10. Examine the allelic ladders.
 - Verify that the allelic ladder is called correctly for each marker.
11. Analyzed samples can be viewed as a group or individually by highlighting the samples to view. After selecting the sample click the **Display Plots** button. There are several options available to view the electropherogram.
12. Edit any labels as appropriate e.g. spike, background, -A
13. Review the remaining sample files. Evaluate the following parameters:
 - Peak shape and height (optimal values between 1000-6000 RFU, although acceptable and type able signals may occur outside of this range).

- Matrix quality (baselines should be relatively flat and there should not be a pattern of pronounced peaks or dips below true DNA peaks in the other four colors).
- Peak profile (examine for artifactual peaks e.g. spikes).

6.2.5.5.3: Yfiler

GeneMapper ID-X analysis software is used to analyze the raw data collected by the 3500xl Genetic Analyzer.

- A matrix file is applied to the raw data to create a single baseline as well as to correct for spectral overlap and produce peaks of the five individual colors.
- A size curve is created using co-injected DNA fragments of known size and the unknown peaks are assigned a size by interpolation.

1. Open the GeneMapper ID-X program with a blank project window or from the GeneMapper ID-X program select **File>Add Samples to Project**.
2. Select the appropriate run folder saved on the DNA drive and click **Add to List**. Once all samples have been added to the list, click **Add** to import the files.
3. In the Sample Type column, assign the correct sample type to each sample (i.e. sample, ladder, control)
4. Select **ASCL_Y_3500** as Analysis Method.
5. Select **Yfiler_v1x** as the Panel.
6. Select **GS600_LIZ** as the Size Standard.
7. Click the green arrow to analyze the project.
8. View the raw data to examine the LIZ. Verify that the analysis range is between 60bp and 600bp and the peaks are correctly labeled.
9. Review controls
 - Display each control (including positive and negative amplification controls, and blank controls).
 - If peaks above 125 RFU are observed in the negative controls, the sample can be re-injected.
 - Examine the Positive control and verify the correct calls of the alleles.

10. Examine the allelic ladders.
 - Verify that the allelic ladder is called correctly for each marker.
11. Analyzed samples can be viewed as a group or individually by highlighting the samples to view. After selecting the sample click the **Display Plots** button. There are several options available to view the electropherogram.
12. Edit any labels as appropriate e.g. spike, background, -A
13. Review the remaining sample files. Evaluate the following parameters:
 - Peak shape and height (optimal values between 1000-6000 RFU, although acceptable and type able signals may occur outside of this range).
 - Matrix quality (baselines should be relatively flat and there should not be a pattern of pronounced peaks or dips below true DNA peaks in the other four colors).
 - Peak profile (examine for artifactual peaks e.g. spikes).

6.2.5.6: Analysis with ArmedXpert Software

ArmedXpert is a Mixture analysis tool that assists the analyst in deconvoluting a mixture based on the peak heights detected and parameters set from validation studies. The software calculates the probable genotypes then the analyst selects the genotype(s) to associate with each contributor. All cases & samples are to go through ArmedXpert (AX), except if the sample has a major that will not be used for statistics and the minor is inconclusive, in this case the major will be designated on the call sheet and the JusticeTrax electros become the accurate representation of the calls.

1. Combining 12 and 24 second injections of the same run into one GeneMapper Project
 - a. Add samples to GM project as usual starting with the 12sec injection and analyze as usual with ASCL_PP16HS Analysis Method
 - b. Once you have determined which samples require reinjection at 24sec, import those samples from your 24sec injection data into the same GM Project. This

will show up as a second branch on the Project tree on the left side of the screen. Note that you can view all samples (12 & 24 sec) by selecting the top-most “project” header, or you can view only samples for 12 or 24 sec injection by selecting the respective name from the project tree.

- c. For 24sec samples, change the Sample Name (in the GM Project) to indicate 24sec injection (e.g. add “_24” suffix to the sample name). This will allow you to differentiate between 12 and 24sec data on the AX call sheet. Don’t forget to also do this for all necessary 24sec controls.
 - d. Mixtures that need interpretation should be reanalyzed using ASCL_PP16HS_AXPT Analysis Method. This will remove GM stutter filter before the sample is exported to AX. Non-stutter artifacts should be deleted in GM before export.
2. Importing samples to Excel and ArmedXpert from GeneMapper
- a. In GM, select “ArmedXpert Export” from the Table Setting drop down menu
 - i. In GM, make sure to select the top line of the “Project” tree so that both 12 and 24 sec samples are exported.
 - ii. File -> Export Combined Table
 - iii. In pop up window select “one line per sample” (all other defaults are correct) and export file to your AX folder on the S drive
 - b. Import this data to make your call sheet for 12 & 24 second data in Excel:
 - i. On the “Final” page of the Excel sheet (which has been updated on the S drive) there is a new set of buttons titled “For PP16HS” to the right of the old ones; use these to import your AX export file and print your call sheets. There is no need to check the columns on the left if you are using a combined (12 & 24sec) GM project. For single source samples, this is the sheet that needs to match your JTX electro calls. For samples that will require mixture interpretation, these will be deconvoluted and

additional data will be provided (described further in section 5) to specify major/ minor or other interpretations.

- ii. On the Excel call sheets, use the radio buttons to the right of each sample name to make notations for reinjection, etc. Loci with alleles <500RFU will be shaded in grey automatically.
 - c. In ArmedXpert: Click the helix button, click “import”, click “other” and select file to import. This will open an Excel-style sheet with all of your samples and allele calls listed.
3. Sample Comparison Check. This no longer happens automatically in AX so you need to perform it on the call sheet (described above in 2.d.).
- a. In AX main page on the top bar menu, select “Reporting” tab, click the “Samples Comparison” button.
 - b. In the pop up window, select the name of your text file in both the “Reference” box and the “Comparison” box. (This will compare your project to itself.) On the bottom right of the pop up window, scroll down and select “Staff” (all other defaults are correct). Press the “Match” button.
 - c. An excel-style Comparison sheet will be produced. If there are any TRUE matches (e.g. contamination to staff or other samples) (far right column), the page needs to be printed and documented in the applicable case file(s). If there are no true matches, this page does not need to be printed.
 - d. Save the comparison with your project name (regardless of if there are matches) to your ArmedXpert folder on the S drive.
4. Statistics on single source profiles: on “Interpretation” tab, select the desired stat button (e.g. RMP or Single Source) and use your mouse to select the sample on your table that you’d like to perform this stat on.
- a. Note that “Single Source” should be used for a straight profile and “RMP” should be used for a deconvoluted mixture (even if that is on the major component of the mixture)

- b. Print the statistics for your file: Select the helix button on the top left of the AX screen and select “Print” in the pop up menu. The follow settings are suggested: Portrait, Fit to page 1 wide, 2 tall.
5. Mixture Interpretation: on “Interpretation” tab, select “Begin Mixture Interpretation” and select (via mouse or drop down menu) the sample you’d like to work with.
- a. Check the “Multi PHr” button
 - b. Change the number of contributors if necessary
 - c. Change the Stutter to 100% then check the “Apply Globally” box
 - d. Apply a reference if appropriate (only set apply a reference if it is an intimate source sample or if it is stated to be from that individual)
 - i. Click “References” button
 - ii. Click “Select a reference to apply”
 - iii. Select sample
 - iv. The Autocall reference button can be used to automatically select the reference genotype for each loci.
 - e. Select genotypes for each contributor at each locus (e.g. using Popout calls).
 - i. Note that minor alleles do not necessarily have to be assigned to a contributor (e.g. if you have a minor that will be inconclusive).
 - ii. Name designations for contributors should be the default “Profile 1, Profile 2, etc.” with the major component being Profile 1 and any profiles with a Reference applied designated with a “(K#)” suffix (e.g. Profile 1 (K1) noting the known sample applied to that profile.
 - f. Troubleshooting: Note that the procedures described in steps i. and ii. below will affect only the locus in question – once you have started the interpretation, these changes will not be applied globally (to the entire profile)
 - i. If peak height imbalance is causing the mixture information to advise you that there are no options for this locus, this can be addressed by adjusting the “PHr” button on the Mixture Interpretation page. Usually, toggling that down a bit will help.

