ARKANSAS STATE CRIME LABORATORY

FORENSIC DNA SECTION

QUALITY ASSURANCE MANUAL

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1 FORENSIC DNA 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 & MANAGEMENT

QA PROGRAM, DNA ANALYSIS, LAB OPERATIONS AND MANAGEMENT

This QA Manual has been approved by the DNA Casework Supervisor, CODIS Administration, DNA Technical Leader, Laboratory Quality Assurance Manager, Assistant 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.

2 PERSONNEL QUALIFICATIONS & JOB DESCRIPTIONS

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

2.1.1.3 QUALIFICATIONS

A) Education

The technical leader shall meet the following qualifications:

1) 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.
2) 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.
3) The specific subject areas listed above shall constitute an integral component of any course work used to demonstrate compliance with this Standard.
4) 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.
5) 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 DNA 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 lab-wide 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 FBI QAS and ANAB 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 five (5) 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 FBI QAS and ANAB 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 is recommended to have a minimum of five (5) years of experience as a forensic 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 Casework and CODIS Sections Analyst Training Manual.

D) 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.

2.1.5 FORENSIC DNA PROCESSOR

2.1.5.1 RESPONSIBILITY

The forensic DNA Processor is responsible for processing DNA samples for analysis and specifically delegated QA responsibilities from the Casework Supervisor. The DNA Processor will be authorized to perform individual sample processing tasks specified on Letters of Qualification from the DNA Technical Leader and Casework Supervisor following the satisfactory completion of training and testing. The term Processor may also be used in this manual to refer to any qualified DNA Analyst, or DNA Analyst-in-training qualified for the task being discussed.

2.1.5.2 JOB FUNCTION

A) Implementing the QA program.
B) Handling reagents.
C) Establishing liaisons with colleagues in the field.
D) Processing casework samples for analysis.
E) Providing expert testimony.
F) Interacting with investigative personnel.
G) Assisting in training new employees.
H) All other duties as assigned.

2.1.5.3 QUALIFICATIONS

A) Education
The DNA processor shall have at a minimum, a high school diploma or equivalent.

B) Training
The DNA processor shall complete the DNA training program established by the ASCL DNA Technical Leader and DNA Casework Supervisor. This training entails the processing of a range of samples encountered in the assigned 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 sample processing. A complete list of training requirements can be located in the DNA Casework Section Technician Training Manual.

C) Continuing Education
The DNA processor 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

INITIAL TRAINING

Training will be guided by the appropriate DNA Training Manual. The required six-month training program for a forensic laboratory analyst will be dependent 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: a practical test (such as an expired external proficiency test), a written qualifying test, and an oral discussion of the written test. An analyst will also complete a moot court before performing independent casework. See the Training Manual for the complete training program. The DNA Technical Leader, CODIS Administrator, and Casework Supervisor shall 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.

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 quarterly, at a minimum. An excel sheet located on the shared drive will be filled out to document the articles read. The spreadsheet is able to confirm a quarterly entry by each analyst for a given date range. The analyst can disperse the article to the rest of the Section either by email or manually. A request for articles not freely available to analysts can be forwarded to a supervisor for procurement.

CONTINUING EDUCATION

All Casework employees have access to continuing educational experiences, such as classes, lectures, seminars, conferences, and professional meetings. The DNA TL, DNA Supervisor, CODIS Administrator, and Analysts will attend 8 hours of continuing education per calendar year, at a minimum. Continuing education will be documented in Qualtrax with certificates, agenda, presenter CV, attendance sheets, and TL approval, as available. Any electronic continuing education must be approved by the TL and attendance must be documented. Any internal continuing education must be documented, including title, CV of presenter, attendance, dates, and notes or records of the presentation. The TL will ensure that each analyst has a planned activity for meeting the 8 hour minimum requirement by mid-year. The TL will be responsible for an annual review of all DNA personnel training to ensure quality and completeness of continuing education.

COMPETENCY TESTING

Competency testing for the following activities will be conducted and documented prior to these actions being performed on evidence:

- Laboratory activities (testing and/or sampling)
- Analysis of results
- Review of results
- Authorization of results
- Verification of results
- Technical review
- Expressing an opinion or interpretation

2.3 ACTIONS & APPROVALS

See *ASCL DOC-01 Quality Manual* section 6.2.6 for categories of Authorizations.

2.3.1 DNA TECHNICAL LEADER

A) Can initiate, suspend, and resume DNA analytical operations for the laboratory or an individual.
B) Reviews DNA quality manager's actions in implementing the quality assurance program for the Forensic DNA section.
C) Oversees the technical operations of the Forensic DNA laboratory.
D) Approves method development, modification, verification, and/or validation.

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) Approves DNA quality manager's actions in implementing the quality assurance program for the DNA section.

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.
C) May Analyze and Report Results, Technically Review and Authorize Reports.

2.3.5 DNA PROCESSORS

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.

3 FACILITIES

3.1 OVERALL LABORATORY SECURITY

The Arkansas State Crime Laboratory system has security monitors that cover the external perimeter of the buildings and parking lots. Security cameras are also located on the first floor of the Main 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.

The ASCL currently performs DNA laboratory activities at only one location:

- Main Laboratory: 3 Natural Resources Drive, Little Rock AR 72205

3.2 FORENSIC BIOLOGY LABORATORY SECURITY

The Physical Evidence, CODIS and DNA Casework areas of the laboratory are limited in access to other laboratory personnel through the electronic security system. Each analyst is assigned a unique programmed fob that enables entry into the laboratory. If an area is not monitored by the electronic security system, then access to the area is controlled by physical lock-and-key, with only authorized personnel being issued the key to the area.

3.3 FORENSIC DNA CASEWORK LABORATORY

The Forensic DNA Laboratory spaces are 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. See Section 13.2 for a full discussion of contamination prevention guidelines. Records of critical environmental conditions will be stored in the DNA QC Images folder of the ForensicBiology drive.

3.3.1 DNA PRE-PCR LABORATORY

The Forensic DNA Pre-PCR areas consist 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:

1) Use disposable gloves at all times.
2) Sterilize the bench top before and after each use 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 un-amplifiable.
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 will 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 will be washed on a monthly basis.
13) General housekeeping will be performed as needed (e.g., sweeping, mopping, dusting).

3.3.2 DNA 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. Amplified DNA must be handled carefully. Steps will be taken to avoid dispersing it around the room to reduce the potential for transfer of amplified DNA to other work areas.

SPECIAL PRECAUTIONS:

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 each use 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.

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 number (YYYY-1-XXXXX) or (YYYY-XXXXXX) will be used.*

4.1 EVIDENCE HANDLING PROCEDURES

Evidence is submitted to the Arkansas State Crime Laboratory from investigating agencies only. Evidence submitted to the Evidence Receiving section of the laboratory is 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 request needs to be amended after work has begun, all affected personnel shall be notified.

4.2 CHAIN OF CUSTODY

See the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.4.1.1.3 for Chain of Custody guidelines. Extracts from Forensic DNA testing may be returned to the same packaging as the original item for long-term storage without sub-itemization. If the original item is to be returned, the extracts will be sub-itemized for long-term storage. Sub-items collected for Forensic DNA testing may be retained by the ASCL indefinitely.

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

At times, evidence submitted for DNA testing is not adequately packaged. The analyst may document and correct the deficiency. If there is any concern that the packaging deficiency has affected the integrity or identity of the test item, the analyst's Section Chief and the customer agency shall be advised and consulted with for further instructions. If the analyst discovers an inconsistency between the stated and actual contents of a package, or if there is doubt about the suitability of an evidence item for testing, then the analyst shall attempt to contact the customer before proceeding. All contacts will be documented in the case record (e.g., using an *Agency Contact Form* (ASCL-FORM-06), by email). For minor inconsistencies, the analyst shall use their judgment on whether to contact the customer, but must make a note of the discrepancy in the case file. After analysis, the DNA Analyst re-packages the evidence in a manner that will preserve the evidence while in storage and awaiting trial.

4.5 SEALS

During the evidence processing procedure, the DNA Analyst will, if possible, avoid damaging seals on the evidence made by others. For example, a box or a bag will 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 not already stored electronically in JusticeTrax, 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 DISTRUCTION OF EVIDENCE

The Forensic DNA Section destroys no evidence, except in the case of fetal tissue samples as described in 4.11.10. Any packaging not retained will be documented in the appropriate casefile.

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 the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.4.1.1.2 to find Test Item Packaging and Sealing requirements. Key points are listed below:

- DNA evidence will be sealed so that the contents cannot escape and that opening the container results in obvious damage or alteration.
- A proper seal includes the initial of the person sealing the evidence across the seal. As soon as is practicable once the examination is complete at least one layer of packaging will be properly sealed.
- DNA evidence will be protected from loss, cross-transfer, contamination, and deleterious change.
- If evidence must be stored or conditioned under special environmental conditions (e.g., refrigerated, frozen), then these conditions shall be maintained, monitored, and recorded.
- Whenever practical, the original seal will be left intact when opening a container.
- If the original packaging cannot be kept, complete documentation and a picture of original packaging must be retained in the case record.

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 & 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 & 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 will, with some reasonable exceptions, 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 will 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 analyst initials. The package will 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 a Filemaker Database and an excel spreadsheet (DNA-FORM-1) and the labeling of tubes to ensure the integrity of each sample throughout processing. The software 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 serve to document the location of each sample during the quantitation, amplification, and electrophoretic processes.

4.11.8 LONG TERM STORAGE

See the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.4.1.1 to find Test Item Storage requirements. For evidence storage purposes, DNA mag-locked areas meet the definition of "secure, limited-access area" and key-locked pre-amplification cleanroom areas meet the definition of "short-term storage location".
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 containers 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 will 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

Fetal Tissue Samples fall under specific legislation in Arkansas. Act 725 (2013) amended AR Code 12-18-108 to include language which directs the ASCL to test and dispose of tissue samples from aborted products of conception from juvenile patients. The fetal sample will be examined, documented and processed 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 or email stating that the remaining fetal tissue may be destroyed. The letter or email will be scanned into the case file and the remaining fetal tissue will then be destroyed.

5 VALIDATIONS

The laboratory shall only use validated methodologies for DNA analyses. These include any new methods and procedures for sampling, handling, transport, storage and preparation of items to be tested. There are two types of validation: developmental and internal. See the *ASCL Quality Assurance Manual* (ASCL-FORM-01) section 7.2.2 discussion for Validation procedures.

5.1 DEVELOPMENTAL VALIDATION

Developmental validation is required on any novel methodology for forensic DNA analysis. When method development is performed, a plan is approved by the DNA Technical leader and assigned to personnel approved to perform method validation. Adequate resources are made available.

During the method development, the process is reviewed to ensure that the original goals are still being fulfilled. If a modification to the plan is required, it will be approved and authorized by a revision of the plan document in Qualtrax.

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 & Stability
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 validated procedures shall be evaluated and approved for use if the modifications are covered by the initial validation conditions by the DNA Technical Leader. An additional validation of the modification will be needed if determined by the technical leader.

6 ANALYTICAL PROCEDURES (TEST METHODS)

Following a review of submitted evidence by an Evidence Receiving technician or other approved ASCL personnel, a DNA request will be created in LIMS if appropriate. Requests for non-routine work must be reviewed by the Forensic DNA Section Chief or her designee. If approved, the Section Chief (or designee) must initial and date the *ASCL Evidence Submission Form* or LIMS-generated Submission sheet next to the request. Deviations from normal analytical procedure will be documented on the *Deviations/Non-Conformance Form* (CODIS-FORM-17) to ensure technical justification and authorization.

The laboratory shall only use validated methodologies for DNA analyses (see section 5).

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
Quantiplex Pro	Qiagen
PowerPlex Fusion 6C	Promega
YfilerPlus	ThermoFisher

Miscellaneous Items:

2800M	Promega
Buffer G2	Qiagen
Buffer MTL	Qiagen
3M Sodium Acetate, pH 5.2	ThermoFisher
0.5M EDTA, pH 8.0	ThermoFisher
GeneScan 600 Liz	ThermoFisher

6.1.1.1 SOURCES OF MATERIALS, REAGENTS, CHEMICALS, & 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. Only suitable externally-provided products will be used.

6.1.1.2 SUPPLY & 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 SAFETY DATA SHEETS (SDS, PREVIOUSLY MSDS)

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

6.1.1.4 LABORATORY PREPARED REAGENTS & 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 ForensicBiology 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 including dilutions and aliquots will be clearly labeled. Labels will include at a minimum: identity of reagent; date of preparation or expiration; and identity of individual preparing reagent. Lot number, and storage requirements (as appropriate) may also be included. Labels may be placed on the individual reagent aliquots or on the specific container of the aliquots. A barcode may represent the lot number. Labels or records will also include identity of preparing analyst, components used, and expiration date.

6.1.1.6 STORAGE & DISPOSAL

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

6.1.1.7 CRITICAL REAGENTS & SUPPLIES

Critical consumables, supplies, and services which affect the quality of testing will be obtained from reliable suppliers. 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 & COMPONENTS

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 checked PowerPlex Fusion 6C 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 QUANTITATION 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 AMPLIFICATION KITS & COMPONENTS

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 will 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. Critical Reagents purchased as a component of a kit may only be used with a kit lot for which it has passed a Quality Check. Critical Reagents not purchased as a component of a kit are not restricted to use with only the lot used to perform the Quality Check.

6.1.1.7.4 BONE & TOOTH EXTRACTION REAGENTS

The reagents will be marked with the receive date and initials of the individual who receives the kit. An appropriate positive control will be extracted along with a reagent blank. The positive control 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 reagents. If the reagent does not produce the expected profile, the samples will 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. Critical Reagents not purchased as a component of a kit are not restricted to use with only the lot used to perform the Quality Check.

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 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 will 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 in the regent blank will be noted in the report. If the extraneous DNA is present in both the reagent blank and associated sample 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 Fusion 6C and Y23. 007 is the positive control for Yfiler Plus. 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 to 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 will be amplified and analyzed concurrently in the same instrument with the same samples and same PCR kit.

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 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 INTERNAL SIZE MARKER & 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.

6.1.2.5 NIST STANDARD

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

6.1.2.5.1 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). All internal NIST traceable standards will be labeled with a lot designator and will be maintained as labeled.

6.1.2.5.2 NIST STANDARDS HANDLING, STORAGE, & PREVENTION OF DETERIORATION

NIST SRM samples will be maintained as the manufacturer recommends. 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.

6.1.2.5.3 NIST QUANTITATION STANDARDS

NIST Quantitation Standard samples will be maintained as the manufacturer recommends. The NIST quantitation standard may be used to adjust analysis settings for the sequence detection software v.1.2.3 and the expected IPC and Y-intercept value ranges in use for casework quantitation.

6.1.2.6 QUANTITATION STANDARDS

Quantitation standard consists of a series of dilutions made from the standard stock provided with each quantitation kit.

The quantitation standard is used to create a standard curve to allow for the quantitation of the samples amplified concurrently. A usable standard curve must consist of at least one replicate in 4 of the 5 dilutions.

6.1.3 DETECTION & 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, and the amplification blank detects contamination during the setup of amplification. 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 *Deviations/Non-Conformance Form* (CODIS-FORM-17). If a DNA employee 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 Quality Assurance Concern (QAC) may be necessary.

6.2 STANDARD OPERATING PROCEDURES

Note: Due to the high variety of DNA samples submitted, the sampling plan for each extraction type is at best a recommendation based on typical amounts of DNA obtained from each sample type in a typical case scenario under optimal conditions. It is the responsibility of the authorized DNA personnel to determine for each sample the appropriate manor and amount of sample collected based on sample condition, sample volume, case scenario, etc. DNA samples are not homogenous and the sampling methods will be considered non-statistical. Therefore, Forensic DNA findings may only be applied to the portion of the sample consumed.

In some circumstances, deviation in methods and procedures may be necessary. At such times the *Deviations/Non-Conformance Form* (CODIS-FORM-17) must be completed and signed by the CODIS Administrator and/or the DNA Technical Leader to ensure the proposed deviation is within validated guidelines.

6.2.1 SAMPLING PROCEDURES

6.2.1.1 PACKAGING AND SEALING

See the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.4.1.1.2 to find Test Item Packaging and Sealing requirements. Key points are listed below:

DNA evidence will be sealed so that the contents cannot escape and that opening the container results in obvious damage or alteration.

A proper seal includes the initial of the person sealing the evidence across the seal. As soon as is practicable once the examination is complete at least one layer of packaging will be properly sealed.

DNA evidence will be protected from loss, cross-transfer, contamination, and deleterious change.

Whenever practical, the original seal will be left intact when opening a container.

If the original packaging cannot be kept, complete documentation and a picture of original packaging must be retained in the case record.

See the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.4.1.1.3 and the *DNA Quality Assurance Manual* (DNA-DOC-01) sections 4.2 for Chain-of-Custody guidelines.

6.2.1.2 SAMPLING AND TECHNICAL RECORDS

Sampling and Technical Records will be maintained whenever sampling or examination is performed, including (where relevant):

- Identification of the sampling and examination methods used
- The date and time of sampling or examination
- The identification and description of the sample
- Identification of the person performing the sampling or examination
- Identification of any equipment used
- Environmental or transport conditions
- Identification of the sampling or examination location
- Any deviation, addition, or exclusion from the sampling or examination method and plan

The forensic DNA section will use approved forms and methods for collecting the above listed sampling and examination records. These may include the DNA Filemaker evidence reports or the paper-based equivalents.

Deviations from the sampling plan and procedures outside of normal variation may be requested by the customer or deemed appropriate by the analyst. At such times the *Deviations/Non-Conformance Form* (CODIS-FORM-17) must be completed and signed by the DNA Supervisor and/or the DNA Technical Leader and maintained in the case record.

All examination records (or copies of printouts) will be stored in the "Request" folder in the LIMS case file. As the analytical instrument data is generally incompatible with JusticeTrax storage, the electronic data will be stored on the ForensicBiology shared drive or the FileMaker server.

If data, an observation, or a calculation is rejected, the reason and date of the rejection and the identity of the person rejecting must be recorded on the records.

6.2.1.3 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 will 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) will be developed and entered into Specimen Manager by a CODIS Analyst for the Casework Analyst to obtain. This can be changed on a case-by-case basis upon approval of the CODIS Administrator and/or Casework Supervisor.

6.2.1.4 SEXUAL ASSAULT KITS PROCESSING (SA KITS)

Sexual Assault Kits fall under specific legislation in Arkansas. Act 839 (2019) amended AR Code 12-12-406 to include language which directs the ASCL to test all newly submitted SA Kits with a goal of 60 days for completion. Act 1168 (2015) directs the ASCL to provide a report and plan to address unsubmitted SA kits (SAFER). In an effort to deliver results to investigating agencies within a given timeframe, swabs from sexual assault kits in active investigations will not be screened for serological results prior to DNA extraction. Rather, Autosomal- and Y-STR processing will be based on the quantitation results. Bulk evidence such as bedding and clothing items will be screened by the Physical Evidence section if deemed necessary. In a further material modification, it has been

determined that unless indicated through case specific information, SA kit oral swabs will not be immediately processed. Oral swabs may be included with supplemental requests. Active SA kits will be given priority over SAFER SA Kits.

PROCESSING OF THE SEXUAL ASSAULT KIT IN PHYSICAL EVIDENCE

1) Outer and inner packaging of the kit will be documented per the SER-DOC-01 Quality Manual. Samples of SA Kit swabs will be placed into appropriately labeled DNA extraction tubes and placed into secure storage for extraction and analysis by a DNA Processor.

PROCESSING OF THE SEXUAL ASSAULT KIT IN DNA

1) If not processed by Physical Evidence, the outer packaging of the kit will be documented in Filemaker Pro (front and back photos showing seals are sufficient, notes are optional); a scale is preferred in all photographs. If Filemaker Pro is not available, DNA-FORM-05 forms may be used.
2) The kit will be opened and the inner contents of the kit will be documented with the same specificity as the outer packaging (front and back, seals, etc.).
3) Individual photos or descriptions of the samples will be documented in Filemaker Pro.
4) The extraction information will be documented in Filemaker Pro, DNA-FORM-01, or DNA-FORM-05.
5) Samples will be quantitated with the current validated Quantitation kit to screen for suitable DNA template. Only a qualified DNA analyst will interpret the Quantitation data to make the determination of which samples will be amplified.
6) Suitable samples will be amplified with current validated Amplification kit and analyzed on the 3500xl Genetic Analyzer. A qualified DNA analyst will interpret the run data for reporting.
7) When analysis is complete and the reports have been reviewed, the final reviewer will mark that testing is completed for SA Kits tracked in the Arkansas Sexual Assault Kit Tracking website.

JUSTICETRAX DOCUMENTATION OF SEXUAL ASSAULT KITS:

The items within the kit will be itemized in JusticeTrax. The sexual assault kit will be returned to Secure Storage by the processors. Any retained sample items will remain in the possession of the processor or in DNA laboratory secure storage. Maintenance and storage of the DNA extracts will be the responsibility of the DNA Processors recorded in each step of the process. Once processing is finished, retained samples and processing extracts will be transferred to DNA long term storage.

QUANTITATION:

Qiagen Quantitation kits will be utilized as a screening tool for total human autosomal and Y-chromosomal DNA. No further processing is necessary if samples fail to have sufficient amount of DNA or sufficient amount of a male contributor. Cases that are considered “Stop at Quant” will be assessed for additional evidence and assigned to Physical Evidence as necessary. Cases with samples containing the adequate amount of DNA for further processing will proceed to data analysis. If multiple samples are submitted, STR processing may be further restricted to the sample

deemed to have the highest probability of producing a suspect profile (highest total DNA with largest percentage of male contributor).

DATA ANALYSIS:

The DNA analyst will examine the data and determine if there are usable profiles. If not or if there are multiple perpetrators, the analyst will further assess the case and determine if additional evidence or Y-STR processing is needed. The Physical Evidence section will be contacted if there is additional evidence to be examined.

6.2.1.5 ROUTINE CASEWORK PROCESSING

Examination of DNA evidence items is restricted to DNA Analysts or Processors specifically authorized to process samples for DNA extraction.

PROCESSING OF THE SUBMITTED SAMPLES

1) Outer packaging of the submitted samples will be documented in Filemaker Pro (front and back photos showing seals are sufficient, notes are optional); a scale is preferred in all photographs. If Filemaker Pro is not available, DNA-FORM-05 forms may be used.
2) The packaging will be opened and the inner contents of the package will be documented with the same specificity as the outer packaging (front and back, seals, etc.).
3) Individual photos or descriptions of the samples will be documented in Filemaker Pro or DNA-FORM-05.
4) The extraction information will be documented in Filemaker Pro, DNA-FORM-01, or DNA-FORM-05.
5) Samples will be quantitated with the current validated Quantitation kit to screen for suitable DNA template. Only a qualified DNA analyst will interpret the Quantitation data to make the determination of which samples will be amplified. Quantitation, while preferred for all samples, may be omitted for Known Reference samples; it is recommended that more than one concentration of extract be amplified. Likewise, Extraction Reagent Blanks (RB) may proceed without quantitation, but must be amplified at the maximum concentration with a currently validated amplification kit.
6) Suitable samples will be amplified with current validated Amplification kit and analyzed on the 3500xl Genetic Analyzer. A qualified DNA analyst will interpret the run data for reporting.

