The Arkansas State Crime Laboratory

Forensic DNA Section

Quality Assurance Manual

Table of Contents

DNA

Forensic DNA Section

QUALITY ASSURANCE MANUAL

SECTION 1: INTRODUCTION

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

A. Provide the users of laboratory services access to forensic analysis including biological fluid identification and DNA typing of selected biological materials associated with official investigations.

B. Ensure the quality, integrity and accuracy of the DNA typing data and its presentation through the implementation of a detailed Quality Assurance/Quality Control program.

C. Provide the criminal justice system with a functional DNA database (CODIS) to help law enforcement agencies solve criminal cases.

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

A. Monitor on a routine basis the analytical testing procedure for DNA typing by means of Quality Control (QC) standards, proficiency test and audits.

B. Verify that the entire DNA typing procedure is operating within the established performance criteria, as stated in the Analytical section of the Quality Manual and that the quality and validity of the analytical data are maintained.

C. Ensure that problems are noted and that corrective action is taken and documented.

D. Ensure the overall quality as outlined in the DNA Advisory Board Guidelines.

1.1: Organization and Management

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

This QA Manual has been approved by the appropriate management authorities and as such forms part of the laboratory Quality Manual 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 through formal chain of command processes, with affected manual pages and files updated. Previous versions of revised documents are maintained in a separate Historical Archive Manual. All DNA Analysts must be notified of the changes and must be given any necessary training.

1.1.2: Relationship of Individuals and Job Responsibilities:
Forensic DNA Section's Organizational Chart is located in DNA-DOC-03.

SECTION 2: PERSONNEL QUALIFICATIONS AND JOB DESCRIPTIONS

The following establishes the job function, responsibility and qualifications for each position. This includes specification and description of lines of responsibility for developing, implementing, recording and updating the QA program.

2.1: Personnel

2.1.1: DNA Technical Leader

2.1.1.1: Responsibility

The technical leader is ultimately responsible for 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 CODIS Section personnel.
g. Review, on an annual basis, the procedures of the laboratory and document such review.

h. Analyzing samples, providing expert testimony, and performing other routine duties of a CODIS Analyst.

i. Review and approve training, quality assurance, and proficiency testing programs in the laboratory.

2.1.1.3: Qualifications

Education

The technical leader shall meet the following qualifications:

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

Training

The technical leader shall have three years of forensic, databasing or human identification DNA laboratory experience obtained at a laboratory where DNA testing was conducted for identification, databasing or forensic purposes. 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 database or 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.

Continuing Education

The technical leader must stay abreast of developments within the field of DNA typing by reading current scientific 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.

Other

Must additionally meet the requirements specified for a DNA Analyst.

2.1.2: Casework Supervisor

2.1.2.1: Responsibility

The casework supervisor is responsible for the daily operation, including the supervision and management of personnel and casework flow through the Forensic DNA Section.

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.

2.1.2.3: Qualifications

a. Education

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

b. Training

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

c. Experience

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

d. Continuing Education

The Casework Supervisor must stay abreast of developments within the field of DNA typing by reading current scientific 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. 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.

2.1.3.3: Qualifications

a. Education

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

b. Training

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

c. Experience

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

d. Continuing Education

The CODIS administrator must stay abreast of developments within the field of DNA typing by reading current scientific 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, chemistry, statistics, biochemistry, and molecular biology (molecular genetics or recombinant DNA technology). With a minimum of nine (9) cumulative semester hours or equivalent that cover the required subject areas.

b. Training

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

c. Experience

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

d. Continuing Education

The DNA analyst must stay abreast of developments within the field of DNA typing by reading current scientific 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: DNA Quality Manager

2.1.5.1: Responsibility

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

2.1.5.2: 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.

2.1.6: DNA Safety Officer

2.1.6.1: Responsibility

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

2.1.6.2: Job Function

a. Test safety equipment and complete required documentation.

b. Maintain chemical inventory within the section as well as maintain MSDS binder.

c. Responsible for the disposal of any chemical/biological waste.

d. Complete safety survey on a semi-annual basis.

e. Insures incident reports are completed and returned when an accident occurs.

f. Maintain first aid kit.

g. Provide safety orientation for new employees and manage the overall safety of the section.

2.2: Training

Training will be guided by the DNA Training Manual.

The required six-month training program for forensic laboratory personnel will depend upon previous training and experience. The training period may consist of continuous training or it may consist of a period of training plus time spent in supervised casework. The DNA technical leader will assess and document any adjustments to the established training program.

2.3: Actions and Approval

2.3.1: DNA Technical Leader

a. Can initiate, suspend, and resume DNA analytical operations for the laboratory or an individual.

b. Must approve DNA quality manager's action.

2.3.2: DNA Quality Manager

a. Can reject any chemical, reagent, supply or material which fails to meet the specifications set forth in the Quality Manual. The rejection of any such item must be documented in the Reagent Preparation Manual.

b. Can terminate DNA testing if a technical problem is identified and is not resolved by the Technical Leader. The CODIS Administrator and the rest of the DNA Section must be notified and the specific problem(s) must be documented in the QA manual where the Casework Supervisor, CODIS Administrator and/or Technical Leader will initial to signify approval.

2.3.3: Casework Supervisor

a. Can reject materials or suspend testing in the same manner as the DNA Quality Manager, following the same unit notification and problem documentation specifications.

b. Must approve the DNA Quality Manager's actions.

2.3.4: DNA Analysts (Other than DNA Quality Manager)

a. May recommend rejection of chemicals, reagents, supplies or materials that are found to be inadequate.

b. May recommend termination of DNA testing if a technical problem is found.

SECTION 3: FACILITIES

3.1: Overall Laboratory Security

The Arkansas State Crime Laboratory building has security monitors that cover the external perimeter of the building and parking lots. Security cameras are also located on the first floor of the Crime Laboratory. Only authorized personnel are allowed access to the 2nd and 3rd floor unless accompanied by authorized personnel. All access to areas is established through a key-card electronic tracking system to ensure security and limited access.

Refer to the Arkansas State Crime Laboratory Quality Manual for comprehensive details regarding laboratory wide security.

3.2: Forensic Biology Laboratory Security

(Physical Evidence, CODIS and Casework DNA analysis areas)

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

3.3: Forensic DNA Casework Laboratory Set-up

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

3.3.1: DNA Pre-PCR Laboratory

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

Special Precautions (DNA)

1. Use disposable gloves at all times.
2. Sterilize the beach top before and after you use it with diluted bleach solution.
3. Sterilize those solutions which 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

6. Centrifuge all 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 which may contaminate the sample.
9. Use disposable bench paper to prevent the accumulation of human DNA on permanent work surfaces. Bleach should be used to decontaminate exposed work surfaces after each use.
10. Wear a dedicated lab coat for pre-amplification sample handling when working in the pre-PCR DNA extraction work area.
11. Face masks and/or face shields must be worn when working with evidence and setting up amplifications.
12. Lab coats should be washed on a monthly basis.

3.3.2: DNA Casework Post-PCR Laboratory

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

Special Precautions

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

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

SECTION 4: EVIDENCE CONTROL

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

4.1: Evidence Handling Procedures

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

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

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

4.2: Chain of Custody

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

4.3: Prioritizing

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

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

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

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

4.4: Packaging

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

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

4.5: Seals

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

4.6: Release of Evidence

No evidence will be released from the laboratory, unless to the submitting agency, a police property custodian, or to a person with a court order or search warrant. Statute 12-12-312 Records confidential and privilege.

4.7: Release of Information

See the Arkansas State Crime Laboratory Quality Manual for the policy on the release of information.

4.8: Disposition

All evidence remaining after analysis will be retained in the Forensic DNA Section.

* *note** The Forensic DNA Section will not store amplified DNA products after the case has been administratively reviewed.

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.
Starting in 2008 the case file is stored electronically within Justice Trax, our case file management system. Any case submitted prior to 2008 the paper case files can be stored in a secure location.

4.10: Destruction of Evidence

The Forensic DNA Section destroys no evidence.

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.

**See NRC 1996 recommendations*

4.11.1: Acceptance for DNA

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

4.11.2: Identification of Evidence and Work Product

DNA extracts excluding known samples are considered as evidence and will be dried, sealed, and stored in DNA storage after the completion of the case. Work products are all other products 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, any requests for additional testing must be approved by the Casework Supervisor.

4.11.4: Evidence Marking

The Evidence Receiving Section of the 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: 2007- 01234). 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: Sample Type Identification (Pre-PCR)

Colored micro-centrifuge tubes should be used during chelex and organic extraction procedures in the following manner to ensure sample integrity:

1. Green tubes = Female fraction (used for differential extraction procedures)
2. Blue tubes = Suspect (known reference sample)
3. Yellow tubes = Victim (known reference sample)
4. Clear tubes = Items of evidence or misc. samples
5. Pink tubes = Used for paternity (child) samples
6. Amber tubes = Used for master mix components

4.11.6: Evidence Collection and Examination

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

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

Preparation for the examination:

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

2. Items which are submitted and are transferred directly for DNA testing, should be assigned the same identifier in which it was submitted under , so not to assign redundant item numbers.

3. Prepare the work area. The bench space must be clean and free of clutter. The work area should be covered with white paper to prevent loss of small evidence and to prevent the cross transfer of trace evidence from one item to another. The necessary tools and reagents for examination should be conveniently placed. Adequate lighting should be provided to allow close visual inspection of evidence. Lab work sheets should be at hand to note observations.

4. A lab coat must be worn to protect ones clothing from contamination. Gloves must be worn to protect one from infectious diseases that could be present in biological material or for protection from toxic chemicals. Mask must be worn over nose and mouth to prevent contamination of evidence.

4.11.7: Examination

1. Examine one item at a time, making sure the work area and tools are cleaned between examinations.

2. Mark evidence for future identification with your initials. The package should be checked to ensure that the lab case number and item number is present.

3. If items are known to be disease contaminated, handle cautiously yet expeditiously. It is always good laboratory practice to handle all evidence with universal precautions as though the evidence was disease contaminated. Clean hood or work area and instruments with bleach solution.

4.11.8: Long Term Storage

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

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

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 swabs. Unless the entire reference sample swab was consumed during extraction. In that case 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 collected by the Medical Examiner's Office, spotted on FTA paper, can be stored at room temperature in file cabinets dedicated for long term evidence storage.

SECTION 5: VALIDATION

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

5.1: Developmental Validation

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

1. Characterization of genetic markers.
2. Species specificity.
3. Sensitivity.
4. Stability.
5. Reproducibility.

6. Case-type samples.
7. Population.
8. Mixture.
9. Precision.
10. Accuracy.
11. PCR-based studies.
    a. Reaction conditions.
    b. Assessment of differential amplification.
    c. Assessment of preferential amplification.
    d. Effects of multiplexing.
    e. Assessment of appropriate controls.
    f. Product detection.

5.2: Internal Validation

Internal validation is required on any methodologies that are utilized for forensic DNA analysis in the laboratory. A developmentally validated methodology can not 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 and contain the following studies where applicable:

1. Accuracy.
2. Precision.
3. Reproducibility.
4. Sensitivity.
5. Mixture.

Before an analyst can begin using an internally validated procedure for DNA casework, the analyst must successfully complete a qualifying test.

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

SECTION 6: ANALYTICAL PROCEDURES

6.1: Generic Guidelines

6.1.1: Reagents

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

Commercial Kits:

DNA Investigator Kits	Qiagen
Quantifiler-Duo Kits	Applied Biosystems
Identifiler DNA Kits	Applied Biosystems
Minifiler DNA Kits	Applied Biosystems
Y-filer DNA Kits	Applied Biosystems

Miscellaneous Items:

Taq Gold Polymerase	Applied Biosystems

6.1.1.1: Sources of Materials, Reagents, Chemicals and Supplies

A listing of commercial sources for all materials, reagents, chemicals, and supplies will be maintained in the Reagent Preparation Manuals.

6.1.1.2: Supply and Materials Inventory

Reagents and supplies, which have passed their expiration date, will not be used in casework. However, they may be designated for use on non-critical samples, i.e., training samples, test gels, etc. The Purchasing Agent will keep all receipts.

6.1.1.3: Material Safety Data Sheets (MSDS)

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

6.1.1.4: Laboratory Prepared Reagents and Solutions

All laboratory prepared reagents and solutions will be made with great care and using good laboratory practices.

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 log book:

- Identity
- Date of preparation
- Date of expiration
- Instructions on preparation of reagent
- Lot numbers of solvents and/or chemicals used in preparation of reagent

- A method to verify the reagent's reliability (if applicable)
- Initials of the person preparing reagent
- Initials of the person verifying reagent (if applicable)

6.1.1.5: Labeling Requirements

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

6.1.1.6: Storage and Disposal

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

6.1.1.7: Critical reagents and supplies

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 Binder.