- ii. When AX accounts for stutter, it reduces the allele peak height by the stutter percentage. Occasionally, this will cause true minor peaks to fall below analytical threshold. If you believe that has happened with your sample, you can back off of the 100% stutter. Keep in mind that stutter ratios applied are the max values validated at that locus and can vary between alleles within a locus)
- iii. If you have other adjustments to your interpretation of that locus it should be noted using the “Add Comment” button (use the button itself, not it’s drop-down menu)
- iv. Notes that apply to the entire mixture interpretation (e.g. assumptions) should be noted using the “Add Comment” drop-down menu option “Add comment to Mixture Interpretation”
- v. These modifications & comments will show up in the final “Mixture Interpretation” notes generated by AX.
- g. Recording your mixture deconvolution: On mixture interpretation page, click the “view call report” button. This will open an excel-style workbook.
 - i. The “Calls” page shows your mixture in different formats; the entire mixture, and the deconvoluted components.
 - 1. The allele calls for the un-deconvoluted profile on this sheet are what needs to match your electros in JTX. It is recommended that you re-analyze mixture sample electros using the usual ASCL_PP16HS Analysis Method (which will remove most stutter) and add back in any true peaks as determined by AX before imaging your electro into JTX.
 - 2. Print this page for your case file. Recommended settings: Landscape; Fit to pages 1x1
 - ii. The “Mix Interp” page shows your mixture interpretation details including any comments or adjustments that you may have made. Print this for your file if necessary. Note that printing “Mix Interp” is not necessary in all circumstances (e.g. if you have a major but minor will be reported as

- inconclusive). Recommended settings: Portrait, Zoom to 100%
- iii. Before exiting, save your Mixture Interpretation to your AX folder on the S drive (AX will prompt you to save when you try to exit)
 - h. Statistics on a deconvoluted mixture: Start from the “Calls” page of the call report (see above). From top bar menu, select “Interpretation” and then the stats that you want to use (e.g. “RMP”) and use the mouse to highlight what you want to do stats on (e.g. the major or minor component of your deconvoluted mixture). This will open an excel-style window with your statistics.
 - i. The “Frequencies” page will list your statistics.
 1. Adjust the number of contributors (box at the bottom) if necessary.
 2. Make any other changes that you determined / commented on during mixture deconvolution (e.g. homozygote not a viable option for X locus)
 3. Print the stats for your file. Recommended settings: Portrait; Fit to pages 1x5 (it will automatically scale back if it can fit it in less pages)
 - ii. The “Calculations” page is for your reference and does not need to be printed.
 - iii. If you made any changes, it is recommended that you save the Frequency Analysis to your AX folder on the S drive (AX will prompt you before you close that window)
 - iv. Compare deconvoluted mixture to known samples if applicable (see 6.b. below). Note that this page can substitute for your “calls” page described in 8.g.i. since it contains redundant information.
 6. Checking References: on the AX main page top bar menu, select “Match & Comparison”
 - a. “Find where reference is included” should be used when comparing a known to a batch or a sample
 - b. “Find Included in the Reference” should be used to compare deduced profiles to known samples
 7. Committing Samples to the database: On main AX top bar menu, select “Data” tab, click “Commit Samples to Data Source”. In the pop-up window highlight your data file from the list on the left and

use the “>” to add it to the right side of the window. Click the “Commit” button.

8. Accessing ME sample profiles in AX: Under the “Data” tab on the main page, select “Load samples from data source”. In the pop-up window “Search:” field, type your ME sample # (or just “ME” and check the “search profiles” box. A list will show on the left side of the window. Select the sample(s) that you want and move them to the right side of the window using the “>” button. Press the “Load” button.

6.2.5.7: Autosomal STR Interpretation Guidelines

The purpose of these guidelines is to establish a general framework and outline minimum standards to ensure that:

- Conclusions in casework reports are scientifically supported by the analytical data, including that obtained from appropriate standards and controls;
- Interpretations are made as objectively as possible, consistently from analyst to analyst, and within established limits.

The goal of the evaluation and interpretation of amplified STR data is to determine the DNA profile(s) of the donor(s) of the questioned samples for comparison to reference sample profiles.

- A peak is defined as a distinct, triangular section of an electropherogram.
- Genotypes are determined from the diagnostic peaks of the appropriate color and size range for a particular locus.

6.2.5.7.1: Analytical Threshold

The minimum peak height threshold will be set at 175 (Relative Fluorescent Unit) RFU for PowerPlex 16 HS for software recognition of a peak. The analytical threshold is the value that denotes a signal is above the background noise and is considered a true peak and it is set at 175 RFU for PowerPlex 16 HS. Optimal peak height values range between 1000-4000 RFU, although acceptable and typeable signals may occur outside of this range.

6.2.5.7.2: Stochastic Threshold

The stochastic threshold is the value that denotes both peaks for a heterozygous locus will be detected and it is set at 500 RFU for PowerPlex 16 HS.

6.2.5.7.3: Peak Height Ratio

Peak height ratios of heterozygote alleles are defined as the ratio of the lower peak's height to the higher peak's height, expressed as a percentage. Peak height ratios were examined in the validation data and threshold were determined based on peak heights. Peak height of the lower sister allele dictates which ratio to use below.

Peak Height	Ratio
>2500 RFU	60%
1500-2500 RFU	50%
<1500 RFU	30%

Homozygote allele peak heights are approximately twice that of heterozygotes as a result of a doubling of the signal from two alleles of the same size.

6.2.5.7.4: Off Ladder Variants

Off ladder (OL) calls are first converted to size in base pairs (bp), then compared to the size of the appropriate ladder alleles and the allelic designation is determined. If the OL is not a "perfect" repeat, but rather varies by 1, 2 or 3 bp from a ladder allele, then it will be designated as an integer of that variation. For example, if a green OL peak size is 238.39 bp, and the 36 allele of the **D21S11** ladder is 236.32 bp, then the peak will be designated a **D21S11** 36.2. If an allele falls above the largest or below the smallest peak of the sizing ladder, the allele will be designated as either greater than (>) or less than (<) the respective ladder allele.

The analyst will re-amplify or re-inject, then type any sample containing a peak not properly interpreted as an allele by the software, especially if it is not appropriately balanced with an associated allele or at a height expected for a homozygote.

An off ladder variant which has been seen and confirmed at least two times in the population sampled at the Arkansas State Crime Laboratory is no longer considered a rare variant. These

peaks can be confidently and accurately called without confirmation.

6.2.5.7.5: Tri-Allele

A tri-allelic system is one which contains three distinct alleles, rather than the normal one or two. In order to insure that the sample is a true tri-allelic specimen, the sample should be re-amplified and run a second time. However, if observed in overlapping systems or in multiple samples from the case, tri-allelic loci may be considered confirmed. If there is not enough extract left for re-amplification, the sample may be re-loaded. However, if the tri-allelic sample cannot be confirmed, the locus may be reported as inconclusive or a technical note may be recorded in the case file (the Casework Supervisor or Technical Leader may need to be notified to determine how to report the locus).

6.2.5.7.6: Artifacts

Artifacts can occur and need to be recognized. These may include, but are not limited to, the following: spikes, pull-up, stutter and non-template nucleotide addition.

6.2.5.7.6.1: Spikes

Spikes are artifactual peaks usually observed in at least two colors. Spikes can be caused by urea crystals in the capillary, power surges, or other instrument related issues. A spike will not exhibit the same morphology as a peak, but will be sharper or "spike" shaped. Spikes are unique to fragments analyzed using capillary electrophoresis. Spikes will have fragment sizes which vary only slightly in the 3500xl data. Above threshold spikes should be noted and may be re-injected.

6.2.5.7.6.2: Stutter

In addition to an allele's primary peak, artifactual minor "stutter" peaks can occur at four-base intervals. The most common stutter peaks observed in all loci are four bases smaller than the primary peak ("n-4"). It is also possible to

see additional “n+4” peaks (four bases larger), especially when excessive amounts of DNA are amplified.

Stutter peaks are evaluated by examining the ratio of the stutter peak height to the height of the appropriate adjacent allele, expressed as a percentage. The height of stutter peaks can vary by locus, and longer alleles within a locus generally have a higher percentage of stutter. The maximum expected percentage of stutter is less than 25% for any locus. Peaks in the stutter positions greater than this value may indicate the presence of a mixture. In addition to a mixed sample, stutter peaks may be elevated above established thresholds by the following:

Analyzed peak heights above the optimal range may be “off-scale” in the raw data, meaning that the CCD camera may be saturated. While the GeneMapper ID-X software will alert the analyst to any off-scale raw data peaks, the analyzed peak may be assigned a lower value due to smoothing and base-lining functions. Therefore, the observed percent stutter will be inaccurately high. If the stutter peak is greater than the maximum allowed and the primary peak is above 20,000 RFU and/or has been labeled off-scale, the analyst should interpret the results with caution. The sample may be re-amplified with less input DNA or re-injected.

PowerPlex 16 HS Loci Stutter Ratios

LOCUS	-4 STUTTER RATIOS	+4 STUTTER RATIOS
D3S1358	.13	.023
TH01	.06	
D21S11	.22	.045
D18S51	.13	
Penta E	.13	
D5S818	.11	.028
D13S317	.12	.031
D7S820	.1	.042
D16S539	.13	.03
CSF1PO	.1	.021
Penta D	.06	
AMEL	0	
vWA	.14	.014
D8S1179	.11	.025
TPOX	.06	.028

FGA	.14	.031
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6.2.5.7.6.3: Non-Template Nucleotide Addition (-A)

Amplification conditions have been set to maximize the non-template addition of a 3' terminal nucleotide by DNA polymerase. Failure to attain complete terminal nucleotide addition results in "band splitting", visualized as two peaks one base apart. This is most often seen when an excessive amount of DNA is amplified or amplification is performed under sub-optimal PCR conditions.

6.2.5.7.6.4: Pull-Up

Small artifactual peaks can appear in other colors under true peaks. This phenomenon is termed "pull-up". Pull-up is a result of spectral overlap between the dyes, which is normally corrected for by the spectral calibration. If a pull-up peak is above the minimum peak height detection threshold, it will be sized at approximately the same size as the true peak. Pull-up can occur as a result of the following:

- Application of a sub-optimal spectral can cause pull-up. If necessary, spectral standards can be injected on the same capillary after the analytical run and a new spectral can be made and applied.
- Amplification using excess input DNA can lead to off-scale peaks. The matrix may not perform properly with off-scale data.