JUSTICETRAX DOCUMENTATION:

Most items submitted will already be itemized in JusticeTrax. Additional itemization may be recorded in JusticeTrax as needed. DNA extracts will remain in the DNA cleanrooms with the remaining samples, to be dried and stored with the original sample when testing is complete. No subitemization of the extracts is needed. DNA samples and extracts will remain the processor's responsibility until final storage; this person will assure the proper storage of the extracts and the original samples. Once processing is finished, retained samples and extracts will be transferred to DNA long term storage.

QUANTITATION:

Cases that are considered "Stop at Quant" will be assessed for additional evidence and assigned to Physical Evidence as necessary. Cases with samples containing the adequate amount of DNA for further processing will proceed to data analysis.

DATA ANALYSIS:

The assigned DNA analyst is responsible for the examination of the data and will determine if there are usable profiles. If there is no profile obtained or there is an inconclusive profile, the assigned analyst will further assess the case and determine if Y-STR processing is needed and/or the Physical Evidence section will be contacted if there is additional evidence to be examined.

6.2.2 EXTRACTION PROTOCOLS

**Note: All extraction steps must be performed in the Pre-PCR Laboratory. Use of the Pre-PCR lab requires Personal Protective Equipment in the form of Lab coat, disposable gloves, and disposable face mask. Access to the Pre-PCR lab space is restricted to authorized individuals due to the presence of evidence and the cleanroom nature of the lab space. Use separate reagents and pipettes dedicated to each area and clean each area thoroughly after use. Extraction tubes are stored in the Pre-PCR laboratory refrigerators until cases are completed and samples are stored long term.*

**Note: Known reference samples must be extracted at a different time and/or space than questioned samples. Extracts from reference samples are not routinely dried down, however it is recommended if the entire sample was used during extraction.*

6.2.2.1 WHOLE BLOOD

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

1) Label appropriate 2.0 mL EZ1 sample tube.
2) Add 200 µL of whole blood to the labeled sterile 2.0 mL EZ1 sample tube.
3) Add 190 µL 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 µL of liquid in the tube.
4) Add 10 µL of Proteinase K, mix by vortexing. (DO NOT add additional Proteinase K, even if increased volume of G2 is used.)
5) Incubate at 56 ºC for a minimum of 15 minutes.
6) If necessary, centrifuge briefly.
7) Process on EZ1 with Trace protocol. (see protocol below, 6.2.4.12)
8) Estimate the amount of DNA in the sample. (See Quantitation SOP)
9) The samples are now ready for PCR amplification.

6.2.2.2 TRACE SAMPLES

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

1) Label 2.0 mL EZ1 sample tube.
2) Add appropriate sample to the sample tube (as listed below):
   - Dried 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. Cut cotton swab from stick and place into the labeled sterile 2.0 mL EZ1 sample tube. Cuttings may also be used for this extraction.
   - 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.
3) Add 190 µL 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 µL of liquid in the tube.
4) Add 10 µL of Proteinase K, mix by vortexing. (DO NOT add additional Proteinase K, even if increased volume of G2 is used)
5) Incubate at 56 ºC for a minimum of 15 minutes.
6) If necessary, centrifuge briefly.
7) Process on EZ1 with Trace TD protocol. (see protocol below, 6.2.4.12)
8) Estimate the amount of DNA in the sample. (See Quantitation Kit SOP)
9) The samples are now ready for PCR amplification.

6.2.2.3 SEMEN-CONTAINING STAINS, QIACUBE (2-6 SAMPLES)

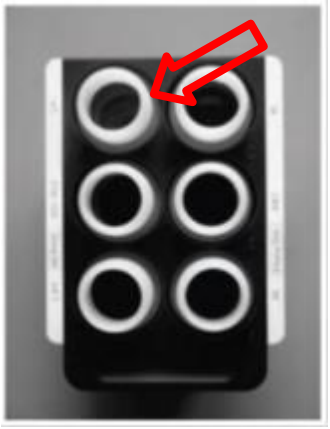
1) Label appropriate 1.5 mL Qiacube sample tube as Semen Fraction (SF) and label appropriate 2.0 mL EZ-1 sample tube as Epithelial Fraction (EF).
2) Cut an appropriate-size sample and place into a labeled 1.5 mL Qiacube sample tube.
3) Add 480 µL G2 buffer, then 20 µL of Proteinase K, mix by vortexing.
4) Incubate at 56 ºC for a minimum of 15 minutes.
5) Centrifuge tube briefly to remove drop from lid.

6) Remove any solid material from tube. Use toothpick to remove cloth or swab from tube, twisting to remove excess fluid. Discard solid material in appropriate waste.
7) Samples are 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 tip racks with 1000µL 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 2mL screw top tube and place in Position A in the Tip Rack.
        v. Load 1.5 mL Qiacube tubes with samples from step 6 into Position 3 in the Rotor Adapters.
        vi. Place rotor adaptors in rotor according to chart below (also found 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.

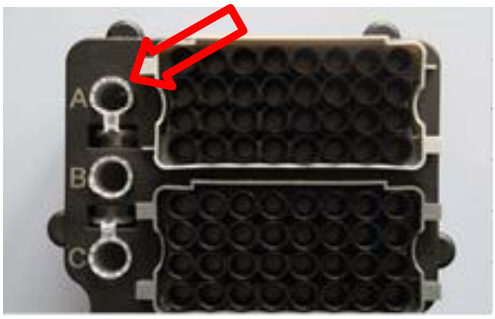
QIAcube Rotor Loading Chart (2-6 samples)

Sperm Lysis Buffer (µL)		G2 (µL)	ProK (µL)	DTT (µL)
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

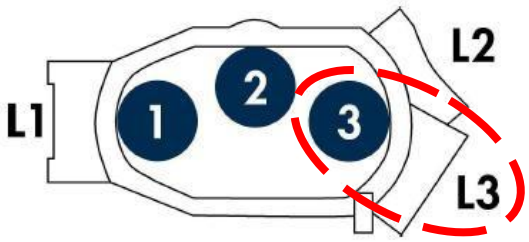
Reagent Bottle Rack – Position 1

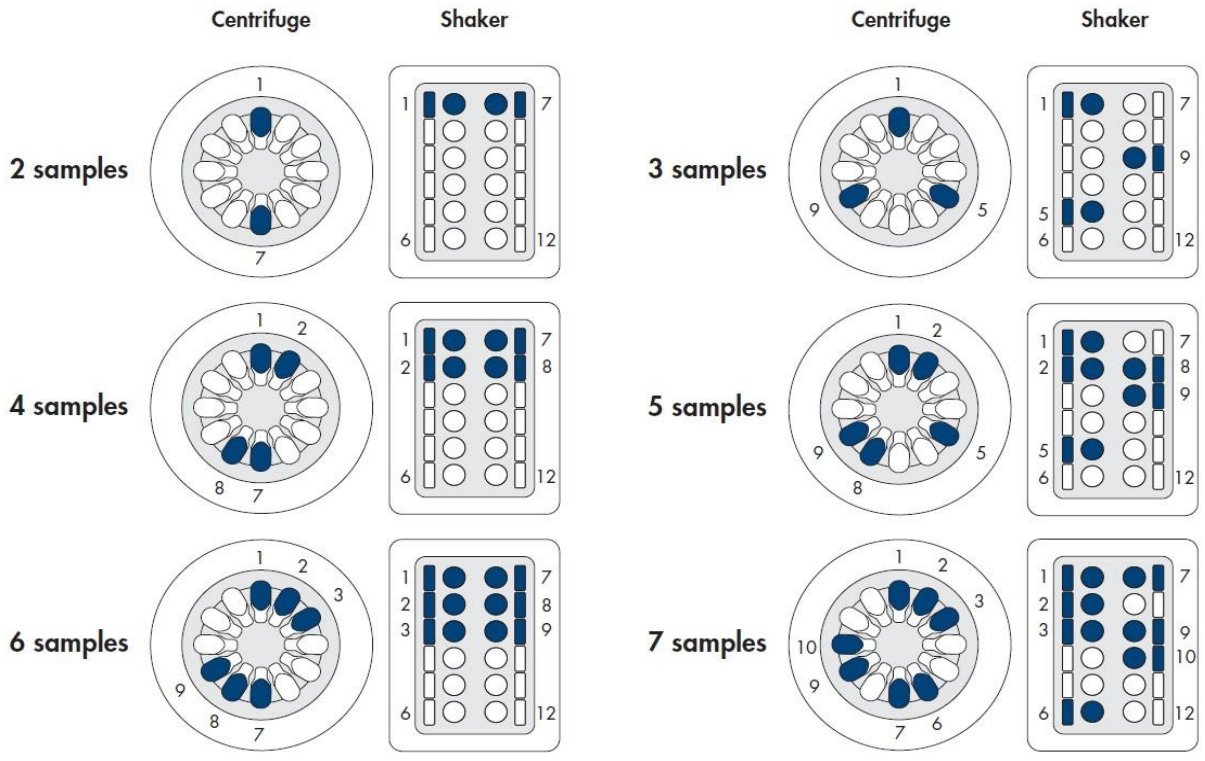


Tip Rack – Position A



Rotor Adapter – Position 3





8) Process Epithelial Fraction on EZ1 with Trace protocol. (See protocol below, 6.2.4.12) and save samples for step 15.
9) Remove 1.5 mL tube from the rotor adapter and close tube.
10) Incubate at 56 ºC for a minimum of 10 minutes.
11) Centrifuge tube briefly to remove drops from lid.
12) Transfer sample from the 1.5 mL tube to a labeled 2.0 mL EZ1 sample tube.
13) Process Sperm Fraction on EZ1 with Trace protocol. (see protocol below, 6.2.4.12)
14) Estimate the amount of DNA in the sample. (See Quantitation Kit SOP)
15) The samples are now ready for PCR amplification.

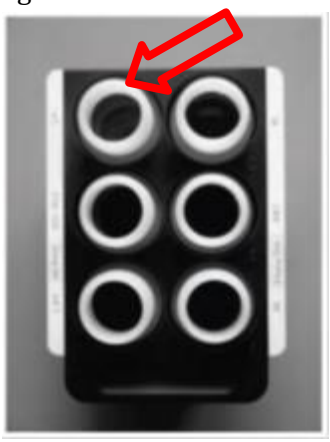
6.2.2.4 SEMEN-CONTAINING STAINS, QIACUBE (7-12 SAMPLES)

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 appropriate-size sample and place into a labeled 1.5 mL Qiacube sample tube.
3) Add 480 µL G2 buffer, then 20 µL of Proteinase K, mix by vortexing.
4) Incubate at 56 ºC for a minimum of 15 minutes.
5) Centrifuge tube briefly to remove drop from lid.
6) Remove any solid material from tube. Use toothpick to remove cloth or swab from tube, twisting to remove excess fluid. Discard solid material in appropriate waste.
7) Samples are now ready for Qiacube separation:
   g) Ensure Qiacube is on.
   h) Press DNA.
   i) Select PIPETTING and Press SELECT.
   j) Select EPITHELIAL AND SPERM CELL and Press SELECT.
   k) Select SEPARATION AND LYSIS 12 A and Press SELECT.
   l) Follow steps on screen to setup workstation:
      i. Press START.
      ii. Empty waste drawer and fill tip racks with 1000µL 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 2mL screw top tube and place in Position A in the Tip Rack.
      v. Load 1.5 mL QIAcube tubes with samples from step 6 into Position 3 in the Rotor Adapters.
      vi. Place rotor adaptors in rotor according to chart below (also found 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.

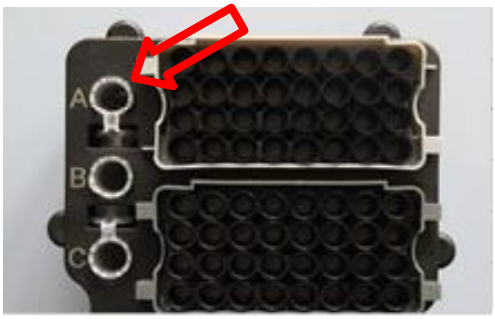
QIAcube Rotor Loading Chart (7-12 samples)

Sperm Lysis Buffer (µL)		G2 (µL)	Pro K (µL)	DTT (µL)
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

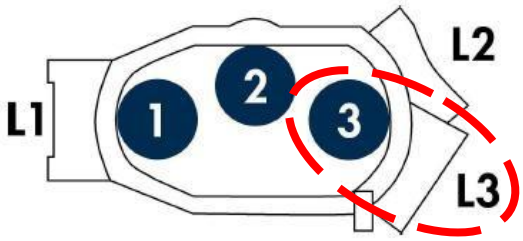
Reagent Bottle Rack – Position 1

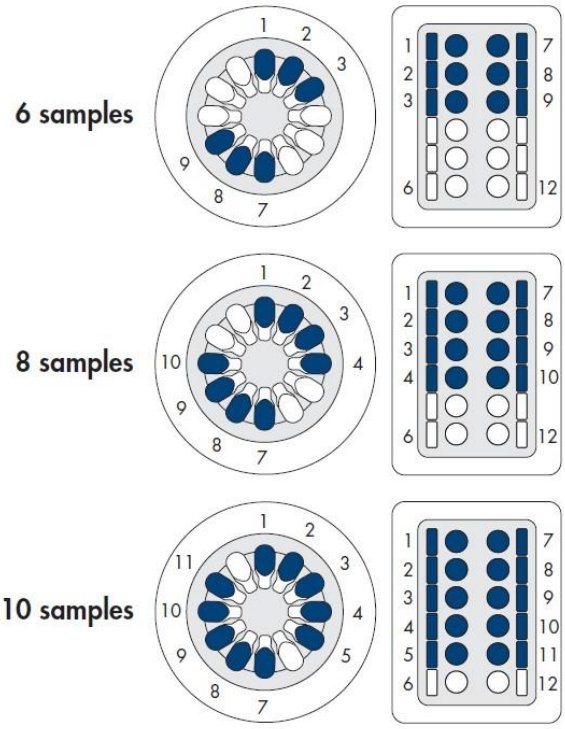


Tip Rack – Position A



Rotor Adapter – Position 3





8) Process Epithelial Fraction on EZ1 with Trace protocol. (See protocol below, 6.2.4.12) and save samples for step 16. (This step can be done alongside step 15)
9) 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 1000µL wide-bore tips.
        iii. Press Start.
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. (see protocol below, 6.2.4.12)
15) Estimate the amount of DNA in the sample. (See Quantitation Kit SOP)
16) The samples are now ready for PCR amplification

6.2.2.5 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 µL G2 buffer. Additional G2 buffer may be added to absorbent samples to ensure ~190 µL of liquid in the tube.
4) Add 10 µL of Proteinase K, mix by vortexing. (***DO NOT*** add additional Proteinase K, even if increased volume of G2 is used.)
5) Incubate at 56 ºC 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 and save samples for step 18. (see protocol below, 6.2.4.12)
10) Wash sperm cell pellet by adding 500 µL 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 µL G2 buffer to the sample tube.

13) Add 10 µL Proteinase K and 10 µL 1 M DTT.
14) Vortex for 10 seconds.
15) Incubate at 56 ºC for a minimum of 10 minutes.
16) Centrifuge tube briefly to remove drops from lid.
17) Process Sperm Fraction on EZ1 with Trace protocol. (see protocol below, 6.2.4.12)
18) Estimate the amount of DNA in the sample. (See Quantitation Kit SOP)
19) The samples are now ready for PCR amplification.

6.2.2.6 MODIFIED SEMEN-CONTAINING STAIN EXTRACTION

(Samples may contain undetected sperm cells e.g., p30(+) samples, or tape-lifts from sperm(+) and p30(+) samples, etc.)

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. Additional G2 buffer may be added to absorbent samples to ensure ~190 µL of liquid in the tube.
4) Add 10 µL Proteinase K and 10 µL 1M DTT, mix by vortexing. (DO NOT add additional Proteinase K, even if increased volume of G2 is used.)
5) Incubate at 56 ºC 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. (see protocol below, 6.2.4.12)
9) Estimate the amount of DNA in the sample. (See Quantitation Kits SOP)
10) The sample is now ready for PCR amplification.

6.2.2.7 HAIR EXTRACTION

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 ºC for a minimum of 30 minutes.
6) Centrifuge tube briefly to remove drops from lid.
7) Process on EZ1 with Trace TD protocol. (see protocol below, 6.2.4.12)
8) Estimate the amount of DNA in the sample. (See Quantitation Kits SOP)
9) The sample is now ready for PCR amplification

6.2.2.8 NAIL CLIPPINGS & 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 ºC 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. (see protocol below, 6.2.4.12)
9) Estimate the amount of DNA in the sample. (See Quantitation Kits SOP)
10) The sample is now ready for PCR amplification.

6.2.2.9 TISSUE EXTRACTION

**Note* Separation of fetal tissue from maternal tissue may be aided by rinsing and then submerging the tissues in PBS (phosphate-buffered saline) in a disposable petri dish in a cleanroom hood. Chorionic villi may be visualized around the fringes of the fetal tissues. These villi can be collected with a sterile scalpel for testing. See Johnson, et.al, JFS 2Nov2010.pdf in the Literature folder of the ForensicBiology drive for further clarification.*

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 ºC for a minimum of 3 hour.
6) Centrifuge tube briefly to remove drops from lid.
7) Process on EZ1 with Trace TD protocol. (see protocol below, 6.2.4.12)
8) Estimate the amount of DNA in the sample. (See Quantitation Kits SOP)
9) The sample is now ready for PCR amplification.

6.2.2.10 TOOTH EXTRACTION

1) Label an appropriate number of 2.0 mL EZ1 sample tubes (typically 3 to 5).
2) Remove dirt and debris, if present, from the exterior of the tooth by rinsing with sterile water or scrubbing with a disposable toothbrush.
3) Grind the tooth to a fine powder in an appropriate grinder.
4) Divide the total volume of tooth powder equally into the 2.0 mL EZ1 sample tubes. It is not recommended to exceed 150mg of powdered product per tube.
5) To each sample tube:
   a) Add 225 µL of G2 buffer.
   b) Add 25 µL of Proteinase K.
   c) Add 250 µL 0.5M EDTA, pH 8.0.

d) Mix by inverting the tube several times and incubate at 56 ºC in a heated orbital incubator for 24 hours.
e) Centrifuge at 6000 rpm for 4 minutes to pellet the remaining debris.
f) Transfer the supernatant to a labeled 2.0 mL EZ1 sample tube. Discard tube with pellet.
g) Add 400µL MTL buffer to the supernatant tube.
h) Add 50µL 3M NaOAc, pH 5.0 to each sample tube.
i) Add 1µL carrier RNA to each sample tube (optional).

6) Process all extraction tubes on an EZ1 with "Large Volume" protocol with 40µL. (see protocol below, 6.2.4.12)
7) Label a Microcon® DNA centrifugal device tube. And insert a Microcon® filter device into the tube.
8) Combine eluted DNA from duplicate tubes into a single Microcon® filter. (0.5 mL maximum volume). Seal with the attached cap.
9) Centrifuge at 500 x g until an appropriate liquid volume remains on top of the filter.
10) Place a new, labeled tube over the top of the Microcon® filter device and invert the filter in the assembly.
11) Spin at 1,000 x g for three minutes to transfer concentrate to tube.
12) Remove Microcon® filter device from the tube and discard.
13) Estimate the amount of DNA in the sample. (See Quantitation Kit SOP)
14) The samples are now ready for PCR amplification.

6.2.2.11 BONE EXTRACTION

1) Inspect the sample visually, noting amount available and the state of the bone. Determine if there is sufficient sample available for any follow-up testing (if needed). If not, consult with the DNA Supervisor, DNA Technical Leader, or the CODIS Administrator.
2) Label an appropriate number of 2.0 mL EZ1 sample tubes.
3) Prepare Bone powder:
   Remove dirt and debris, if present, from the exterior of the bone by rinsing with sterile water and/or abrading a section of the outer surface with the Dremel in the hood with the sash opened for maximum airflow.

Dremel Tool with Drill Bit

a. Using the Dremel with a fresh 1/8 inch drill bit, bore numerous holes along a 1 inch long section of the cleaned bone in the hood positioned so that the ground bone can be collected on a weigh boat or weigh paper.
b. Discard drill bit after use.

SPEX Freezer Mill

a. Using the Dremel with a cutting disc, remove a 1 inch long quarter-section window of the cleaned bone in the hood.
b. Place the section of bone in a freshly cleaned freezer mill vial and chill by submersion in the liquid nitrogen.

c. Grind the bone in the freezer mill.
d. Remove the vial from the mill and quickly transfer the contents into labeled sample tubes. Thoroughly clean the vial and vial components with hot water and dishwashing detergent using a non-scratch silicone scrub brush, followed by heavy soaking with 10% bleach spray. The parts will be rinsed with sterile dH20 and allowed to dry.
e. Once dry, the parts will be swabbed with a moist swab, which will then be extracted to ensure the cleaning has successfully decontaminated all parts before reuse.
    4) Divide the total volume of bone powder equally into the 2.0 mL EZ1 sample tubes. Do not exceed 150µl of powdered bone in a single tube.
    5) Add 225 µL of G2 buffer.
    6) Add 25 µL of Proteinase K.
    7) Add 250 µL of 0.5M EDTA, pH 8.0.
    8) Mix by inverting the tube(s) several times.
    9) Incubate at 56 ºC in a heated orbital incubator with constant motion for 24 hours.
    10) Centrifuge at 6000 rpm for 4 minutes to pellet the remaining debris.
    11) Transfer the supernatant to a 2.0 mL EZ1 sample tube.
    12) Add 400 µL MTL buffer to each 2.0 mL EZ1 sample tube.
    13) Add 50 µL of 3M NaOAc, pH 5.0 to each 2.0 mL EZ1 sample tube.
    14) Add 1 µL carrier RNA to each 2.0 mL EZ1 sample tube (optional).
    15) Process all extraction tubes on an EZ1 with "Large Volume" protocol with 40µL. (see protocol below, 6.2.4.12)
    16) Label a Microcon® DNA centrifugal device tube. And insert a Microcon® filter device into the tube.
    17) Combine eluted DNA from duplicate tubes into a single Microcon® filter. (0.5 mL maximum volume). Seal with the attached cap.
    18) Centrifuge at 500 x g until an appropriate liquid volume remains on top of the filter.
    19) Place a new, labeled tube over the top of the Microcon® filter device and invert the filter in the assembly.
    20) Spin at 1,000 x g for three minutes to transfer concentrate to tube.
    21) Remove Microcon® filter device from the tube and discard.
    22) Estimate the amount of DNA in the sample. (See Quantitation Kit SOP)
    23) The samples are now ready for PCR amplification.

6.2.2.12 PROCESSING SAMPLES ON EZ1

1) Ensure EZ1 workstation is on.
2) Press START to start protocol setup.
3) Press ESC (for no report).
4) Press 1 or 2 (for Trace or Trace TD protocol), or 3 (for Large-Volume).
5) Press 2 (for elution in TE buffer).
6) Choose elution volume (most applications will be 50 µL, but highly concentrated samples can be diluted in larger volumes).

7) Press any key to continue.
8) Follow steps on screen to setup workstation:
9) Load cartridges into the rack.
10) Load opened 1.5 mL elution tubes in Row 1 of tip rack.
11) Load tip holders and tips in Row 2 of tip rack.
12) Load opened 2.0 mL sample tubes in Row 4 of tip rack.
13) Close workstation door.
14) Press START to start protocol.
15) Once display show PROTOCOL FINISHED, remove elution tubes. Discard waste appropriately.
16) Estimate the amount of DNA in the sample. (See Quantitation Kits SOP)
17) The sample is now ready for PCR amplification.