6.1.1.7.1: DNA Investigator Kits

DNA investigator kits will be marked with the receive date and initials of the individual who receives the kit. A known blood sample will be processed through the extraction kit to check the quality of the reagents. The DNA extract will be amplified with a QCed Identifiler 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 will be informed. The Technical Leader will examine the problem and contact the manufacturer if necessary.

6.1.1.7.2: Quantifiler-Duo Kits

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

6.1.1.7.3: Identifiler, Minifiler, and Y-filer DNA Kits

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

6.1.1.7.4: Taq Gold Polymerase

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

6.1.2: Controls and Standards

It is essential that proper control samples are included when samples are extracted, amplified and typed. The typing results obtained from these controls are important for the interpretation of the profiles obtained.

6.1.2.1: Reagent Blank (RB)

The reagent blank tests 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 100 RFUs 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 should be considered inconclusive for analysis and re-extracted. If the DNA sample has been consumed and re-extraction is not possible, then the DNA technical leader, Casework Supervisor and Laboratory Director will be consulted to analyze the samples and reagent blank. If after analysis the source of the contaminating DNA does not

appear to be in the samples, then the contamination will be noted in the report. If the extraneous DNA is present in both the reagent blank and associated sample, then the sample will be reported as inconclusive.

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6.1.2.1: 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. The positive control provided with each amplification kit serves as the appropriate positive control. 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.

6.1.2.2: Negative Control (AMP-)

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

The negative control tests for contamination of samples during the setup of the amplification reactions. If the negative control exhibits unexplainable peaks above 100 RFUs 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.

6.1.2.3: Quantification Standards

Quantification standard consists of 8 dilutions made from the standard stock provided with each quantification kit.
The quantification standard is used to create a standard curve to allow for the quantification of the samples amplified concurrently. The standard curve must consist of at least 6 of the 8 dilutions.

6.1.2.4: Internal Size Marker and Allelic Ladder

Internal size marker is added to each sample and ladder prior to electrophoresis. The internal size marker allows the genetic analysis software to determine the size (in basepairs) 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 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; 2391b for autosomal STRs and 2395 for Y-STRs) annually or whenever substantial changes are made to the procedures. The standard will be carefully handled to prevent contamination and deterioration. The standard will be discarded at the end of the certification period.

6.1.3: Detection and Control of Contamination

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

If contamination has been discovered, the laboratory will try to discover the source of the contamination. The incident will be documented in the Contamination Log Form. If a DNA analyst is found to be the source of the contamination, the Casework Supervisor will be notified and take the necessary corrective actions. If the contamination is from outside the DNA section, the appropriate supervisor will be notified to address the contamination source.

6.2: Standard Operating Procedures

6.2.1: Extraction Protocols

Note:All extracted sample (non-reference) must be concentrated (microcon) prior to any quantitation.

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

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

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

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

6.2.1.1: Chelex

6.2.1.1.1: Reagents

6.2.1.1.1.1: Phosphate Buffered Saline (PBS)

Dissolve 1 g potassium chloride ACS reagent, 40 g sodium chloride, 1 g potassium phosphate monobasic, and 5.5 g anhydrous disodium phosphate (sodium phosphate dibasic) in 4000 mL DI water. Check pH, adjust to 7.4 if necessary with concentrated HCL.
Adjust final volume to 5 liters. Autoclave. Store at room temperature. Aliquot amount needed for appropriate number of extractions into sterile, disposable plastic tube and discard unused portion of aliquot.
Expiration Date: 1 year

6.2.1.1.1.2: Tris EDTA Buffer (TE)

Add 10 mL of 1 M Tris-HCL, pH 8.0 and 0.2 mL of 0.5 M EDTA to 990 mL of DI water and mix. Dispense 100 mL aliquots and sterilize by autoclaving.
Expiration Date: 1 year

6.2.1.1.1.3: 1 M Tris - HCL, pH 8.0

WARNING: Hydrochloric acid (HCL) causes severe burns and is irritating to the eyes. When preparing this reagent, use a fume hood and avoid inhalation and contact with the skin. Wear lab coat, gloves, and protective eyewear when handling.

Dissolve 121.1 g of Tris base in 800 ml of DI water. Adjust to pH 8.0 (+/- 0.2) at room temperature by adding approximately 45 ml of concentrated HCL. Adjust the final volume to 1 liter with DI water and mix thoroughly. The solution should be autoclaved or filtered through a 0.2 um Nalgene filter.
Expiration Date: 1 year

6.2.1.1.1.4: Chelex Solution

5% - Weigh out 5 g. Chelex 100 Resins (100-200 mesh, sodium form, BioRad 143-2832) into a bottle which has been autoclaved with a stir bar in

it. Add 100 mL of sterile DI water. Adjust pH to 10 with 5 N NaOH. Store at room temperature.

10% - Weigh out 20 g. Chelex 100 Resin into a bottle which has been autoclaved with a stir bar in it. Add 100 mL of sterile DI water. Adjust pH to 10 with 5 N NaOH. Store at room temperature.

Agitate Chelex solutions with stir bar while aliquoting.
Expiration Date: 1 year

6.2.1.1.1.5: DTT (1 M Dithiotheritol, 10 mL)

Dissolve 1.54 g Dithiothreitol in 10 mL of sterile DI water. Use a 15 mL disposable tube. Do not autoclave. Store 100 ul aliquots in sterile 0.5 ml microfuge tubes at -20 deg.C. Discard any unused portion of thawed tube.
Expiration Date: N/A

6.2.1.1.1.6: Proteinase K 10mg/mL, 10 mL

Dissolve 100 mg Proteinase in 10 mL sterile DI water, in a sterile disposable plastic 15 mL tube. Store 100 ul aliquots in sterile 0.5 mL microcentrifuge tubes at -20 deg. C. Thaw tubes as needed for appropriate number of extractions.
Caution: powdered Proteinase K and solutions of Proteinase K can be irritating to mucous membranes. Wear safety glasses and gloves when handling.
Expiration Date: N/A

6.2.1.1.1.7: Sperm Wash Buffer pH 7.5, 500 mL

Add 5 mL 1M Tris-HCl, pH 7.5, 10 mL 0.5 M EDTA, 5 mL 5 M NaCl (or 5N NaCl), and 50 mL 20% SDS to 430 mL DI water. Check pH . Autoclave. Store at room temperature.
Expiration Date: 1 year

6.2.1.1.1.8: Stain Extraction Buffer

Dissolve 5.84 g NaCl in 500 DI water with stirring. To this solution add 10 mL of 1 M Tris, 20 mL of 0.5 M EDTA, and 100 mL 20% SDS. Titrate to pH 8.0 with HCl. Bring to a final volume of 1 L with DI water. Store at room temperature.

Supplement with DTT before use. To 100 mL of the above solution, add 601.4 mg DTT and stir until disolved. Store at room temperature. The complete solution is good for no more than two weeks.
Expiration Date: 1 year (stain extraction buffer only-no DTT added)

6.2.1.1.2: Whole Blood / Bloodstains

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

1. Add 10 ul whole blood or a bloodstain approximately 3mm x 3mm in size to a sterile 1.5 ml microcentrifuge tube. Pipet 1 ml sterile PBS into the tube. Vortex approximately 2 seconds.

2. Incubate at room temperature approximately 30 min. Vortex 5 sec.

3. Spin in a microcentrifuge for 2 min. at approximately 15,000 x g.

4. Without disturbing the pellet, carefully remove and discard the supernatant, leaving all but 20 to 30 ul behind being careful to cover the pellet. If the sample is a bloodstain, leave the fabric substrate in the tube with the pellet.

5. Add 200 ul 5% Chelex. Be sure to mix chelex before withdrawing the 200ul.

6. Vortex approximately 10 seconds.

7. Incubate at 56 degrees C. for 30 minutes.

8. Vortex for approximately 10 seconds.

9. Incubate in a boiling water bath using beaker buddy for 8 minutes.

10. Vortex tube for approximately 10 seconds.

11. Spin in a microcentrifuge for 3 minutes, at approximately 15,000 x g.

12. Estimate the amount of DNA in the sample. (See Quantifiler Kits SOP)

13. The sample is now ready for amplification.

6.2.1.1.3: Dried Blood Spotted on FTA

**The following procedure is utilized with known samples submitted from the Medical Examiner's Office or any blood dried on FTA paper is submitted.

1. Label appropriate 1.5ml extraction tube.

2. Cut the stain, approximately 3mm x 3mm in size and place into a sterile 1.5 ml microcentrifuge tube.

3. Wash the sample three (3) times with FTA Purification Reagent (approximately 150 ul). Discard reagent after each wash.

4. Rinse the sample two (2) times with TE^{-1} (approximately 150 ul) discarding the TE after each rinse.

5. Pipet 1 ml sterile PBS into the tube. Vortex approximately 2 seconds.

6. Incubate at room temperature approximately 30 min.

7. Vortex 5 sec. Then spin in a microcentrifuge for 2 min. at approximately 15,000 x g.

8. Without disturbing the pellet, carefully remove and discard the supernatant, leaving all but 20 to 30 ul behind to cover the pellet . If the sample is a bloodstain, leave the fabric substrate in the tube with the pellet.

9. Add 200 ul 5% Chelex. Be sure to mix chelex before withdrawing the 200ul.

10. Vortex approximately 10 seconds.

11. Incubate at 56 degrees C. for 30 minutes.

12. Vortex for approximately 10 seconds.

13. Incubate in a boiling water bath using beaker buddy for 8 minutes.

14. Vortex tube for approximately 10 seconds.

15. Spin in a microcentrifuge for 3 minutes. at approximately 15,000 x g.

6.2.1.1.4: Semen-containing Stains

1. Using a clean cutting surface for each different sample, dissect swab or fabric into the appropriate sample size.

2. Add swab or fabric cutting to a sterile 1.5 ml microcentrifuge tube. Pipet 700 ul PBS into tube and vortex for 2 seconds.

3. Incubate at room temperature for approximately 30 min.

4. Twirl the swab or fabric with a sterile toothpick for at least 2 min. to agitate the cells off of the substrate.

5. Remove the swab or fabric using a sterile toothpick or Pipet tip, and place into a spin-x basket.

6. Centrifuge the sample in a microcentrifuge for 1 min. at approximately 15,000 x g and discard the swab cutting or fabric.

7. Without disturbing the pellet, carefully remove and discard all but approximately 50 ul of the supernatant (or leave behind twice the volume of the pellet, if this would amount to more than 50 ul). *Note: this pellet is called the cell pellet.*

8. Add 150 ul sterile distilled, deionized water to the cell pellet. Add 2 ul of proteinase K (10 mg/ml) and vortex for 1 second.

9. Incubate at 56 deg.C for 1 hr. (No more than 2 hrs.) to lyse non-sperm cells.

10. Spin in a microcentrifuge for 5 minutes at approximately 15,000 x g. The resultant pellet is called the sperm pellet.

11. Add the supernatant to 50 ul of 20% Chelex in a fresh, sterile 1.5 ml microcentrifuge tube. This sample is called the "female fraction" and contains non-sperm DNA. Save the female fraction in a green 1.5 ml tube for later DNA analysis, which begins at step 16.

12. Wash the sperm pellet as follows: Resuspend the pellet in 0.5 ml sperm wash buffer (10mM Tris-HCl, 10mM EDTA, 50 mM NaCl, 2% SDS, pH 7.5). Vortex briefly. Spin in a microcentrifuge for 5 minutes at approximately 15,000 x g. Remove approximately 450 ul of the supernatant and discard. This should leave about 50 ml remaining.

13. Repeat wash steps 12 an additional 4 times, for a total of 5 washes.

14. Wash sperm pellet once with sterile distilled, deionized water as follows: Resuspend the pellet in 1 ml water. Vortex briefly. Spin in a microcentrifuge for 5 minutes at approximately 15,000 x g. Remove and discard approximately 950 ul of the supernatant. This should leave about 50 ul remaining.

15. Add 150 ul of 5%Chelex to the approximately 50 ul sperm cell pellet. Add 2ul of Proteinase K (10 mg/ml) and 7 ul 1M DTT.

16. Vortex female fraction and sperm samples for approximately 10 seconds. Pulse spin in microcentrifuge at approximately 15,000 x g.