6.2.5.7.6.5: Other

In addition to amplification artifacts described above the following anomalies can arise during electrophoresis and analysis:

Significant room temperature fluctuation may result in size variation between injections such that allelic ladder peaks differ by more than 0.5 bp from allelic peaks in other injections. This will disrupt sample analysis using the GeneMapper ID-X program. Analyzing samples with an injection of allelic ladder nearest the questioned samples

may alleviate this problem. If desired, the sample(s) and an allelic ladder may be re-injected to confirm the typing.

Artifactual peaks of a single color will not display the typical spectral overlap characteristic of the five fluorescent dyes in the raw data. Peak width may not be similar to the peaks resulting from dye-labeled DNA. These peaks can be shown to be artifactual by re-injection of the sample.

6.2.5.7.7: Detection of Single Source Samples

A sample is consistent with being from a single source if each locus typed has only one or two alleles. For an apparent single-source questioned sample, compare the results obtained from the questioned sample with the results from the known samples in the case. The determination of inclusions or exclusions is the responsibility of the analyst working the case. This determination is based on all tests and observations made for that sample.

6.2.5.7.8: Detection of Mixtures

Samples may contain DNA from more than one individual. A sample may be consistent with being a mixture if it exhibits one or more of the following characteristics at more than one locus:

- More than two alleles are present at a locus after stutter and other artifact considerations have been evaluated and dismissed as possible causes.
- A peak is present at a stutter location and its height is greater than the pre-determined stutter ratios of the height of the appropriate adjacent allele.
- Severely unbalanced peak height ratios exist for sister alleles of heterozygous genotypes within the profile. With the possible exception of low template amplifications, ratios less than 60% are rare in normal unmixed samples.

6.2.5.7.9: Interpretation of Mixed Samples

The interpretation applied to a mixed sample by the analyst in each particular case should be based upon all relevant information.

- If there is a dominant profile present in the mixture, one can determine any inclusions or exclusions by comparing the profiles from known reference samples with the dominant DNA types. Dominant types of a mixture can be assigned based on peak heights as well as peak height ratios.
- Profile frequency estimates and/or source attribution can be reported for the dominant DNA profile.
- Information can be gained from minor profiles observed in mixtures as to possible inclusion or exclusions of sources. Mixture statistics should be applied to the entire profile where the minor contributor cannot be clearly determined. Use of the ArmedXpert software can assist in the separating the mixture. RMP stats should be applied to deduced minors.
- When using ArmedXpert and more than one source of DNA is detected but cannot be separated, then all likely genotype combinations must be considered for each locus. (restricted RMP)
- In the event of a complex mixture where the number of contributors is determined to be 3 or more the sample can be marked as inconclusive. The interpretation and use of a complex mixture of this nature requires approval of the DNA Casework Supervisor, DNA Technical Leader, or the CODIS Administrator.

6.2.5.7.10: Incomplete / Partial STR Profiles

The possibility exists that not every locus will amplify. This can occur if the DNA is of limited quantity, severely degraded or if the DNA sample contains PCR inhibitors. Since loci are independent, any locus that shows results can be evaluated. Statistical applications can be used on the loci that gives results above 175 RFU for PowerPlex 16 HS. However there is a decrease in peak height heterozygosity with lower levels of DNA. One must interpret a homozygote near the threshold of interpretation with caution.

6.2.5.7.11: Partial Mixed STR Profiles

It is possible in a mixture that the entire profile from one of the contributors is not represented. This may be due to allele drop out or allele masking in a stutter position. In this situation an individual cannot necessarily be excluded from contributing to the mixed DNA profile and should be reported as “cannot be excluded from contributing to the mixed profile from ...” The affected areas will be left out of the statistical calculation.

6.2.5.7.12: STR Profile Interpretation

Following GeneMapper analysis, each sample is evaluated at each locus by visual identification of amplified product(s). The alleles are recorded and comparisons are made between the question samples and reference standards. Determining whether the genotype of one sample is consistent with the genotype of another sample is based on the analyst’s professional, trained judgment.

Inclusion: If the reference standard sample genotype(s) is/are present in the evidentiary or questioned sample(s), at all loci tested, the reference individual is included as a possible source of the DNA. If the evidence sample is degraded or limited alleles are obtained, the suspect can still be included if his/her alleles are consistent with that of the evidence. If the evidence sample is a mixture, and the suspect’s alleles are included in that mixture, he/she can still be included.

Exclusion: If at any locus tested, the reference standard sample genotype(s) is/are not present in the evidentiary or questioned single source sample(s), the reference individual is excluded as a possible source of the DNA. Exclusions in mixtures or degraded (partial) profiles will be determined by locus to locus comparison. Exclusions cannot be made on profile that have been deemed too limited for interpretational value.

Inconclusive: It is possible to obtain a STR profile that contains a complex mixture from multiple individuals, contains alleles that do not meet the analytical or stochastic threshold, or have poor peak morphology. In these cases the result may be reported as uninterpretable or inconclusive. If a sample is reported as inconclusive then a reason must be given in the report, (a. the complexity of the mixture, b. insufficient quality of data, c. limited amount of DNA) Example "The DNA profile obtained from Q1 is inconclusive for comparative purposes due to the complexity of the mixture

."

Criminal Paternity: In criminal paternity and missing person's cases (identity), exclusions are only reported if there are two or more loci inconsistent with the unknown individual when compared to relatives to account for mutations. The report must reflect when there is a one locus mismatch. It is noted that mutation rates can be obtained from STRbase and must be listed on the report. Any mutation occurring from father to male child should have Y-STR analysis performed for confirmation.

6.2.5.7.13: Statistical Calculations

The frequency of occurrence between allele fragments of samples reported as being consistent is determined for each polymorphic locus within a racial group.

The frequency associated with a particular pattern of alleles from a sample is based upon principles of Hardy-Weinberg equilibrium.

If the sample under analysis demonstrates two alleles, the frequency is determined by the equation $2pq$, where p and q represent the frequencies of allele #1 and #2.

If the sample under analysis consists of a single allele, the frequency is determined by the equation $p^2 + p(1-p)\emptyset = 0.01$ and p represents the frequency of the allele.

If a known sample consists of more than two alleles at a particular locus, no frequency data will be generated for that locus.

The frequency for the overall DNA pattern resulting from the alleles detected at different loci, termed a profile, is determined by multiplying the genotype frequency obtained from each locus.

Procedure for calculating allele and genotype frequencies:

The following represents an example of data collected from a PCR database and the procedures used to determine the allele and genotype frequencies.

Example: TH01 locus in Caucasian population (n = 209)

Allele frequency:

Frequency of allele = Number of times the allele was observed out of all possible alleles for a particular locus/2n.

Minimum Allele Frequency (NRC II, 1996)

NOTE: This method requires that a minimum of 5 copies of an allele before the allele frequency can be used for calculation of genotype frequency.

Example:

This estimate is strictly driven by database size:

Minimum allele frequency = 5/2N
N = the number of individuals in the database

For the 13 allele at vWA:
Actual allele frequency = 2/392 = .0051
Minimal allele frequency = 5/392 = .0128

This method is conservative and also addresses some substructure effects.

Expected Genotype Frequency:

Based on the assumption that the TH01 genetic locus is in Hardy-Weinberg equilibrium, the expected genotype frequencies are calculated from the allele frequencies, as in the following examples:

TH01 Genotype 7, 7:

$$(\text{Frequency of 7 allele})^2 + \text{Frequency of the 7 allele} (1 - \text{Frequency of 7 allele}) = (0.141)^2 + 0.141(1 - 0.141) = 0.021$$

OR

TH01 Genotype 7, 9.3:

$$2(\text{Frequency of 7 allele})(\text{Frequency of 9.3 allele}) = 2(0.141)(0.340) = 0.096$$

6.2.5.7.13.1: ArmedXpert

ArmedXpert software allows you to determine the probability of drawing a specific DNA profile at random from a given population. The calculations show whether the probability of a random match with the DNA profile in question is high or low.

- a. A high probability indicates that the profile's characteristics are quite common within the population. Thus, the profile does not distinguish itself from others in the population
- b. A low probability indicates that the profile's characteristics are quite rare. This strongly indicates that the profile represents the individual in question.

6.2.5.7.13.2: Single Source Stain

Statistical significance for single source stains will be calculated according to the following:

- a. Statistical significance will be expressed as an inverse probability of inclusion. (profile frequency = 0.00020 = 1 in 5,000)
- b. For homozygotes (AA),

Recommendation 4.1 – National Research Council report “The Evaluation of Forensic DNA Evidence” 1996, the following formula will be used:

$$p^2 + p(1-p)\theta$$

$\theta = 0.01$ for general US population

$\theta = 0.03$ for isolated groups (American Indians)

- c. For heterozygotes (AB), the formula : $F = 2pq$
- d. For all loci to identify the match: $F = (f_1 \times f_2 \times f_3 \dots)$
- e. Inverse probability = $1/F$

6.2.5.7.13.3: Mixed DNA Stains

If the profile is to be treated as a DNA mixture, then the profile can be analyzed in one of the following manners:

- a. **Mixture formula:** *based on the genotypes selected for calculation. Formula used for calculation can be seen on the calculation page in ArmedXpert.*

The inverse probability (1/F) will be reported.