6.2.3 EXCEL SAMPLE MANAGEMENT (DNA-FORM-01)

1) Open form from ForensicBiology\Excel Form\
2) Enable macros (if needed)
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 information as queried.
6) Enter Case number and Samples Name on the *SAMPLES* tab.
7) Press the *CONTINUE TO QUANT* 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 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 *HYres or QuantPro* as template.
16) Click *FILE IMPORT SAMPLE SETUP*, select the text file saved on the 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 the flash drive and click *SAVE*.
20) Go to *QUANT SETUP* in Excel sheet and click *IMPORT QUANT DATA* button.
21) Select the file saved from the 7500 (It will be a .cvs file) click *OK*.

22) Now go to *TOTAL DNA QUANTITY TAB* and examine the data.
23) Adjust any dilutions as needed and then Press the *CONTINUE TO STR AMP PAGE*.
24) Record the Thermocycler used, add the controls to the end of the list. Change the amount of DNA to the appropriate amount if need.
25) Press *PRINT SHEETS FOR AMPLIFICATION* button.
26) Select injections needed from list.
27) Select location and then 'save file as' XXXX(Run Name),
28) Take flash drive to 3500xl.
29) On the 3500xl in the Plate manager, click the *IMPORT PLATE BUTTON*.
30) Select the txt file saved on the flash drive and click *OK*.
31) 3500xl will say that it imported OK. If gives an error of invalid symbol or other issue with the name, there is possibly had a space in a sample name on the first page.
32) Start *RUN*.
33) Press PRINT WORKSHEETS TO JUSTICETRAX IMAGING and Select *JUSTICETRAX IMAGING*.
34) Once the 3500xl run is completed, copy the files and analyze with GeneMapper ID-X.
35) Once all files have been analyzed, go back to the main GeneMapper windows and change the table settings to the export selection.
36) Next, click *FILE*, *EXPORT COMBINED TABLE*.
37) Select *ONE LINE PER SAMPLE* on the right
38) Place the file where it can be found later.
39) Click *EXPORT COMBINED TABLE*.
40) In the Excel file on the *FINAL* tab select the samples for each injection time, if needed.
41) Click *GENERATE CALL SHEETS*.
42) Press the injection time that needs to have data imported to, if needed
43) Select the file and click *OK*.
44) Correct all calls on the call sheets
45) For Y-23 statistics, select desired profiles on the *FINAL* tab, click the *Y-STATS* button.
46) Open, then select and copy the desired Y23 profile. Press the open *Y23_STATS* button, and paste the selection into the desired cells.

6.2.4 DNA QUANTITATION PROTOCOLS

The QIAGEN Investigator Quantiplex Pro (Quant Pro) system is used for the quantification of amplifiable total human and human male DNA in a sample. The DNA quantitation assay combines a target-specific human DNA assay, target-specific human male DNA assay, and an internal PCR control (IPC) assay. Quant Pro also includes a human DNA degradation assay.

6.2.4.1 PREPARING THE DNA QUANTIFICATION STANDARD

Quant Pro Qiagility Preparation

- Label 0.2ml tubes A-E, and NTC. Vortex the Kit DNA Standard 3-5 sec.
- Transfer 120 uL of the standard into each of the 0.2ul tubes labeled A.
- Transfer 77 uL of the diluent into each of the 0.2ul tubes labeled NTC.
- Transfer the remaining Diluent into one of the remaining 1.5ml tubes
- Prepare Master Mix with 144 uL Reaction Mix and 144 uL Primer into the last 1.5ml tube.
- Turn on the Qiagility instrument, open the template called QuantPro Standards on the computer desktop.
- Set up the instrument deck by replacing the 50ul tips (top left position) with the 200ul tips from the cabinet.
- Place the Master Mix tube in the center well and the Diluent tube in the bottom left well of the 5 tube holder block.
- Place all 12 of the tubes labeled A-E, and NTC in the location for Standards.
- Place a labeled plate on the instrument deck.
- Run the program. When done, cover plate with a clear adhesive cover.
- Continue below:

Quant Pro Manual Preparation

- Label 0.2ml tubes A-E, and NTC. Vortex the Kit DNA Standard 3-5 sec.
- Transfer 99 uL of the standard into each of the 0.2ul tubes labeled A.
- Transfer 99 uL of the diluent into each of the 0.2ul tubes labeled B-E and NTC.
- Transfer 9 uL of STD A into each STD B tube. Vortex to mix.
- Transfer 9 uL of STD B into each STD C tube. Vortex to mix.
- Transfer 9 uL of STD C into each STD D tube. Vortex to mix.
- Transfer 9 uL of STD D into each STD E tube. Vortex to mix.
- Prepare Master Mix with 144 uL Reaction Mix and 144 uL Primer into the last 1.5ml tube.
- Label a 96-well optical amplification plate.
- Aliquot 18 uL reaction mix in wells 1A-1F and 2A-2F.
- Load 2 uL of each STD per the default QuantPro plate setup. Cover plate with clear adhesive cover.
- Continue below:

- Place plate on the 7500 instrument. Run the default template for *QuantPro*; there is no need to import a sample setup.
- Export the run results.
- Open the *QuantPro QC Check.xlsm* file from the S drive. Instructions can be found on the *Instructions* sheet if needed. Go to *Input_CSV* sheet.
- Delete all present data if needed. Click *Yes*.
- Go to the *Data* tab and select *From Text*. Import your exported results file.
- Select *Delimited*, then *Next*. Check *Tab* and *Comma*, then click *Finish* and *OK*.
- Go to *QC Check* sheet and make sure there are green "Pass" boxes for standards A-E listed. If passing, check/update the lot

number and preparation and expiration dates on *QC Check*. Click *Export* button to export as a .pdf and record the new standards in the *Quant STD QC.xls*.

- Hyperlink information from *QuantPro QC* excel file to *QuantPro QC Check* data in the *QCSheets* folder.
- Remove old Standard Curve dilutions and place one set of new labeled standards in each clean room.

6.2.4.2 PREPARING THE QUANTITATION REACTION

Manual Preparation

- Prepare Quantitation kit master mix per the instructions on the DNA Quantitation worksheet (DNA-FORM-1a):

  - # wells (+ 3-5 extra) x 9 µL PCR Reaction Mix
  - # wells (+ 3-5 extra) x 9 µL Primer Mix

- Vortex and dispense 18 µL of the master mix into each well of a 96-well reaction plate.
- Add a total of 2 µL of sample, standard or control to the appropriate well.
- Seal the reaction plate with the optical adhesive cover.
- Centrifuge the plate at 3000rpm for about 20 seconds.

Qiagility Preparation

- Prepare Quantitation kit master mix per the instructions on the DNA Quantitation worksheet (DNA-FORM-1a):

  - # wells (+ 3-5 extra) x 9 µL PCR Reaction Mix
  - # wells (+ 3-5 extra) x 9 µL Primer Mix

- Turn on the Qiagility.
- Start the program called [*Kit*] *7500*.
- Put the Master Mix in the center well of the 5 tube holder block.
- Place the Standard Curve Tubes in the block for Standards.
- Put a labeled plate on the machine.
- Place sample tubes in the sample blocks according to the Qiagility Setup sheet that is printed from the excel sheet.
- Press the *GREEN TRIANGLE* Button to start run.
- Seal the reaction plate with the optical adhesive cover.
- Centrifuge the plate at 3000rpm for about 20 seconds.

6.2.4.3 RUNNING THE REACTIONS AND ANALYSIS OF RESULTS

1) Running the Reactions:
   a) Turn on the computer and then turn on the 7500 instrument.

b) Position the plate in the 7500 instrument thermal block so that well A1 is in the upper-left corner.
c) Initialize the ABI data collection software.
d) Select *New* from the *File* menu.
e) From the drop-down menu under *Template*, select *Quant Pro*.
f) Select *Import Samples Setup* from File menu.
g) *Save As*- with the file name (i.e., Init_MMDDYY.sds)
h) On Instrument tab, press start when ready to run.

2) Data Analysis:
a) Press the *Green Triangle* icon to automatically analyze the 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 R2 ≥ 0.98. If not, outlying standard results can be eliminated to a minimum of one set of 5 standard measurements. If it still does not have a R2 ≥ 0.98, then the DNA quantities will be used with caution.

3) 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/µL) can be diluted appropriately and amplified.
c) Stopping further analysis based on quantitation results:
i. If total DNA quantitation result is .0025 ng/µL or below then the sample does not need to be further processed for STR analysis. (See report sections for report wording)
ii. If a sexual assault sample contains 5% or less of male contributor (Quant Pro: Male quantitation result / Total quantitation result * 100) then the sample does not need to be further processed for STR analysis. (See report sections for report wording)
iii. If the semen fraction of a sexual assault case is processed for STR analysis then the corresponding epithelial fraction will be processed regardless of the percent male contribution in the epithelial fraction.
iv. If all question (Q) items are stopped because of quantitation then victim knowns do not need to be processed for STR analysis. Suspect knowns will be processed for STR analysis.
d) Y-STR analysis is not currently stopped due to low or undetectable male quantitation results. All question items may therefore be eligible for analysis with the current Y-STR amplification kit. However, case specific factors may dictate that some samples not be processed for Y-STR analysis. These include:
i. In cases with multiple samples, low or undetected male samples may be reserved pending Y-STR results from samples with good male quantitation results,
ii. Number of suspected male perpetrators,
iii. Number of male contributors to the autosomal mixture exceeds two.

6.2.5 DNA AMPLIFICATION PROTOCOLS

6.2.5.1 AMPLIFICATION KIT BACKGROUND

PowerPlex 16 HS

The PowerPlex 16 HS System allows co-amplification and three-color detection of sixteen loci (15 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. (See Table 1) It is noted that PowerPlex 16 HS is no longer in active use by the ASCL.

Table 1. The PowerPlex 16 HS PCR Amplification System

STR Locus	Label	Chromosomal Location	Alleles Included in PowerPlex 16 HS Allelic Ladder	Control 2800M
Penta E	FL	15q	5 - 24	7,14
D18S51	FL	18q21.3	8-10, 10.2, 11-13, 13.2, 14-27	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, 33.2, 34, 34.2, 35, 35.2, 36-38	29,31.2
TH01	FL	11p15.5	4-9, 9.3, 10-11, 13.3	6,9.3
D3S1358	FL	3p	12-20	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	20,23
TPOX	TMR	2p24-2pter	6-13	11,11
D8S1179	TMR	8q	7-18	14,15
vWA	TMR	12p12-pter	10-22	16,19
Amelogenin	TMR	Xp22.1-22.3 and Y	X, Y	X,Y
Penta D	JOE	21q	2.2, 3.2, 5, 7-17	12,13
CSF1PO	JOE	5q33.3-34	6-15	12,12
D16S539	JOE	16q24-qter	5, 8-15	9,13
D7S820	JOE	7q11.21-22	6-14	8,11
D13S317	JOE	13q22-q31	7-15	9,11
D5S818	JOE	5q23.3-32	7-16	12,12

PowerPlex Fusion 6C

The PowerPlex Fusion 6C System is a 27-locus multiplex for HID applications including forensic analysis, relationship testing and research use. This six-color system allows co-amplification and detection of the 18 autosomal loci in the expanded CODIS core loci (CSF1PO, FGA, TH01, vWA, D1S1656, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11) with Amelogenin and DYS391 for gender determination. The Penta D, Penta E, D22S1045, TPOX and SE33 loci are included to increase discrimination and allow searching of databases that include these loci. Finally, two rapidly mutating Y-STR loci, DYS570 and DYS576, are included in the multiplex. This extended panel of STR markers is intended to satisfy both CODIS and ESS recommendations. (Table 2)

Table 2. The PowerPlex Fusion 6C PCR Amplification System

STR Locus	Label	Chromosomal Location	Alleles in PowerPlex Fusion6C Allelic Ladder	Control 2800M
Amelogenin	FL-6C	Xp22.1–22.3 and Y	X, Y	X, Y
D3S1358	FL-6C	3p21.31 (45.557Mb)	9–20	17, 18
D1S1656	FL-6C	1q42 (228.972Mb)	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19, 19.3, 20.3	12, 13
D2S441	FL-6C	2p14 (68.214Mb)	8–11, 11.3, 12–17	10, 14
D10S1248	FL-6C	10q26.3 (130.567Mb)	8–19	13, 15
D13S317	FL-6C	13q31.1 (81.62Mb)	5–17	9, 11
Penta E	FL-6C	15q26.2 (95.175Mb)	5–25	7, 14
D16S539	JOE-6C	16q24.1 (84.944Mb)	4–16	9, 13
D18S51	JOE-6C	18q21.33 (59.1Mb)	7–10, 10.2, 11–13, 13.2, 14–27	16, 18
D2S1338	JOE-6C	2q35 (218.705Mb)	10, 12, 14–28	22, 25
CSF1PO	JOE-6C	5q33.1 (149.436Mb)	5–16	12, 12
Penta D	JOE-6C	21q22.3 (43.88Mb)	2.2, 3.2, 5–17	12, 13
TH01	TMR-6C	11p15.5 (2.149Mb)	3–9, 9.3, 10–11, 13.3	6, 9.3
vWA	TMR-6C	12p13.31 (5.963Mb)	10–24	16, 19
D21S11	TMR-6C	21q21.1 (19.476Mb)	24, 24.2, 25, 25.2, 26–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38	29, 31.2
D7S820	TMR-6C	7q21.11 (83.433Mb)	5–16	8, 11
D5S818	TMR-6C	5q23.2 (123.139Mb)	6–18	12,12
TPOX	TMR-6C	2p25.3 (1.472Mb)	4–16	11,11
D8S1179	CXR-6C	8q24.13 (125.976Mb)	7–19	14,15
D12S391	CXR-6C	12p12 (12.341Mb)	14–17, 17.3, 18, 18.3, 19–27	18,23
D19S433	CXR-6C	19q12 (35.109Mb)	5.2, 6.2, 8–12, 12.2, 13, 13.2, 14, 14.2,15, 15.2, 16, 16.2, 17, 17.2, 18, 18.2	13,14
SE33	CXR-6C	6q14 (89.043Mb)	4.2, 6.3, 8–20, 20.2, 21, 21.2, 22, 22.2, 23.2, 24.2, 25.2, 26.2,27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35–37, 39	15,16
D22S1045	CXR-6C	22q12.3 (35.779Mb)	7–20	16,16
DYS391	TOM-6C	Y	5–16	10
FGA	TOM-6C	4q28 (155.866Mb)	14–18, 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, 32.2, 33.2,42.2, 43.2, 44.2, 45.2, 46.2, 48.2, 50.2	20,23
DYS576	TOM-6C	Y	11–23	18
DYS570	TOM-6C	Y	10–25	17

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 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 (Table 3). PowerPlex Y23 is no longer the primary Y-STR amplification kit in active use by the ASCL and will no longer be included in ASCL DNA proficiency tests. Once current proficiency tests expire, analysts must retake a proficiency test with Y23 to process new samples. Reanalysis of existing Y23 data is permitted by an analyst within two years of the last Y23 proficiency with expressed authorization by the Technical Leader.

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

Yfiler Plus

The Yfiler Plus PCR Amplification Kit is a 6-dye, short tandem repeat (STR) multiplex assay that amplifies 27 Y-STR loci in a single PCR reaction, see Table 4. The Yfiler Plus Kit Allelic Ladder is used to genotype the analyzed samples when using GeneScan™ 600 LIZ™ Size Standard v2.0. The genotype of the Control DNA 007 and the alleles contained in the allelic ladder are listed in the table. (Table 4)

Table 4: The Yfiler Plus PCR Amplification System

Locus designation	Dye label	Alleles included in Allelic Ladder	DNA Control 007
DYS576	6-FAM™	10-25	19
DYS389I		9-17	13
DYS635		15-30	24
DYS389II		24-35	29
DYS627		11-27	21
DYS460	VIC™	7-14	11
DYS458		11-24	17
DYS19		9-19	15
YGATAH4		8-15	13
DYS448		14-24	19
DYS391		5-16	11
DYS456	NED™	10-24	15
DYS390		17-29	24
DYS438		6-16	12
DYS392		4- 20	13
DYS518		32-49	37
DYS570	TAZ™	10-26	17
DYS437		10-18	15
DYS385 a/b		6-28	11,14
DYS449		22-40	30
DYS393	SID™	7-18	13
DYS439		6-17	12
DYS481		17-32	22
DYS387S1		30-44	35,37
DYS533		7-17	13

6.2.5.2 AMPLIFICATION SET UP

PowerPlex Fusion 6C

Manual Plate Set Up:

- Prepare Fusion 6C master mix per the instructions on the STR Master Mix worksheet (DNA-FORM-1d):
  - # wells (+ 3-5 extra) x 5 µL PCR Reaction Mix
  - # wells (+ 3-5 extra) x 5 µL Primer Mix
- Dispense 10 µL of master mix and 15 µL of sample into each well on a 96-well amp plate as indicated by the STR Amplification Worksheet (DNA-FORM-1c).
- Cover the plate with PCR septa.

Qiagility Plate Set Up:

- Prepare Fusion 6C master mix per the instructions on the STR Master Mix worksheet (DNA-FORM-1d):
  - # wells (+ 3-5 extra) x 5 µL PCR Reaction Mix
  - # wells (+ 3-5 extra) x 5 µL Primer Mix
- Export the sample setup data from the STR Amplification Worksheet (DNA-FORM-1c) onto a USB memory stick.
- Turn on the Qiagility.
- Start the Fusion setup program.
- Import the sample setup .QAS file as indicated on the Qiagility Amp Setup Sheet that is printed from the STR Amplification Worksheet.
- Put the Master Mix in the appropriate well of the 5 tube holder block.
- Place the 2800M Positive PCR control in the appropriate location in the Standards block.
- Put a labeled plate on the machine.
- Place sample tubes in the sample blocks according to the Qiagility Amp Setup Sheet.
- Press the Green Triangle Button to start run.
- Cover the plate with a PCR septa.

Briefly spin the plate in the centrifuge and place into the thermocycler. To start the Amplification 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.
5) Wait for confirmation that the program has begun before leaving.

The following are the thermocycler parameters used for amplification of PowerPlex Fusion 6C:

96°C	1min	
96°C	5sec	
60°C	1min	29cycles
60°C	10min	
4°C	forever	

Yfiler Plus

Manual Plate Set Up (no Qiagility Set Up available):

- Prepare Yfiler Plus master mix per the STR Master Mix worksheet instructions (DNA-FORM-1d):
  - # wells (+ 3-5 extra) x 10 µL Master Mix
  - # wells (+ 3-5 extra) x 5 µL Primer Set
- Dispense 15 µL of master mix and 10 µL of sample into each well on a 96-well amp plate as indicated by the STR Amplification Worksheet (DNA-Form-1c).
- 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.
  5) Wait for confirmation that the program has begun before leaving.

The following are the thermocycler parameters used for amplification of Yfiler Plus:

Initial Incubation Step	Optimum Cycle Number: 29		Final Extension	Final Hold
	Denature	Anneal/Extend		
95°C	94°C	61.5°C	60°C	4°C
1 minute	4 seconds	1 minute	22 minutes	∞

6.2.5.3 3500XL SAMPLE SET UP

After amplification is complete, samples are set up for the 3500xl. A 96 Opti-Well plate is used. It is best to pre-warm the oven approximately 15 minutes before the run starts.

PowerPlex Fusion 6C & Yfiler Plus

1) Create a master mix solution in the following ratios:

PowerPlex Fusion 6C	Yfiler Plus
0.5 µL Internal Lane Standard (WEN_ILS_500)	0.4 µL GeneScan™ 600 LIZ™ Size Standard v2.0
9.5 µL of HiDi Formamide	9.6 µL of HiDi Formamide

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

10) On the 3500xl computer, go to Library, and click on Import.
11) Select the .txt file to import. The typical setup is for a single 24 second injection at 1.2 kV Fusion6C or Yfiler Plus by default. Edit settings as necessary and click Okay.
12) Go to the Dashboard and press the Pre-Heat Button.
13) Link the appropriate plate to the plate map under the Load Plates for Run.
14) To start the run, click on the Start Run Button.

6.2.6 STR ANALYSIS

6.2.6.1 ANALYSIS OF RAW DATA/GENEMAPPER ID-X

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

***Note: PowerPlex 16HS and Powerplex Y23 information is restricted to reanalysis of historical data files by Technical Leader-approved analysts. A memorandum of authorization and competency test is required if this reanalysis is more than 2 years from the date of completion of the last proficiency test with this technology.*

- 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 and select FILE>ADD SAMPLES TO PROJECT.
  2) Select the appropriate run folder saved on the ForensicBiology drive and click ADD TO LIST. Once all appropriate 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:
     ASCL_Fusion6C/ASCL_Fusion6C_AXPT for PowerPlex Fusion 6C data.
     ASCL_Yfiler+_v1.0 for Yfiler Plus data.
     ASCL_PP16HS/ASCL_PP16HS_AXPT/ASCL_Y23 for historical PP16HS/PPY23 data.
  5) Select Panel:
     PowerPlex_Fusion_6C_Panels_IDX_v1.1 for PowerPlex Fusion 6C data.
     Yfiler_Plus_Panel_v4_ASCL for Yfiler Plus data.
     PowerPlex_16_IDX_Alpha/ PowerPlexY23_IDX_v2.0 for historical PP16HS/PPY23 data.
  6) Select Size Standard:
     WEN_ILS_500_CS for PowerPlex Fusion 6C data.
     GS600_LIZ_(60-460) for Yfiler Plus data.
     ILS 600/WEN_ILS_500_IDX_Y23 for historical PP16HS/PPY23 data.
  7) Click the GREEN ARROW to analyze the project.

8) View the raw data to examine the sizing standards. 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 analytical threshold 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).

6.2.6.2 ANALYSIS OF AUTOSOMAL PROFILES 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 mixtures are to go through ArmedXpert (AX) interpretation, 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. Appendix B is a Generalized Guideline for Mixture Interpretation Workflow. The workflow and the guidelines below allow for the interpretation of the majority of samples in the DNA section of the Arkansas State Crime Laboratory. Any significant deviation from the workflow or guidelines below needs to be discussed and approved by the DNA Technical Leader.

***NOTE: PowerPlex 16 HS is restricted to reanalysis of historical data files by Technical Leader-approved analysts. A memorandum of authorization is required if this reanalysis is more than 2 years from the date of completion of the last proficiency test with this technology*

Combine 12 and 24 second injections of a run into one GeneMapper Project.

- Add samples to GM project as usual starting with the 12sec injection and analyze as usual with ASCL_PP16HS Analysis Method
- Once it has been determined which samples require reinjection at 24sec, import those samples from the 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 all samples (12 &

24 sec) can be viewed by selecting the top-most "project" header, or samples for only 12 or 24 sec injection can be viewed by selecting the respective name from the project tree.

- If both 12 second and 24seconds samples are analyzed on the same GM project, change the Sample Name in the GM Project to indicate 24sec injection (e.g., add "_24" suffix to the sample name). This allows differentiation between 12 and 24 sec data on the electropherogram and call sheets. Also do this for all necessary 24sec controls.