17. Incubate at 56 deg. C. For 1 hr.

18. Vortex tubes for approximately 10 seconds.

19. Incubate the samples in a boiling water bath (beaker buddy) for 8 minutes.

20. Vortex tubes for approximately 10 seconds.

21. Spin in a microcentrifuge for 3 minutes at approximately 15,000 x g.

22. To the top of the concentrator, add all of the extract, being careful not to transfer any Chelex beads. Close the cap (using scotch tape might be necessary to hold the cap in place), and spin in a microcentrifuge for 15 to 25 minutes at 500 x g. Remove the concentrator from the microcentrifuge and discard the bottom microtube. Invert the concentrator basket on the appropriately labeled clean microcentrifuge tube. Add approximately 20 to 100 ul of TE buffer to the top of the concentrator and place into a microcentrifuge for approximately 15 to 25 minutes at 500 x g.

23. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kits SOP)

24. The samples are now ready for PCR amplification.

6.2.1.1.5: Modified Extraction of Semen-Containing Stains

1. Using a clean cutting surface for each different sample, dissect swab or fabric into thirds or is sample permits, utilize one entire swab in analysis.

2. Add swab or fabric cutting to a sterile 1.5ml microcentrifuge tube. Pipette 700µL PBS into tube and vortex for 2 seconds.

3. Incubate at room temperature for 30 minutes.

4. Twirl the swab or fabric with a sterile toothpick for at least 2 minutes to agitate the cells off of the substrate.

5. Remove the swab or fabric with a sterile toothpick or Pipette tip, and place into a spin-x basket.

6. Centrifuge the sample in a microcentrifuge for 1 minute at approximately 15,000 x g and discard the swab cutting of fabric.

7. Without disturbing the pellet, carefully remove and discard all but approximately 50µL of the supernatant (or leave behind twice the volume of the pellet, it this would amount to more than 50µL). Note: this pellet is called the cell pellet.

8. Add 150µL of 5% Chelex to the approximate 50µL sperm cell pellet. Add 2µL of Proteinase K (10mg/mL) and 7µL 1M DTT.

9. Vortex the cell pellet for 10 seconds. Spin in the microcentrifuge for 3 seconds.

10. Incubate the cell pellet at 56 ºC. For approximately 1 hour.

11. Vortex tubes for approximately 10 seconds.

12. Incubate the samples in a boiling water bath (beaker buddy) for 8 minutes.

13. Vortex tubes for approximately 10 seconds.

14. Spin in a microcentrifuge for 3 minutes at approximately 15,000 x g.

15. Place the sample extract into a Microcon 100 unit. To the top of the concentrator, add all of the extract, being careful not to transfer any Chlelex beads. Close the cap and spin in a microcentrifuge for 15 to 25 minutes at approximately 500 x g. Remove the concentrator from the microcentrifuge and discard the bottom microtube. Invert the concentrator basket on the appropriately labeled clean microcentrifuge tube. Add approximately 20 to 100 µL of TE buffer to the top of the concentrator and place into a microcentrifuge for approximately 15 to 25 minutes at approximately 500 x g.

16. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kits SOP)

17. The samples are now ready for PCR amplification

6.2.1.1.6: Epithelial Cell Extractions (Including oral swabs, fingernails, etc.)

*note- Oral Swabs do not require concentration

*This procedure may also be used for samples that may contain limited amounts of DNA (shirt collars, dilute blood stains, touch evidence etc...)

1. Using a clean cutting surface for each different sample, cut a 3 mm x 3mm portion of the gauze or filter paper, or an appropriate portion of a swab. Place in a sterile 1.5 ml microcentrifuge tube.

***If larger tube is needed due to material, a 10 ml conical tube may be used and appropriate concentrations of solution (chelex, etc...) must be used.*

2. Add approximately 400 ul 5% Chelex to sample. Volume of Chelex may be increased or decreased due to differences in samples size.

3. Vortex tubes for approximately 10 seconds.

4. Incubate at 56 deg.C for 30 min.

5. Vortex tubes for 10 sec.

6. Incubate in a boiling water bath for 8 minutes.

7. Vortex tubes for approximately10 seconds.

8. Spin in a microfuge at approximately 15,000 x g for 3 minutes.

9. To the top of the concentrator, Microcon 100 (filter concentrator - amicon # 42413) unit, add all of the extract, being careful not to transfer any Chelex beads. Close the cap (using scotch tape might be necessary to hold the cap in place), and spin in a microcentrifuge for 15 to 25 minutes at approximately 500 x g. Remove the concentrator from the microcentrifuge and discard the bottom microtube. Invert the concentrator basket on the appropriately labeled clean microcentrifuge tube. Add approximately 20 to 100 ul of TE buffer to the top of the concentrator and place into a microcentrifuge for approximately 15 to 25 minutes at approximately 500 x g.

10. Estimate the amount of DNA in the samples.

11. The sample is now ready for PCR amplification.

6.2.1.1.7: Envelope Flaps / Stamps

1. Carefully open envelope flap or remove stamp using steam and clean tweezers.

2. Using a sterile cotton swab moistened in sterile, distilled, deionized water, swab gummed envelope flap or stamp. Cuttings may also be used for this extraction. Cut cotton swab from stick and transfer the cotton to a sterile 1.5 ml microcentrifuge tube. ***If larger tube is needed due to material, a 10 ml conical tube may be used and appropriate concentrations of solution (chelex, etc...) must be used.*

3. Add 450 ul 5% Chelex to the swab. Volume of Chelex may be increased due to differences in samples size.

4. Add 30 ul Proteinase K (10 mg/ml) to each tube; vortex 2 seconds to mix.

5. Incubate at 56 deg.C for 90 minutes.

6. Vortex tubes for approximately 10 seconds.

7. Incubate in a boiling water bath for 8 minutes.

8. Vortex at high speed for approximately 10 sec.

9. Spin in a microcentrifuge at approximately 15,000 x g for 3 minutes.

10. To the top of the concentrator, Microcon 100 (filter concentrator - amicon # 42413) unit, add all of the extract, being careful not to transfer any Chelex beads. Close the cap (using scotch tape might be necessary to hold the cap in place), and spin in a microcentrifuge for 15 to 25 minutes at approximately 500 x g. Remove the concentrator from the microcentrifuge and discard the bottom microtube. Invert the concentrator basket on the appropriately labeled clean microcentrifuge tube. Add approximately 20 to 100 ul of TE buffer to the top of the concentrator and place into a microcentrifuge for approximately 15 to 25 minutes at approximately 500 x g.

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

12. The sample is now ready for amplification.

6.2.1.1.8: Tape Lifts

1. Using a sterile, moistened cotton swab, swab the entire tape lift to remove any epithelial cells which may be present.

2. Cut cotton swab from stick and transfer the cotton to a sterile 1.5 ml microcentrifuge tube. ***If larger tube is needed due to material, a*

10 ml conical tube may be used and appropriate concentrations of solution (chelex, etc...) must be used.

3. Add 450 ul 5% Chelex to the swab. Volume of Chelex may be increased or decreased due to differences in samples size.
4. Add 30 ul Proteinase K (10 mg/ml) to each tube; vortex 2 seconds to mix.

5. Incubate at 56 deg.C for 90 minutes.

6. Vortex tubes for approximately 10 seconds.

7. Incubate in a boiling water bath for 8 minutes.

8. Vortex at high speed for approximately 10 sec.

9. Spin in a microcentrifuge at approximately 15,000 x g for 3 minutes.

10. To the top of the concentrator, Microcon 100 (filter concentrator - amicon # 42413) unit, add all of the extract, being careful not to transfer any Chelex beads. Close the cap (using scotch tape might be necessary to hold the cap in place), and spin in a microcentrifuge for 15 to 25 minutes at approximately 500 x g. Remove the concentrator from the microcentrifuge and discard the bottom microtube. Invert the concentrator basket on the appropriately labeled clean microcentrifuge tube. Add approximately 20 to 100 ul of TE buffer to the top of the concentrator and place into a microcentrifuge for approximately 15 to 25 minutes at approximately 500 x g.

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

12. The sample is now ready for amplification.

6.2.1.1.9: Cigarette Butts

1. Using a new, sterile, scalpel blade, slice and approximately 5 mm wide strip from the cigarette butt (including filter) in the area which would have been in contact with the mouth.

2. Cut into smaller pieces, and put pieces into a sterile, 1.5 ml microcentrifuge tube.

3. Add 1 ml 5% Chelex to tube. Volume of Chelex may be increased or decreased due to differences in samples size.

4. Vortex tubes for approximately 30 sec.

5. Incubate at 56 deg.C for 30 minutes.

6. Vortex tubes for approximately 30 sec.

7. Incubate in a boiling water bath for 8 min.

8. Vortex tubes for approximately 30 sec.

9. Spin tube in a microcentrifuge at approximately 15,000 x g for 3 min.

10. To the top of the concentrator, Microcon 100 (filter concentrator - amicon # 42413) unit, add all of the extract, being careful not to transfer any Chelex beads. Close the cap (using scotch tape might be necessary to hold the cap in place), and spin in a microcentrifuge for 15 to 25 minutes at approximately 500 x g. Remove the concentrator from the microcentrifuge and discard the bottom microtube. Invert the concentrator basket on the appropriately labeled clean microcentrifuge tube. Add approximately 20 to 100 ul of TE buffer to the top of the concentrator and place into a microcentrifuge for approximately 15 to 25 minutes at approximately 500 x g.

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

12. The sample is now ready for amplification.

6.2.1.1.10: Hair Extractions

1. Label two 1.5 ml microcentrifuge tubes. One tube is for the hair root and the other is for the hair shaft.

2. Pipet 200 ul of 5% Chelex into each tube. Add 2 ul Proteinase K (10mg/ml).

3. Wash the hair containing the sheath material to reduce surface dirt and contaminants by immersing the hair in 0.2 ml digest buffer (sperm wash buffer)

4. Add the root portion of the hair to one of the 1.5 ml tubes containing Chelex.

5. Add the shaft portion of the hair to the other 1.5 ml tube. This will act as a control.

6. Incubate at 56 deg.C at least 6 to 8 hours or overnight.

7. Vortex at high speed for approximately 5 to 10 seconds.

8. Pulse spin samples in microcentrifuge.

9. Incubate in boiling water bath of 8 minutes.

10. Vortex at high speed for approximately 5 to 10 seconds.

11. Spin in a microcentrifuge for 2 to 3 minutes at approximately 15,000 x g.

12. To the top of the concentrator, Microcon 100 (filter concentrator - amicon # 42413) unit, add all of the extract, being careful not to transfer any Chelex beads. Close the cap (using scotch tape might be necessary to hold the cap in place), and spin in a microcentrifuge for 15 to 25 minutes at approximately 500 x g. Remove the concentrator from the microcentrifuge and discard the bottom microtube. Invert the concentrator basket on the appropriately labeled clean microcentrifuge tube. Add approximately 20 to 100 ul of TE buffer to the top of the concentrator and place into a microcentrifuge for approximately 15 to 25 minutes at approximately 500 x g.

13. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kits SOP)

14. The sample in now ready for PCR amplification.

6.2.1.1.11: Organic and Tissue Extraction

1. Activate Stain Extraction Buffer: To 100 mL of stain extraction buffer, add 601.4mg DTT. Stir until dissolved. Store refrigerated for up to 2 weeks.

2. Using a new, sterile scalpel blade, cut a piece of tissue approximately 3 mm squared.

3. With a scalpel blade, mince tissue into small pieces and transfer the minced tissue to a 1.5ml microcentrifuge tube.

4. To the sample add 300 ul of activated stain extraction buffer and 7.5 ul proteinase K solution. Vortex on low speed (be alert for fluid leakage from the tube cap) for 1 second and spin in a microcentrifuge for 2 seconds to force the cutting into the extraction fluid.

5. Incubate the tube at 56 deg.C for a minimum of overnight.

6. Spin in a microcentrifuge for 2 seconds to force condensate into the bottom of the tube.

7. In a fume hood, add 300 ul phenol/chloroform/isoamyl alcohol to the stain extract. Vortex (low speed) the mixture briefly to attain a milky emulsion. Spin the tube in a microcentrifuge for 3 minutes.

8. To a Microcon 100 concentrator add 100 ul TE. Transfer the aqueous phase from the tube in step 7 to the concentrator. Avoid pipetting solvent (bottom layer) from the tube into the concentrator.

9. Place a spin cap on the concentrator and spin in a microcentrifuge at approximately 500 x g for 10 minutes.

10. Carefully remove the concentrator unit from the assembly and discard the fluid from the filtrate cup. Return the concentrator to the top of the filtrate cup.

11. Remove the spin cap and add 200 ul TE to the concentrator. Replace the spin cap and spin the assembly in a microcentrifuge at approximately 500 x g for 10 minutes.