OR:

- b. **Likelihood ratio:** For each mixed DNA profile E, the likelihood ratio,

$$L = P(E|C_x) / P(E|C_y)$$

For comparing two explanations, C_x and C_y , this mixed profile is calculated where $P(E|C_x)$ is the probability of the profile E to have arisen under explanation C_x . An input screen with three grids allows the user of Popstats to enter the mixed profile; the alleles of the mixed profile which are believed to come from x unknown contributors under explanation C_x ; and the alleles of the mixed profile which are believed to come from y unknown contributors under explanation C_y . The user also has to specify what the number of unknowns, x and y , are for explanations C_x and C_y , respectively. The number of unknown contributors must be strictly greater than half of the number of those bands/alleles they contribute for each locus. **For example:** If there are 3 bands for locus Th01, 4 bands for locus vWA, and 2 alleles for locus TPOX from x contributors, then x has to be strictly greater than $3/2$, $4/2$ and $2/2$. Therefore, $x \geq 2$.

OR:

- c. **Probability of Exclusion (PE)**

Note CPE / CPI can only be performed on loci that do not show signs of dropout (peaks below the stochastic threshold, unless all expected peaks are present)

Example: STR Alleles in a mixture 11, 13, 15

Allele 11 = 0.122
Allele 13 = 0.176
Allele 15 = 0.041

- P (probability) = 0.339 , Q = 1 – P = 0.661
- P.E. (probability of exclusion)
- CPE (Combined Probability of Exclusion)
- $CPE = 1(1-PE_i)(1-PE_j)(1-PE_k)$
- $CPE = 1-(1-.885)(1-.398)(1-.505)$
- $CPE = 1-(.115)(.602)(.459)$
- $CPE = 1-(0.034)$
- $CPE = 0.966$

With a CPE of 0.966, 96.6% of unrelated (Caucasians) would be expected to be excluded as contributors to the observed DNA Mixture.

- CPI (Combined Probability of Inclusion)
- 3.4% of unrelated (Caucasians) could not be excluded as contributors to the observed DNA mixture.

6.2.5.7.13.4: Population Database

The Arkansas State Crime Laboratory utilizes the FBI database with is reference below.

Reference: Budowle B., Moretti TR, Baumstark AL, Defenbaugh DA, Keys KM

“Population Data on the Thirteen CODIS Core Short Tandem Repeat Loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians” J Forensic Science

6.2.5.8: Y-STR Interpretation Guidelines

The interpretation of results in casework is a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-set rule. However, it is important that the laboratory develops and adheres to minimum criteria for interpretation of analytical results. These criteria are based on validation studies, literature references, and casework. It is to be expected that these interpretation guidelines will continue to evolve as the technology and collective experience of the laboratory grows.

The purpose of these guidelines is to establish a general framework and outline minimum standards to ensure that:

- Conclusions in casework reports are scientifically supported by the analytical data, including that obtained from appropriate standards and controls;
- Interpretations are made as objectively as possible, consistently from analyst to analyst, and within established limits.

The goal of the evaluation and interpretation of amplified STR data is to determine the DNA profile(s) of the donor(s) of the questioned samples for comparison to reference sample profiles.

- A peak is defined as a distinct, triangular section of an electropherogram.
- Haplotypes are determined from the diagnostic peaks of the appropriate color and size range for a particular locus.

6.2.5.8.1: Threshold

The minimum peak height threshold will be set at 175 (Relative Fluorescent Unit) RFU for Y23 software recognition of a peak. The interpretation threshold is set at 175 RFU for Y23. Optimal peak height values range between 1000-4000 RFU, although acceptable and typeable signals may occur outside of this range.

If no alleles are detected in a locus then a single dash (-) will be placed on the call sheet for the locus.

6.2.5.8.2: Off Ladder Variants

Off ladder (OL) calls are first converted to size in base pairs (bp), then compared to the size of the appropriate ladder alleles and the allelic designation determined. If the OL is not a "perfect" repeat, but rather varies by 1, 2 or 3 bp from a ladder allele, then it will be designated as an integer of that variation. For example, if a green OL peak size is 238.39 bp, and the 13 allele of the **DYS19** ladder is 236.32 bp, then the peak will be designated a **DYS19** 13.2. If an allele falls above the largest or below the smallest peak of the sizing ladder, the allele will be designated as either greater than (>) or less than (<) the respective ladder allele.

The analyst will either re-amplify or re-inject, then type any sample containing a peak not properly interpreted as an allele by the software.

An off ladder variant which has been seen and confirmed at least two times in the population sampled at the Arkansas State Crime Laboratory is no longer considered a rare variant. These peaks can be confidently and accurately called without confirmation.

6.2.5.8.3: Artifacts

Artifacts can occur and need to be recognized. These may include, but are not limited to, the following: spikes, pull-up, stutter and non-template nucleotide addition.

6.2.5.8.3.1: Spikes

Spikes are artifactual peaks usually observed in at least two colors. Spikes can be caused by urea crystals in the capillary, power surges, or other instrument related issues. A spike will not exhibit the same morphology as a peak, but will be sharper or "spike" shaped. Spikes are unique to fragments analyzed using capillary electrophoresis. Spikes will have identical fragment sizes in the ABI 3500xl data, and fragment sizes which vary only slightly in the ABI 3500xl data. Above threshold spikes should be noted and may be re-injected.

6.2.5.8.3.2: Stutter

These are the artifacts of the amplification process. These bands will be observed in the n-4, n-2 and n+3 positions of major peaks and will have a smaller peak height. GeneMapper ID will usually calculate the stutter percentage and factor out the stutter peaks. If a peak is in the stutter position and is called, the profile should be carefully studied to ensure that the peak is not from a mixture.

PowerPlex Y23 Stutter Ratio

Y-23 Stutter	-8	Standard	-2	+2, +3, +4
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Ratios		negative Stutter (-3,-4,-5)		
DYS576	0.059	0.163		0.034
DYS389 I		0.081		
DYS448		0.047		
DYS389 II		0.163		
DYS 19		0.107	0.102	0.038
DYS391	0.014	0.124		0.027
DYS481	0.072	0.298		0.055
DYS549		0.114		0.02
DYS533		0.107		0.031
DYS438		0.05		
DYS437		0.084		
DYS570	0.029	0.159		0.018
DYS635		0.161		
DYS390		0.144		
DYS439		0.114		
DYS392	0.031	0.171		0.105
DYS643		0.039		
DYS393	0.021	0.151		0.022
DYS458	0.016	0.147		0.023
DYS385	0.025	0.16		0.017
DYS456		0.154		
YGATAH4		0.111		

6.2.5.8.3.3: Non-Template Nucleotide Addition (-A)

Amplification conditions have been set to maximize the non-template addition of a 3' terminal nucleotide by AmpliTaq Gold DNA polymerase. Failure to attain complete terminal nucleotide addition results in "band splitting", visualized as two peaks one base apart. This is most often seen when an excessive amount of DNA is amplified or amplification is performed under sub-optimal PCR conditions.

6.2.5.8.3.4: Pull-Up

Small artifactual peaks can appear in other colors under true peaks. This phenomenon is termed “pull-up”. Pull-up is a result of spectral overlap between the dyes, which is normally corrected for by the spectral. If a pull-up peak is above the minimum peak height detection threshold, it will be sized at the same size as the true peak. Pull-up can occur as a result of the following:

- Application of a sub-optimal spectral can cause pull-up. If necessary, spectral standards can be injected on the same capillary after the analytical run and a new spectral can be made and applied.
- Amplification using excess input DNA can lead to off-scale peaks. The matrix may not perform properly with off-scale data.

6.2.5.8.3.5: Other

In addition to amplification artifacts described above the following anomalies can arise during electrophoresis and analysis:

Significant room temperature fluctuation may result in size variation between injections such that allelic ladder peaks differ by more than 0.5 bp from allelic peaks in other injections. This will disrupt sample analysis using the GeneMapper ID program. Analyzing samples with an injection of allelic ladder nearest the questioned samples may alleviate this problem. If desired, the sample(s) and an allelic ladder may be re-injected to confirm the typing.

Artifactual peaks of a single color will not display the typical spectral overlap characteristic of the five fluorescent dyes in the raw data. Peak width may not be similar to the peaks resulting from dye-labeled DNA. These peaks can be shown to be artifactual by re-injection of the sample.

6.2.5.8.4: Detection of Single Male Contributor

A sample may be considered to represent a single male haplotype when the observed number of alleles at each locus is one and the signal intensity ratio of alleles at a duplicated locus is consistent with a profile from a single

contributor. All loci should be evaluated in making this determination. It should be noted that individuals have been typed who exhibit multiple locus duplications at loci other than DYS385.

6.2.5.8.5: Detection of Mixtures

Mixed DNA samples are commonly encountered. All loci must be taken into consideration when interpreting a mixture. Less intense peaks that fall in the $n-4$, $n-2$ or $n+3$ positions should be interpreted with caution based on the examiner's training and experience. As a guideline, the maximum expected stutter percentages for each locus are listed in a table (see Stutter Bands). For loci where two distinct genotypes are observed and no peaks could be considered stutter, then both genotypes can be reported.

6.2.5.8.6: Interpretation of Mixed Samples

- Mixtures with Major/Minor Male Contributors

A sample may be considered to consist of a mixture of major and minor male contributors if a distinct contrast in signal intensity exists among the alleles. All loci should be evaluated in making this determination.

- Mixtures with a Known Male Contributor(s)

In some cases, when one of the male contributors (e.g., the victim) is known, the genetic profile of the unknown male contributor may be inferred. Depending on the profiles in the specific instance, this can be accomplished by subtracting the contribution of the known male donor from the mixed profile.