PowerPlex Fusion 6C and PowerPlex 16 HS:

1) Mixtures that need interpretation will be reanalyzed using ASCL_Fusion6C_AXPT or 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) Exporting samples to Excel and ArmedXpert from GeneMapper:
    a) In GM, select ARMEDXPERT EXPORT from the Table Setting drop down menu (for PowerPlex 16HS, make sure to select the top line of the PROJECT tree so that both 12 and 24 sec samples are exported.)
        i. FILE -> EXPORT COMBINED TABLE
        ii. In pop up window select ONE LINE PER SAMPLE (all other defaults are correct) and export file to the AX folder on the ForensicBiology drive
    b) Import this data to make the call sheet in Excel:
        i. On the "Final" tab of the DNA-FORM-1 Excel workbook (located on the ForensicBiology drive) there is a set of buttons titled For PP16HS and For PPF6C to the right of the old ones; use these to import the AX export file and print the call sheets. For single source samples, this is the sheet that needs to match the 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. PowerPlex Fusion 6C Loci with alleles <600RFU (and PowerPlex 16HS 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 the samples and allele calls listed.
3) Sample Comparison Check. This not triggered automatically in AX so it must be performed manually it on each 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 the text file in both the REFERENCE box and the COMPARISON box. (This will compare the 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 the project name (regardless of if there are matches) to the ArmedXpert folder on the ForensicBiology drive.

4) Statistics on single source profiles: on "Interpretation" tab, select the desired stat button (e.g., RMP or Single Source) and use the mouse to select the sample on the table desired for stats.

    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 the 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 desired.

    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 a minor will be called 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 such as 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 the interpretation is started, these changes will not be applied globally (to the entire profile)

        i. If peak height imbalance is causing the mixture information to advise 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 this has happened with the sample, stutter can be reduced from 100% at the loci. (Keep in mind that stutter ratios applied are the max expected values from the validation at that locus and can vary between alleles within a locus) Typically if the peak height of an stutter allele that has had 100% stutter removed is between 60-174 rfu it indicates that it could be a true minor peak and the stutter percentage should be lowered to 60% to see if the peak is recovered. If it is not recovered by lowering stutter percentage, but it has been determined it to be a true minor allele, select CONSIDER ALLELE from the pop-out calls window and make a note in the ADD COMMENTS box. If it is not possible to determine if an allele is real or artifact, it may be included as a possible allele in the interpretation, with an appropriate note in the ADD COMMENT box ("Considered X, possible allele, 165 rfu @ 60%). If the obligate sister allele is below stochastic threshold, the possible allele is included in the 2p (allele, any) statistic. If however, the obligate sister is above stochastic threshold, then the possible allele should be included as one of the options in the statistics, but the possible allele should not also be an obligate allele.

iii. If other adjustments have been made to the 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.

vi. When selecting genotype possibilities in ArmedXpert variation in the relative contribution should remain consistent for the entire profile. Any given loci should not deviate more than 10% points (eg. If it is an 80% / 20% mix the minor genotypes combinations should be listed as 10-30% of the mixture). If the sample is have degradation or inhibition issues than 15% points can be used. Greater than 15% points is possible, but should only occur in a small number of loci, otherwise it is an indication of possibly more contributors or other issues with the sample.

g) Recording the mixture deconvolution: On mixture interpretation page, click the "view call report" button. This will open an excel-style workbook.

i. The CALLS page shows the 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 the electros in JTX. It is recommended that the mixture sample electros be re-analyzed using the usual Analysis Method (which will remove most stutter) and add back in any true peaks as determined by AX before imaging the electro into JTX.
2. Print this page for the case file. Recommended settings: LANDSCAPE; FIT TO PAGES 1X1

ii. The MIX INTERP page shows the mixture interpretation details including any comments or adjustments that may have been made. Print this for the file if necessary. Note that printing MIX INTERP is not necessary in all circumstances (e.g., if a major is useable but minor will be reported as inconclusive). Recommended settings: Portrait, Zoom to 100%

iii. Before exiting, save the Mixture Interpretation to the AX folder on the ForensicBiology drive (AX will prompt to save on 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 desired (e.g., "RMP") and use the mouse to highlight the sample desired (e.g., the major or minor component of the deconvoluted mixture). This will open an excel-style window with the statistics.

i. The FREQUENCIES tab will list the statistics. (separate tab in ArmedXpert v3.0.7 only)
   1. Adjust the number of contributors (box at the bottom) if necessary.
   2. Make any other changes needed / commented on during mixture deconvolution (e.g., homozygote not a viable option for X locus)
   3. Print the stats for the file. Recommended settings: Portrait; FIT TO PAGES 1X5 (it will automatically scale back if it can fit it in less pages)

iv. The CALCULATIONS tab is for reference and does not need to be printed. (ArmedXpert v3.0.7 only)

v. If any changes were made, it is recommended that the Frequency Analysis be saved to the AX folder on the ForensicBiology drive (AX will prompt before closing that window)

vi. Compare deconvoluted mixture to known samples if applicable (see 6.b. below).

6) Checking References: on the AX main page top bar menu, select MATCH & COMPARISON
   a) FIND WHERE REFERENCE IS INCLUDED may be used when comparing a known to a batch or a sample
   b) FIND INCLUDED IN THE REFERENCE may 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 the 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 the 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) desired and move them to the right side of the window using the ">" button. Press the "LOAD" button.

6.2.6.3 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 unknown will be evaluated for suitability for comparison and (if applicable) for statistical rarity calculations, prior to comparison to one or more known reference items.

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.

6.2.6.3.1 ANALYTICAL THRESHOLD

The minimum peak height threshold will be set at 175 (Relative Fluorescent Unit) RFU for PowerPlex 16 HS and Fusion 6C 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 and Fusion 6C. Optimal peak height values range between 1000-4000 RFU, although acceptable and typeable signals may occur outside of this range.

6.2.6.3.2 STOCHASTIC THRESHOLD

The stochastic threshold is the value that denotes both peaks for a heterozygous locus will be detected and it is set at 600 RFU for PowerPlex Fusion 6C. For PowerPlex 16HS reanalysis, the stochastic threshold is set at 500 RFU.

6.2.6.3.3 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 thresholds were. Peak height of the higher sister allele dictates which ratio to use below.

PowerPlex 16HS (reanalysis)

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

Fusion 6C

Peak Height	Ratio
>1500 RFU	60%
<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.

6.2.6.3.4 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 is observed for the first time with a size of 273.44 bp, and the 22 allele of the **D2S1338** ladder is 271.45 bp, then the peak will be designated a **D2S1338** 22.2 OL. 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.

Following the confirmation as a true Off Ladder allele, the allele will be added to the appropriate Ladder(s) as a virtual allele with the OL designation. Any second observation of the same allele will also be reconfirmed.

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.

6.2.6.3.5 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 will be re-amplified and run a second time. 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 needs to be notified). Confirmed triallelic loci will not be used for statistical calculations.

6.2.6.3.6 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.

6.2.6.3.6.1 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 will be noted and may be re-injected.

6.2.6.3.6.2 STUTTER

In addition to an allele's primary peak, artifact peaks can occur at 2-, 3-, 4-, or 5-base intervals. The most common stutter peaks observed in all loci are 1 repeat smaller than the primary peak ("n-4"). It is also possible to see additional peaks 1 repeat larger ("n+4"), especially when excessive DNA template is 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 will interpret the results with caution. The sample may be re-amplified with less input DNA or re-injected.

Approved STR Stutter Ratios can be seen in table form in Appendix E.

6.2.6.3.6.3 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.

6.2.6.3.6.4 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.

6.2.6.3.6.5 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.

6.2.6.3.7 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.

6.2.6.3.8 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 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-determine 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.

6.2.6.3.9 INTERPRETATION OF MIXED SAMPLES

The interpretation applied to a mixed sample by the analyst in each particular case will be based upon all relevant information. Appendix B is a Generalized Guideline for Mixture Interpretation Workflow. The workflow and the guidelines below allow for the interpretation of the majority of samples in the DNA section of the Arkansas State Crime Laboratory. Any significant deviation from the workflow or guidelines below needs to be discussed and approved by the DNA Technical Leader.

- **Note – For PowerPlex 16 HS** All mixture samples will have been injected for 24 seconds on the 3500xl. The two exceptions to this rule in PowerPlex 16 HS are if the minor is already too complex in the 12 second injection data, and if the minor profile is the victim that will be applied to the mixture.

- Determination of the number of contributors is performed by examining all loci for the number of alleles present. (Caution should be taken when the potential contributors are related due to the sharing potential of sharing alleles)

- If any locus has greater than 6 alleles the sample has four or more contributors and is deemed inconclusive.

- If any locus has greater than 4 alleles then the number of total autosomal alleles needs to be counted.

- If there are greater than 97 total autosomal alleles (63 in PP16HS) the sample is most likely a four person mix and is deemed inconclusive.

- If there are between 90 and 97 (between 59 and 63 in PP16HS) total autosomal alleles then the mixture could be a three or four person mixture and will be interpreted with caution.

- If there are less than 90 (59 in PP16HS) total autosomal alleles then the mixture is most likely a 3 person mixture and may be analyzed.

- If no locus has more than 4 alleles then the peak height ratios will be examined to insure they are consistent with a two person mixture.

- 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 will 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 may 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.

6.2.6.3.10 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 give results above 175 RFU for PowerPlex 16 HS and PowerPlex Fusion 6C. 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. Incomplete samples may be reamplified with increased template amounts up to 5ng per reaction.

6.2.6.3.11 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 will be reported as "cannot be excluded from contributing to the mixed profile from …" The affected areas will be left out of the statistical calculation.

6.2.6.3.12 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 profiles 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 STR-Base and must be listed on the report. Any mutation occurring from father to male child will have Y-STR analysis performed for confirmation.

6.2.6.3.13 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 $p^2 + p(1-p)Ø = 0.01$ 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.

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:

(Frequency of 7 allele)^2 + Frequency of the 7 allele (1-Frequency of 7 allele)Θ = $(0.141)^2 + 0.141(1-0.141)0.01 = 0.021$

OR

TH01 Genotype 7, 9.3:

2(Frequency of 7 allele)(Frequency of 9.3 allele) = 2(0.141(0.340) = 0.096

6.2.6.3.14 ARMEDXPERT

ArmedXpert software allows an analyst 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.

6.2.6.3.15 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 <u>homozygotes (AA),</u>

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 <u>heterozygotes</u> (AB), the formula : $F = 2pq$
d) For all loci to identify the match: $F = (f_1 \times f_2 \times f_3 \ldots)$
e) Inverse probability = $1/F$

6.2.6.3.16 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) **<u>Mixture formula</u>***: 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.

b) **<u>Likelihood ratio</u>:** 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 Tho1, 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, $x \geq 2$.

c) Probability of Exclusion (PE)

**Note* Probability of Exclusion is retained primarily as a statistical calculation for kinship and historical data that cannot be reanalyzed with more current methods. CPE / CPI can only be performed on loci that do not show signs of dropout (peaks below the stochastic threshold, or otherwise indicated on historical data.)*

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

Given the allele frequencies in the Caucasian population below:

Allele Frequency 11 = 0.122
Allele Frequency 13 = 0.176
Allele Frequency 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.

6.2.6.3.17 KINSHIP

Statistical significance for kinship will be calculated using Popstats native to the CODIS v.8.0 software package. (Explanation of Popstats calculations as listed in CODIS 8.0 Help files can be seen in Appendix C). The typical calculations include:

- Paternity Trio and Reverse Paternity Trio
- Single Parent Kinship
- Full-Sibling Kinship

- Additional comparisons may be used but reporting guidelines have not been defined and will require consultation with the Technical leader and DNA Supervisor.

6.2.6.3.18 POPULATION DATABASE

With the update to CODIS v8.0 software, the Arkansas State Crime Laboratory utilizes the 2017 Revised National Institute of Standards and Technology U.S. Population Dataset (NIST SRD 1036). Available populations include African American, Asian, Caucasian, and Hispanic, as well as a Combined dataset.

References:

- Hill, C.R., Duewer, D.L., Kline, M.C., Coble, M.D., Butler, J.M. (2013) U.S. population data for 29 autosomal STR loci. Forensic Sci. Int. Genet. 7: e82-e83.
- Steffen, C.R., Corrigendum to 'U.S. Population Data for 29 Autosomal STR Loci' (2017) Forensic Science International: Genetics

6.2.6.4 Y-STR INTERPRETATION GUIDELINE

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.

6.2.6.4.1 ANALYTICAL THRESHOLD (AT)

A minimum peak height threshold will be set for GeneMapper ID-X software recognition of a peak. 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 (-) may be placed on the call sheet for the locus.

PowerPlex Y23: 175 RFU in all channels (*historical data only*)

Yfiler Plus: 100 RFU in all channels

6.2.6.4.2 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, in Y23 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.

6.2.6.4.3 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.

6.2.6.4.3.1 SPIKES

Spikes are artifact 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 will be noted and may be re-injected.

6.2.6.4.3.2 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 two peaks, one base apart. This is most often seen when an excessive amount of DNA is amplified or the amplification is performed under sub-optimal PCR conditions.

6.2.6.4.3.3 PULL-UP/PULL-DOWN (PU)

Small artifact 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.

6.2.6.4.3.4 STUTTER (ST)

These are artifacts due to template slippage during the amplification process. These peaks may be observed in the 2bp, 3bp, 4bp or 5bp positions of major peaks and will have a smaller peak height. GeneMapper ID-X will apply a filter based on the observed stutter percentage seen in validation. As there is potential for true peaks to be filtered and not all stutter peaks will always be filtered, the analyst must examine all profiles carefully.

Approved YSTR Stutter Ratios can be seen in table form in Appendix E.

6.2.6.4.3.5 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 will be noted and may be re-injected.

6.2.6.4.3.6 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.

6.2.6.4.3.7 PULL-UP

Small artifact 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.

6.2.6.4.3.8 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.

Artifact 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 of an artifact nature by re-injection of the sample.

6.2.6.4.4 MULTI-ALLELIC LOCI

A multi-allelic system is one which contains several distinct alleles, rather than the normal one (or two in DYS385, DYS389, or DYS387S1). In order to insure that the sample is a true multi-allelic specimen, the sample will be re-amplified and run a second time. However, if observed in overlapping systems or in multiple samples from the case, multi-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 multi-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). Confirmed multi-allelic areas will not be used for statistical calculations.

6.2.6.4.5 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 (except DYS385, DYS389, or DYS387S1) and the signal intensity ratio of alleles at a duplicated locus is consistent with a profile from a single contributor. All loci will be evaluated in making this determination. It will be noted that individuals have been typed who exhibit multiple locus duplications.

6.2.6.4.6 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 common stutter positions will be interpreted with caution based on the analyst's training and experience. As a guideline, the maximum expected stutter percentages for each locus are listed in tables above.
If a profile is determined to have more than one contributor, then the maximal number of contributors should also be determined. The validation of PowerPlex Y23 examined mixtures with two contributors. For this reason, any historical Y23 mixture with more than two contributors must be considered inconclusive. The validation for Yfiler Plus examined mixtures with up to three contributors and for this reason, any mixtures with more than three contributors must be considered inconclusive.

6.2.6.4.7 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 from loci with good amplification quality. A ratio between contributors will be estimated, with all loci of good quality will be evaluated in making this determination. If a major component cannot be fully deconvoluted from the mixture at all loci, the remaining loci with alleles from both contributors may still be used in the statistical calculator by including both alleles and searching in the mixture configuration.

PPY23: Based on the Y23 validation, a Mixture Interpretation Guideline (MIG) of 175 rfu above analytical threshold has been established for the interpretation of loci with only one allele. For an example using a 2:1 ratio between contributors, the minor component should be expected to be at least 350rfu. The actual allele must therefore be over 1050 rfu (700rfu+350rfu) before the minor contributor may be considered.
Based on the Mixture Interpretation Guideline, loci with only one allele, less than 350rfu, should not be assigned to either contributor due to the possibility of drop-out for both major and minor contributors.
Yfiler Plus: Based on the Yfiler validation data, any two-male mixture for which the major contributor is estimated to exceed 75% of the total template, the largest allele(s) at each loci are recommended to be designated as part of the major contributor profile. Additionally, alleles should also be designated as part of the major profile based on a minimum 2:1 ratio at each loci if the majority of loci in a two-male mixture shows a minimum 2:1 ratio.
For the three-male mixtures, the major alleles may be identified when the peak heights are approximately twice the remaining allele peak heights for each loci, and may be further deconvoluted by the assumption of either the middle or minor contributor's profile. Alleles from the middle or minor contributor are more challenging to attribute and should not be attempted unless assisted by the assumption of either the middle or minor contributor's profile (obligate peaks or over 500 rfu).

- Mixtures with Known Male Contributor(s)

In some cases such as when a male may be an assumed contributor (e.g., the victim), 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 (YSTR_STATS_v4.3). 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 and the percent of the population that would be expected to be excluded.

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

6.2.6.4.8 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 based upon information provided by the other markers, results can generally still be interpreted from the loci that do amplify. For both Y23 and Yfiler Plus, profiles with 9 or more loci are generally acceptable for interpretation, profiles with less than 6 should generally be considered very marginal and only be used when the specific case warrants it (such as for exclusion or number of contributors considerations).

6.2.6.4.9 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 profiles 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. 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."

6.2.6.4.10 STATISTICAL CALCULATIONS

Recommendations of the *SWGDAM Interpretation Guidelines for Y-Chromosomal Typing*, *2014*, are followed for the interpretation of YSTR profiles. The counting method is the basis of the preferred way to perform statistical calculations for Y-STRs due to the lack of independent recombination between loci. The counting method involves searching a given haplotype against a database to determine the number of times the haplotype was observed in that database. The search of the haplotype and all statistical calculations are performed using the YSTR_STATS excel sheet which originated from the California DOJ Y-Mix Database Filter Tool utilizing the US Y-STR Database and adapted for use by the ASCL. The US Y-STR Database is located at the following location: http://www.usystrdatabase.org/newdefault.aspx**.**

In most circumstances, single-source and fully deconvoluted mixture profiles will be searched in the YSTR_STATS_vX tool as single source samples. If the reference profile cannot be excluded from the evidence profile, the results will be reported as the number of observations in the database for each population. Any conclusion will include the likelihood of exclusion, with the match probability reported as a likelihood ratio for each population.

Under typical circumstances, indistinguishable mixture profiles may be searched in the YSTR_STATS_vX tool as multiple source samples. If the reference profile cannot be excluded from the mixture profile, the results will be reported as the number of observations in the database for each population. Any conclusion will include the likelihood of exclusion, with the match probability for each population reported as an expected profile frequency.

Due to the different sizes of the Yfiler Plus, and Y23 databases, which make up the US-Y-STR Database, differing strategies are needed to maximize the potential value of the differing profiles. When comparing a Y23 profile, the standard method is to use the entire profile in a limited search against the other Y23 profiles in the database. These results should be

compared to a limited search against the original Yfiler core loci or even an unlimited search against the entire database. The Y23 search results can then be safely reported. However, the Yfiler Plus profile cannot likewise be searched against the more limited Yfiler Plus profile database, as the database is smaller and the appropriate theta values have not been experimentally derived. Therefore, the most appropriate search method for Yfiler Plus is to limit the entered loci to the original Yfiler core and use a limited search setting. This is also the most appropriate result to report when comparing Yfiler or Yfiler Plus profile to a Y23 profile. See Appendix D for further discussion of Y-STR Statistical considerations.

6.3 REPORTS

The policies regarding laboratory reports conform to the lab-wide policies and may be found in the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.8. All current case reports and records are stored electronically with the aid of JusticeTrax LIMS-plus software program. Once review is complete, the electronic version is considered the official case record, and the paper file can be destroyed.

Prior to authorizing 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.

Per the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.8.7.3, when opinions or interpretations are directly communicated by dialogue to a customer, a record of the communication will be retained.

Per the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.8.8, when an error is found on the original report, an "Amended Report" will be created in LIMS. The edit will be identified and the reason for the change will be included on the report. When the error causes the change of analytical results, the DNA Technical Leader, DNA Supervisor, or DNA Quality Manager will perform the technical review on the amended request.

See the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.8.1.2 for a list of instances not requiring a laboratory report.

6.3.1 ELEMENTS OF THE CASE REPORT

All reports must contain the following, as recommended by SWGDAM. For a list of additional ASCL report elements, see the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.8.2.1.

a) Case identifier
b) Description of evidence examined
c) A description of methodology
d) Loci examined (if STR analysis is performed)
e) Results and/or conclusions
f) An interpretative statement (either quantitative or qualitative)

g) Date issued
h) Disposition of evidence
i) A statement regarding the initial entry or search against CODIS, if needed.
j) A signature and title or equivalent identification of the person(s) accepting responsibility of the content of the report.
k) A statement that addresses the fact that only samples listed on the report were tested.

6.3.2 AUTOSOMAL-STR REPORT GUIDELINES

General templates are listed below. Case situations may require additional discussion statements or even deviations from the general templates as specific assumptions and considerations are made. New statements and deviations must be approved by the Technical Leader and/or DNA supervisor. Approved statements will be stored with the more extensive templates for the most common DNA reports currently available on the ForensicBiology drive.

6.3.2.1 SAMPLES WITH QUANTITATION VALUES ≤ .0025 NG/µL

RESULTS
Q#, (*evidence*), did not contain a sufficient amount of DNA for further processing.

6.3.2.2 SEXUAL ASSAULT SAMPLE WITH MALE CONTRIBUTION ≤ 5%

RESULTS
Q#, (*evidence*), did not contain a sufficient amount of a male contributor for autosomal processing.

6.3.2.3 SEXUAL ASSAULT SAMPLE WITH NO MALE CONTRIBUTION DETECTED

RESULTS
No male contributor to Q1# was indicated. Therefore, Q# will not be processed for autosomal testing.

6.3.2.4 NO DNA OBTAINED

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

6.3.2.5 SINGLE SOURCE STAINS

Condition 1: DNA profile obtained from evidence item, no reference standards are available for comparison.

RESULTS
A DNA profile was obtained from Q#.

Condition 2: Expected contributor is consistent with the item of evidence. No indications of a mixture or foreign contributor.

RESULTS

Considering the presence of K#, (*assumed contributor*), no foreign DNA profile was obtained from Q#. (*no further comparisons to this sample are needed.)*

Note *Statistical values for the match must be given unless a reason for an assumed contributor is documented, such as collected in an SA Kit, description of collection directly from the person, or conversation with officers.*

Condition 3: Reference standard K2 (*suspect*) is consistent with the Q1 (*evidence*). No indication of a mixture. (*no further comparisons to this sample may be needed if the comparison is of no probative value*.)

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 African American population, 1 in *X* in the Asian population, 1 in *Y* in the Caucasian population and 1 in *Z* in the Hispanic population.

Note *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.

6.3.2.6 MIXTURES

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

RESULTS

The DNA profile obtained from Q1 (*evidence*) indicates the presence of DNA from two individuals. 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 African American population, 1 in *X* in the Asian population, 1 in *Y* in the Caucasian population and 1 in *Z* in the Hispanic population.

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

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

Condition 2: Persons in the case, such as K1, are expected to be present on the item for non-case specific reasons (such as an intimate sample), and a foreign profile can be determined from the DNA that is consistent with the K2 (*suspect*).

RESULTS

A DNA mixture consistent with originating from two individuals was obtained from Q1 (evidence). Considering the presence of K1, (assumed), [as a partial contributor], the DNA profile foreign to K1 (assumed) matches the DNA profile from K2, (foreign). The probabilities of selecting an unrelated individual at random having a DNA profile matching the profile foreign to ASSUMED from the DNA mixture obtained from Q1 are approximately: 1 in *W* in the African American population, 1 in *X* in the Asian population, 1 in *Y* in the Caucasian population and 1 in *Z* in the Hispanic population.

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

Conclusion:

The DNA profile foreign to K1, (assumed), identified on Q1 originated from K2, (foreign), within all scientific certainty.