12. Remove the concentrator from the microcentrifuge and discard the bottom microtube. Invert the concentrator basket on the appropriately labeled clean microcentrifuge tube. Add approximately 20 to 100 ul of TE buffer to the top of the concentrator.

13. Spin the assembly in a microcentrifuge at approximately 500 x g for 5 minutes.

14. Discard the concentrator and cap the tube.

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

16. The sample in now ready for PCR amplification.

6.2.1.2: EZ1 Robot Extractions

6.2.1.2.1: Whole Blood

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

1. Label appropriate 2.0 ml EZ1 sample tube.

2. Add 200 ul 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 diH_2O) 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 you increase the volume in step 3.)

5. Incubate at 56 ºC for 15 minutes.

6. If necessary, centrifuge briefly.

3. Process on EZ1 with Trace protocol.

    a. Ensure EZ1 workstation is on.
    b. Press "Start" to start protocol setup.
    c. Press "Esc" (for no report)
    d. Press "2" (for Trace TD protocol)
    e. Press "2" (for elution in TE buffer)
    f. Choose elution volume, "1" for 50 µl, "2" for 100 µl, "3" for 200 µl. (most applications will be 50 µl, but highly concentrated forensic known samples can be diluted in larger volumes).
    g. Press any key to continue.
    h. Follow steps on screen to setup workstation/
        i. Load cartridges into the rack.
        ii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
        iii. Load tip holders and tips in Row 2 of tip rack.
        iv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
        v. Close workstation door.
        vi. Press "Start" to start protocol.
        vii. Once display show "Protocol finished", remove elution tubes. Discard waste appropriately.

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

5. The samples are now ready for PCR amplification.

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

1. Label appropriate 2.0 ml EZ1 sample tube.

2. ***Blood stains**

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

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

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

   ***Cigarette Butts**

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

   ***Stamps / Envelope Flaps**

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

   ***Tape Lift Swabs**

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

7. Add 190 µl of ***DILUTED*** G2 buffer. (Diluted G2 buffer is a 1:1 dilution with diH_2O) Additional ***DILUTED*** G2 buffer may be added to absorbent samples to ensure ~190 µl of liquid in the tube.

8. Add 10 µl of Proteinase K, mix by vortexing. (***DO NOT*** add additional Proteinase K, even if you increase the volume in step 3.)

9. Incubate at 56 ºC for 15 minutes.

10. If necessary, centrifuge briefly.

11. Process on EZ1 with Trace TD protocol.

    a. Ensure EZ1 workstation is on.
    b. Press "Start" to start protocol setup.
    c. Press "Esc" (for no report)
    d. Press "2" (for Trace TD protocol)
    e. Press "2" (for elution in TE buffer)

f. Choose elution volume, "1" for 50 µl, "2" for 100 µl, "3" for 200 µl. (most applications will be 50 µl, but highly concentrated forensic known samples can be diluted in larger volumes).
g. Press any key to continue.
h. Follow steps on screen to setup workstation/
    i. Load cartridges into the rack.
    ii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
    iii. Load tip holders and tips in Row 2 of tip rack.
    iv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
    v. Close workstation door.
    vi. Press "Start" to start protocol.
    vii. Once display show "Protocol finished", remove elution tubes. Discard waste appropriately.

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

13. The samples are now ready for PCR amplification.

6.2.1.2.3: Semen-containing Stains

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 you increase the volume in step 3.)

5. Incubate at 56 ºC for 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.

a. Ensure EZ1 workstation is on.
b. Press “Start” to start protocol setup.
c. Press “Esc” (for no report)
d. Press “2” (for Trace TD protocol)
e. Press “2” (for elution in TE buffer)
f. Choose elution volume, “1” for 50 µl.
g. Press any key to continue.
h. Follow steps on screen to setup workstation/
    i. Load cartridges into the rack.
    ii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
    iii. Load tip holders and tips in Row 2 of tip rack.
    iv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
    v. Close workstation door.
    vi. Press “Start” to start protocol.
    vii. Once display show “Protocol finished”, remove elution tubes. Discard waste appropriately.
i. Save samples for step

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 three (3) times.

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 1 hour.

16. Centrifuge tube briefly to remove drops from lid.

17. Process Sperm Fraction on EZ1 with Trace protocol.

    a. Ensure EZ1 workstation is on.
    b. Press “Start” to start protocol setup.
    c. Press “Esc” (for no report)
    d. Press “2” (for Trace TD protocol)
    e. Press “2” (for elution in TE buffer)
    f. Choose elution volume, “1” for 50 µl, “2” for 100 µl, “3” for 200 µl. (most applications will be 50 µl, but highly concentrated samples can be diluted in larger volumes).
    g. Press any key to continue.
    h. Follow steps on screen to setup workstation/
        i. Load cartridges into the rack.

ii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
iii. Load tip holders and tips in Row 2 of tip rack.
iv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
v. Close workstation door.
vi. Press "Start" to start protocol.
vii. Once display show "Protocol finished", remove elution tubes. Discard waste appropriately.

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

19. The samples are now ready for PCR amplification.

6.2.1.2.4: Hair Extractions

1. Label appropriate 2.0 ml EZ1 sample tube. One tube is for the hair root and the other is for the hair shaft.

2. Cut an appropriately size sample and place into the labeled sterile 2.0 ml EZ1 sample tube.

3. Add 180 µl G2 buffer to the sample tube.

4. Add 10 µl Proteinase K and 10 µl 1 M DTT.

5. Incubate at 56 ºC for a minimum of 6 hour.

6. Centrifuge tube briefly to remove drops from lid.

7. Process on EZ1 with Trace TD protocol.

   a. Ensure EZ1 workstation is on.
   b. Press "Start" to start protocol setup.
   c. Press "Esc" (for no report)
   d. Press "2" (for Trace TD protocol)
   e. Press "2" (for elution in TE buffer)
   f. Choose elution volume, "1" for 50 µl, "2" for 100 µl, "3" for 200 µl. (most applications will be 50 µl, but highly concentrated forensic known samples can be diluted in larger volumes).
   g. Press any key to continue.
   h. Follow steps on screen to setup workstation/
      i. Load cartridges into the rack.
      ii. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
      iii. Load tip holders and tips in Row 2 of tip rack.
      iv. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
      v. Close workstation door.

vi. Press "Start" to start protocol.
vii. Once display show "Protocol finished", remove elution tubes. Discard waste appropriately.

8. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kits SOP)

9. The sample in now ready PCR amplification.

6.2.1.2.5: Tissue Extraction

1. Label appropriate 2.0 ml EZ1 sample tube.

2. Cut an appropriately size sample and place into the labeled sterile 2.0 ml EZ1 sample tube.

3. Add 190 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.

   a. Ensure EZ1 workstation is on.
   b. Press "Start" to start protocol setup.
   c. Press "Esc" (for no report)
   d. Press "2" (for Trace TD protocol)
   e. Press "2" (for elution in TE buffer)
   f. Choose elution volume, "1" for 50 µl, "2" for 100 µl, "3" for 200 µl. (most applications will be 50 µl, but highly concentrated forensic known samples can be diluted in larger volumes).
   g. Press any key to continue.
   h. Follow steps on screen to setup workstation/
      viii. Load cartridges into the rack.
      ix. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
      x. Load tip holders and tips in Row 2 of tip rack.
      xi. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
      xii. Close workstation door.
      xiii. Press "Start" to start protocol.
      xiv. Once display show "Protocol finished", remove elution tubes. Discard waste appropriately.

8. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kits SOP)

9. The sample in now ready for PCR amplification.

6.2.1.2.6: Nail Clippings and Gum Extraction

1. Label appropriate 2.0 ml EZ1 sample tube.

2. Cut an appropriately size sample and place into the labeled sterile 2.0 ml EZ1 sample tube.

3. Add 190 µl G2 buffer to the sample tube.

4. Add 10 µl Proteinase K.

5. Incubate at 56 ºC for 15 minutes.

6. Centrifuge tube briefly to remove drops from lid.

7. Remove any solid material from tube with tweezers or a toothpick. Try to remove as much liquid from the material as possible.

8. Process on EZ1 with Trace protocol.

    a. Ensure EZ1 workstation is on.
    b. Press "Start" to start protocol setup.
    c. Press "Esc" (for no report)
    d. Press "2" (for Trace TD protocol)
    e. Press "2" (for elution in TE buffer)
    f. Choose elution volume, "1" for 50 µl, "2" for 100 µl, "3" for 200 µl. (most applications will be 50 µl, but highly concentrated forensic known samples can be diluted in larger volumes).
    g. Press any key to continue.
    h. Follow steps on screen to setup workstation/
        xv. Load cartridges into the rack.
        xvi. Load opened 1.5 ml elution tubes in Row 1 of tip rack.
        xvii. Load tip holders and tips in Row 2 of tip rack.
        xviii. Load opened 2.0 ml sample tubes in Row 4 of tip rack.
        xix. Close workstation door.
        xx. Press "Start" to start protocol.
        xxi. Once display show "Protocol finished", remove elution tubes. Discard waste appropriately.

9. Estimate the amount of DNA in the sample. (See Quantifiler-Duo Kits SOP)

10. The sample in now ready for PCR amplification.

6.2.2: Excel Sample Management Form

1. Open form from S:\Identifiler Excel Form\
2. Enable macros
3. Select the type of run.
4. Select location to save and then save the file with the unique identifier (AA_MMDDYYYY)(AA = initials)
5. Enter Analyst's Name, Initials, Last Name, and the Run name in Samples Tab.
6. Enter Case number and Samples Name on the Samples tab.
7. Press the Continue to Quant duo setup button.
8. Enter the Date.
9. Press Print page button and select the printer to print to.
10. Press the Save Txt for 7500 button.
11. Select location to save and then save the file with the run name.
12. Write in Lot Numbers and Standard prep. info on printed sheet.
13. Setup Quant. in clean room
14. Go to Post room, and start the 7500 program, open a new run select DUO as template.
15. Click File Import Sample Setup, select text file you saved on your flash drive.
16. Save the file then Start Quant.
17. While Quant is running, enter lot numbers and standard prep info into Excel file.
18. After Quant is complete Click file, export, results. Then select your flash drive and click save.
19. Go to Form 1a Quantifiler Duo Setup in Excel sheet and click Import Quantifiler Duo Data button.
20. Input your target DNA amount.
21. Select the file you save from the 7500 (It will be a .cvs file) click OK.
22. Now go to Form 1b Total DNA Quantity Tab and examine your data.
23. Adjust any dilutions you would like to make and then Press the Continue to Identifiler STR AMP Page.
24. Type in your Thermocycler used, add your controls to the end of the list. Changing the amount of DNA to the appropriate amount.

25. Press Print Sheets for Amplification button.
26. Select injections needed from list.
27. Select location and then save file as File name to XXXX(Run Name),
28. Take flash drive to 3130xl.
29. On the 3130xl in the Plate manager click the import plate button.
30. Select the txt file that you saved on the flash drive and click either ok or
31. 3130 should say that it imported ok. If gives an error of invalid symbol or something about the name you probably had a space in your sample name on the first page.
32. Start run.
33. Press Print Worksheets to Justice Trax Imaging and Select Justice Trax imaging.
34. Once the 3130xl run is completed copy your files and analyze with GeneMapper ID.
35. Once all files have been analyzed go back to the main GeneMapper windows and change your table settings to the export selection.
36. Next click FILE, Export Combined Table.
37. *MAKE SURE TO SELECT ONE LINE PER SAMPLE* on the right
38. Place the file anywhere you can find it later.
39. Click export combined table.
40. In the Excel file on the Final tab select the samples for each injection time.
41. Click Generate Call Sheets.
42. Press the injection time that needs to have data imported to.
43. Select the file and click OK.

6.2.3: DNA Quantification Protocols

6.2.3.1: Quantifiler Duo Kit

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

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

***note* Thaw the reagents completely before using**

6.2.3.1.1: Preparing the DNA quantification standard

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

6.2.3.1.2: Preparing the Reactions

a. Prepare Quantifiler master mix:
   - # wells (+ 3-5 extra) x 12.5ul PCR Reaction Mix
   - # wells (+ 3-5 extra) x 10.5ul Primer Mix

b. Vortex and dispense 23ul of the master mix into each well a 96-well reaction plate.
c. Add a total of 2ul of sample, standard or control to the appropriate well
d. Seal the reaction plate with the optical adhesive cover
e. Centrifuge the plate at 3000rpm for about 20 seconds

6.2.3.1.3: Running the Reactions

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

h. On **Instrument** tab, press **start** when you are ready to run

6.2.3.1.4: Data Analysis

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

6.2.3.1.5: Results of analysis

a. If samples results are negative, amplify using 10ul of extract.
b. Overblown samples (<10ng/ul) can be diluted appropriately and amplified.