- Mixtures with Indistinguishable Male Contributors

When major or minor male contributors cannot be distinguished because of similarity in signal intensities or the presence of shared or masked alleles, individual males may still be included or excluded as possible contributors. Indistinguishable mixtures can be processed using the Y mixture tool (excel sheet). The mixture is copied or typed into the sheet and all possible Y-STR profiles are compared against the US Y-STR database. The results are reported as the number of profiles that could be included in the

mixture.

Any result discrepancies between two qualified examiners must be mediated and interpreted by a third qualified DNA examiner. It is recommended that a Supervisor or Technical Leader render all final allele determinations when consensus cannot be reached.

6.2.5.8.7: Incomplete Y-STR Profiles

The possibility exists that not every locus will amplify. This can occur if the DNA is degraded, if the DNA sample contains PCR inhibitors or if a very small quantity of DNA has been amplified. Since each locus is an independent marker whose results are not abased upon information provided by the other markers, results can generally still be interpreted from the loci that do amplify.

6.2.5.8.8: Y-STR Profile Interpretation

Following GeneMapper analysis, each sample is evaluated at each locus by visual identification of amplified product(s). The alleles are recorded and comparisons are made between the question samples and reference standards. Determining whether the genotype of one sample is consistent with the genotype of another sample is based on the analyst's professional, trained judgment.

Inclusion: If the reference standard sample genotype(s) is/are present in the evidentiary or questioned sample(s), at all loci tested, the reference individual is included as a possible source of the DNA. If the evidence sample is degraded or limited alleles are obtained, the suspect can still be included if his/her alleles are consistent with that of the evidence. If the evidence sample is a mixture, and the suspect's alleles are included in that mixture, he/she can still be included.

Exclusion: If at any locus tested, the reference standard sample genotype(s) is/are not present in the evidentiary or questioned single source sample(s), the reference individual is excluded as a possible source of the DNA. Exclusions in mixtures or degraded (partial) profiles will be determined by locus to locus comparison. Exclusions cannot be made on profile that have been deemed too limited for interpretational value.

Too Limited For Comparative Purposes: This may occur when the allele signals are weak or only very limited genetic

information is obtained. Example: Only results from one or two loci are obtained with very light signal, possibly allelic drop out, allelic drop in, or perhaps the weak single allele cannot be ruled out as a heterozygote. This type of genetic information would be too limited for interpretational value.

Inconclusive: It is possible to obtain a STR profile that contains a complex mixture from multiple individuals and contains alleles that do not meet the analytical threshold or peak morphology. In these cases the result may be reported as uninterpretable or inconclusive. If a sample is reported as inconclusive then a reason must be given in the report, (a. the complexity of the mixture, b. insufficient quality of data, c. limited amount of DNA) Example "The DNA profile obtained from Q1 is inconclusive for comparative purposes due to the complexity of the mixture".

6.2.5.8.9: Statistical Calculations

Since Y-STRs are paternally inherited the counting method is the preferred way to perform statistical calculations. All statistical calculations are performed using the mixture tool excel sheet. Created by CALDOJ adapted by ASCL based on the US Y-STR Database. This database is located at the following address:
<http://www.usystrdatabase.org/newdefault.aspx>.

Enter each allele in at the appropriate loci and search the database for the obtained profile. Once the search is completed print out the summary and place in the case file.

6.3: Reports

The policies regarding case records conform to the lab-wide policies and may be found in the lab-wide quality manual. All current cases are stored electronically with the aid of Justice Trax LIMS-plus software program. Once review is complete, the electronic version is considered the official case record, and the paper file can be destroyed.

6.3.1: Elements of the Case Report

*All reports must contain the following items as recommended by SWGDAM.

- a. Case identifier
- b. Description of evidence examined
- c. A description of methodology

- d. Loci examined
- e. Results and/or conclusions
- f. An interpretative statement (either quantitative or qualitative)
- g. Date issued
- h. Disposition of evidence
- i. A signature and title or equivalent identification of the person(s) accepting responsibility of the content of the report.
- j. A statement that addresses the fact that only samples listed on the report were tested.

6.3.2: Report Guidelines

Templates for the most common DNA reports are available on the S drive.

6.3.2.1: No DNA Obtained

1. RESULTS

No DNA profile was obtained from Q1 (*evidence*).

6.3.2.2: Single Source Stains

Condition 1: Reference standard (victim / suspect) is consistent with the item of evidence. No indication of a mixture.

1. RESULTS

The DNA identified on Q1 (*evidence*) is consistent with that of K1 (*victim*). No foreign DNA profile was found.

Or

2. RESULTS

The DNA identified on Q1 (*evidence*) is consistent with K1 (*victim / suspect*).

Note If evidence (Q) was recovered from an item of evidence other than those removed directly from the person(s), statistical values for the match must be given.

Condition 2: Reference standard K2 (*suspect*) is consistent with the Q1 (*evidence*). No indication of a mixture.

1. RESULTS

The DNA extracted from K2 (*suspect*) is consistent with the DNA extracted from Q1 (*evidence*). The probability of selecting an individual at random from the general population having the same genetic markers as those identified in K2 and Q1 is approximately 1 in W in the Caucasian population, 1 in X in the Black population, 1 in Y in the Southeastern Hispanic population and 1 in Z in the Southwestern Hispanic population.

Statistical data that indicates uniqueness (values equal to or greater than 1 in 300 billion), will include the conclusion stated below:

CONCLUSION

The DNA identified on Q1 (*evidence*) originated from K2 (*suspect*) within all scientific certainty.

Note If statistical data does not render uniqueness, no conclusion statement is given. Only results.

6.3.2.3: Mixtures

Condition 1: The results from the Q1 (*evidence*) show more than one source of DNA, however, a dominant (*major*) profile can be determined from the DNA that is consistent with the K2 (*suspect*).

1. RESULTS

The DNA profile obtained from Q1 (*evidence*) indicates the presence of DNA from more than one individual. It is noted that the major component of DNA identified in Q1 (*evidence*) is consistent with the DNA profile obtained from K2 (*suspect*). The probability of selecting an individual at random from the general population having the same genetic markers as those identified in Q1 and K2 is approximately 1 in W in the Caucasian population, 1 in X in the Black population, 1 in Y in the Southeastern Hispanic population and 1 in Z in the Southwestern Hispanic population.

K1 (*victim*) cannot be excluded as the minor contributor to the DNA profile obtained from Q1 (*evidence*).

Statistical data that indicates uniqueness (values equal to or greater than 1 in 300 billion), will include the conclusion stated below:

CONCLUSION

The major component of DNA identified on Q1 (*evidence*) originated from K2 (*suspect*) within all scientific certainty.

Note If statistical data does not render uniqueness, no conclusion statement is given. Only results.

Note If evidence (Q) was removed from the victim's person and contains a semen stain, then the following interpretations will be followed.

- If the victim's profile is in the epi fraction and the semen fraction contains a male profile with no indication of mixture, then the victim does not need to be addressed in the report.
- If the victim's profile is in the epi fraction and the semen fraction contains a mixture, then the victim needs to be addressed (included or excluded) from the mixture.

Condition 2: More than one reference standard could have contributed to a mixed stain result. *Cannot* separate the major and minor components of the DNA profile. *Cannot* exclude either reference sample as being contributors to the DNA profile.

1. RESULTS

(Probability of Exclusion / Inclusion)

The DNA extracted from Q1 (*evidence*) indicates a mixture from more than one individual at 8 of 12 loci. This mixture is consistent with a mixture of DNA from K1 (*victim*) and K2 (*suspect*). 96.99% of unrelated (Caucasians) would be expected to be excluded as contributors to the observed DNA mixture. Conversely, 1 in 34,000 randomly chosen Caucasian individuals would be expected to be included as contributors to the observed DNA mixture.

Note In sexual assault cases, if the race of the perpetrator is known, only the statistical data from that race may be given. If the origin of the sample is unknown then all statistical data will be given.

Note If a homozygous peak falls below the stochastic threshold, then the loci cannot be used in the statistical calculations. If either or both heterozygous peaks both fall below the stochastic threshold then the loci cannot be used in the statistical calculations unless the expected allele numbers are accounted for.

Or

2. RESULTS

(Likelihood Ratio)

The DNA extracted from Q1 (*evidence*) indicates a mixture from more than one individual. This mixture is consistent with a mixture of DNA from K1 (*victim*) and K2 (*suspect*). The mixture profile from Q1 (*evidence*) is 100 billion times more likely if it came from a mixture of DNA from K1 (*victim*) and K2 (*suspect*) than if it came from two random Caucasian individuals, 1 trillion times more likely if it came from two random Black individuals and 2 trillion times more likely if it came from two random Hispanic individuals.

6.3.2.4: Forensic Paternity Case

Condition 1: Cannot exclude the alleged father as the true biological father.

**All alleles are designated on report.

1. RESULTS

Single Parentage Index: 5,140

Based on the above results, K3 (Alleged Father) cannot be excluded as the biological father of K2 (child). The probability of paternity in this case is 99.99%.

Condition 2: The father is excluded as the true biological father.

1. RESULTS

Based on the above result, K3 (Alleged Father) is excluded as being the biological father of K2 (Child).

Note In paternity cases, if the race of the mother and alleged father is known and similar, then only the statistical data from that race may be given. If the race of the mother and alleged father is known but different, then only the statistical data from those two races may be given. If the origin of the sample is unknown then all statistical data will be given.