Note *If the underlying understanding is that a contributor's DNA is expected to be present on the evidence (Q) and reasons are documented, then the following reporting will be followed.*

- *The assumed contributor(s) will be designated in the results by "Considering the presence of…"*
- *The degree to which an assumed contributor is present may be indicated, such as "…as a partial contributor"*
- *Additional considerations may be included in the results to explain an assumption of contribution, such as consensual partner or mother-child relationship.*

If the evidence contains a semen stain, then the following reporting will be followed. (Male victim/ female suspect and same-sex victim/suspect cases may need approval of the Technical leader and/or DNA supervisor):

- *The epithelial (non-sperm, or 'epi') fraction will be run and analyzed along with the probative sperm fraction to ensure sample integrity. As such, the epithelial fraction will be compared to other samples in the case and on the run plates, but will not be reported unless it is determined to have a probative value not found in the sperm fraction.*
- *If the female victim's profile is in the epi fraction and the sperm fraction contains a male profile with no indication of mixture or only the male major contributor of a mixture is useable (minor is inconclusive), then the victim does not need to be addressed in the comparison to the sperm fraction profile.*

- *If the female victim's profile is in the epi fraction and the semen fraction contains a mixture, then the victim needs to be addressed (considered, included, or excluded) from the mixture unless the victim's contribution is inconclusive.*
- *If the female victim's profile is not on the epi or sperm fraction, the victim can be excluded from reported profiles.*
- *If additional female profiles are present in the sample, then the victim may need to be addressed (considered, included, or excluded).*
- *If a consensual partner's known has been tested and documentation exists that intercourse between the victim and the consensual partner occurred in the 96 hours prior to the sample collection, the partner's profile may also be assumed and applied to the mixture. No statistics are needed.*

If the sample is an intimate sample from the female victim, a differential extraction was not performed, and the sample contains a male profile with no indication of mixture or only the male major contributor of a mixture is useable (minor is inconclusive), then the victim does not need to be addressed in the comparison to the reported profile.

The assumption of a contributor should be determined prior to mixture analysis, not based on whether a mixture interpretation is bettered by an assumption. All assumed contributors should be verified as a contributor prior to further mixture interpretation steps. If an assumed contributor (such as a consensual partner) is excluded from a sample, that exclusion may be reported if probative. It is noted that not all exclusions of assumed contributors need to be reported, such as from intimate samples from the assumed. The objective in assuming a known contributor to a mixture is to clarify the other possible contributors in a manner that is both reasonable and helpful to an investigator and a trier of facts.

Condition 3: More than one reference standard could have contributed to a mixed stain result and the major and minor components of the DNA profile *cannot* be separated. Neither reference sample can exclude as being contributors to the DNA profile.

> RESULTS (Restricted/Modified Random Match Prob.)
>
> A DNA mixture consistent with originating from two individuals was obtained from Q1 (evidence). K1 (victim) and K2 (suspect) cannot be excluded as contributors to the DNA mixture. The probabilities of selecting an unrelated individual at random that cannot be excluded as a contributor to the DNA mixture obtained from Q1 are approximately: 1 in W in the African American population, 1 in X in the Asian population, 1 in Y in the Caucasian population and 1 in Z in the Hispanic population.

Note *Mixtures in which each allele can be assigned to either reference may include the conclusion stated below:*

> CONCLUSION

The DNA mixture obtained on Q1, *Evidence*, is consistent with originating from K1, *Known*, and K2, *Known*.

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

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 African American individuals, 1 trillion times more likely if it came from two random Asian individuals, 1 trillion times more likely if it came from two random Caucasian individuals and 2 trillion times more likely if it came from two random Hispanic individuals.

6.3.2.7 KINSHIP CASES

Kinship statistics may be performed any time there is a question of paternity or familial relations. These cases include but are not limited to: criminal paternity, body identification, and missing persons.

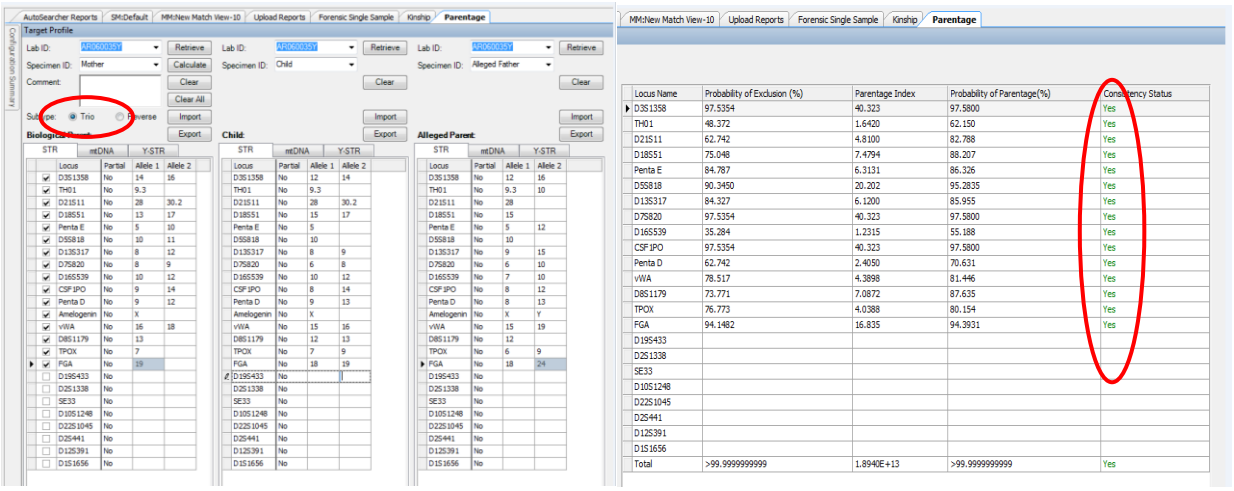
1) Criminal Paternity
   a) Known reference samples from Mother, Child, and Alleged Father submitted:

Note *The three profiles associated with the reference samples from the Mother, Child, and Alleged Father will be entered into Popstats / Parentage. Please be aware that 2 mismatches (per parent) are allowed with a paternity match due to the possibility of mutation(s)* occurring.*

i. Enter the appropriate profiles into the target profiles for Biological Mother; Child; and Alleged Father. (see Parentage Trio Data Entry Tab below)
ii. Select the Calculate button. (Explanation of Popstats calculations as listed in CODIS 7.0 Help files can be seen in Appendix C, reformatted to fit page).
iii. Check to ensure “Consistency Status” is “Yes” at all loci. (see Parentage Trio Report Tab below)
iv. Print Parentage Trio Calculations for reporting. (see Parentage Trio Statistics below)
v. Refer to the Normal Paternity report template.

Example of Paternity:

Parentage Trio Data Entry Tab Parentage Trio Report Tab



Parentage Trio Statistics

Parentage Trio Calculations

Database: \\AR060035YS70\CODIS\Popstats\POPDATA\FBI\Expanded FBI STR 2015
Prior Probability: 0.5

	Biological Parent	*Child*	*Alleged Parent*
Lab ID:	AR060035Y	AR060035Y	AR060035Y
Specimen ID:	Mother (Keyboard)	Child (Keyboard)	Alleged Father (Keyboard)
Comment:			

Population Group: Caucasian

Locus	Probability Of Exclusion (%)	Parentage Index	Probability Of Parentage (%)	Match?	Mutation Rate	Mean Power Of Exclusion
D3S1358	97.5354	40.323	97.5800	Yes		
TH01	48.372	1.6420	62.150	Yes		
D21S11	62.742	4.8100	82.788	Yes		
D18S51	75.048	7.4794	88.207	Yes		
Penta E	84.787	6.3131	86.326	Yes		
D5S818	90.3450	20.202	95.2835	Yes		
D13S317	84.327	6.1200	85.955	Yes		
D7S820	97.5354	40.323	97.5800	Yes		
D16S539	35.284	1.2315	55.188	Yes		
CSF1PO	97.5354	40.323	97.5800	Yes		
Penta D	62.742	2.4050	70.631	Yes		
Amelogenin				Inconclusive		
vWA	78.517	4.3898	81.446	Yes		
D8S1179	73.771	7.0872	87.635	Yes		
TPOX	76.773	4.0388	80.154	Yes		
FGA	94.1482	16.835	94.3931	Yes		
Total	>99.9999999999	1.8940E+13	>99.9999999999	Yes		

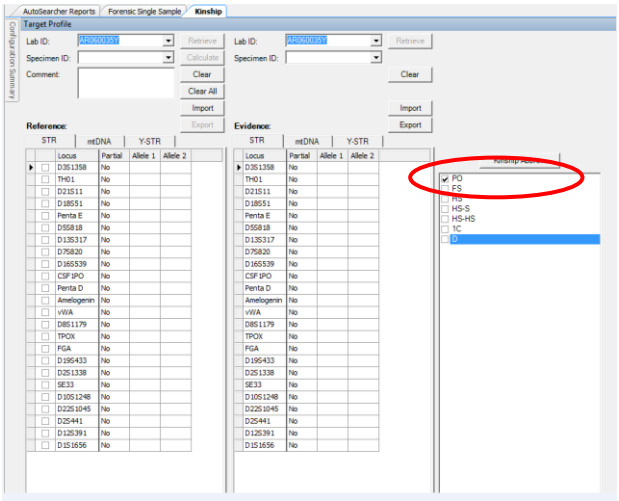
b) Known reference samples from Child and only one Alleged Parent submitted.

Note *In the instance that only known samples from one alleged parent are received, kinship statistics will be performed. If known samples from the mother are received at a later time additional statistics can be performed.*

i. The Reference sample is the known from the alleged parent; the Evidence sample is the child.
ii. Select only "PO" (parent/offspring) in the kinship box in the Kinship Data Entry Tab. (see example below)
iii. Print the Popstats Single Parentage Statistics for reporting. (see example below)
iv. Refer to the Single Parentage Reporting template.

Example of Single Parentage:

Kinship Data Entry Tab



Single Parentage Statistics

Popstats Single Parentage Statistics
DNA Testing Results

Database: \\AR060035YS70\CODIS\Popstats\POPDATA\FBI\Expanded FBI STR 2015

	Reference	*Evidence*
Lab ID:	AR060035Y	AR060035Y
Specimen ID:	alleged father (Keyboard)	child (Keyboard)
Comment:		

Population Group: Caucasian (Continued)

Locus	Allele	Frequency	Probability Of Exclusion (%)	Parentage Index	Mutation Rate	Mean Power Of Exclusion
vWA			40.158	2.5253		
	Allele 1: 12	1.2400E-02(M)				
	Allele 2: 14	9.9000E-02				
	Allele 3: 17	2.6730E-01				
D8S1179			44.329	2.9922		
	Allele 1: 13	3.3420E-01				
TPOX			10.837	0.45704		
	Allele 1: 8	5.4700E-01				
	Allele 2: 9	1.2380E-01				
	Allele 3: 10	3.7100E-02				
FGA			61.953	4.5872		
	Allele 1: 18	2.9700E-02				
	Allele 2: 19	5.4500E-02				
	Allele 3: 23	1.5840E-01				

Single Parentage Probability of Exclusion for Population Group, Caucasian : 99.98826%
Single Parentage Index for Population Group, Caucasian : 46.030

Mutations – *if there is a mutation between the child and alleged father or the biological mother the analyst may remove that locus from the target profile window and perform the statistics without that data, but the possibility of a mutation will be noted in the case report.*

c) Fetal Mixtures

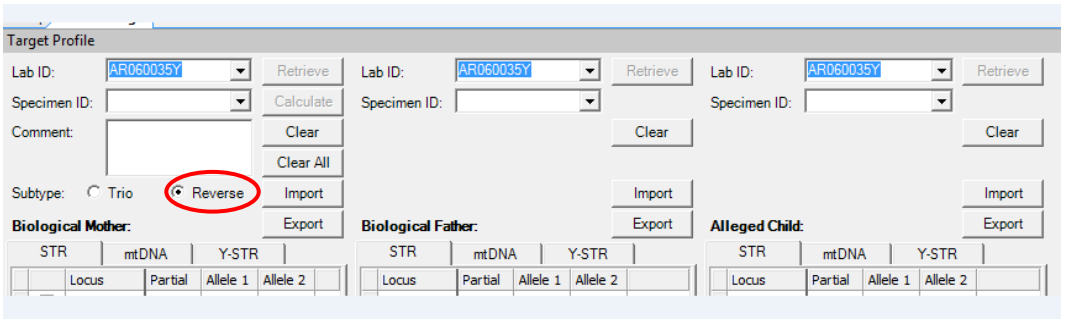
Fetal mixes that indicate a Y chromosome will have Y-STR analysis performed in addition to the autosomal STR testing. If a mother's reference sample has been submitted, these mixtures will be deconvoluted with ArmedXpert, if possible, and paternity statistics will be performed.

2) Body Identification

a) Known reference samples from Mother and Father submitted (reverse paternity)

i. Follows the same guidelines as normal paternity testing.
ii. Select the "Reverse" Button on the Parentage Trio Data Entry Tab. (see example below)
iii. Enter the appropriate profiles for target profiles of "Biological Mother"; "Biological Father"; and "Alleged Child".
iv. Print Reverse Parentage Statistics for reporting.

Example of Reverse Parentage:



Reverse Parentage Statistics

Database: \\AR060035YS70\CODIS\Popstats\POPDATA\FBI\Expanded FBI STR 2015

	Biological Mother	Biological Father	Evidence
Lab ID:	AR060035Y	AR060035Y	AR060035Y
Specimen ID:	Mother (Keyboard)	Father (Keyboard)	Alleged Child (Keyboard)
Comment:			

Population Group: Caucasian (Continued)

Locus	Allele Frequency	Probability Of Exclusion (%)	Parentage Index
Penta D		97.1471	30.733
Allele 1: 8	1.7300E-02		
Allele 2: 9	2.3510E-01		
Allele 3: 13	2.0790E-01		
Allele 4: 15	1.4900E-02		
Amelogenin			
Allele 1: X	L		
Allele 2: Y	L		
vWA		98.1832	101.82
Allele 1: 12	1.2400E-02 M		
Allele 2: 13	1.2400E-02 M		
Allele 3: 14	9.9000E-02		
Allele 4: 17	2.6730E-01		
D8S1179		79.159	3.6419
Allele 1: 13	3.3420E-01		
Allele 2: 14	2.0540E-01		
Allele 3: 15	1.0640E-01		
TPOX		90.0453	6.1595
Allele 1: 8	5.4700E-01		
Allele 2: 9	1.2380E-01		
Allele 3: 10	3.7100E-02		
Allele 4: 12	3.7100E-02		
FGA		92.0748	84.168
Allele 1: 19	5.4500E-02		
Allele 2: 23	1.5840E-01		
Allele 3: 24	1.3860E-01		

Reverse Parentage Probability of Exclusion for Pop Group Caucasian: >99.9999999999%
Reverse Parentage Index for Pop Group Caucasian: 3.4890E+17

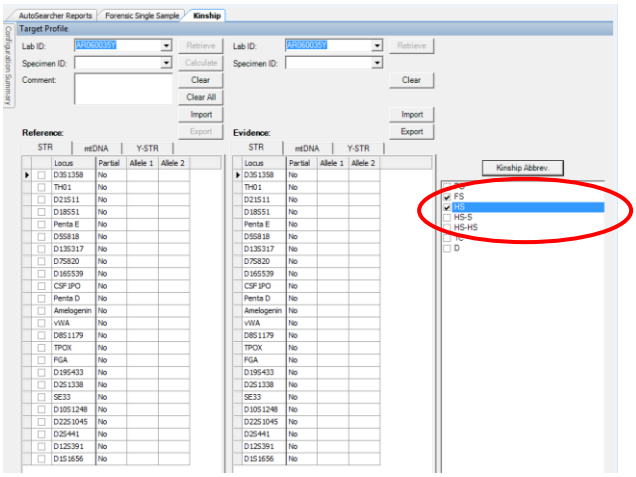
b) Known reference sample from Mother or Father only*
   i. The Reference sample is the known from the family member; the Evidence sample is the unidentified remains.
   ii. Select only "PO" (parent/offspring) in the kinship box.
   iii. Print the Popstats Single Parentage Statistics.
   iv. Refer to the Offspring (Parent Reference) report template.

Note *This process is also the same for when a biological child's known sample is submitted to compare to the unidentified remains - refer to the Offspring report template. Care should be used when looking at relationships outside of parentage and full-siblings. These can be addressed on a case-by-case basis to ensure accurate reporting of statistics if there is a familial match.*

c) Known reference sample from Full Sibling
   i. The Reference sample is from the known family member.
   ii. The Evidence sample is the unidentified remains.
   iii. Select "FS" (full siblings) in the kinship box of the Kinship Data Entry Tab. (see example below)
   iv. Print the Popstats Kinship Statistics Summary. (see example below)
   v. There is no probability of exclusion associated with this statistic.
   vi. Refer to the Sibling report template.

Example of Full Sibling Kinship:

Kinship Data Entry Tab



Full Sibling Statistics

Popstats Kinship Statistics Summary

Database: \\AR060035YS70\CODIS\Popstats\POPDATA\FBI\Expanded FBI STR 2015

	Reference	*Evidence*
Lab ID:	AR060035Y	AR060035Y
Specimen ID:	Sibling (Keyboard)	unidentified remains (Keyboard)
Comment:		

Population Group: Caucasian

Kinship	Kinship Conditional Probability	Unrelated Conditional Probability	Likelihood Ratio
Full Sib (FS)	5.813E-23	1.183E-26	4,916

Population Group: AfricanAmerican

Kinship	Kinship Conditional Probability	Unrelated Conditional Probability	Likelihood Ratio
Full Sib (FS)	6.456E-24	2.280E-28	28,320

Population Group: SoutheastHispanic

Kinship	Kinship Conditional Probability	Unrelated Conditional Probability	Likelihood Ratio
Full Sib (FS)	2.751E-23	2.542E-27	10,820

Population Group: SouthwestHispanic

Kinship	Kinship Conditional Probability	Unrelated Conditional Probability	Likelihood Ratio
Full Sib (FS)	1.786E-24	8.860E-29	20,150

6.3.2.8 CODIS-ELIGIBLE PROFILE SEARCH AND ENTRY

Sample profiles deemed eligible for searching against or for entry into the CODIS database(s) will be denoted in the report body. These notes will follow the templates available on the ForensicBiology shared drive. Additional information regarding the reporting of CODIS-eligible sample profile searches, associations, entries, and removals will be included in the standard report footer to ensure compliance with ISO17025:2017/ANAB AR3125 standards 7.1.9.

This single communication is sufficient to meet the requirement to report the extent of the search or entry of a profile as the CODIS Procedures and the CODIS software are configured to automatically advance a profile to the most extensive level for which it is eligible. If an entered sample is determined to be ineligible, it shall be removed per the *CODIS DOC-01 Quality Manual* and an additional communication with the investigating agency explaining the removal will be made. Documentation of this communication will be included in the casefile for future reference.

6.3.3 Y-STR REPORT GUIDELINES

6.3.3.1 NO PROFILE REPORT

Condition 1: No Y-Chromosomal DNA profile obtained from Q1.

Results:

No Y-STR profile was obtained from Q1.

6.3.3.2 PROFILE-ONLY REPORT

Condition 1: The Y-Chromosomal DNA profile obtained from Q1 is useable but no known reference standard is available for comparison.

Results:

A Y-STR profile was obtained from Q1.

If further testing is needed, two oral swabs are required from any developed suspect(s).

6.3.3.3 INCONCLUSIVE REPORTS

These basic report templates include profiles too limited for comparisons, mixtures too complex for comparison, and data which is not interpretable due to other factors such as artifacts.

Condition 1: The single source Y-Chromosomal DNA profile obtained from Q1 is too limited for comparison, or is low quality data.

Results:

The partial Y-STR profile obtained from Q1 is inconclusive for comparative purposes due to the limited amount of DNA (or, due to insufficient quality of data).

Condition 2: The Y-Chromosomal DNA mixture obtained from Q1 has indications of more than 2 contributors, is too limited for comparison, or is low quality data (confounding artifacts, unable to determine # of contributors, etc.)

Results:

The Y-STR mixture obtained from Q1 is inconclusive for comparative purposes due to the complexity of the mixture (or, due to insufficient quality of data, or, due to the limited amount of DNA).

6.3.3.4 EXCLUSION REPORT

Condition 1: The Y-Chromosomal DNA profile obtained from Q1 does not match the Y-Chromosomal DNA profile obtained from the known reference standard, K1.

Results:

K1, Known 1, is excluded as a contributor to the Y-STR profile obtained from Q1.

6.3.3.5 INCLUSION REPORTS

These basic report templates include comparisons to both single-source and isolated contributors. It will typically be most reasonable to report the likelihood of exclusion for a randomly selected individual from the a given population, and the likelihood that a Y-STR DNA profile obtained from evidence occurred due to an origin within the K1 patrilineage than if the source of the evidence is a randomly selected individual from the a given population. It may be necessary to report

Observations within the Database or the resultant Frequency estimates (based on 95% CI) and reports may be altered to suit.

Condition 1: The Y-STR profile obtained from Q1 matches the Y-STR profile obtained from K1.

Results:

The Y-STR profile obtained from Q1 matches the Y-STR profile obtained from K1, Known 1. 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 xxx of xxx African American individuals, xxx of xxx Asian individuals, xxx of xxx Caucasian individuals, xxx of xxx Hispanic individuals, and xxx of xxx Native American individuals within the database.

Conclusion:

The DNA profile obtained from Q1 is xxx times more likely to occur if Known 1 or his paternally related male relatives are the contributor than if the source of the evidence is a randomly selected individual from the African American population, xxx times more likely than a randomly selected individual from the Asian population, xxx times more likely than a randomly selected individual from the Caucasian population, xxx times more likely than a randomly selected individual from the Hispanic population, and xxx times more likely than a randomly selected individual from the Native American population. Conversely, xxxx% of unrelated African American individuals, xxxx% of unrelated Asian individuals, xxxx% of unrelated Caucasian individuals, xxxx% of unrelated Hispanic individuals, and xxxx% of unrelated Native American individuals would be expected to be excluded from the observed DNA profile.

Condition 2: The Y-STR profile obtained from Q1 indicates the presence of DNA from two males. K1, Known 1, can be assumed. K2, Known 2, is consistent with the foreign contributor. (see Autosomal-STR Report Guidelines 6.3.2.6 for directives on assuming contributors.)

Results:

The Y-STR profile obtained from Q01 indicates the presence of DNA from two males. Considering the presence of K1, Known 1, the DNA profile foreign to Known 1 matches the DNA profile from K2, Known 2. Therefore, neither K2, nor any of his paternally related male relatives can be excluded as the contributor of this DNA. The Y-STR profile foreign to Known 1 has been observed in xxx of xxx African American individuals, xxx of xxx Asian individuals, xxx of xxx Caucasian individuals, xxx of xxx Hispanic individuals, and xxx of xxx Native American individuals within the database.

Conclusion:

The DNA profile foreign to K1 obtained from Q01 is xxx times more likely to occur if Known 2 or his paternally related male relatives are the contributor than if the source of the evidence is a randomly selected individual from the African American population, xxx times

more likely than a randomly selected individual from the Asian population, xxx times more likely than a randomly selected individual from the Caucasian population, xxx times more likely than a randomly selected individual from the Hispanic population, and xxx times more likely than a randomly selected individual from the Native American population. Conversely, xxxx% of unrelated African American individuals, xxxx% of unrelated Asian individuals, xxxx% of unrelated Caucasian individuals, xxxx% of unrelated Hispanic individuals, and xxxx% of unrelated Native American individuals would be expected to be excluded from the observed DNA profile.