6.2.4: Amplification Protocols

6.2.4.1: AmpFℓSTR® Identifiler ™ PCR Amplification Kit

6.2.4.1.1: Background

The AmpFℓSTR Identifiler ™ PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies 15 tetranucleatide repeat loci and the Amelogenin gender determining marker in a single PCR amplification. All thirteen of the required loci for CODIS are included in this kit for known-offender databasing in the United States (Budowle *et al.,* 1998a). Two additional loci, D2S1338 and D19S433, are included. These loci are consistent with the AmpFℓSTR ™ SGM Plus™ PCR Amplification Kit. The combination of the 15 loci is consistent with several worldwide database recommendations.

Advantages:

The Identifiler kit uses a five-dye fluorescent system for automated DNA fragment analysis. By adding an additional dye, more loci can be multiplexed in a single PCR amplification as compared to the previous 4-dye system. Applied Biosystems PET™ and LIZ™ dyes expand the spectral detection range that can be used on the ABI Prism® genetic analysis instrumentation. Together with 6-FAM™, VIC™ and NED™ dyes, the spectral emission for this five-dye set extends to 660nm. The AmpFℓSTR® Identifiler kit employs the same primer sequences as used in all previous AmpFℓSTR® kits. A

degenerate unlabeled primer for the D8S1179 locus was added to the AmpFℓSTR® Identifiler™ Primer Set in order to address a mutation observed in a population of Chamorros and Filipinos from Guam (Budowle *et al.,* 1998b and Budowle *et al.*, 2000). The addition of the degenerate primer allows for the amplification of those alleles in the samples containing this mutation without altering the overall performance of the AmpFℓSTR Identifiler PCR Amplification kit.

Non-nucleotide linkers are used in primer synthesis for the following loci: CSF1PO, D2S1338, D13S317, D16S539 and TPOX. For these primers, non-nucleotide linkers are placed between the primer and the flourescent dye during oligonucleotide systhesis (Grossman *et al.,* 1994 and Baron *et al.,* 1996). Non-nucleotide linkers enable reproducible positioning of the alleles to facilitate inter-locus spacing. By combining the five-dye system with the non-nucleotide linkers for selected loci, the same primer sequences developed for previous AmpFℓSTR kits are used without modification.

Multi-component analysis is the process that separates the five different fluorescent dye colors into distinct spectral components. The four dyes used in AmpFℓSTR® Identifiler™ PCR Amplification Kit to label samples are 6-FAM™, VIC™, NED™, and PET™ dyes. The fifth dye, LIZ ™, is used to label the GeneScan™-500 Size Standard.

Each of these fluorescent dyes emits its maximum fluorescence at a different wavelength. During data collection, with the ABI Prism® instruments, the fluorescent signals are separated by a diffraction grating according to their wavelengths and projected onto a charge-coupled device (CCD) camera in a predictably spaced pattern. 6-FAM dye emits at the shortest wavelength and is displayed as blue; followed by the VIC dye (green), NED dye (yellow), PET dye (red) and LIZ dye (orange).

Although each of these dyes emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes. The goal of multi-component analysis is to effectively correct for spectral overlap.

Table 1. The AmpFℓSTR® Identifiler™ PCR Amplification System

STR Locus	Label	Chromosomal Location	Alleles Included in Identifiler Allelic Ladder	Control 9947a
D2S1338	VIC	2q35-37.1	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28	19,23

D18S51	NED	18q21.3	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27	15, 19
D21S11	6-FAM	21q11.2-q21	24, 24.2, 25, .26, 27, 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, 37, 38	30, 30
THO1	VIC	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3	8, 9.3
D3S1358	VIC	3p	12, 13, 14, 15, 16, 17, 18, 19	14, 15
FGA	PET	4q28	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2	23, 24
TPOX	NED	2p23-2per	6, 7, 8, 9, 10, 11, 12, 13	8, 8
D8S1179	6-FAM	8	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	13, 13
VWA	NED	12p12-pter	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24	17, 18
Ameogenin	PET	X:p22.1-22.3 Y: p11.2	X, Y	X
D19S433	NED	19q12-13.1	9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2	14, 15
CSF1PO	6-FAM	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15	10, 12
D16S539	VIC	16q24-qter	5, 8, 9, 10, 11, 12, 13, 14, 15	10, 11
D7S820	6-FAM	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15	10, 11
D13S317	VIC	13q22-q31	8, 9, 10, 11, 12, 13, 14, 15	11, 11
D5S818	PET	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16	11, 11

6.2.4.1.2: Amplification Setup

Samples amplified using AmpFℓSTR Identifiler chemistries.

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

AmpFℓSTR PCR Reaction Mix 10.5µL

AmpliTaq Gold DNA Polymerase 0.5 µL

AmpFℓSTR Identifiler Primer Set 5.5 µL

Dispense 15 µL of master mix and 10 µL of sample into a 96-opti well plate, cover the plate with PCR septa. Briefly spin the plate in the centrifuge and place into the 9700 AB thermocycler. To start the run, follow these steps:

1. Turn on the power to 9700 thermocycler

2. Press Run

3. Scroll to the appropriate program

4. Press Start

5. Ensure the proper volume is entered

6. Press Start again

The following is the 9700 thermocycler parameters that are used during amplification of Identifiler:

95°C	11min	
94°C	1min	28cycles
59°C	1min	28cycles
72°C	1min	28cycles
60°C	60min	
4°C	forever	

6.2.4.1.3: Sample Setup for the 3130xl Instrument

After amplification is complete, samples are set up for the 3130xl. A 96 opti-well plate is used. Create a master mix solution in the following ratios:

0.3µℓ of LIZ Size Standard
8.7µℓ of HiDi Formamide

1. Pipette 9µℓ of master mix into each well used.

2. Ensure that all the wells of an injection contain master mix. The 3130xl should never inject sample from a dry well.

3. Add 1 µℓ of sample to each well (a multi-channel pipette is beneficial).

4. Add 1 µℓ of ladder to each ladder sample. At minimum, 1 ladder per plate must be present.

5. Briefly spin the plate in the centrifuge.

6. Place the plate into the 3130xl instrument. The plate only fits into the instrument in one direction.

6.2.4.1.4: 3130xl Instrument Setup

1. Go to Plate Manager

2. Click on Import

3. Select the txt file to import.

4. Click 'OK'

5. Verify the sample names.

6. Verify the 'Results Group' and an 'Instrument Protocol'.

7. Click 'OK"

8. It is best to start the oven approximately 15 minutes before the run starts.

   a. Click on Manual Control
   b. From the pull down menu select Oven On
   c. Click Send command
   d. From the pull down menu select Set Oven Temperature
   e. Set temperature to 60º
   f. Click Send Command

9. Link the appropriate plate to the plate map under the 'Run Scheduler' menu.

10. To start the run Click on the green arrow or File→Start Run

11. Click OK in popup window.

6.2.4.1.5: Analysis of Raw Data / GeneMapper ID

GeneMapper ID analysis software is used to analyze the raw data collected by the 3130 Genetic Analyzer.

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

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

2. Select the appropriate run folder saved on the USB stick and click **Add to List**. Once all samples have been added to the list, click **Add** to import the files.

3. In the Sample Type column, assign the correct sample type to each sample (i.e. sample, ladder, control)

4. Select Analysis Method.

5. Select **Identifiler _v2** as the Panel.

6. Select **CE_G5_HID_GS500** as the Size Standard.

7. Click the green arrow to analyze the project.

8. View the raw data to examine the LIZ size standard. Verify that the analysis range is between 75bp and 450bp and the peaks are correctly labeled. The 250bp peak should not be labeled.

9. Review controls
- Display each control (including positive and negative amplification controls, and blank controls).
- If peaks above 100 RFU are observed in the negative controls, the sample can be re-injected.
- Examine the Positive control and verify the correct calls of the alleles.

10. Examine the allelic ladders.
- Verify that the allelic ladder is called correctly for each marker.

11. Analyzed samples can be viewed as a group or individually by highlighting the samples to view. After selecting the sample click the **Display Plots** button. There are several options available to view the electropherogram. Refer to the GeneMapper ID Software Version 3.2.1 Human Identification Analysis Tutorial for specific information on plot views.

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 1000-4000 RFU, although acceptable and type able signals may occur outside of this range).
- Matrix quality (baselines should be relatively flat and there should not be a pattern of pronounced peaks or dips below true DNA peaks in the other four colors).

- Peak profile (examine for artifactual peaks e.g. spikes).

6.2.4.2: AmpFℓSTR® Minifiler ™ PCR Amplification Kit

6.2.4.2.1: Background

The AmpFℓSTR Minifiler ™ PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies 8 tetranucleatide repeat loci and the Amelogenin gender determining marker in a single PCR amplification. Seven of the thirteen required loci for CODIS are included in this kit for known-offender databasing in the United States (Budowle *et al.,* 1998a). One additional loci, D2S1338 is included.

Advantages:

The Minifiler kit employs primers closely flanking the STR repetitive regions (miniSTRs) of the DNA. This amplification results in amplicons that are significantly shorter in length than those produced in AmpFℓSTR® Identifiler™ and AmpFℓSTR® SGM Plus™ PCR Amplification Kits. Several labs have confirmed that MiniSTRs have a higher success rate for DNA analysis of degraded DNA samples (Butler et al., 2003; Chung et al., 2004; Coble and Butler, 2005; Drabek et al., 2004; Grubwieser et al., 2006; Wiegand et al., 2001).

Non-nucleotide linkers are used in primer synthesis for the following loci: CSF1PO, FGA, D16S539, D18S51, Amelogenin, D2S1338,, D21S11 and D7S820. For these primers, non-nucleotide linkers are placed between the primer and the flourescent dye during oligonucleotide systhesis (Grossman *et al.,* 1994 and Baron *et al.,* 1996). Non-nucleotide linkers enable reproducible positioning of the alleles to facilitate inter-locus spacing

Multi-component analysis is the process that separates the five different fluorescent dye colors into distinct spectral components. The four dyes used in AmpFℓSTR® Minifiler™ PCR Amplification Kit to label samples are 6-FAM™, VIC™, NED™, and PET™ dyes. The fifth dye, LIZ ™, is used to label the GeneScan™-500 Size Standard.

Each of these fluorescent dyes emits its maximum fluorescence at a different wavelength. During data collection, with the ABI Prism® instruments, the fluorescent signals are separated by a diffraction grating according to their wavelengths and projected onto a charge-coupled device (CCD) camera in a predictably spaced pattern. 6-FAM dye emits at the shortest wavelength and is displayed as blue;

followed by the VIC dye (green), NED dye (yellow), PET dye (red) and LIZ dye (orange).

Although each of these dyes emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes. The goal of multi-component analysis is to effectively correct for spectral overlap.

Table 1. The AmpFℓSTR® Minifiler™ PCR Amplification System

STR Locus	Label	Chromosomal Location	Alleles Included in Identifiler Allelic Ladder	Control 9947a
D2S1338	VIC	2q35-37.1	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28	19,23
D18S51	NED	18q21.3	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27	15, 19
D21S11	VIC	21q11.2-q21	24, 24.2, 25, .26, 27, 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, 37, 38	30, 30
FGA	PET	4q28	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2	23, 24
Ameogenin	VIC	X:p22.1-22.3 Y: p11.2	X, Y	X
CSF1PO	PET	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15	10, 12
D16S539	NED	16q24-qter	5, 8, 9, 10, 11, 12, 13, 14, 15	10, 11
D7S820	6-FAM	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15	10, 11
D13S317	6-FAM	13q22-q31	8, 9, 10, 11, 12, 13, 14, 15	11, 11

6.2.4.2.2: Amplification Setup

Samples amplified using AmpFℓSTR Minifiler chemistries.