6.3.2.5: Y-STR Report Guidelines

Exclusion Report

The evidence listed below has been analyzed utilizing PCR (Polymerase Chain Reaction) DNA technology. The following loci have been examined: DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385, DYS393, DYS391, DYS439, DYS635, DYS392, Y-GATA-H4, DYS437, DYS438, and DYS448.

The Y-Chromosomal DNA profile obtained from *Item 1* does not match the Y-Chromosomal DNA profile obtained from the blood of *John Doe*. Therefore, *John Doe* is excluded as the contributor of this DNA.

Inclusion Report

The evidence listed below has been analyzed utilizing PCR (Polymerase Chain Reaction) DNA technology. The following loci have been examined: DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385, DYS393, DYS391, DYS439, DYS635, DYS392, Y-GATA-H4, DYS437, DYS438, and DYS448.

Items Examined:

Q1 Vaginal swabs
K1 Known oral swabs from suspect

Results:

The Y-STR profile obtained from Q1 matches the Y-STR profile obtained from K1. Therefore, neither K1, nor any of his paternally related male relatives can be excluded as the contributor of this DNA.

The Y-STR profile obtained from K1 and Q1 has been observed in 0 of ____ African American individuals, 0 of ____ Asian individuals, 0 of ____ Caucasian individuals, 0 of ____ Hispanic individuals, and 0 of ____ Native American individuals within the database.

Samples have been retained.

6.3.2.6: SDIS Forensic Hit Supplemental Reports

The DNA Casework Section will be notified by the CODIS Section once a Forensic hit has occurred between a forensic unknown and suspect known. The DNA analyst will generate a report based on this information.

SECTION 7: EQUIPMENT CALIBRATION AND MAINTENANCE

Only suitable and properly operating equipment will be employed and only authorized personnel should operate the equipment. The purpose of the procedures in this section is to ensure that the parameters of the testing process are routinely monitored in the manner necessary to maintain the success and reliability of the testing procedures.

It is possible to verify “after the fact” that the equipment, materials and reagents used in an analysis have not significantly affected the reliability of the results. For example, controls utilized during each phase of the testing procedure are designed to signal

potential problems in the analysis. If acceptable results are obtained on these controls, it is reasonable to assume that the results from other samples analyzed simultaneously are also reliable. If the controls indicate a problem with the analysis, it may be possible to determine the source of the problem and make corrections. Depending on the nature of the problem, re-analysis of the samples may be required.

However, where the samples are irreplaceable and/or limited in amount, it is highly desirable to minimize the need for repeat analysis due to failure of equipment, materials or reagents. To that end, quality control (QC) procedures should focus as much as possible on preventing problems before they occur rather than dealing with them after they happen.

7.1: Instrument and Equipment

The following Category 1 equipment is considered to be critical for the forensic DNA section:

Pipettes	Thermocyclers
Thermometers	Refrigerators
Freezers	7500
Heat Blocks	pH Meter
EZ-1 Robots	3500xls Qiagility

7.2: Inventory

An inventory log will be maintained on the S drive for each instrument or piece of equipment considered to be essential for DNA analysis. This log may include the manufacturer, model number, serial number, purchase date, replacement date, and if present, asset number and all additional requirements of the Arkansas State Crime Laboratory Quality Manual.

7.3: Operating Manuals

Warranty information and operating manuals will be filed in the laboratory and readily available to all operators of instruments and equipment.

7.4: Calibration / Maintenance / Repair Records

Anytime an instrument or piece of equipment requires calibration, service or maintenance, that information will be documented. Maintenance logs will be maintained either on the DNA "S" drive or on the applicable instrument control computer(s).

- ** In the event that any piece of equipment fails or does not pass its specific requirements, the equipment must be taken out of service until it can be maintained properly.
- All equipment failing must be documented in instrument log
 - A sign must be placed on the equipment as "Out of Service"
 - No equipment will be placed back into service until proper performance is demonstrated.

- d. The DNA Quality Manager must inform the Technical Leader and Casework Supervisor of all equipment failure.

7.5: Calibration and Maintenance Schedules

Each instrument/piece of equipment considered essential for DNA typing will be maintained (may include calibration) or verified on an appropriate schedule. Preventative maintenance on the 7500, 3500xl, EZ1 advanced, EZ1xl advanced and the Qiagility will be performed, at a minimum, yearly, by the manufacturer. Schedule for maintenance is found in the DNA "S" drive on the computer. A maintenance log is maintained on any instrument or piece of equipment in which the following has occurred: damage, malfunction or modification or repair to equipment. Schedule and description for maintenance is found in the DNA drive on the computer. The date all equipment is removed from service is recorded and maintained on the DNA drive for a minimum of one full ASCLD/LAB accreditation cycle.

As Needed or Annually at a Minimum

- pH meter - solution is probe checked and replaced as necessary by laboratory personnel.
- Centrifuges - cleaning by laboratory personnel with 10% bleach solution.
- Spatial and Spectral for 3500xls (whenever array door is opened a spatial and spectral (G and F dyes) must be performed according to the manufacturer.
- Tachometer - will be sent for calibration

7.5.1: Annually

- Pipettes – performance checks and calibrated by an outside company.
- Drift-con- Thermal cycler calibration system must be sent out annually for calibration.
- NIST Traceable Thermometer – A NIST traceable thermometer will be calibrated or a new thermometer purchased.

7.5.2: Semi-Annually

- Thermometer – All thermometers will be verified by a laboratory personnel using a NIST traceable thermometer. In addition, prior to being placed into service, thermometers will be verified using a NIST traceable thermometer (unless the thermometer being placed into service is NIST traceable).

7.5.3: Quarterly

- Biological safety hoods – serviced and calibrated by outside company, if needed

At the current time, the fume hoods in the laboratory are monitored through a software program called WinControl. The Software receives data from control points throughout the hood system and displays them on a monitor in the office of Rick Gallagher. Another monitor is located in the Arkansas Building Authority's (ABA) office located in the Natural Resources Building adjacent to the Arkansas State Crime Laboratory. The HVAC system is monitored by both ABA Engineering and Operations Sections. The monitoring is through a web based remote entry software program provided by the HVAC controls vendor. Currently, if an alarm goes off in a hood, it will display an alarm message which will trigger a computer response and if needed, an on-site visit from maintenance personnel. If the problem persists, an outside company will be brought in to handle the problem.

- Balances are performance-checked using NIST traceable weights per instructions detailed on DNA-FORM-06. The NIST traceable weights are calibrated every 10 years.

7.5.4: Bi-Monthly

- Thermocyclers and Quantitative PCR Thermocyclers – Drifcon temperature verification test performed by laboratory personnel. If test fails, an outside company is called for service and unit is taken out of service. (See DNA "S" drive)

7.5.5: Monthly

- Swipe tests: A swipe test of the DNA and Physical Evidence Section is on a scheduled rotation. Cotton swabs are moistened with distilled water and rubbed on the analyst's bench top. Results will be submitted to DNA Technical Leader to analyze and produce a report. If contamination is detected, the work area with contamination will be wiped down with a 10% bleach solution and retested immediately and also placed on the next month's swipe test schedule. This area will not be used until the results are deemed acceptable by the Technical Leader. The DNA Supervisor, CODIS Administrator, Physical Evidence Supervisor, Quality Managers for CODIS, DNA, and Physical Evidence will receive the report. All documentation is kept on the DNA "S" drive on the computer.
- The 3500xl and computers restarted.
- Wet the seals on the 3500xl

7.5.6: Weekly (by laboratory personnel as needed)

- Polymer is changed on the 3500xl.
- Conditioning wash is performed on the 3500xl.
- Change buffer containers, septas, and reagents on the 3500xl.

- Grease O-rings on the EZ1 robots

7.5.7: Each Day of Use (by laboratory personnel as needed)

- Autoclave – check water levels before use.
- Check temperature of refrigerators and freezers in both pre-amp and post-amp rooms on DNA-FORM-17a,b and DNA-FORM-7
- Qiagility is decontaminated and documented on DNA-FORM-38
- Heat Blocks – temperature checked prior to use.
- Bench tops – CODIS DNA (pre): After each use, the bench tops must be cleaned with a 10% bleach solution and documented on DNA-FORM-11
- End of day (after last protocol) on the EZ1
 - Clean Piercing unit
 - Close Door
 - Press “2” MAN (Manual Function)
 - Press “3” Clean
 - Press “Start”
 - Open door and clean piercing units with a soft cloth and ethanol. Piercing unit is sharp.
 - Wipe piercing unit with di water.
 - Close Door and Press “ENT”
 - Press “ESC”
 - Check that the tray and racks are clean, if needed clean with ethanol and then di water.
 - UV for 20 minutes.
 - Documented on DNA-FORM-41

7.5.8: Instrument or Equipment Cleaning Procedures

- Centrifuges
Wipe out the inside of the centrifuge with 10% bleach solution as needed, or appropriate cleaner as recommended by manufacturer.
- Biological Safety Hood
After each use, wipe down inside of hood with 10% bleach.

7.5.9: Transport/Storage of Equipment

In the event the equipment needs to be stored or transported the following precautions will be taken to ensure proper functioning and to prevent contamination and deterioration.

- Storage
Equipment will be decontaminated and processed for storage according to manufacturer recommendations.

- Transport
Equipment will be prepared for movement if necessary according to manufacturer's recommendations. Equipment sensitive to movement (eg. 3500xl) will be, at a minimum, performance checked according to Section 7.6.

7.6: Performance Checks

Any new critical instruments or equipment that has been serviced requires a performance check to ensure it is operating properly before being used for casework analysis. The performance check will be documented and approved by the DNA technical leader.