Condition 3: The Y-STR profile obtained from Q1 indicates the presence of DNA from two males. This mixture can be deconvoluted into a major and a minor contributor. K1, Known 1, is consistent with a contributor.

Results:

The Y-STR profile obtained from Q1 indicates the presence of DNA from two males (at # of # loci). It is noted that the major component of the Y-STR mixture obtained from Q1 matches the Y-STR profile obtained from K1, Known 1. Therefore, neither K1 nor any of his paternally related male relatives can be excluded as the contributor of the major component of DNA. The Y-STR profile obtained from K1 and the major component of Q1 has been observed in xxx of xxx African American individuals, xxx of xxx Asian individuals, xxx of xxx Caucasian individuals, xxx of xxx Hispanic individuals, and xxx of xxx Native American individuals within the database.

Conclusion:

The major component of the DNA profile obtained from Q01 is xxx times more likely to occur if Known 1 or his paternally related male relatives are the contributor than if the source of the evidence is a randomly selected individual from the African American population, xxx times more likely than a randomly selected individual from the Asian population, xxx times more likely than a randomly selected individual from the Caucasian population, xxx times more likely than a randomly selected individual from the Hispanic population, and xxx times more likely than a randomly selected individual from the Native American population. Conversely, xxxx% of unrelated African American individuals, xxxx% of unrelated Asian individuals, xxxx% of unrelated Caucasian individuals, xxxx% of unrelated Hispanic individuals, and xxxx% of unrelated Native American individuals would be expected to be excluded from the major component of the observed DNA profile.

6.3.3.6 MIXTURE REPORTS

These basic report templates include comparisons to mixtures which cannot be fully deconvoluted. It will typically be most reasonable to report the likelihood of exclusion and the match probability for a randomly selected individual from a given population. It may be necessary to report Observations within the Database or the resultant Frequency estimates (based on 95% CI) and reports may be altered to suit. Note

Condition 1: The Y-STR profile obtained from Q1 indicates the presence of DNA from two males. This mixture is consistent with a mixture of DNA from K1, Known 1, and an unknown male.

Results:

The Y-STR profile obtained from Q1 indicates the presence of DNA from two males. K1, Known 1, cannot be excluded as a contributor to the observed Y-STR mixture. Therefore, neither K1, nor any of his paternally related male relatives can be excluded as contributors of this DNA. The profiles consistent with having been a possible contributor to the Y-STR mixture obtained from Q1 have been observed in xxx of xxx African American individuals, xxx of xxx Asian individuals, xxx of xxx Caucasian individuals, xxx of xxx Hispanic individuals, and xxx of xxx Native American individuals within the database.

Conclusion:

Based on the results from the mixture obtained on Q1, 1 in xx randomly chosen African American individuals, 1 in xxx randomly chosen Asian individuals, 1 in xxx randomly chosen Caucasian individuals, 1 in xxx randomly chosen Hispanic individuals, and 1 in xxx randomly chosen Native American individuals would be expected to be included as contributors to the DNA mixture obtained from Q1. Conversely, xxxx% of unrelated African American individuals, xxxx% of unrelated Asian individuals, xxxx% of unrelated Caucasian individuals, xxxx% of unrelated Hispanic individuals, and xxxx% of unrelated Native American individuals would be expected to be excluded from the observed DNA mixture.

Condition 2: The Y-STR profile obtained from Q1 indicates the presence of DNA from two males. This mixture is consistent with a mixture of DNA from K1, Known 1, and K2, Known 2. Neither can be assumed.

Results:

The Y-STR profile obtained from Q1 indicates the presence of DNA from two males. This mixture is consistent with a mixture of DNA from K1, Known 1, and K2, Known 2. Therefore, neither Known 1 nor Known 2, nor any of their paternally related male relatives can be excluded as contributors of this DNA. The profiles consistent with having been a possible contributor to the Y-STR mixture obtained from Q1 have been observed in xxx of xxx African American individuals, xxx of xxx Asian individuals, xxx of xxx Caucasian individuals, xxx of xxx Hispanic individuals, and xxx of xxx Native American individuals within the database.

Conclusion:

Based on the results from the mixture obtained on Q1, 1 in xx randomly chosen African American individuals, 1 in xxx randomly chosen Asian individuals, 1 in xxx randomly chosen Caucasian individuals, 1 in xxx randomly chosen Hispanic individuals, and 1 in xxx randomly chosen Native American individuals would be expected to be included as contributors to the DNA mixture obtained from Q1. Conversely, xxxx% of unrelated African American individuals, xxxx% of unrelated Asian individuals, xxxx% of unrelated Caucasian

individuals, xxxx% of unrelated Hispanic individuals, and xxxx% of unrelated Native American individuals would be expected to be excluded from the observed DNA mixture.

6.3.3.7 CODIS-ELIGIBLE PROFILE SEARCH AND ENTRY

Sample profiles deemed eligible for searching against or for entry into the CODIS database(s) will be denoted in the report body. These notes will follow the templates available on the ForensicBiology shared drive. Additional information regarding the reporting of CODIS-eligible sample profile searches, associations, entries, and removals will be included in the standard report footer to ensure compliance with ISO17025:2017/ANAB AR3125 standards 7.1.9.

This single communication is sufficient to meet the requirement to report the extent of the search or entry of a profile as the CODIS Procedures and the CODIS software are configured to automatically advance a profile to the most extensive level for which it is eligible. If an entered sample is determined to be ineligible, it shall be removed per the *CODIS DOC-01 Quality Manual* and an additional communication with the investigating agency explaining the removal will be made. Documentation of this communication will be included in the casefile for future reference.

7 EQUIPMENT

Only suitable and properly operating equipment will be employed and only authorized personnel will 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. The ASCL Forensic DNA section does not use equipment outside of ASCL permanent control.

In order to safeguard irreplaceable and/or limited samples, quality control (QC) procedures will focus as much as possible on preventing problems before they occur rather than dealing with them after they happen. As such, it is the responsibility of all DNA personnel to report quality issues to the DNA Quality Manager, DNA Supervisor, and/or DNA Technical Leader. In the event a quality issue is found which may affect analyzed samples, it is preferable that effected samples be reprocessed. However, where the samples are irreplaceable and/or limited in amount, reprocessing may not be a viable option. In such a case, 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.

7.1 INSTRUMENT & EQUIPMENT

New employees shall be trained on the appropriate equipment during their training program and be authorized to operate the equipment. This authorization will be documented on Analyst & Technician Competency Authorization, ASCL-FORM-62, and shall be maintained in Qualtrax. Validation of new equipment, procedures, and software shall require training of personnel before authorization. Only individuals trained in the proper use of the equipment shall be authorized to operate it independently. Instructions on the use and maintenance of equipment shall be available for use.

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

Pipettes	Thermocyclers	EZ-1
3500xl	7500	Qiagility
Qiacube	NIST-Thermometer	Drift-Con

7.2 INVENTORY

An inventory log will be maintained on the ForensicBiology 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 *ASCL DOC-01 Quality Manual* section 6.4.

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 in either paper or electronic form.

7.4 SERVICE 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 ForensicBiology 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.

a) All equipment failures must be documented in the instrument log.
b) A sign must be placed on the equipment as "Out of Service".
c) 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.
e) A Quality Assurance Concern (QAC) workflow will be initiated if needed as noted in ASCL-DOC-01 6.4.9.
f) If an adjustment/repair is performed because a calibration does not meet specifications, then pre- and post-adjustment/repair data will be retained

7.5 CALIBRATION & MAINTENANCE SCHEDULES

DNA testing methods do not result in reports of metrological data. The DNA sections are not required to establish an Uncertainty based on measurements. However, it is desirable that equipment which can influence laboratory activities be treated as critical to the overall findings.

Each instrument or piece of equipment considered essential for DNA typing will be maintained and calibrated or verified with appropriate schedule. A schedule for maintenance is found in the *DNA-FORM-12 DNA Equipment Care Schedule*. A maintenance log entry is maintained for any instrument or piece of equipment in which the following has occurred: damage, malfunction or modification or repair to equipment. After expiration of any initial warranty period, the AB7500, AB3500xl, EZ1xl advanced, Qiacube, and Qiagility will have Annual Maintenance contracts with the manufacturers which will include an annual Preventative Maintenance visit. At a minimum, each instrument will pass a performance check annually. Any calibrated or verified equipment with a defined period of

validity will be labeled to indicate the verification status. The date any equipment is removed from service is recorded and maintained on the ForensicBiology drive for a minimum of one full accreditation cycle.

7.5.1 ANNUALLY (OR AS NEEDED)

- Spatial for 3500xls (whenever array window door is opened a spatial must be performed according to the manufacturer)
- Spectral for 3500xls must be performed in the following instances:
  - Use of a dye set that does not have a valid calibration on the instrument
  - Change the capillary array
  - Maintenance involves an optical service procedure (realignment of optics, replacement of laser or CCD camera)
  - An increase in pull-up peaks is seen in DNA profiles
- Pipettes – performance-check and calibration for traceability, no accuracy verification or MOU estimation needed.
  - Calibration and performance check to be performed by an outside vendor meeting ASCL-DOC-01 6.5.1.1 specifications.
  - Traceability criteria are based on ISO 8655:

Nominal volume	Maximum permissible systematic error		Maximum permissible random error	
µl	± %	± µl [a]	± % [b]	± µl [c]
1	5,0	0,05	5,0	0,05
2	4,0	0,08	2,0	0,04
5	2,5	0,125	1,5	0,075
10	1,2	0,12	0,8	0,08
20	1,0	0,2	0,5	0,1
50	1,0	0,5	0,4	0,2
100	0,8	0,8	0,3[d]	0,3[d]
200	0,8	1,6	0,3[d]	0,6[d]
500	0,8	4,0	0,3	1,5
1 000	0,8	8,0	0,3	3,0
2 000	0,8	16	0,3	6,0
5 000	0,8	40	0,3	15,0
10 000	0,6	60	0,3	30,0

- NIST Traceable Thermometer – A NIST-traceable thermometer will be purchased annually to ensure continuous traceability for all DNA thermometers. No performance check needed.
- Thermometer – performance check and verification for traceability.
  - Verification to be performed by laboratory personnel.
  - Annually or prior to being placed into service (unless currently NIST-traceable)
  - Traceability criteria: detailed on DNA-Form-19
- Drift-con – (Thermocycler calibration system) performance-check and calibration for traceability.
  - Calibration and performance check to be performed by an outside vendor meeting ASCL-DOC-01 6.5.1.1 specifications.

    - Traceability criteria: t_{90}-$t \leq 0.25^{o}C$ @ 30 °C, 60 °C, 90 °C, and 95 °C
- Thermocyclers and Quantitative PCR Thermocyclers – Drift-con temperature verification
    - Verification to be performed by laboratory personnel
    - Traceability criteria:
      Accuracy: +/- 1°C @ 30 °C, 50 °C, 60 °C, 70 °C, 90 °C, & 95 °C @ 15, 30, & 90 seconds
      Spread: +/- 1°C @ 30 °C, 50 °C, 60 °C, 70 °C, 90 °C, & 95 °C @ 15, 30, & 90 seconds
    - If test fails, an outside company is called for service and unit is taken out of service.
- Balances – performance check and verification
    - Verification to be performed by laboratory personnel
    - NIST-traceable weights are calibrated or replaced every 10 years
    - Traceability criteria: detailed on DNA-FORM-06
      Accuracy (BAL-2): +/- 1% @ 1g, 2g, 3g, 5g, 10g, 20g, 30g, 50g, and 100g
      Accuracy (BAL-1): +/- 5% @ 1g, 2g, 3g, 5g, 10g, 20g, 30g, 50g, 100g, and 500g

7.5.2 QUARTERLY

- Biological safety hoods – serviced and calibrated by outside company, if needed. Monitoring and Management is performed on a lab-wide basis beyond the scope of this manual.

7.5.3 MONTHLY

- None currently

7.5.4 BI-WEEKLY (BY LABORATORY PERSONNEL, AS NEEDED)

- The 3500xl and computers shall be restarted.
- Wet the seals on the 3500xl.
- Polymer is changed on the 3500xl.
- Conditioning wash is performed on the 3500xl.
- Change buffer containers, septa, and reagents on the 3500xl.

7.5.5 WEEKLY (BY LABORATORY PERSONNEL, AS NEEDED)

- Grease O-rings on the EZ1 robots, if used.

7.5.6 EACH DAY OF USE (BY LABORATORY PERSONNEL, AS NEEDED)

- Autoclave – check water levels before use.
- Check temperature of refrigerators and freezers in pre-amp and post-amp rooms (DNA-FORM-17) if lab space is used.
- Qiagility is decontaminated and documented on DNA-FORM-38, if used.
- Heat Blocks – temperature checked prior to use (DNA-FORM-17).
- Bench tops – CODIS & DNA (pre-amp): After each use, the bench tops must be cleaned with a 10% bleach solution and documented on DNA-FORM-11.
- EZ1 - End of day (after last protocol), if used:

- Clean Piercing unit
  - Close Door
  - Press “2” MAN (Manual Function), then press “3” Clean
  - Press “Start”, then open door.
  - Clean piercing units with a soft wipe and alcohol. **Piercing unit is sharp!**
  - Wipe piercing unit with deionized water.
  - Close Door and Press “ENT”, then press “ESC”
- Check that the tray and racks are clean, if needed, clean with ethanol and then deionized water.
- Run UV decontamination cycle for 20 minutes.
- Document decontamination on DNA-FORM-41

7.5.7 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.

7.5.8 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. Non-portable 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.

a) 7500: Following the maintenance, repair, or moving of a 7500, a performance check will be performed. The performance check requires a set of standards be run (that have already been QC checked) and have a passing R2 value of .98 or above and the top standard be within 2 standard deviations of the validation values.

b) 3500xL: Following the maintenance, repair, or moving of a 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.

c) Thermocycler: Following maintenance, repair, or moving, a performance check will be performed. The performance check requires a set (minimum of 2) of positive controls (2800M, CG, etc) and an AMP_Neg to be amplified according to the current Autosomal STR amplification protocol. The samples will then be run on the 3500xl and analyzed in GeneMapper ID-X to ensure the samples 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.

d) Qiagility: Following maintenance, repair, or moving, a performance check will be performed. The performance check requires a set of standards be run on the 7500 (that have already been QC checked) and have a passing R2 value of .98 or above and the top standard be within 2 standard deviations of the validation values.

e) Qiagen EZ1: Following maintenance, repair, or moving, a performance check will be performed. The performance check requires a set (minimum of 2) of blood with known profiles 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/µL and the sample produces the expected DNA profile.

f) Qiagen Qiacube: Following maintenance, repair, or moving, 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 will show a useable male contribution in the semen fraction by analyzing the quantitation data.

g) NIST-Traceable Thermometer (new), Drift-Con, and Pipettes: performance checks will be performed by calibrating vendor and documented in the associated vendor records as the "As Left" value.

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. When possible, the Arkansas State Crime Laboratory utilizes proficiency testing offered from approved ISO/IEC 17043 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 personnel actively engaged in DNA testing. It is mandatory that the DNA personnel conduct all portions of a test up to the limit of their qualification, alone and without selecting or accepting any assistance from other persons. Violation may result in disciplinary action for those receiving and those rendering assistance. If the personnel have any questions or require assistance, they will contact the DNA Technical Leader. In order to avoid unfair advantages to other personnel 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 personnel 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 submission to the proficiency provider beginning January 1, 2017. Previously, the date test is performed has been recorded as the date of technical review. All analysts, technical reviewers, and processors 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

See the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.7.8 for additional information.

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 any discrepancies will be submitted to the Casework CODIS 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 will be collected and submitted to the Technical Leader. The Technical Leader (or their appointed person) will be responsible for providing to the external test source the required data 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 also review the results of the Technical Leader, Casework Supervisor, or CODIS Administrator and document it in the proficiency workflow. This review will be conducted in a timely manner. The electronic copy of the proficiency test is the official copy.

8.5 EVALUATION OF PROFICIENCY TESTS

PROFICIENCY TEST REVIEW GUIDELINES

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 DNA-FORM-36 (DNA Proficiency Review Form) 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 action taken to minimize the error in the future.
      i. All reported major and minor alleles are correct according to ASCL DNA interpretation guidelines.
      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.
      v. Minor allele calls: If there is a discrepancy between the provider results verses 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 Quality Assurance Concern (QAC) in Qualtrax.
4) Proficiency tests are documented in Qualtrax in the Proficiency Testing Workflow. The date that the PT results are submitted to the proficiency provider is considered the Date of Completion. The date under Results Review indicates the date the results from the proficiency provider are reviewed.
5) 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.
6) All discrepancies/errors and subsequent corrective actions must be documented.
7) 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) Submission Review - In addition to the normal technical and administrative casefile reviews, a specific review of the proficiency results paperwork will be performed and documented on DNA-FORM-36 (DNA Proficiency Review Form) by the TL, Casework Supervisor, or CODIS Administrator, whichever is not assigned a test in the set. This review will ensure that the correct electronic paperwork will be submitted to the proficiency provider.
4) Consensus Assessment - When the results are available from the proficiency provider, the submitted results will be compared to the consensus results by the TL and another non-tested analyst, typically the Casework Supervisor or the CODIS Administrator to ensure a complete and thorough review. This review will be documented on DNA-FORM-36 (DNA Proficiency Review Form). Any discrepancies will be noted and any explanations or Corrective Actions will be documented.

8.6 CORRECTIVE ACTION FOR PROFICIENCY TEST ERRORS

The following are the specific policies, procedures and criteria for any corrective action taken as a result of a discrepancy in a proficiency test. These terms as used in this section are limited to proficiency testing in the Forensic DNA and CODIS sections.

8.6.1 AUTHORITY & ACCOUNTABILITY

It is the responsibility of the Casework Supervisor and Technical Leader 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 (PT TESTING ONLY)

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 will 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 (PT TESTING ONLY)

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 will be made aware of the appropriate corrective action in order to minimize the recurrence of the discrepancy.

8.6.2.3 ANALYTICAL / INTERPRETATIVE

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 Qualtrax Proficiency Testing Workflow. 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 may serve as training samples until it is consumed or destroyed.

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 staff member's name or initials included on the worksheet.

Prior to authorizing 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.

Per the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.8.7.3, when opinions or interpretations are directly communicated by dialogue to a customer, a record of the communication will be retained.

A master list for abbreviations will be placed on the ForensicBiology drive. Novel abbreviations may be added by staff as needed and the "secure" list will be updated when significant changes are warranted.

9.1 REVIEWS

The Forensic DNA section complies with all *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.7.1.2 lab wide review requirements.

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 JusticeTrax. 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 CASE FILE

The case file is maintained electronically and contains the following information (as applicable):

- Submission Sheet
- Extraction Sheet
- Worksheets (* When appropriate)
  - Quantitation Sheets

  - STR AMP Sheets
  - Master Mix Sheet
  - Plate Loading Sheet *
  - Call Sheets
  - PopStats and/or ArmedXpert*
  - CODIS *
- GeneMapper Data
- Review Sheet
- Case Report

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

9.1.1.2 TECHNICAL REVIEW SHEET

The technical review for regular casework 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 and/or ArmedXpert Samples 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, appropriate samples(s), etc.?

9.1.2 ADMINISTRATIVE REVIEW

An administrative reviewer 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 will 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 JusticeTrax. The administrative reviewer will electronically initial that the administrative review was completed in JusticeTrax 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 test, 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 JusticeTrax consistent with the date evidence was submitted for DNA analysis?
- Has the Technical Review been documented completely?

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.

9.1.4 REVIEW FOR OUTSOURCED CASES

A review of data from samples outsourced to a vendor laboratory by the ASCL must be performed prior to the release of the vendor laboratory report to the submitting agency. This review will ensure the necessary analysis has been performed and documented by the vendor laboratory. If the reviewing analyst determines that additional analysis or re-analysis is not needed based on the vendor documentation, then they will complete the DNA Contract Case Review form (see DNA-FORM-43), roll the administrative milestone in JusticeTrax, and release the vendor report to the submitting agency. The criteria are listed below:

- Were all necessary analyses performed and documented according to established guidelines?
- Have all controls, internal lane standards, and allelic ladders been verified for expected results?
- Are all genotypes correct and do all called peaks meet the required threshold(s)?

- Are all genotypes called and transcribed correctly?
- Are the conclusions (both inclusions and exclusions) and statistics (if applicable) correct?
- Does the vendor report address all items sent to the vendor laboratory?
- Has the vendor noted and corrected all errors, both technical and administrative?
- Is the vendor report free of all errors and understandable to person(s) who will read the report?
- Have the vendor report, all vendor casefile notes, and corrective action documentation been securely stored?
- Are all CODIS requirements addressed: eligibility, appropriate specimen category; appropriate sample(s) etc.?
- If CODIS eligible, has the outsourcing request in JusticeTrax been administratively reviewed and has the vendor report been released to the agency?

If data is CODIS eligible, then the analyst will open a new JusticeTrax request to document and report the CODIS entry, which will be reviewed by another CODIS-approved analyst. The criteria are listed below:

- Have the appropriate specimens with correct allele calls and specimen categories been entered into CODIS?
- Has an ASCL report been created accurately reflecting which profile(s) have been entered into CODIS?

9.2 CORRECTIVE ACTIONS

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

9.2.1 NONCONFORMING WORK

Nonconforming testing is testing in which Forensic DNA procedures are not followed or the agreed-upon requirements of the customer (e.g., testing of standards and controls, test precision and accuracy, the care and handling of evidence, instrument performance) are not met. All Forensic DNA staff, including analysts and supervisory personnel, must be vigilant for any indication of nonconforming testing.

For Forensic DNA, there are three key levels of non-conforming work, each of which requires a different response:

- Simple corrections in which an isolated incident can be resolved immediately and documented in the casefile or record, when appropriate.
- Simple nonconformities requiring *Non-Conformance Form* (CODIS-FORM-17) to ensure technical justification, supervisor acknowledgement, and authorization for performing or reporting the deviation.

- Level 1 & 2 Nonconformities which require a *Quality Assurance Concern* (QAC) workflow be initiated. See the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.10 for more information

9.2.2 AUTHORITY & ACCOUNTABILITY

The DNA 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 Supervisor 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.

10 TESTIMONY REVIEW

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

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 will be maintained and will 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.

12 COMPLAINTS

See the *ASCL Quality Assurance Manual* (ASCL-DOC-01) section 7.9

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. The Arkansas State Crime Laboratory tries to maintain a safe working environment. It is the responsibility of the DNA/CODIS staff to familiarize themselves with all exit doors, safety showers and fire extinguishers. The crime lab provides training in chemical hygiene, blood borne pathogens, CPR, and first aid to all of the employees.

13.2 DNA LABORATORY CONTAMINATION PREVENTION

The Arkansas State Crime Laboratory Forensic DNA and CODIS Databasing sections share several laboratory spaces for analytical processes. In an effort to ensure that all Databasing and Forensic DNA staff consistently employ work habits that minimize the risk of DNA contamination — either sample to sample or laboratory staff to sample— the following list of basic preventive measures will be employed as necessary by analysts when performing DNA analysis. Some of the measures listed below may not be practicable or relevant at all times, but the principles of contamination prevention will apply. Shared analytical spaces include:

Cleanrooms:	301 (SAK Room), 309 (Window Room), 311(Windowless Room), and 340 (Bone Room)
Post-PCR rooms:	317 (DNA Post), 312 (CODIS Post)
Other rooms:	313 (Reagent Room), 272 (CODIS Alternate)

13.2.1 PERSONAL PROTECTIVE EQUIPMENT

Lab coats with cuffs that can be covered with disposable gloves will be worn in Cleanrooms.