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

AmpFℓSTR Master Mix 10.0µL
AmpFℓSTR Minifiler Primer Set 5.0µL

Dispense 15 µL of master mix and 10 µL of sample into a 96-opti well plate, cover the plate with PCR septa. Briefly spin the plate in the centrifuge and place into the 9700 AB thermocycler. To start the run, follow these steps:

1. Turn on the power to 9700 thermocycler

2. Press Run

3. Scroll to the appropriate program

4. Press Start

5. Ensure the proper volume is entered

6. Press Start again

The following is the 9700 thermocycler parameters that are used during amplification of Minifiler:

95°C	11min	
94°C	20sec	30cycles
59°C	2min	30cycles
72°C	1min	30cycles
60°C	45min	
4°C	forever	

6.2.4.2.3: Sample Setup for the 3130xl Instrument

After amplification is complete, samples are set up for the 3130. A 96 opti-well plate is used. Create a master mix solution in the following ratios:

0.3µℓ of LIZ Size Standard
8.7µℓ of HiDi Formamide

1. Pipette 9µℓ of master mix into each well used.

2. Ensure that all the wells of an injection contain master mix. The 3130xl should never inject sample from a dry well.

3. Add 1 µℓ of sample to each well (a multi-channel pipette is beneficial).

4. Add 1 µℓ of ladder to each ladder sample. At minimum, 1 ladder per plate must be present.

5. Briefly spin the plate in the centrifuge.

6. Place the plate into the 3130xl instrument. The plate only fits into the instrument in one direction.

6.2.4.2.4: 3130xl Instrument Setup

1. Go to Plate Manager
2. Click on Import
3. Select the txt file to import.
4. Click 'OK'
5. Verify the sample names.
6. Verify the 'Results Group' and an 'Instrument Protocol'.
7. Click 'OK"
8. It is best to start the oven approximately 15 minutes before the run starts.
   a. Click on Manual Control
   b. From the pull down menu select Oven On
   c. Click Send command
   d. From the pull down menu select Set Oven Temperature
   e. Set temperature to 60º
   f. Click Send Command
9. Link the appropriate plate to the plate map under the 'Run Scheduler' menu.
10. To start the run Click on the green arrow or File→Start Run
11. Click OK in popup window.

6.2.4.2.5: Analysis of Raw Data / GeneMapper ID

GeneMapper ID analysis software is used to analyze the raw data collected by the 3130 Genetic Analyzer.

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

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

2. Select the appropriate run folder saved on the USB stick and click **Add to List**. Once all samples have been added to the list, click **Add** to import the files.

3. In the Sample Type column, assign the correct sample type to each sample (i.e. sample, ladder, control)

4. Select Analysis Method.

5. Select **Minifiler_GS500_v1** as the Panel.

6. Select **CE_G5_HID_GS500** as the Size Standard.

7. Click the green arrow to analyze the project.

8. View the raw data to examine the LIZ size standard. Verify that the analysis range is between 75bp and 450bp and the peaks are correctly labeled. The 250bp peak should not be labeled.

9. Review controls
- Display each control (including positive and negative amplification controls, and blank controls).
- If peaks above 100 RFU are observed in the negative controls, the sample can be re-injected.
- Examine the Positive control and verify the correct calls of the alleles.

10. Examine the allelic ladders.
- Verify that the allelic ladder is called correctly for each marker.

11. Analyzed samples can be viewed as a group or individually by highlighting the samples to view. After selecting the sample click the **Display Plots** button. There are several options available to view the electropherogram. Refer to the GeneMapper ID Software Version 3.2.1 Human Identification Analysis Tutorial for specific information on plot views.

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 1000-4000 RFU, although acceptable and type able signals may occur outside of this range).
- Matrix quality (baselines should be relatively flat and there should not be a pattern of pronounced peaks or dips below true DNA peaks in the other four colors).
- Peak profile (examine for artifactual peaks e.g. spikes).

6.2.4.3: Controls and Guidelines for AmpFℓSTR® Identifiler and Minifiler ™ PCR Amplification Kits

6.2.4.3.1: Controls

6.2.4.3.1.1: 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. A reagent blank must be included with each extraction set. The reagent blank will be amplified at full strength (10 µl of undiluted reagent blank).
The reagent blank tests 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 100 RFUs 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 should be considered inconclusive for analysis and re-extracted. If the DNA sample has been consumed and re-extraction is not possible, then the DNA technical leader, Casework Supervisor and Laboratory Director will be consulted to analyze the samples and reagent blank. If after analysis the source of the contaminating DNA does not appear to be in the samples, then the contamination will be noted in the report. If the extraneous DNA is present in both the reagent blank and associated sample, then the sample will be reported as inconclusive.

6.2.4.3.1.2: 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. 9947a is the positive control that is supplied with the AmpFℓSTR® Identifiler™ PCR Amplification Kit. 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.

6.2.4.3.1.3: Negative Control (AMP-)

The negative control (amplification blank) contains all the reagents for the amplification mix but no DNA.
The negative control tests for contamination of samples during the setup of the amplification reactions. If the negative control exhibits unexplainable peaks above 100 RFUs 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.

6.2.4.3.1.4: Internal Size Marker and Allelic Ladder

Internal size marker is added to each sample and ladder prior to electrophoresis. The internal size marker allows the genetic analysis software to determine the size (in basepairs) 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 to assign an allele call to any peaks observed based on their size.

6.2.4.3.2: Interpretation Guidelines

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

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

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

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

6.2.4.3.2.1: Threshold

The minimum peak height threshold will be set at 100 (Relative Fluorescent Unit) RFU for software recognition of a peak. The interpretation threshold is set at 100 RFU. Optimal peak height values range between 1000-4000 RFU, although acceptable and typeable signals may occur outside of this range.

6.2.4.3.2.2: 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 lower than 60% may indicate a mixture. Occasionally a non-mixed sample will be outside of this range. Depending upon the sample source, the loci in question, the number of loci affected and the percent disparity between alleles, the sample may need to be re-amplified and typed.

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.

6.2.4.3.2.3: Off Ladder Variants

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

The analyst will re-amplify or re-inject, then type any sample containing a peak not properly interpreted as an allele by the software, especially if it is not appropriately balanced with an associated allele or at a height expected for a homozygote.
An off ladder variant which has been seen and confirmed at least two times in the population sampled at the Arkansas State Crime Laboratory is no longer considered a rare variant. These peaks can be confidently and accurately called without confirmation.

6.2.4.3.2.4: Tri-Allele

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

6.2.4.3.2.5: 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.

6.2.4.3.2.5.1: Spikes

Spikes are artifactual peaks usually observed in at least two colors. Spikes can be caused by urea crystals in the capillary, power surges, or other instrument related issues. A spike will not exhibit the same morphology as a peak, but will be sharper or "spike" shaped. Spikes are unique to fragments analyzed using capillary electrophoresis. Spikes will have identical fragment sizes in the ABI Prism 3130xl data, and fragment sizes which vary only slightly in the ABI Prism 3130xl data. Above threshold spikes should be noted and may be re-injected.

6.2.4.3.2.5.2: Stutter

In addition to an allele's primary peak, artifactual minor "stutter" peaks can occur at four-base intervals. The most common stutter peaks observed in all loci are four bases smaller than the primary peak ("n-4"). It is also possible to see additional "n+4" peaks (four bases larger), especially when excessive amounts of DNA are amplified.

Stutter peaks are evaluated by examining the ratio of the stutter peak height to the height of the appropriate adjacent allele, expressed as a percentage. The height of stutter peaks can vary by locus, and longer alleles within a locus generally have a higher percentage of stutter. The maximum expected percentage of stutter is less than 20% 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 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 6000 RFU and/or has been labeled off-scale, the analyst should interpret the results with caution. The sample may be re-amplified with less input DNA or re-injected.

Identifiler Loci Stutter Ratios

LOCUS	STUTTER RATIOS APPLIED
D3S1358	0.08
TH01	0.08
D13S317 *	0.06
D16S539 *	0.06
D2S1338 *	0.09
D19S433	0.11
vWA	0.10
TPOX	0.03
D18S51 *	0.16
AMEL *	0.0
D5S818	0.06
FGA *	0.13
D8S1179	0.07
D21S11 *	0.06
D7S820 *	0.05

CSF1PO *	0.05

* Minifiler loci stutter ratios

6.2.4.3.2.5.3: Non-Template Nucleotide Addition (-A)

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

6.2.4.3.2.5.4: Pull-Up

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

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

6.2.4.3.2.5.5: Other

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

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

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

6.2.4.3.3: 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.

6.2.4.3.4: Detection of Mixtures

Samples may contain DNA from more than one individual. A sample is 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 20% 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.

6.2.4.3.5: Interpretation of Mixed Samples

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

- If there is a dominant profile present in the mixture, one can determine any inclusions or exclusions by comparing the profiles from known reference samples with the dominant DNA types.

- Profile frequency estimates and/or source attribution can be reported for the dominant DNA profile.

- Information can be gained from minor profiles observed in mixtures as to possible inclusion or exclusions of sources. Mixture statistics should be applied to the entire profile where the minor contributor cannot be clearly determined. In a mixture that is consistent with only two contributors, where a single source minor profile can be determined based on heterozygote peaks at any particular locus, single source statistics can be applied to the minor profile at these loci.

- When more than one source of DNA is detected and one cannot be singled out, all possible combinations must be considered for each locus.

- Where activity is observed below 100 RFU and above approximately 40 RFU it will be notated in the analysts notes for the particular locus as an asterisk (*). Loci containing activity below 100 RFU will not be used in the mixture calculation.

6.2.4.3.6: 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 100 RFU. However there is a decrease in peak height heterozygosity with lower levels of DNA. One must interpret a homozygote near the threshold of interpretation with caution.

6.2.4.3.7: 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 can not necessarily be excluded from contributing to the mixed DNA profile and should be reported as "cannot be excluded from contributing to the mixed profile from …" The affected areas will be left out of the statistical calculation.

6.2.4.3.8: 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 it 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.

Too Limited For Interpretational Value: It is possible to obtain a partial STR profile that contains enough genetic information to exclude and individual but not include individuals. 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 inconclusive, or perhaps the weak single allele cannot be ruled out as a heterozygote. This type of genetic information would be too limited for interpretational value.

Inconclusive: It is possible to obtain a STR profile that contains a complex mixture from multiple individuals and contains alleles that do not meet the minimum rfu threshold or peak morphology. In these cases the result may be reported as uninterruptable or inconclusive.

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

analysis performed for confirmation.

6.2.4.3.9: 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.

Example:

This estimate is strictly driven by database size:

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

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

This method is conservative and also addresses some substructure effects.

Expected Genotype Frequency:

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

TH01 Genotype 7, 7:

(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

6.2.4.3.9.1: PopStats

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

a. A high probability indicates that the profile's characteristics are quite common within the population. Thus, the profile does not distinguish itself from others in the population

b. A low probability indicates that the profile's characteristics are quite rare. This strongly indicates that the profile represents the individual in question.

6.2.4.3.9.2: Single Source Stain

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

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

b. For homozygotes (AA),

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

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

$\theta = 0.01$ for general US population
$\theta = 0.03$ for isolated groups (American Indians)

c. For heterozygotes (AB), the formula : $F = 2pq$

d. For all loci to identify the match: $F = (f_1 \times f_2 \times f_3 \ldots)$

e. Inverse probability = 1/F

6.2.4.3.9.3: Mixed DNA Stains

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

a. **Mixture formula**: $f = (p_1 + p_2 + \ldots p_k + m.p_{null})^2$
where p_1 , p_2 , …, p_k are occurrence frequencies of the band/alleles of the locus, $k \geq 1$ is the number of bands/alleles of the profile at the locus, and p_{null} is the **"null allele frequency" = 0 .**

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

All evidence mixed profiles will be calculated using the Popstats formula listed above.
OR:

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 grater than 3/2, 4/2 and 2/2. Therefore, $x \geq 2$.

OR:

c. **<u>Probability of Exclusion</u> (PE)**

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

Allele 11 = 0.122
Allele 13 = 0.176
Allele 15 <u>= 0.041</u>

- 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.

6.2.4.3.9.4: Population Database

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

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

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

6.2.4.4: AmpFℓSTR® Yfiler ™ PCR Amplification Kit

6.2.4.4.1: Background

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

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

The following table shows the loci amplified by the Yfiler kit and the corresponding dyse used. The AmpFℓSTR Yfiler Kit Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder and the genotype of the Control DNA 007 are listed in the table.