1. 7500: Following the maintenance or moving of either 7500 a performance check will be performed. The performance check requires a set of standards be run (that have already been QC'd) and have a passing R2 value of .98 or above and the top standard be within 2 standard deviations of the validation values.
2. 3500xL: Following the maintenance or moving of either 3500xl a performance check will be performed. The performance check requires a ladder to be injected using the standard protocol. The run will then be analyzed in GeneMapper ID-X to ensure that the ladder passes the requirements setup in GeneMapper ID-X.
3. Thermocycler: Following the maintenance or repair a performance check will be performed. The performance check requires a set (minimum of 2) of 2800M and an AMP – to be amplified according to standard PowerPlex 16HS protocol. The samples will then be run on the 3500xl and analyzed in GeneMapper ID-X to ensure the sample amplified properly. All samples are required to amplify properly to pass the performance check. The DNA Technical Leader can override this requirement if there are documented reasons for the failure.
4. Qiagility: Following the maintenance or repair a performance check will be performed. The performance check requires a set of standards be run on the 7500 (that have already been QC'd) and have a passing R2 value of .98 or above and the top standard be within 2 standard deviations of the validation values.
5. Qiagen EZ1: Following the maintenance or repair a performance check will be performed. The performance check requires a set (minimum of 2) of NIST traceable blood on FTA to be extracted, quantified, amplified, and run on a 3500xl. The set will then be analyzed with GeneMapper ID-X to ensure the extraction occurred properly. A passing performance check is when the amount of DNA extracted is at least 0.05 ng/ul and the sample produces the expected DNA profile.

6. Qiagen Qiacube: Following the maintenance or repair a performance check will be performed. The performance check requires a set (minimum of 2) of Semen containing sample. These samples will be prepared by mixing saliva swab with semen. A passing performance check is showing a good separation of male and female in the semen fraction by analyzing the quantitation data.

SECTION 8: PROFICIENCY

Proficiency testing is used periodically to demonstrate the quality performance of the DNA laboratory and serves as a mechanism for critical self-evaluation. This is accomplished by the analysis and reporting of results from appropriate biological specimens, submitted to the laboratory as open and/or blind case evidence.

All specimens submitted as part of a proficiency test must be analyzed and interpreted according to the DNA analysis protocol approved by the laboratory at the time of the proficiency test.

Since the proficiency-testing program is a critical element of a successful QA program, it is an essential requirement. The Arkansas State Crime Laboratory utilizes proficiency testing from approved ASCLD-LAB providers.

Open proficiency test specimens are presented to the laboratory and its staff as proficiency specimens and are used to demonstrate the reliability of the laboratory's analytical methods as well as the interpretive capability of the DNA Analyst. Participation in the open proficiency test program is the primary means by which the quality performance of this DNA laboratory is judged and is an essential requirement since this laboratory performs casework.

8.1: Personnel

Proficiency testing pertains to those DNA Analysts actively engaged in DNA testing. It is mandatory that the DNA Analyst conduct the entire test alone without selecting or accepting any assistance from other persons. Violation may result in disciplinary action for those receiving and those rendering assistance. If the examiners have any questions or require assistance, they should contact the DNA Technical Leader. In order to avoid unfair advantages to other examiners at different stages of analyzing the same proficiency test samples, they may not consult one another with regard to their samples, procedures, analysis or interpretations. To do so defeats the purpose of proficiency testing for the individual and the laboratory. Newly qualified analysts will complete a proficiency test within 6 months of their qualification.

8.2: Frequency

Proficiency tests are performed semi-annually such that each DNA Analyst is tested at least twice a year, (once in the first six months of the year and a second in the second six months of the year). There must be at least four months between each test, and not more than eight months between tests. For the purpose of tracking the time between tests, the date the test is performed has been designated as the date of the proficiency review. All analysts, technical reviewers and technicians shall be proficiency tested at least once per year in each of the DNA technologies including test kits for DNA typing and each platform in which they perform analysis.

8.3: Specimen

Each proficiency test may consist of dried specimens of blood and/or other physiological fluids, either singly or as a mixture. Each sample to be tested should contain an amount sufficient so that a conclusion can be drawn from the results of the analysis.

8.4: Documentation of Proficiency Test Results

When the proficiency test is complete, all results (proficiency test case file) will be given to the Technical Leader or designee. The official case file is stored in JusticeTrax. The official electronic version must include all administration, examination documentation, how samples were obtained or created (if internal test), results from provider, and any corrective action reports.

The Technical Leader will provide a yearly summary of who was tested and status of their performance. This information will be documented in a separate secure filing system. Documentation of this is also submitted to the NDIS Administrator.

*It is noted that all proficiency tests must be processed consistent with the normal processing of casework, including all associated documentation (technical and administrative review.)

Data Documentation

Upon the completion of a proficiency test, at a minimum, the following proficiency test data and information should be collected and submitted to the Technical Leader and the outside test source for evaluation:

1. Proficiency Test Set Identifier
2. Identity of DNA Analyst
3. Dates of Analysis and Completion
4. Copies of all Work Sheets/Notes and supporting conclusions
5. GeneMapper ID worksheets
6. Any discrepancies noted
7. Corrective actions taken (if applicable)
8. Test Results

Report Format for DNA Analyst's Test Findings

Some conclusion is required as to whether the unknown and known specimens could have a common origin or whether an exclusion can be demonstrated.

Adequate and correct discrimination must be demonstrated in order to pass the proficiency test.

Review and Reporting of Proficiency Test Results

The Technical Leader and either the Casework Supervisor or CODIS Administrator (depending on proficiency cycle) reviews all test materials and compares results to the information from the test manufacturer and informs the DNA Analysts of the tests results and documents their performance. The Scientific Operations Director will review the results of the Technical Leader and Casework Supervisor and document it on the proficiency log. This review should be conducted in a timely manner. The electronic copy of the proficiency test is the official copy.

8.5: Evaluation of Proficiency Test

1. No analyst performing/assigned to a proficiency test will be involved in the proficiency review process. Except for the technical leader see #2.
2. The technical leader must review and initial on the review sheet that any inconclusive result complies with the laboratory's guidelines.
3. All final reports are graded as satisfactory or unsatisfactory.
 - a. A satisfactory grade is attained when there are no analytical errors for the DNA profile typing data. Administrative errors shall be documented and corrective action taken to minimize the error in the future.
 - i. All reported major and minor* alleles are correct
 - ii. All reported inclusions and exclusions are correct.
 - iii. All reported genotypes and/or phenotypes are correct according to consensus genotypes/phenotypes or within established empirically determined ranges.
 - iv. All reports reported as inclusive or un-interpretable are consistent with written laboratory guidelines. The basis for inconclusive interpretations in proficiency tests must be documented.

*Minor allele calls: If there is a discrepancy between the provider results versus the analyst's results, the test can be graded satisfactory if the minor alleles meet interpretational guidelines (refer to Section 6.2.2.2).

- b. An unsatisfactory grade is attained when any of the above satisfactory criteria are not met. The Section Chief must initiate a Corrective Action Request (CAR) in Qualtrax.
4. If there is a discrepancy between the expected results and the experimental results, the Casework Supervisor and/or DNA Technical Leader must notify the labwide QA Manager. Minor discrepancies may be deemed satisfactory based on the following factors with approval of the labwide QA Manager: Discipline interpretation guidelines or Consensus results.

5. All discrepancies/errors and subsequent corrective actions must be documented.
6. All proficiency test participants shall be informed of the final test results.

Proficiency Test Review Procedure

1. All proficiency tests will be reviewed the same as casework. See section 9 for technical and administrative review procedures.
2. Since reports do not include the locus and alleles, the proficiency test documentation to be sent to the proficiency provider must be technically reviewed to eliminate transcription errors. As a further measure to additionally eliminate any transcription errors, the Administrative Reviewer must also examine the locus and alleles that are being transcribed onto the proficiency provider's worksheets.
3. When proficiency test reviews are documented in the analyst's Employee History Binder on the Proficiency Testing Log (ASCL-FORM-01), the *Date Completed* indicates the date that the administrative review occurred. The *Date under Results Review* indicates the date the results from the proficiency provider are reviewed.

8.6: Corrective Action for Proficiency Test Errors

The following clearly defines the specific policies, procedures and criteria for any corrective action taken as a result of a discrepancy in a proficiency test.

8.6.1: Authority and Accountability

It is the responsibility of the Casework Supervisor to assure that discrepancies are acknowledged and that any corrective action is documented.

8.6.2: Types of Errors

8.6.2.1: Administrative Error (Level 2 Nonconformity)

Any significant discrepancy in a proficiency test determined to be the result of administrative error (clerical, sample mix-up, improper storage, documentation, etc.) may be corrected as follows:

1. A second sample set may be submitted to an individual within one week if the Casework Supervisor believes discrepancies occurred in the first test sample set. The second sample or test material will be different than the first sample but will apply to the same subject matter under testing. The individual will immediately examine the second sample upon receipt.
2. If an error of this type is not detected until the Analyst has concluded their analysis, and therefore negates their work, they must be issued an additional proficiency test set. The duplication

of analysis due to administrative error in no way reflects negatively on the analyst. However, the cause of the error should be found and eliminated from future proficiency tests.

3. If an error is due to any clerical or administrative error (typographical or otherwise – not including analyst sample mix-up or improper storage), the internal review processing steps must be evaluated to eliminate or reduce errors.