Lab coats will be changed and laundered on a regular basis. If the risk of contamination is heightened based upon the activities that have occurred while wearing a lab coat, it should be replaced with a newly laundered one. Examples of when to change a lab coat include but are not limited to: after the processing of a case consisting of several items of bloody clothing or after an analyst knows that the exterior surfaces of a lab coat may have become contaminated with DNA, such as after a sneeze.

Lab coats will be worn when collecting biohazardous waste for disposal. They will be decontaminated properly when the activity is complete.

Face masks will be used for all analytical activities in the Cleanroom spaces. Face masks will be worn such that the nose and mouth will be completely covered. Facemasks and other personal effects such as glasses should not be handled directly during the course of evidence examination and special care should be taken to avoid the use of gloved hands to manipulate such items.

Personnel should avoid talking over evidence during evidence screening and/or sampling.

Personnel should only touch items of evidence with fresh, clean gloves.

Prior to use, personnel should visually inspect gloves for defects; if any damage is observed before or during examination of evidence, new gloves should be used.

Gloves should be changed with high frequency. Generally, if an analyst cannot recall when they last put on fresh gloves, the gloves should be changed.

Personnel should not use gloves that have come in contact with evidence to also touch computer keyboards, iPads, pens, pencils, etc., unless those items are specifically designated for use during the analytical process.

Cellular phone calls should not be answered and text messages/emails should not be exchanged during the processing and examination of evidence.

13.2.2 TOOLS AND REAGENTS

Computers in the laboratory spaces will be decontaminated regularly by wiping mice and keyboards (or keyboard covers) with a bleach-saturated towel. A "gloves-off" policy for all shared computers in laboratory spaces will be implemented.

For electronic equipment shared between administrative and analytical workspaces, special care should be taken to avoid cross contamination. At a minimum, a washable cover of sufficient size to cover input controls, such as touchscreen or keys and mouse pad, should be used.

"Analytical-use only" pens, pencils, markers, rulers, etc. will be used during evidence examination. When not in use, they should be stored in a location that is less susceptible to contamination. These items should be decontaminated on a regular basis.

Water dropper bottles used during analysis should only be handled with gloves and should be stored in a location that is less susceptible to contamination. The bottles should be regularly decontaminated and refreshed with clean, autoclaved deionized water. Water dropper bottles shall not leave the Cleanroom spaces except for sterilization. In addition, the analyst should avoid touching the swab with the dropper bottles.

Clean gloves shall be used when handling swab packaging when the swabs will be used for DNA sample collection. Swab heads should not touch anything other than the item being swabbed.

13.2.3 DECONTAMINATION

A dilute bleach solution (or product containing bleach) will be used to clean workspaces and tools prior to evidence processing. Alcohol (ethanol and isopropanol are both acceptable) or clean deionized water should be used to rinse residual bleach from those items that will come in direct contact with the evidence. Commercial disinfectant products (such as Clorox wipes) will not be used for the purposes of decontamination unless they contain bleach or are designed specifically for laboratory surface decontamination.

Analysts will adopt a "bleach-in-bleach-out" approach. Prior to initiating evidence screening and/or sampling at the beginning of a given work day, work surface(s) and tools will be decontaminated. Additional decontamination will continue as appropriate throughout the day. A final decontamination should occur at the close of the work-session.

A regular workspace decontamination schedule will be established. This will include benches, cabinets, drawer pulls, computer keyboards, mice, exteriors of reagent bottles, cameras, etc.

13.2.4 GENERAL EVIDENCE HANDLING

An enclosed biohood must be available in every Cleanroom. Analysts should use these hoods for the processing of liquid blood or other biohazardous samples.

Clean paper or other bench covering shall be used under items of evidence. Paper or other covering material will be changed between items. Bench covering material should not be stored uncovered outside of the Cleanroom workspaces.

Evidence should not be placed directly on top of external packaging and or in a location that will come into direct contact with external packaging. Evidence packaging is often handled without gloves and cross-contamination from the packaging to the evidence may occur.

13.3 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. The accreditation documentation will be stored in Qualtrax. All vendor laboratories must also comply with standards set forth in the Arkansas State Crime Laboratory quality manual. Prior to any outsourcing of data generation, 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 may be technically and administratively reviewed by the vendor laboratory, or may be re-analyzed by a qualified, proficient DNA analyst in the methodology used by the vendor laboratory, depending on the approved technical specifications and specific scenario of the case. The re-analysis and/or CODIS eligibility review will give ownership of the data to the analyst performing the task. If re-analyzed, a new report will be generated by the ASCL analyst, and the data must be technically reviewed prior to being searched in the CODIS system. If no reanalysis is performed, the vendor laboratory report will be forwarded to the requesting agency and any associated CODIS entry will be reported by the ASCL in a new DNA request. After CODIS entry, the casefile will then get an administrative review before the 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 may accept the findings of an on-site visit conducted by another NDIS participating laboratory in lieu of conducting an on-site visit in person. See DNA-Form 21 for the on-site visit checklist.

If the Arkansas State Crime Laboratory finds it necessary to transfer evidence to an outside laboratory (e.g. FBI, UNT), an *Inter-Laboratory Evidence Transfer Form* (see ASCL-FORM-07) must be completed and entered into the case file. The Inter-Laboratory Evidence Form may be waived for items funded out of a grant and/or items under a contract. Any cost incurred by the laboratory must be approved by the Fiscal Officer. If there will be a cost incurred to the customer, the customer

must be notified and approve of the arrangement. This must be documented and placed in the case file. The Quality Assurance Manager maintains a register of all subcontractors used for testing and/or calibrations and maintains documentation of their competency and compliance as described in section 4.5.1.

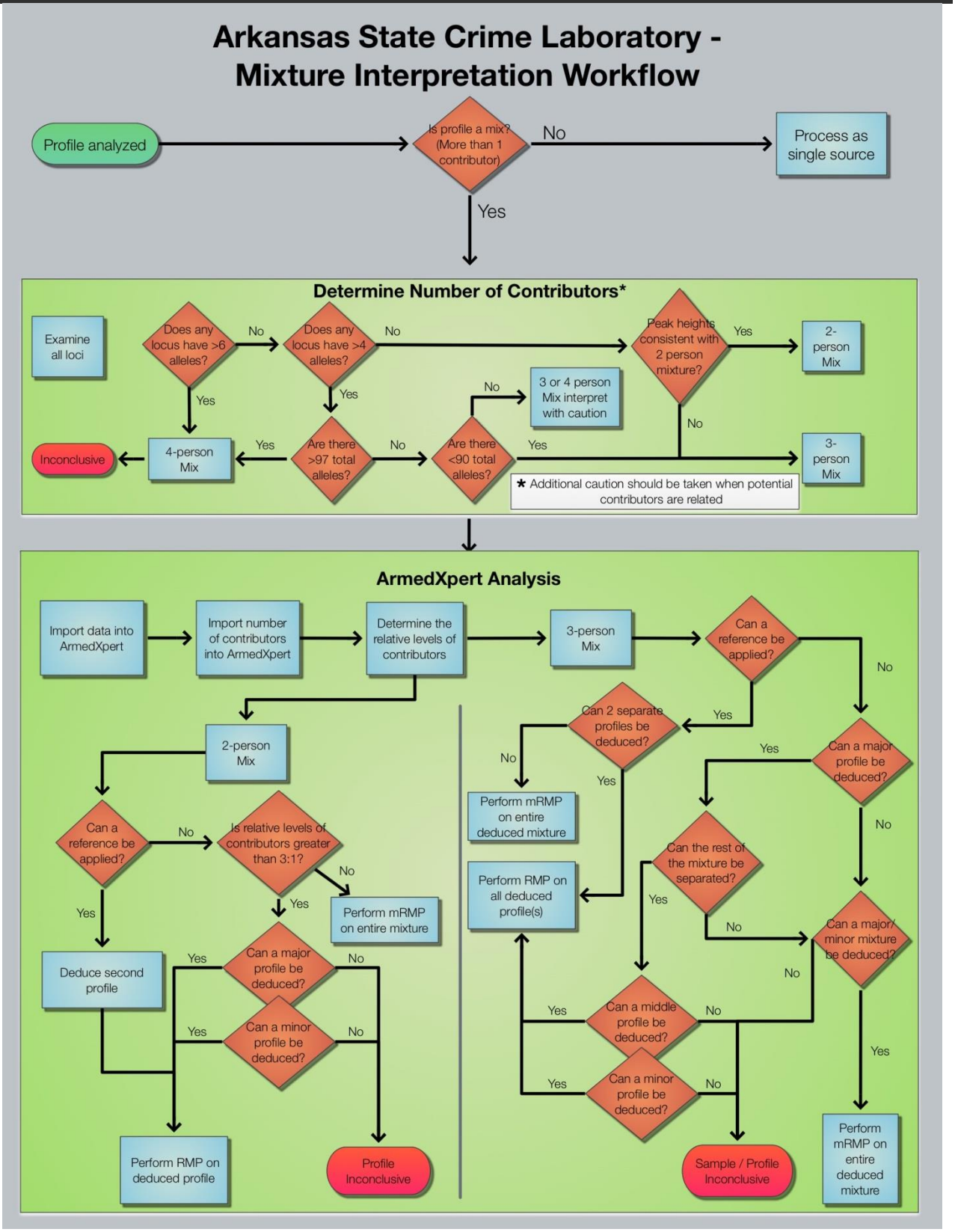
APPENDIX A TECHNICAL LEADER CONTINGENCY PLAN

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.

APPENDIX B ARMEDXPERT MIXTURE INTERPRETATION WORKFLOW



APPENDIX C KINSHIP CALCULATIONS (AS FOUND IN CODIS V8.0)

APPENDIX C.1 PROBABILITY OF EXCLUSION

The Probability of Exclusion (PE) is defined as the probability of excluding a random individual as a parent, given the alleles of the child's profile and the biological parent's profile. The DNA profile of the alleged parent is not considered in calculating the PE. The Probability of Exclusion is equal to the frequency of all the people in the population who do not contain an allele that matches the obligate parental allele of the child. If the genotypes of the biological parent, child, and alleged parent do not match at one locus, then it is declared "no match" at that locus and the entire parentage test is declared "inconclusive." If the genotypes of the known parent, child, and alleged parent do not match at two or more loci, then "no match" is declared for the entire parentage test.

A population group-specific mean power of exclusion for a locus can be calculated using the corresponding allele frequencies of the locus and the formula given by Butler as shown: $\bar{A} = PEavg = H^2(1-2H(1-H)^2)$

where $H \equiv 1 - \sum_{j=1}^{n} p_j^2$ denotes the degree of heterozygosity of a locus, defined as follows:

where n denotes the number of detectable alleles at a locus for a population, and p_j denotes the frequency of the j^{th} allele. The PI for an inconsistent locus is then: $PI = \mu / \bar{A}$.

The Combined Parentage Index (CPI) for the profile is calculated by multiplying together the individual locus PI, as shown: $CPI = \prod_{locus} PI$

The combined probability of exclusion (CPE) is: $CPE = 1 - \prod_{locus}(1 - PE_{locus})$.

For a locus with inconsistency, $\bar{A}$ is used for PE_{locus}.

All matched cases and their corresponding formulas for PI and PE are listed in the Parentage Formula Table.

PE for Parentage Trio

The Probability of Exclusion (PE) of parentage for a DNA profile is determined by the following equation:

PE = 1 - $\prod_{loci}$ [1 - (PE_{locus})].

Note that the expression of (1-PE_{locus}) is simply PI_{locus}, the locus probability of inclusion, and that **PI + PE = 1** always.

The Probability of Parentage (*W*) for the alleged parent in a parentage trio mode is determined from the CPI by the following equation: $W = \frac{CPI \cdot p_{prior}}{CPI \cdot p_{prior} + (1 - p_{prior})}$ with the default of value of 0.5 for *Pprior*. *Pprior* is the prior probability of parentage. The prior probability is user-configurable and its default value is set to the neutral value of 0.5 for every locus of the profile. That is, Popstats assumes there is a 50% probability that the alleged parent is the biological parent of the child, and a 50% probability that the alleged parent is not the biological parent of the child.

Note: The profile *W* is not calculated by multiplying the individual locus *W*s. The values arrived would not be the same as those arrived at using the CPI method. Thus, the individual locus *W* is not calculated.

APPENDIX C.2 PARENTAGE TRIO

Parentage Trio in Popstats calculates the Parentage Index and the Probability of Exclusion by using the equations shown in the following table that map to the applicable genotypes of the Biological Parent, Child, and Alleged Parent:

	Known Parent (KP)	Child (C)	Alleged Parent (AP)	Parentage Index (PI)	Probability of Exclusion (PE)
1	AA	AA	AA	$1 / a$	$(1: a)^2$
	AB	AA	AA		
	BB	AB	AA		
	BC	AB	AA		
2	AA	AA	AB	$1 / 2a$	$(1: a)^2$
	BB	AB	AB		
	BB	AB	AC		
	BC	AB	AD		
	BC	AB	AB		
	AB	AA	AB		
3	AA	AB	BB	$1 / b$	$(1: b)^2$
	AC	AB	BB		
4	AA	AB	AB	$1 / 2b$	$(1: b)^2$
	AA	AB	BC		
	AC	AB	BD		
	AC	AB	AB		
5	AB	AB	AB	$1 / a + b$	$[1 - 1(a + b)]^2$
6	AB	AB	AA	$1 / a + b$	$[1 - 1(a + b)]^2$
	AB	AB	BB		
7	AB	AB	AC	$1 / 2(a + b)$	$[1 - 1(a + b)]^2$
	AB	AB	BC		

Where a and b are the allele frequencies of A and B, respectively.

***Note**: If a or b is less than the minimum allele frequency, then the minimum allele frequency is used for calculation instead.*

In the case where the alleged parent has an inconsistent genotype at one locus, then $\boldsymbol{\mu / \bar{A}}$ will be calculated for the Parentage Index, where $\boldsymbol{\mu}$ is the mutation rate of the locus and $\boldsymbol{\bar{A}}$ is the mean power of exclusion of the locus. If there are more than two loci with inconsistencies, then the parentage case is an exclusion.

Once the Parentage Index of each locus is determined for both the case of consistent genotypes and the case of mutation, the probability of parentage (W), the Combined Parentage Index (CPI), and the Combined Probability of Exclusion (CPE) can be calculated as before. Namely, the probability of parentage for each locus is:

$$W = \frac{PI \cdot (\text{prior probability})}{PI \cdot (\text{prior probability}) + [1 - (\text{prior probability})]}$$

The Combined Parentage Index is: **CPI =** $\prod_{loci}$ **PI**

The Combined Probability of Parentage is: **CW =** $\prod_{loci}$ **W**

The Combined Probability of Exclusion is: **CPE = 1:** $\prod_{loci}$ (1 : PE)

APPENDIX C.3 REVERSE PARENTAGE

The probability of exclusion of Reverse Parentage can be interpreted as the proportion of all of those individuals in the population that do not contain an allele that matches the biological mother and a second allele that matches the biological father. Similarly, note that a, b, c, and d denote the allele frequencies of alleles A, B, C, and D, respectively. The following table shows the RPI and PE formula for various Mother-Father-alleged child genotype combinations.

Biological Parent 1	Biological Parent 2	Alleged Child	Reverse Parentage Index	Probability of Exclusion	Genotype Combination Characteristics
AA	AB	AB	$1 / 4ab$	$1 - a(a + 2b)$	The evidence is heterozygous and is identical to one biological parent's genotype; the other biological parent is homozygous. Possible children's genotypes AA(50%), AB(50%) for AA, AB parents; and BB(50%), AB(50%) for BB, AB parents.
AB	AB	AB	$1 / 4ab$	$1 - a(a + b)^2$	All profiles are heterozygous and identical. Possible children's genotypes AA(25%), AB(50%), B(25%).
AA	AB	AA	$1 / 2a^2$	$1 - a(a + 2b)$	The evidence is homozygous and is identical to one biological parent's genotype; the other known biological is heterozygous.
AB	AB	A	$1 / 4a^2$	$1 - a(a + b)^2$	The evidence is homozygous; the known parents' genotypes are both heterozygous and identical. Possible children's genotypes are AA(25%), AB(50%), and BB(25%).
AA	BB	AB	$1 / 2ab$	$1 - 2ab$	The evidence is heterozygous and the parents' profiles are homozygous. Possible children's genotype is AB.
AA	BC	AB	$1 / 4ab$	$1 - 2a(b + c)$	The evidence is heterozygous; one biological parent is heterozygous; the other is homozygous; and the profiles of the biological parents do not share any alleles. For AB and CC, the possible children's genotypes are AC(50%) and BC(50%); for A and BC, the possible children's genotypes are AB(50%) and AC(50%); for B and AC, the
AB	CD	AC	$1 / 8ac$	$1 - 2(a + b)(c + d)$	The evidence is heterozygous; both biological parents are heterozygous; and the biological parents' profiles do not share any alleles. The possible children's genotypes are AC (25%), BC (25%), AD (25%), BD (25%).
AB	BC	AC	$1 / 8ac$	$1 + b^2 - 2(a + b)(b + c)$	The evidence is heterozygous; both biological parents are heterozygous; and the biological parents' profiles share one allele. For AB and BC, the possible children's genotypes are AB (25%), BB (25%), AC (25%), BC (25%).
AB	BC	BC	$1 / 8bc$		
AB	BC	BB	$1 / 4b^2$	$1 + b - 2(a + b)(b + c)$	The evidence is homozygous; both of the biological parents are heterozygous; and the biological parents' profiles share one allele. The evidence must have the allele shared between the biological parents. Possible children's genotypes are AB(25%), BB(25%), AC(25%) & BC(25%)
AA	AA	AA	$1 / a^2$	$1 - a^2$	All profiles are homozygous and identical. The possible children's genotype is AA.

After the RPI for each locus is calculated, the Combined Reverse Parentage Index for the entire profile is then calculated by the product rule: $\mathrm{CRPI} = \prod_{\text{locus}} \mathrm{RPI}_{locus}$

The Combined Probability of Exclusion for Reverse Parentage for the entire profile is:

$$\mathrm{CPE} = 1 - \prod_{\text{locus}} (1 - \mathrm{PE}_{locus}).$$

APPENDIX C.4 GENERAL KINSHIP FORMULA

I-T-O kinship statistics evaluate the likelihood that the pair of given DNA profiles are associated by kinship as opposed to chance.

Popstats Kinship supports the following kinship relationships:

- Parent-Offspring
- Full Sibling
- First Cousin
- Double first cousin
- Half Sibling: Half Sibling, Uncle/Aunt-Nephew/Niece, Grandparent-Grandchild (parents are not related)
- Half Sibling-Sibling: Half Sibling, one of each person's parents are siblings with each other
- Half Siblings Half Sibling: Half Sibling, one of each person's parents are half-siblings with each other

Given a pair of DNA profiles, the likelihood ratio of the two DNA profiles being from relatives vs. the two DNA profiles being unrelated is calculated for each locus as follows:

Relationship Notation		**Φ_2**	**Φ_1**	**Φ_0**
Parent-Offspring	PO	0	1	0
Full Sibling	FS	1 / 4	1 / 2	1 / 4
Half-Sibling	HS	0	1 / 2	1 / 2
Half-Sibling Sibling	HS-S	1 / 8	1 / 2	3 / 8
Half-Sibling-Half-Sibling	HS-HS	1 / 16	1 / 2	1 / 16
First Cousin	1C	0	1 / 4	3 / 4
Double First Cousin	D	1 / 16	3 / 8	9 / 16

Where:

θ_0 is the probability that the locus genotypes of the individuals share no allele by descent for the specified kinship.

θ_1 is the probability that the locus genotypes of the individuals share one allele by descent for the specified kinship.

θ_2 is the probability that the locus genotypes of the individuals share two alleles by descent for the specified kinship.

	G_x (Reference)	G_y (Evidence)	Conditional Probability of G_y given G_x under:		
			I (*2 alleles IDB*) $P_2(X,Y)$	T (*1 allele IDB*) $P_1(X,Y)$	O (*0 alleles IBD*) $P_0(X,Y)$
1	AA	AA	1	a	a^2
2	AA	BB	0	0	b^2
3	AA	AB	0	b	$2ab$
4	AA	BC	0	0	$2bc$
5	AB	AA	0	$a / 2$	a^2
	AB	BB		$b / 2$	b^2
6	AB	CC	0	0	c^2
7	AB	AB	1	$a + b / 2$	$2ab$
8	AB	AC	0	$c / 2$	$2ac$
	AB	BC		$c / 2$	$2bc$
9	AB	CD	0	0	$2cd$

Where:

$P_2(X,Y)$ = probability of G_y (reference) given G_x (evidence) with 2 of their alleles identical by descent (IBD);

$P_1(X,Y)$ = probability of G_y (reference) given G_x (evidence) with 1 of their alleles identical by descent (IBD);

$P_0(X,Y)$ = probability of G_y (reference) given G_x (evidence) with 0 of their alleles identical by descent (IBD);

The probability of observing the locus genotype G_y (reference) given G_x (evidence) for a specified kinship is:

$$\mathbf{P(kinship) = [P_2(X,Y) \times \theta_2] + [P_1(X,Y) \times \theta_1] + [P_0(X,Y) \times \theta_0]}$$

The likelihood of the locus genotype G_y (reference) given G_x (evidence) being related by specified kinship vs. the locus genotypes being unrelated is: **LR(kinship) = P(kinship) / P(unrelated)**

To summarize, for each locus:

	Kinship Likelihood Ratio	**Kinship Probability**
Locus Formula	P(Kinship) / P(Unrelated)	P(Kinship)

The combined probability for the specified kinship is: $\mathbf{CP(kinship) = \prod_{loci} P(kinship)}$

and the combined likelihood ratio for the profiles to be related by the specified kinship vs. being unrelated is:

$$\mathbf{CLR(kinship) = \prod_{loci} LR(kinship)}$$

In a Kinship case with the single parentage calculation, if the genotypes of the reference and the evidence do not match at one or two loci, then the mutation analysis is applied in the calculation of Parentage Index (PI) for these loci. The Parentage index for a non-matching or inconsistent locus is: $\boldsymbol{\mu / \bar{A}}$

Where $\boldsymbol{\mu}$ is the mutation rate of the locus and $\boldsymbol{\bar{A}}$ is the mean power of exclusion of the locus. Popstats applies mutation analysis for up to two non-matching or inconsistent loci.

APPENDIX C.5 SINGLE PARENTAGE KINSHIP

In the special case where the relationship is Parent-Offspring (PO), the calculation of the Parentage Index (PI) as stated in the General Kinship Formula can be simplified. This is the so-called "single parent" case, or the "motherless paternity" case. In addition to the Parentage Index, the probability of exclusion can be derived for the special case.