Table 2. The AmpFℓSTR® Yfiler™ PCR Amplification System

LOCUS DESIGNATION	ALLELES INCLUDED IN Yfiler KIT ALLELIC LADDER	DYE LABEL	DNA 007 GENOTYPE
DYS456	13-18	6-FAM™	15
DYS389I	10-15	6-FAM™	13
DYS390	18-27	6-FAM™	24
DYS389II	24-34	6-FAM™	29

DYS458	14-20	VIC®	17
DYS19	10-19	VIC®	15
DYS385a/b	7-25	VIC®	11,14
DYS393	8-16	NED™	13
DYS391	7-13	NED™	11
DYS439	8-15	NED™	12
DYS635	20-26	NED™	24
DYS392	7-18	NED™	13
Y GATA H4	8-13	PET®	13
DYS437	13-17	PET®	15
DYS438	8-13	PET®	12
DYS448	17-24	PET®	19

Applied Biosystems fluorescent multi color dye technology allows the analysis of multiple loci, including loci that have alleles with overlapping size ranges. Alleles for overlapping loci are distinguished by labeling locus-specific primers with different colored dyes.
Multicomponent analysis is the process that separates the five different fluorescent dye colors into distinct spectral components. The four dyes used in the AmpFℓSTR® Yfiler™ PCR Amplification Kit to label samples are 6-FAM™, VIC®, NED™, PET® dyes. The fifth dye, LIZ®, is used to label the GeneScan™-500 Size Standard.

6.2.4.4.2: Case Acceptance Policy

The approach for using Y-STR analysis in casework will be case dependant and generalized step-by-step procedure is not appropriate. However the following will be considered when determining whether or not Y-STR analysis will be attempted in a case

1. Case scenario

2. P-30 results

3. Autosomal STR results

6.2.4.4.3: Amplification Setup

Samples amplified using AmpFℓSTR Yfiler chemistries.

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

AmpFℓSTR PCR Reaction Mix	9.2µL
AmpliTaq Gold DNA Polymerase	0.8 µL
AmpFℓSTR Primer Mix	5.0 µL

Dispense 15 µL of master mix and 10 µL of sample into a 96-opti well plate, cover the plate with PCR septa. Briefly spin the plate in the centrifuge and place into the 9700 AB thermocycler. To start the run, follow these steps:

1. Turn on the power to 9700 thermocycler

1. Press Run

2. Scroll to the appropriate program

3. Press Start

4. Ensure the proper volume is entered

5. Press Start again

The following is the 9700 thermocycler parameters that are used during amplification of Yfiler:

95°C	11min	
94°C	1min	30cycles
61°C	1min	30cycles
72°C	1min	30cycles
60°C	80min	
4°C	forever	

6.2.4.4.4: Samples Setup for the 3130xl Instrument

After amplification is complete, samples are set up for the 3130. A 96 opti-well plate is used. Create a master mix solution in the following ratios:

0.3µℓ of LIZ Size Standard
8.7µℓ of HiDi Formamide

1. Pipette 9µℓ of master mix into each well used.

2. Ensure that all the wells of an injection contain master mix. The 3130xl should never inject sample from a dry well.

3. Add 1 µℓ of sample to each well (a multi-channel pipette is beneficial).

4. Add 1 µℓ of ladder to each ladder sample. At minimum, 1 ladder per plate must be present.

5. Briefly spin the plate in the centrifuge.

6. Place the plate into the 3130xl instrument. The plate only fits into the instrument in one direction.

6.2.4.4.5: 3130xl Instrument Setup

1. Go to Plate Manager

2. Click on Import

3. Select the txt file to import.

4. Click 'OK'

5. Verify the sample names.

6. Verify the 'Results Group' and an 'Instrument Protocol'.

7. Click 'OK"

8. It is best to start the oven approximately 15 minutes before the run starts.

a. Click on Manual Control
b. From the pull down menu select Oven On
c. Click Send command
d. From the pull down menu select Set Oven Temperature
e. Set temperature to 60º
f. Click Send Command

9. Link the appropriate plate to the plate map under the 'Run Scheduler' menu.

10. To start the run Click on the green arrow or File→Start Run

11. Click OK in popup window

6.2.4.4.6: Analysis of Raw Data / GeneMapper ID

1. All data is analyzed using GeneMapper ID

2. Open GeneMapper ID

3. Add samples to project (File→Add Sample to Project)

4. Find correct sample files

5. Click: Add to List

6. Click: Add or Add & Analyze

7. By using the pull down menus ensure the Sample type, Analysis Method and Specimen Category are correct.

8. Click on the green arrow or Analysis→Analyze to begin the process.

9. Check the Size Match Editor and Display plots for each sample analyzed.

10. If needed, the raw data can be accessed to confirm calls.

11. Describe the reason for each artifact deleted (ex; pull-up, spike, etc…).

12. Save the GeneMapper project for review. This project will be saved as a '.ser' file.

- To export the project out of GeneMapper, open GeneMapper Manager.
- Highlight the project(s) to be exported
- Click 'Export'
- Save to the appropriate location. The exported projects will be saved as '.ser'.

6.2.4.4.7: Controls

6.2.4.4.7.1: 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. A reagent blank must be included with each extraction set. The reagent blank will be amplified at full strength (10 µl of undiluted reagent blank).
The reagent blank tests 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

100 RFUs 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 should be considered inconclusive for analysis and re-extracted. If the DNA sample has been consumed and re-extraction is not possible, then the DNA technical leader, Casework Supervisor and Laboratory Director will be consulted to analyze the samples and reagent blank. If after analysis the source of the contaminating DNA does not appear to be in the samples, then the contamination will be noted in the report. If the extraneous DNA is present in both the reagent blank and associated sample, then the sample will be reported as inconclusive.

6.2.4.4.7.2: 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. 007 is the positive control that is supplied with the AmpFℓSTR® Yfiler™ PCR Amplification Kit. 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.

6.2.4.4.7.3: Negative Control (AMP-)

The negative control (amplification blank) contains all the reagents for the amplification mix but no DNA.
The negative control tests for contamination of samples during the setup of the amplification reactions. If the negative control exhibits unexplainable peaks above 100 RFUs 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.

6.2.4.4.7.4: Negative Control (Female DNA)

The 9947a control is used for the female control. The purpose of the female control is to ensure that no female DNA was amplified. Since Yfiler only amplifies male DNA no profile above threshold should be identified. If an allele is detected, all results of samples associated with the amplification should be considered inconclusive. All samples must then be re-injected or re-amplified and analyzed.

6.2.4.4.7.5: Internal Size Marker and Allelic Ladder

Internal size marker is added to each sample and ladder prior to electrophoresis. The internal size marker allows the genetic analysis software to determine the size (in basepairs) 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 to assign an allele call to any peaks observed based on their size.

6.2.4.4.8: Interpretation Guidelines

The interpretation of results in casework is necessarily 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.

6.2.4.4.8.1: Threshold

The minimum peak height threshold will be set at 100 (Relative Fluorescent Unit) RFU for software recognition of a peak. The interpretation threshold is set at 100 RFU. Optimal peak height values range between 1000-4000 RFU, although acceptable and typeable signals may occur outside of this range.

6.2.4.4.8.2: Off Ladder Variants

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

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

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

6.2.4.4.8.3: Artifacts

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

6.2.4.4.8.3.1: Spikes

Spikes are artifactual peaks usually observed in at least two colors. Spikes can be caused by urea crystals in the capillary, power surges, or other instrument related issues. A spike will not exhibit the same morphology as a peak, but will be sharper or "spike" shaped. Spikes are unique to

fragments analyzed using capillary electrophoresis. Spikes will have identical fragment sizes in the ABI 3130xl data, and fragment sizes which vary only slightly in the ABI 3130xl data. Above threshold spikes should be noted and may be re-injected.

6.2.4.4.8.3.2: Stutter

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

LOCUS	**STUTTER RATIOS APPLIED**	**ADDITIONAL STUTTER PEAKS**
DYS456	0.14	N+4 0.02
DYS3891I	0.09	
DYS390	0.10	
DYS389II	0.17	
DYS458	0.13	N+4 0.02
DYS19	0.11	N-2 0.11
DYS385A	0.12	
DYS385B	0.20	
DYS393	0.13	
DYS391	0.18	
DYS439	0.12	
DYS635	0.12	
DYS392	0.13	N+3 0.08
GATA_H4	0.11	
DYS437	0.06	N-5 0.03
DYS438	0.04	
DYS448	0.05	

6.2.4.4.8.3.3: Non-Template Nucleotide Addition (-A)

Amplification conditions have been set to maximize the non-template addition of a 3′ terminal nucleotide by AmpliTaq Gold DNA polymerase. Failure to attain

complete terminal nucleotide addition results in "band splitting", visualized as two peaks one base apart. This is most often seen when an excessive amount of DNA is amplified or amplification is performed under sub-optimal PCR conditions.

6.2.4.4.8.3.4: Pull-Up

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

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

6.2.4.4.8.3.5: Other

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

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

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

6.2.4.4.9: Interpretation of Sample Peaks

Amplified products from samples will be interpreted based on peak quality, peak morphology and RFU values. It is a requirement of the analyst, based on experience, to determine which sample peaks meet the criteria for allele designation. All peaks must meet a minimum RFU threshold of 100. "Out of range" peaks are any peaks not falling within the size range of 98-350 base pairs. The presence of out of range peaks may be noted, but they are not considered part of the Y-chromosomal DNA profile.

In general, a single source profile at each locus will appear as a single peak, with the exception of DYS385.

Inconclusive Allele Calls: In those cases where peaks are not present or are below the minimum 100 RFU threshold, allele calls for that sample at that locus may be designated as inconclusive "INC".

6.2.4.4.9.1: Single Source Samples

If it is determined that a question sample has been deposited by a single male source, comparisons are then made to the known reference samples in the case. A match is declared if all alleles in the question sample match the known sample. The haplotype is then searched in the appropriate database and the number of observations of that haplotype is reported.

6.2.4.4.9.2: Mixed DNA Samples

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

1. A sample that has interpretable peaks at one or more locus may be reported even though no bands are detected in the remaining loci

2. If the mixture is distinguishable (designation can be made between the major and minor alleles) the minor allele must

be designated with ( ). Mixtures that are determined to be distinguishable should contain minor alleles in which the rfu value is approximately 50% of the major allele(s).

**An independent, qualified DNA analyst must review all results obtained

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

6.2.4.4.10: Incomplete STR Profiles

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

6.2.4.4.11: Statistical Calculations

Since Y-STRs are paternally inherited the counting method is the preferred way to perform statistical calculations. All statistical calculations are preformed using US Y-STR Database. This database is located at the following address: =http://www.usystrdatabase.org/newdefault.aspx**.**

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

6.3: Reports

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

6.3.1: Elements of the Case Report

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

a. Case identifier
b. Description of evidence examined

c. A description of methodology
d. Loci examined
e. Results and/or conclusions
f. An interpretative statement (either quantitative or qualitative)
g. Date issued
h. Disposition of evidence
i. A signature and title or equivalent identification of the person(s) accepting responsibility of the content of the report.

6.3.2: Report Guidelines

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

6.3.2.1: No DNA Obtained

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

6.3.2.2: Single Source Stains

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

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

Or

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

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

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

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

6.3.2.3: Mixtures

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

1. RESULTS

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

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

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

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

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

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

1. RESULTS
   (Probability of Exclusion / Inclusion)

   The DNA extracted from Q1 (*evidence*) indicates a mixture from more than one individual at 8 of 12 loci. This mixture is consistent with a mixture of DNA from K1 (*victim*) and K2 (*suspect*). 96.99% of

unrelated (Caucasians) would be expected to be excluded as contributors to the observed DNA mixture. Conversely, 1 in 34,000 randomly chosen Caucasian individuals would be expected to be included as contributors to the observed DNA mixture.

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

Or

2. RESULTS

(Likelihood Ratio)

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

6.3.2.4: Forensic Paternity Case

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

**All alleles are designated on report.

1. RESULTS
   Based on the above results, K3 (Alleged Father) cannot be excluded as the biological father of K2 (child).

   The probability of paternity in this case is 99.99%.

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

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

6.3.2.5: Y-STR Report Guidelines

Exclusion Report

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

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

Inclusion Report

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

The Y-chromosomal DNA profile obtained from Item 1 matches the Y-chromosomal DNA profile obtained from K1, John Doe. Therefore, neither John Doe nor any of his paternally related male relatives can be excluded as the contributor of this DNA.
The Y-chromosomal DNA profile obtained from Item 1 has been observed in the population groups as follows:

Population Group	Observations	Database Size	Upper Limit Frequency (%)
African American			
Asian			
Caucasian			
Hispanic			
Native American			

SECTION 7: EQUIPMENT CALIBRATION AND MAINTENANCE

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

It is possible to verify "after the fact" that the equipment, materials and reagents used in an analysis have not significantly affected the reliability of the results. For example, controls utilized during each phase of the testing procedure are designed to signal potential problems in the analysis. If acceptable results are obtained on these controls, it is reasonable to assume that the results from other samples analyzed simultaneously are also reliable. If the controls indicate a problem with the analysis, it may be possible to determine the source of the problem and make corrections. Depending on the nature of the problem, re-analysis of the samples may be required.