8.6.2.2: Systemic Error (Level 1 Nonconformity)

Any significant discrepancy in a proficiency test determined to be the result of a systematic error (equipment, materials, environment) may require a review of all relevant case work since the DNA unit's last successfully completed proficiency test. Once the cause of the discrepancy has been identified and corrective action taken, all DNA Analysts should be made aware of the appropriate corrective action in order to minimize the recurrence of the discrepancy.

8.6.2.3: Analytical / Interpretative Error

1. Any significant discrepancy in a proficiency test result determined to be the consequence of an analytical /interpretative discrepancy must prohibit the individuals involved in producing the discrepant result from further examination of case evidence until the cause of the problem is identified and corrected. The Technical Leader determines the need to audit prior cases based upon the type of error and its cause.
2. Before resuming analysis or interpretation of casework, an additional set of open proficiency samples must be successfully completed by the individual responsible for the discrepancy.

8.6.3: Documentation

The results of the proficiency tests and corresponding identifiers are kept in the DNA Analyst's personnel manual. Any corrective action needed due to one of the above discrepancies must be documented in Qualtrax.

8.7: Storage

Once the proficiency has been completed it will be transferred to proficiency storage, and will serve as training samples.

SECTION 9: CASE RECORD

The testing period is defined in the examination notes as the date on the extraction worksheet to the date the report is generated.

Any examination records prepared by an individual other than the analyst who interprets the findings and/or authors the case report will have the preparing analyst's initials indicated on the worksheet.

Prior to issuing a formal report, all casework is subject to technical and administrative reviews by qualified individuals.

In the event that the author of the report did not generate all of the data used in the report, the signature on the report will indicate that all data used for reporting was reviewed by the author of the report.

A master list for abbreviations will be placed on the DNA drive and updated periodically.

9.1: Reviews

9.1.1: Technical Review

The technical reviewer shall be or have been an analyst qualified in the methodology being reviewed and not the author of the current report. The technical reviewer will review all documentation in the case file to ensure that there is sufficient basis for the scientific conclusion(s) in the report and then complete and sign the technical review sheet indicating that a technical review has been completed. The technical reviewer will electronically initial that the technical review was completed in Justice Trax. If a discrepancy is found and an agreement is not reached between the DNA analyst and the reviewer(s), the Casework Supervisor will be consulted. The Technical Leader will be notified of all technical issues and consulted for a final decision if there is still a discrepancy.

9.1.1.1: The Case File

The case file is now maintained electronically and contains the following information:

- Submission Sheet
- Extraction Sheet
- Worksheets (* When appropriate)
 - Quantitation Sheets
 - STR AMP Sheets
 - Master Mix Sheet
 - Plate Loading Sheet *
 - Call Sheets
 - PopStats *
 - CODIS *
- GeneMapper Data

- Review Sheet
- Case Report

Any corrections made to a file released for the review process will be documented in our Justice Trax LIMS system.

9.1.1.2: Technical Review Sheet

The technical review must review all of the criteria described on the technical review sheet. The criteria have been duplicated below.

- Are notes present which adequately describe the packaging and description of the evidence?
- Were all necessary analyses performed and documented according to established guidelines?
- Was the request of the agency addressed by the work performed in the case (reasonable)?
- Have all out of bin Microvariants been confirmed?
- Have all controls, internal lane standards and allelic ladders been verified for expected results?
- Are all genotypes correct and all peaks meet the required threshold(s)?
- Are all genotypes correct and transcribed (including proficiency)?
- Have all the necessary sample files been imported into the GeneMapper ID-X project, and are all the imported sample files appropriate and acceptable?
- Are the conclusions (both inclusions and exclusions) and statistics (if applicable) correct?
- Is the report free of all errors and understandable to persons who will read the report?
- Has GeneMapper ID-X Profile Comparison been performed in this case?
- If DNA testing results are inconclusive or of no probative value, has all appropriate evidence been examined or submitted for further testing (i.e. Serology – Tape Lifts, Trace – Retained Hairs, etc.)? Are all CODIS requirements addressed: eligibility, appropriate specimen category, all appropriate samples(s) indicated on CODIS entry sheet, etc.?

9.1.2: Administrative Review

An administrative review does not need to be qualified in the technology used in the case, but the administrative reviewer must be trained and qualified to perform administrative reviews. The administrative reviewer evaluates the report and supporting documentation for completeness and for editorial correctness. If the administrative reviewer finds an error in the case file, the error should be corrected after consultation with the DNA analyst and/or

technical reviewer. Once the error has been corrected, the administrative reviewer will sign the administrative review sheet and scan the review sheet into case images in Justice Trax. The administrative reviewer will electronically initial that the administrative review was completed in Justice Trax and send one (1) copy of the report out to the submitting agency on iResults. The administrative review cannot be performed by the author of the report.

9.1.2.1: Administrative Review Sheet

The administrative review must review all of the criteria described on the administrative review sheet. The criteria have been duplicated below. ♦

- Does all examination documentation have the dates indicating when the work was performed, ASCL case number and is it stored in the appropriate folder in 'Requests'?
- Does all administrative documentation (e.g. contact forms, faxes, subpoena) contain the ASCL case number, and is it stored in 'Case Images'?
- Has the chain of custody been reviewed for disposition of evidence?
- Is the report consistent with laboratory guidelines and editorial correctness?
- If this is a proficiency, are all transcriptions correct on proficiency provider's forms?
- Are all corrections in the case file made consistent with laboratory policy?
- Is the request date in Justice Trax consistent with the date evidence was submitted for DNA analysis?

9.1.3: Review for Non-Authored Case Testimony

Testimony may be rendered by another qualified DNA analyst upon review of the case file. ASCL-FORM-57 shall be filled out and scanned into the case file.

9.2: Corrective Action

Corrective actions will be performed according to the Arkansas State Crime Laboratory quality manual.

9.2.1: Authority and Accountability

The Casework supervisor will be responsible to assure that discrepancies are acknowledged and corrective actions are documented according to the Arkansas State Crime Laboratory Quality Manual. Corrective actions shall not be implemented without the documented approval of the technical leader.

Any deviation from the DNA Quality Manual (DNA-DOC-01) will be approved by the DNA Administrator and DNA Technical Leader. A log will be kept of each deviation from the DNA Quality Manual. The CODIS Administrator will be notified of any corrective action.

SECTION 10: TESTIMONY REVIEW

See the Arkansas State Crime Laboratory Quality Manual for the policy regarding testimony review.

SECTION 11: AUDITS

Audits are an important aspect of the QA program. They are an independent review conducted to compare various aspect of the DNA laboratory's performance with a standard for that performance. The audits are not punitive in nature, but are intended to provide management with an evaluation of the laboratory's performance in meeting its quality policies and objectives

11.1: Frequency

Audits must be conducted once per year, with the interval between audits not less than six (6) months and not exceeding eighteen (18) months. At least one audit must be completed by an outside agency once every two years.

11.2: Records

Records of each inspection should be maintained and should include the date of the inspection, area inspected, name of the person conducting the inspection, findings and problems, remedial actions taken to resolve existing problems and schedule of next inspection. These records are maintained in the DNA Audit Manual.

SECTION 12: COMPLAINTS

Any staff member receiving a complaint should notify their supervisor. The complaint shall be documented and given to the supervisor. The supervisor shall forward the complaint to the Scientific Operations Director who will investigate the situation and notify top management when necessary. When the concern takes on the nature of a complaint about the laboratory's activities or deficiencies in the quality system, the supervisor will investigate the situation and forward all the information to the QA Manager.

See the Arkansas State Crime Laboratory Quality Manual for the policy regarding complaints.

SECTION 13: MISCELLANEOUS

13.1: Safety

All safety protocol and information is contained in the Arkansas State Crime Laboratory Health & Safety Manual (ASCL-DOC-08). The safety manual covers general laboratory safety.

13.2: Outsourcing

The Arkansas State Crime Laboratory will only outsource to a vendor laboratory that complies with Quality Assurance Standards and accreditation requirements of federal law and can provide documentation of the compliance. All vendor laboratories must also comply to standards set forth in the Arkansas State Crime Laboratory quality manual. Prior to any outsourcing of data, the DNA Technical Leader will document the approval of the technical specifications.

The data generated from samples that are outsourced by the Arkansas State Crime Laboratory will be re-analyzed by a qualified proficient DNA analyst in the methodology used by the vendor laboratory. The re-analysis will give ownership of the data to the analyst performing the analysis. The data must be technically reviewed prior to being searched in the NDIS system. The case then gets an administrative review before a report can be released.

The DNA Technical Leader or his/her designee will conduct an initial on-site visit to the vendor laboratory. If the contract extends beyond one year, an annual on-site visit will be required. The laboratory can accept an on-site visit conducted by another NDIS participating laboratory. Please see DNA-Form 21 for the on-site visit checklist.

Appendix A

In the event the technical leader position is vacated, the following contingency plan will be submitted to the FBI within 14 days for approval. Any work that is in progress may be completed during the 14 day period, but new casework shall not be started until the plan is approved by the FBI.

The Arkansas State Crime Laboratory will conduct interviews within the laboratory among any qualified individuals. If there are no interested or qualified individuals the Arkansas State Crime Laboratory will contact the surrounding states to ask for the assistance of their technical leader until the technical leader position can be posted, interviewed and filled.

A newly appointed technical leader shall be responsible for the documented review of the validation studies currently used by the laboratory and educational and training records of currently qualified analysts.

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