Using the tables in the General Kinship Formula topic, the formula for the Parentage Index is simplified as follows:

	Reference	Evidence	Parent-Offspring Index (PI)	Probability of exclusion (PE)
1	AA	AA	$1 / a$	$(1: a)^2$
2	AA	AB	$1 / 2a$	$(1: a)^2$
3	AB	AB	$a + b / 4ab$	$[1: (a + b)]^2$
4	AB	AA	$1 / 2a$	$[1: (a + b)]^2$
5	AB	AC	$1 / 4a$	$[1: (a + b)]^2$

For inconsistent loci (when the genotype of the alleged parent and the genotype of the child do not share any alleles), the formula are as follows:

	Child (C)	Alleged Parent (AP)	Parentage Index (PI)	Probability of Exclusion (PE)
1	AA	BB	$\mu / \bar{A}$	(1: a)2
2	AA	BC	$\mu / \bar{A}$	(1: a)2
3	AB	CD	$\mu / \bar{A}$	[1: (a + b)]2
4	AB	CC	$\mu / \bar{A}$	[1: (a + b)]2

where μ is the mutation rate of the locus and $\bar{A}$ is the mean power of exclusion of the locus.

A population group-specific mean power of exclusion for a locus can be calculated using the corresponding allele frequencies of the locus and the formula given by Butler as shown: $\bar{A} = PE_{avg} = H^2(1\text{-}2H(1\text{-}H)^2)$, where H denotes the degree of heterozygosity of a locus, defined as follows:

$H \equiv 1 - \sum_{j=1}^{n} p_j^2$, where n denotes the number of detectable alleles at a locus for a population, and p_j denotes the frequency of the j^{th} allele. The PI for an inconsistent locus is then: $PI = \mu / \bar{A}$

The Combined Parentage Index (CPI) for the profile is calculated by multiplying together the individual locus PI, as shown below:

$$CPI = \prod_{locus} PI$$

The combined probability of exclusion (CPE) is: $CPE = 1 - \prod_{locus}(1 - PE_{locus})$.

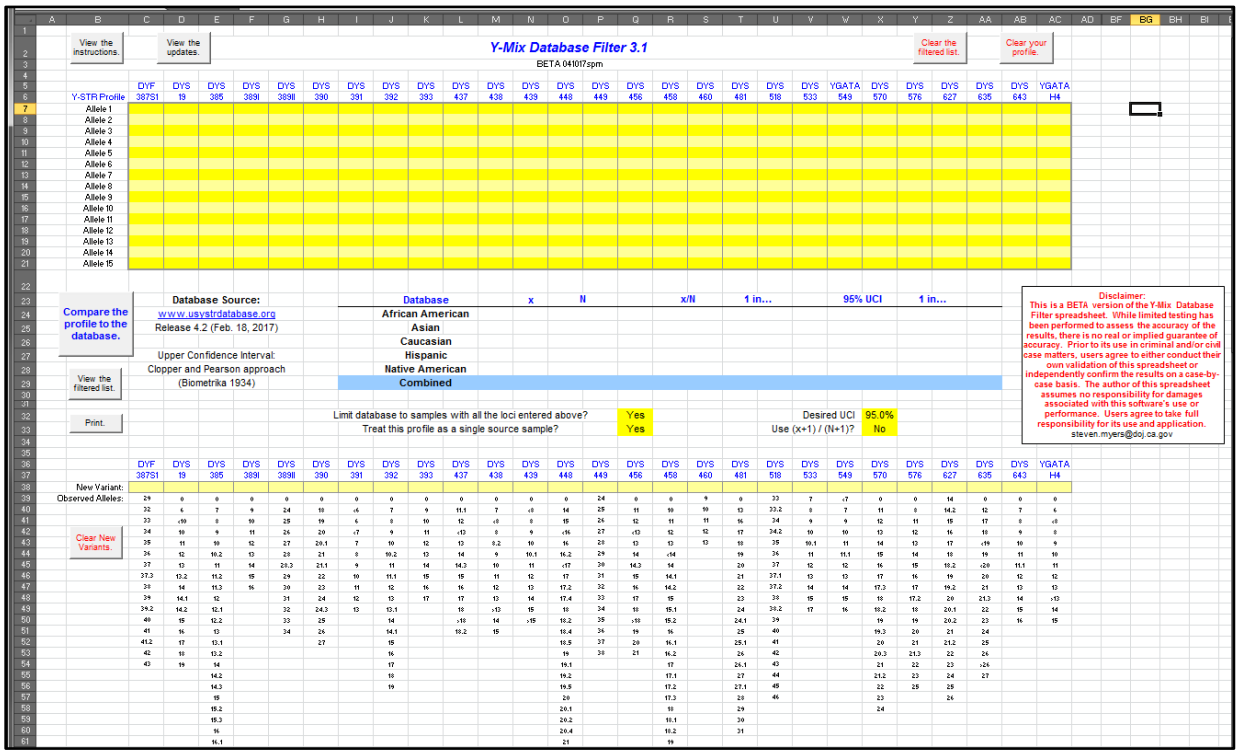
For a locus with inconsistency, $\bar{A}$ is used for the locus PE.

APPENDIX D Y-STR STATISTICAL INTERPRETATIONS

The Arkansas State Crime Laboratory will follow the recommendations in the SWGDAM Interpretation Guidelines for Y-Chromosomal STR Typing, 2014.

Due to the lack of recombination, the entire Y-chromosome haplotype must be treated as a single locus. Haplotype frequencies are estimated using the counting method. The counting method involves searching a given haplotype against a database to determine the number of times the haplotype was observed in that database.

The Arkansas State Crime Laboratory has chosen to utilize a customized version (YSTR_STATS_v4.x) of the California DOJ Y-Mix Database Filter v3.2 (entry screen shown below):



The excel file can be found at ForensicBiology\Excel Form\YSTR_STATS_v4.3.xltm. Profiles can be copied directly from the DNA-FORM-01 project and pasted into the yellow cells. Any settings may be adjusted as needed, and the COMPARE THE PROFILE TO THE DATABASE button pressed. This will filter the database against the entered profile and report any database profiles which have no mismatches.

Settings:

Limit database to samples with all loci entered above?: If Yes, only database profiles with all entered loci will be searched. Any database profiles with missing loci will be ignored. If No, any blank loci in either the entered profile or in the database profiles will not be compared but the rest of the profile will be compared. In general, due to the smaller database set and incomplete theta values for Yfiler Plus, any complete Yfiler Plus profiles should be searched with only the more limited original Yfiler loci with the limit set as YES to maximize the discrimination potential of the search in the largest possible database set. The FILTERED LIST tab will show all profiles found to conform to the entered profile(s). This list should be manually compared to remove any associations to database profiles which would be excluded

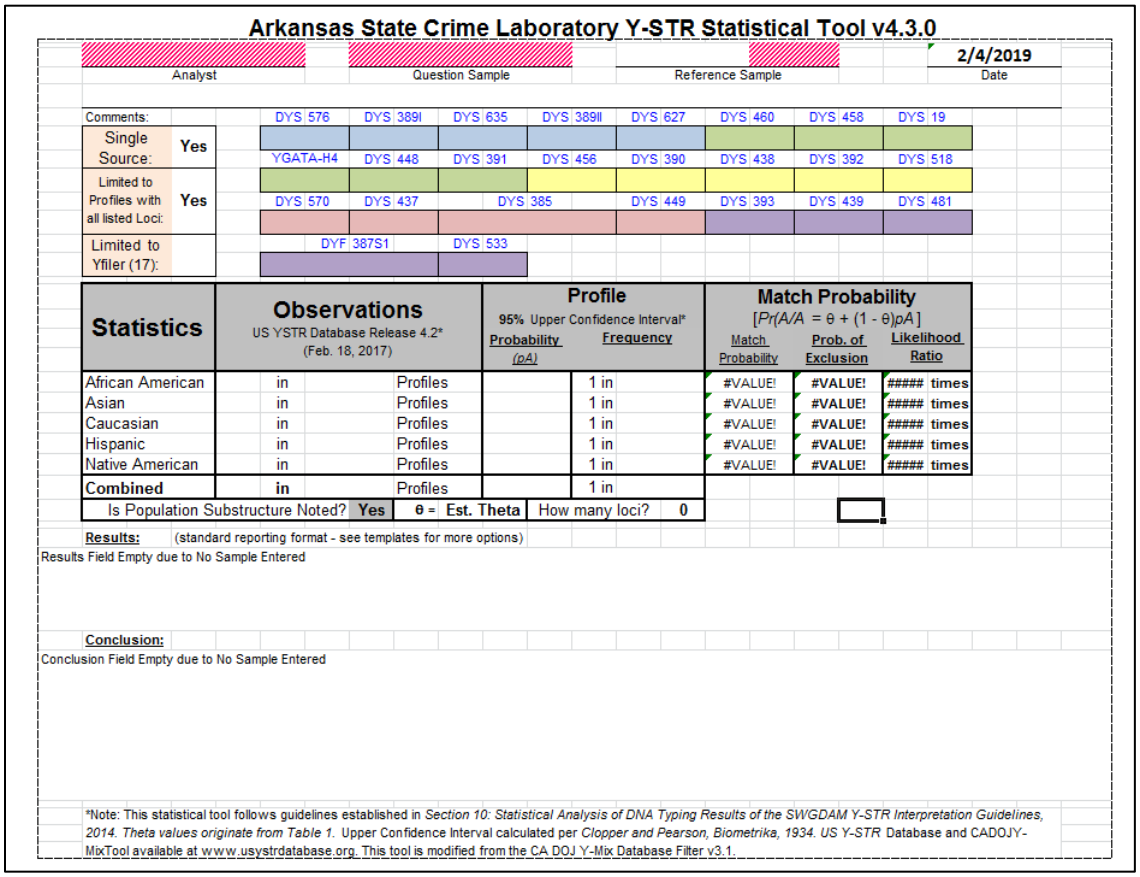
on the expanded Yfiler Plus loci. Be sure to press the CLEAR THE FILTERED LIST button between each search.

Treat this profile as a single source sample?: If Yes, the database profiles must match the entered profile exactly at each loci compared. If No, then the database profiles may match either allele entered for a locus in the entered profile, if more than one is entered. This should be set based on the entered profile.

Desired UCI: This changes the upper confidence interval from the standard at 95%. Only 95% has been performance checked for use in the Arkansas State Crime Laboratory.

Use (x+1)/(N+1)?: This modifies the number of observations to include the entered profile which alters the haplotype frequency estimate. Because a Likelihood Ratio is reported, this should remain No.

The results printout (Yfiler Plus and Y23 shown below) specifies the Y-STR profile searched as well as the selected settings (single-source, limited search, q, Population Substructure, CI).



Arkansas State Crime Laboratory Y-STR Statistical Tool v4.3.0

Analyst | Question Sample | Reference Sample | 2/4/2019 Date

Comments:

Single Source: Yes	DYS 576	DYS 389I	DYS 635	DYS 389II	DYS 627	DYS 460	DYS 458	DYS 19
	YGATA-H4	DYS 448	DYS 391	DYS 456	DYS 390	DYS 438	DYS 392	DYS 518
Limited to Profiles with all listed Loci: Yes	DYS 570	DYS 437	DYS 385		DYS 449	DYS 393	DYS 439	DYS 481
Limited to Yfiler (17):	DYF 387S1		DYS 533					

Statistics	Observations (US YSTR Database Release 4.2* (Feb. 18, 2017))	Profile: 95% Upper Confidence Interval* Probability (pA)	Profile: Frequency	Match Probability [Pr(A/A = θ + (1 - θ)pA]	Prob. of Exclusion	Likelihood Ratio
African American	in Profiles		1 in	#VALUE!	#VALUE!	##### times
Asian	in Profiles		1 in	#VALUE!	#VALUE!	##### times
Caucasian	in Profiles		1 in	#VALUE!	#VALUE!	##### times
Hispanic	in Profiles		1 in	#VALUE!	#VALUE!	##### times
Native American	in Profiles		1 in	#VALUE!	#VALUE!	##### times
Combined	**in** Profiles		1 in			

Is Population Substructure Noted? **Yes** | **θ = Est. Theta** | How many loci? **0**

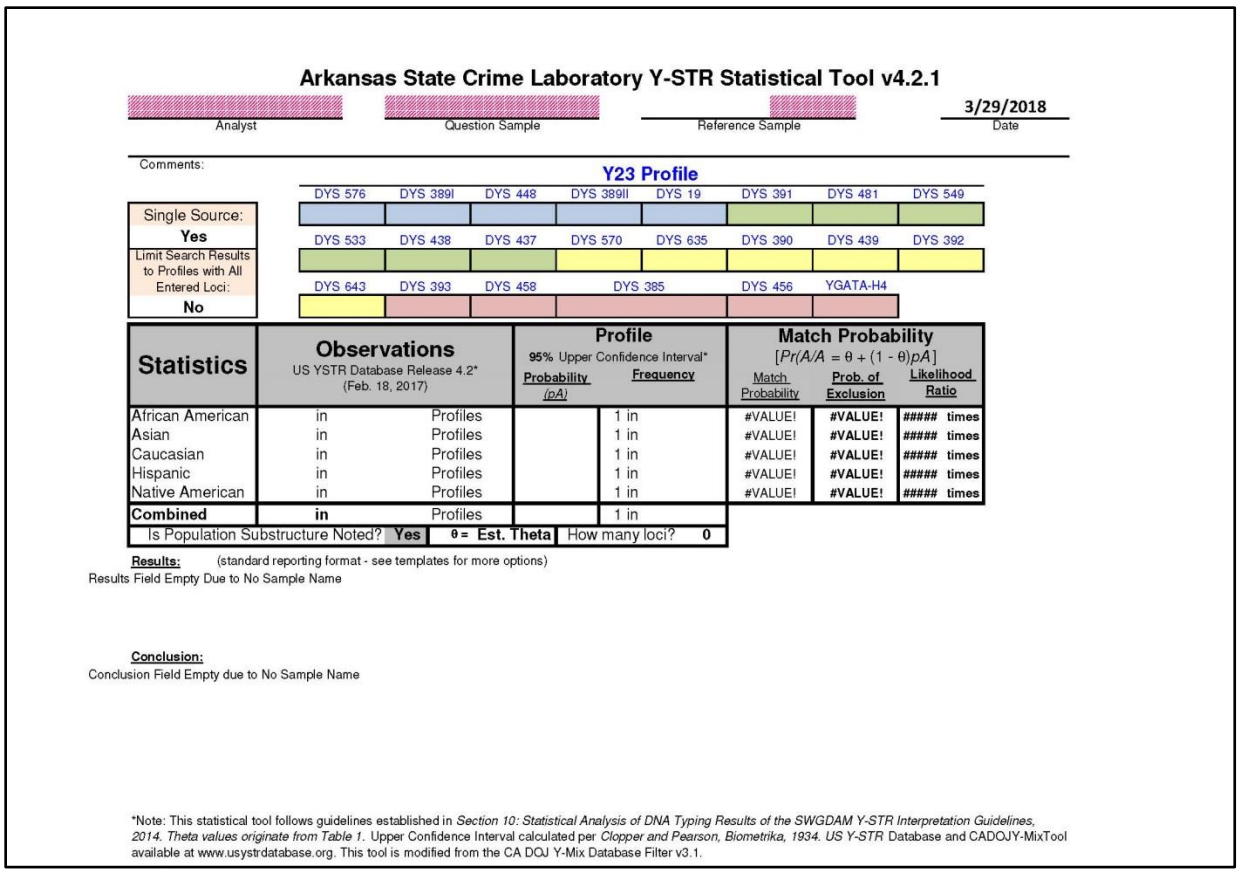
Results: (standard reporting format - see templates for more options)

Results Field Empty due to No Sample Entered

Conclusion:

Conclusion Field Empty due to No Sample Entered

*Note: This statistical tool follows guidelines established in *Section 10: Statistical Analysis of DNA Typing Results of the SWGDAM Y-STR Interpretation Guidelines, 2014. Theta values originate from Table 1.* Upper Confidence Interval calculated per *Clopper and Pearson, Biometrika, 1934. US Y-STR* Database and CADOJY-MixTool available at www.usystrdatabase.org. This tool is modified from the CA DOJ Y-Mix Database Filter v3.1.



Arkansas State Crime Laboratory Y-STR Statistical Tool v4.2.1

Analyst | Question Sample | Reference Sample | 3/29/2018 Date

Comments:

Y23 Profile

DYS 576	DYS 389I	DYS 448	DYS 389II	DYS 19	DYS 391	DYS 481	DYS 549
DYS 533	DYS 438	DYS 437	DYS 570	DYS 635	DYS 390	DYS 439	DYS 392
DYS 643	DYS 393	DYS 458	DYS 385		DYS 456	YGATA-H4	

Single Source: **Yes**

Limit Search Results to Profiles with All Entered Loci: **No**

Statistics	Observations (US YSTR Database Release 4.2* (Feb. 18, 2017))	Profile 95% Upper Confidence Interval* Probability (pA)	Profile Frequency	Match Probability [$Pr(A/A = \theta + (1 - \theta)pA$] Match Probability	Prob. of Exclusion	Likelihood Ratio
African American	in Profiles		1 in	#VALUE!	#VALUE!	##### times
Asian	in Profiles		1 in	#VALUE!	#VALUE!	##### times
Caucasian	in Profiles		1 in	#VALUE!	#VALUE!	##### times
Hispanic	in Profiles		1 in	#VALUE!	#VALUE!	##### times
Native American	in Profiles		1 in	#VALUE!	#VALUE!	##### times
Combined	**in** Profiles		1 in			

Is Population Substructure Noted? **Yes** | θ = **Est. Theta** | How many loci? **0**

Results: (standard reporting format - see templates for more options)

Results Field Empty Due to No Sample Name

Conclusion:

Conclusion Field Empty due to No Sample Name

*Note: This statistical tool follows guidelines established in *Section 10: Statistical Analysis of DNA Typing Results of the SWGDAM Y-STR Interpretation Guidelines, 2014. Theta values originate from Table 1.* Upper Confidence Interval calculated per *Clopper and Pearson, Biometrika, 1934. US Y-STR* Database and CADOJY-MixTool available at www.usystrdatabase.org. This tool is modified from the CA DOJ Y-Mix Database Filter v3.1.

Haplotype Frequency

The US Y-STR Database v4.2 database (http://usystrdatabase.org/) is filtered against the entered profile. The frequency of the entered haplotype in the database is then estimated by dividing the count by the number of haplotypes searched.

95% Upper Confidence Interval-based Profile Probability

A Y-STR profile probability can be estimated from the observed haplotype frequency by attaching a confidence interval (generally 95% or greater) to the haplotype frequency estimate to capture the effect of database size. The ASCL Y23_STATS_v4.2 tool utilizes the Clopper and Pearson (Biometrika, 1934) method for estimating the 95% Confidence Interval.

Match Probability

The match probability addresses the question of a match between the evidentiary and reference samples given that the reference donor is not the source of the evidentiary sample. Match probabilities are the probabilities of observing a profile given that it has already been observed and depend on the evolutionary history of the population. It is calculated by the equation: $[Pr(A/A = \theta + (1 - \theta)pA]$ where pA is the Profile Probability from the 95% Confidence Interval.

This match probability (or its inverse Likelihood Ratio) will be reported as recommended in section 10.3 of the SWGDAM Interpretation Guidelines for Y-Chromosomal STR Typing, 2014.

Population Substructure

Because some population substructure is expected in the Arkansas populations, a theta (θ) value is applied in the calculation of the Match Probability. Theta describes the chance of haplotypes being the same within

populations relative to the chance of them being the same between populations. The theta estimate used varies depending on the number of loci in the profile and is derived from the tables provided in the SWGDAM Interpretation Guidelines for Y-Chromosomal STR Typing, 2014. Theta values for the Yfiler Plus-specific loci have not been determined, so those loci will not be searched against.

Table 1. Theta estimates for all possible subsets of loci for each of three multiplexes

Table 1: ? estimates based on African Americans, Asians, Caucasians, Hispanics & Native Americans	
Loci	Est. Theta
1	0.06
2	0.04
3	0.03
4	0.02
5	0.008
6	0.005
7	0.003
8	0.002
9	0.002
10	0.002
11	0.0009
12	0.0007
13	0.0006
14	0.0005
15	0.0005
16	0.0004
17	0.0004
18	0.0004
19	0.0003
20	0.0003
21	0.0003
22	0.0003

APPENDIX E STUTTER RATIO TABLES

APPENDIX E.1 POWERPLEX 16HS STUTTER RATIOS (RETIRED)

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

APPENDIX E.2 POWERPLEX FUSION 6C STUTTER RATIOS

Locus	Reverse Stutter		Forward Stutter	
	- repeat	ratio	+ repeat	ratio
Amel	-	-	-	-
D3S1358	-4	0.136	+4	0.024
D1S1656	-2	0.056	-	-
	-4	0.174	+4	0.056
D2S441	-4	0.09	+4	0.029
D10S1248	-4	0.166	+4	0.013
D13S317	-4	0.105	+4	0.027
Penta E	-5	0.072	+5	0.026
D16S539	-4	0.12	+4	0.037
D18S51	-4	0.146	+4	0.06
D2S1338	-4	0.136	+4	0.033
CSF1PO	-4	0.117	+4	0.04
Penta D	-5	0.05	+5	0.037
TH01	-4	0.062	+4	0.028
vWA	-4	0.144	+4	0.029
D21S11	-4	0.127	+4	0.036
D7S820	-4	0.099	+4	0.018
D5S818	-4	0.11	+4	0.024
TPOX	-4	0.066	+4	0.021
D8S1179	-4	0.125	+4	0.034
D12S391	-4	0.174	+4	0.027
D19S433	-2	0.014	-	-
	-4	0.126	+4	0.042
SE33	-2	0.066	-	-
	-4	0.195	+4	0.055
D22S1045	-3	0.184	+3	0.114
DYS391	-4	0.094	+4	0.02
FGA	-2	0.012	-	-
	-4	0.165	+4	0.06
DYS576	-4	0.125	+4	0.037
DYS570	-4	0.149	+4	0.038

APPENDIX E.3 POWERPLEX Y23 STUTTER RATIOS (RETIRED)

Locus	-8	-3,-4,-5	-2	+2,+3,+4
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		

APPENDIX E.4 YFILER PLUS STUTTER RATIOS

Locus	n-2.0	n-0.x	n-1.0	n+0.x	n+1.0	n+2.0
DYS576	0.0590		0.1515		0.0338	
DYS389I			0.0916		0.0345	
DYS635			0.1338		0.0331	
DYS389II	0.0161		0.1879		0.0373	
DYS627		0.030 (-0.2)	0.1518		0.0262	
DYS460	0.0068		0.1165		0.0427	
DYS458	0.0178		0.1531		0.0252	
DYS19		0.111 (-0.2)	0.1268	0.034 (+0.2)	0.0372	
YGATAH4			0.1153		0.0227	
DYS448			0.0468		0.0229	
DYS391			0.1000		0.0341	
DYS456	0.0144	0.010 (-0.1)	0.1536		0.0374	
DYS390		0.022 (-0.1)	0.1358		0.0351	
DYS438			0.0586		0.0276	
DYS392	0.0168		0.1694		0.1101	
DYS518	0.0384		0.2550		0.0485	
DYS570	0.0218		0.1565		0.0289	
DYS437			0.0813		0.0165	
DYS385			0.1832		0.037	
DYS449	0.0322		0.2324		0.042	
DYS393	0.0255		0.1407		0.0495	0.0364
DYS439	0.0164	0.023 (-0.1)	0.0989		0.0339	
DYS481	0.0579	0.096 (-0.2)	0.2855		0.0559	
DYF387S1			0.1571		0.1555	
DYS533	0.0188		0.1200		0.046	
note: DYS481 also demonstrated an n-1.3 stutter peak at a ratio of 0.0177						