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

7.1: Instrument and Equipment

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

Pipettes	Thermocyclers
Thermometers	Refrigerators
Freezers	7500
Heat Blocks	pH Meter
EZ-1 Robots	3130xls

7.2: Inventory Calibration and Maintenance Log Book

An inventory log will be maintained on 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, agency inventory number.

7.3: Operating Manuals

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

7.4: Calibration / Maintenance / Repair Records

Anytime an instrument or piece of equipment requires calibration, service or maintenance, that information will be documented on the specific maintenance log sheet. Maintenance logs will be maintained either on the DNA "S" drive or on the applicable instrument control computer(s). Additionally, instruments/equipment on routine service contract will have routine service calls documented.

** 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 failing must be documented in 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 calibration and performance is demonstrated.
d. The DNA Quality Manager must inform the Technical Leader and Casework Supervisor of all equipment failure.

7.5: Calibration and Maintenance Schedules

Each instrument/piece of equipment considered essential for DNA typing will be calibrated on an appropriate schedule. Preventative maintenance will be performed on a schedule recommended by the manufacturer. Schedule for maintenance is found in the

DNA "S" drive on the computer. Anytime an instrument or piece of equipment requires calibration, service or maintenance, and each instrument or piece of equipment considered essential for DNA typing will be calibrated on an appropriate schedule.

As Needed or Annually at a Minimum

- pH meter - solution is probe checked and replaced as necessary by laboratory personnel.
- Vacuum Pumps - clean and flush by laboratory personnel.
- Centrifuges - cleaning by laboratory personnel with 10% bleach solution.
- Spatial and Spectral for 3130xl (whenever array door is opened a spatial and spectral (G and F dyes) must be performed according to the manufacturer.

7.5.1: Annually

- Pipettes – performance checks and calibrated by an outside company.
- Drift-con- Thermal cycler calibration system must be sent out annually for calibration.

7.5.2: Semi-Annually

- Thermometer – calibration check by laboratory personnel. Also check prior to putting into service unless a NIST traceable thermometer. (Calibrated using a NIST Traceable Thermometer).

7.5.3: Quarterly

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

  At the current time, the fume hoods in the laboratory are monitored through a software program called WinControl. The Software receives data from control points throughout the hood system and displays them on a monitor in the office of Rick Gallagher. Another monitor is located in the State Building Service's office located in the Natural Resources Building adjacent to the Arkansas State Crime Laboratory. In the near future, the State Engineer's office will have remote monitoring capability of the hoods and the entire HVAC system. Currently, if an alarm goes off in a hood, it will display an alarm message which will trigger a computer response and if needed, an on-site visit from maintenance personnel. If the problem persists, an outside company will be brought in to handle the problem.

- All test tube racks are cleaned with a 10% bleach solution or by using a stratalinker.
- Balances are performance-checked.

7.5.4: Bi-Monthly

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

7.5.5: Monthly

- Swipe tests: Cotton swabs are moistened with distilled water and rubbed on the analyst's bench top. All documentation is kept on the DNA "S" drive on the computer.
- The 3130xl and computer restarted.
- Burn DVD's with data and back-up the oracle database.

7.5.6: Weekly (by laboratory personnel as needed)

- Pipettes – outside barrel cleaned with water, isopropyl alcohol, bleach or ethanol as needed.
- Water wash is performed on the 3130xl.
- Polymer is changed on the 3130xl.
- Change reservoir septa on 3130xl.
- Wet the seals on the 3130xl.
- Grease O-rings on the EZ1 robots

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

- Autoclave – check water levels before use.
- Check temperature of refrigerators and freezers in both pre-amp and post-amp rooms.
- Heat Blocks – temperature checked prior to use.
- Bench tops – CODIS DNA (pre): After each use, the bench tops must be cleaned with a 10% bleach solution.
- Water and buffer changed in the 3130xl.
- End of day (after last protocol) on the EZ1
    - Clean Piercing unit
        - Close Door
        - Press "2" MAN (Manual Function)
        - Press "3" Clean
        - Press "Start"
        - Open door and clean piercing units with a soft cloth and ethanol. Piercing unit is sharp.
        - Wipe piercing unit with di water.
        - Close Door and Press "ENT"
        - Press "ESC"
    - Check that the tray, and racks are clean, if needed clean with ethanol and then di water.
    - UV for 20 minutes.

7.5.8: Instrument or Equipment Cleaning Procedures

- Centrifuges
  Wipe out the inside of the centrifuge with 10% bleach solution as needed, or appropriate cleaner as recommended by manufacturer.

- Biological Safety Hood
  After each use, wipe down inside of hood with 10% bleach.

7.6: Performance Checks

Any new critical instruments or equipment 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.

SECTION 8: PROFICIENCY

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

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

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

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

8.1: Personnel

Proficiency testing pertains to those DNA Analysts actively engaged in DNA testing. It is mandatory that the DNA Analyst conduct the entire test alone without selecting or accepting any assistance from other persons. Violation may result in disciplinary action for those receiving and those rendering assistance. If the examiners have any questions or require assistance, they should contact the DNA Technical Leader. In order to avoid unfair advantages to other examiners at different stages of analyzing the same proficiency test samples, they may not consult one another with regard to their samples, procedures,

analysis or interpretations. To do so defeats the purpose of proficiency testing for the individual and the laboratory. Newly qualified analysts will complete a proficiency test within 6 months of their qualification.

8.2: Frequency

Proficiency tests are performed semi-annually such that each DNA Analyst is tested at least twice a year, (once in the first six months of the year and a second in the second six months of the year). There must be at least four months between each test, and not more than eight months between tests. For the purpose of tracking the time between tests, the date the test is performed has been designated as the date of the proficiency review.

8.3: Specimen

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

8.4: Documentation of Proficiency Test Results

When the proficiency test is complete, all results (proficiency test case file) will be given to the Technical Leader.

The Technical Leader will provide a yearly summary of who was tested and status of their performance. This information will be documented in a separate secure filing system. Documentation of this is also submitted to the NDIS Administrator.
*It is noted that all proficiency tests must be processed consistent with the normal processing of casework, including all associated documentation (technical and administrative review.)
A *Proficiency Test Summary Form* for each completed proficiency test must be completed and given to the Quality Assurance Manager.

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

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

Report Format for DNA Analyst's Test Findings

Some conclusion is required as to whether the unknown and known specimens could have a common origin or whether an exclusion can be demonstrated. Adequate and correct discrimination must be demonstrated in order to pass the proficiency test.

Review and Reporting of Proficiency Test Results

The Technical Leader and the Casework Supervisor 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. This review should be conducted in a timely manner. The electronic copy of the proficiency test is the official copy.

8.5: Evaluation of Proficiency Test

1. All reported inclusions are correct or incorrect
2. All reported exclusions are correct or incorrect
3. All reported genotypes and/or phenotypes are correct or incorrect according to consensus genotypes/phenotypes or within established empirically determined ranges.
4. All results reported as inconclusive or un-interpretable are consistent with written laboratory guidelines. The basis for inconclusive interpretations in proficiency tests must be documented. The technical leader must review and initial on the review sheet that any inconclusive result complies with the laboratory's guidelines.
5. All discrepancies/errors and subsequent corrective actions must be documented.
6. All final reports are graded as satisfactory or unsatisfactory. A satisfactory grade is attained when there are no analytical errors for the DNA profile typing data. Administrative errors shall be documented and corrective action taken to minimize the error in the future.
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. When proficiency test reviews are documented in the analyst's Personal History Binder, the *Date File Reviewed* indicates the date that the technical review occurred. The *Date Results Reviewed* indicates the date the official results from the proficiency provider are reviewed. Results are deemed satisfactory if all technical review aspects are correct. If, upon receipt of the official results, discrepancies are found, corrective action will be required and documented.

8.6: Corrective Action for Proficiency Test Errors

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

8.7.1: Authority and Accountability

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

8.7.2: Types of Errors

8.7.2.1: Administrative Error

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

1. A second sample set may be submitted to an individual within one week if the Casework Supervisor believes discrepancies occurred in the first test sample set. The second sample or test material will be different than the first sample but will apply to the same subject matter under testing. The individual will immediately examine the second sample upon receipt.
2. If an error of this type is not detected until the Analyst has concluded their analysis, and therefore negates their work, they must be issued an additional proficiency test set. The duplication of analysis due to administrative error in no way reflects negatively on the analyst. However, the cause of the error should be found and eliminated from future proficiency tests.
3. If an error is due to any clerical or administrative error (typographical or otherwise – not including analyst sample mix-up or improper storage), the internal review processing steps must be evaluated to eliminate or reduce errors.

8.7.2.2: Systemic Error

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

8.7.2.3: Analytical / Interpretative Error

1. Any significant discrepancy in a proficiency test result determined to be the consequence of an analytical /interpretative discrepancy must

prohibit the individuals involved in producing the discrepant result from further examination of case evidence until the cause of the problem is identified and corrected. The Technical Leader determines the need to audit prior cases based upon the type of error and its cause.

2. Before resuming analysis or interpretation of casework, an additional set of open proficiency samples must be successfully completed by the individual responsible for the discrepancy.

8.7.3: Documentation

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

8.7: Storage

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

SECTION 9: CASE RECORD

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

9.1: Reviews

9.1.1: Technical Review

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

9.1.1.1: The Case File

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

- Submission Sheet
- Extraction Sheet

- Worksheets (* When appropriate)
    - Quantitation Sheets
    - STR AMP Sheets
    - Master Mix Sheet
    - Plate Loading Sheet *
    - Call Sheets
    - PopStats *
    - CODIS *
- GeneMapper Data
- Review Sheet
- Evidence Retained
- Case Report

9.1.1.2: Technical Review Sheet

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

- Are notes present which adequately describe the packaging and description of the evidence?
- Were all necessary analyses performed and documented according to established guidelines?
- Was the request of the agency addressed by the work performed in the case (reasonable)?
- Have all out of bin Microvariants been confirmed?
- Have all controls, internal lane standards and allelic ladders been verified for expected results?
- Are all genotypes correct and all peaks meet the required threshold of 100rfu?
- Are all genotypes correct and transcribed (including proficiency)?
- Have all the necessary sample files been imported into the GeneMapper 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?
- If this case is an exclusion, are there any profiles consistent with the exclusionary sample noted on the plate?
- If DNA testing results are inconclusive or of no probative value, is there additional evidence suitable for further testing (documentation must be present).
- Is the appropriate specimen category and sample indicated on the CODIS Entry Sheet?

9.1.2: Administrative Review

An administrative review does not need to be qualified in the technology used in the case, but the administrative reviewer must be trained and qualified to perform administrative reviews. The administrative reviewer evaluates the report and supporting documentation for completeness and for editorial correctness. If the administrative reviewer finds an error in the case file, the error should be corrected after consultation with the DNA analyst and/or technical reviewer. Once the error has been corrected, the administrative reviewer will sign the administrative review sheet and scan the review sheet into case images in Justice Trax. The administrative reviewer will electronically initial that the administrative review was completed in Justice Trax and send one (1) copy of the report out to the submitting agency.

9.1.2.1: Administrative Review Sheet

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

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

9.2: Corrective Action

If incorrect results were reported in any CODIS hit, an amended report must be issued reflecting the correction(s).

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

9.2.1: Authority and Accountability

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

9.2.1.1: Administrative Error

Any significant discrepancy in a forensic case determined to be the result of administrative error (clerical or documentation, etc.) may be corrected as follows:

The clerical error will be noted on the official report and initialed. An amended letter will then be sent out to the investigating agency accompanied by a documented phone call.

9.2.1.2: Systematic Error

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

9.2.1.3: Analytical/Interpretative Error

1. Any significant discrepancy in a forensic sample result determined to be the consequence of an analytical/interpretative discrepancy should prohibit the individuals involved in producing the discrepant result from further examination of samples 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.

   Before resuming forensic analysis, an additional set of open proficiency samples must be successfully completed by the individual responsible for the discrepancy.

SECTION 10: TESTIMONY REVIEW

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

SECTION 11: AUDITS

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

11.1: Frequency

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

11.2: Records

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

SECTION 12: COMPLAINTS

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

SECTION 13: MISCELLANEOUS

13.1: Safety

All safety protocol and information is contained in the Arkansas State Crime Laboratory safety manual. The safety manual covers general laboratory safety.

13.2: Outsourcing

The Arkansas State Crime Laboratory will only outsource to a vendor laboratory that complies with Quality Assurance Standards and accreditation requirements of federal law and can provide documentation of the compliance. Prior to any outsourcing of data, the DNA Technical Leader will document the approval of the technical specifications.

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

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

Appendix A

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

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

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