The Arkansas State Crime Laboratory

CODIS Section

Quality Assurance Manual

Table of Contents

CODIS Section

QUALITY ASSURANCE MANUAL

SECTION 1: INTRODUCTION

The Combined DNA Index System (CODIS) is a computerized program designed to house DNA profiles from convicted offender / arrestees, deceased individuals, missing persons and relatives of missing persons, Arkansas State Crime Laboratory staff, forensic cases (both evidence samples and suspect's known reference samples). The purpose of CODIS is to create a national information repository where law enforcement agencies can share DNA information obtained from convicted offender / arrestees and forensic evidence. This system allows agencies to cross reference case evidence profiles with that of other agencies' case evidence profiles.

Currently, there are three levels of CODIS: **National DNA Index System (NDIS), State DNA Index System (SDIS)** and **Local DNA Index System (LDIS**). The Arkansas State Crime Laboratory participates as a State and Local system that has the capability to upload (movement of DNA profiles between systems at different levels) DNA profiles to the National level. The Arkansas State Crime Laboratory is responsible for not only analyzing all convicted offender / arrestee samples for the state, but also to enter and search all crime scene samples obtained from forensic casework. The Federal Bureau of Investigation (FBI) maintains the National level.

As data is entered in the CODIS system it immediately becomes available to search at the State DNA Index System (SDIS).

The National DNA Index System (NDIS) is a centralized index of DNA profiles administered by the FBI. DNA profiles that are allowed by NDIS are contributed to NDIS by participating State CODIS laboratories. The profiles from all forensic cases nationally are searched at this level against the Offender Index and against all the profiles in the Forensic Index. NDIS requires a convicted offender / arrestee profile to contain results from all 13 CODIS core loci (CSF1PO, TPOX, THO1, vWA, D16S539, D7S820, D13S317, D5S818, D3S1358, D8S1179, D21S11, and FGA) and a forensic case profile to contain results from at least 10 of the 13 core loci.

The CODIS software is designed by and provided to the Arkansas State Crime Laboratory by the FBI. Upgrades and modifications to the software are periodically provided to the lab by the FBI through the FBI's contractor. The use of the CODIS system in Arkansas is in accordance with the most current version

of the CODIS User Guide, CODIS Training Reference Manuals, CODIS Installation support documents and CODIS Technical Notes provided to the lab by the FBI and the FBI's contractor. CODIS is a dynamic system and therefore undergoes frequent major and minor software upgrades, which may cause the actual operation of the software to not exactly reflect the policies and procedures in this document. Modifications to this manual will be made to accommodate the changes as necessary.

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 CODIS 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 CODIS Administrator and the CODIS 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 CODIS Analysts must be notified of the changes and must be given any necessary training.

1.1.2: Relationship of Individuals and Job Responsibilities:
CODIS Section's Organizational Chart is located in CODIS-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: CODIS Administrator

2.1.2.1: Responsibility

The CODIS administrator is responsible for the administration of the laboratory's local CODIS network.

2.1.2.2: Job Function

a. Overseeing day-to-day operation of the CODIS Section i.e., scheduling workload, supervising analysts and technicians, monitoring and reviewing. These duties may be distributed among the CODIS Analysts to facilitate case flow.

b. Scheduling and documentation of the CODIS computer training of CODIS analysts.

c. Assurance that the security of data stored in CODIS is in accordance with state and/or federal laws and NDIS operational procedures.

d. Assurance that the quality of data stored in CODIS is in accordance with state and/or federal laws and NDIS operational procedures.

e. Assurance that matches are dispositioned in accordance with NDIS operational procedures.

f. 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.

2.1.2.3: 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.

2.1.3: CODIS Analyst

2.1.3.1: Responsibility

The CODIS analyst is responsible for performing DNA analysis and specifically delegated QA responsibilities from the CODIS Administrator.

2.1.3.2: 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.

2.1.3.3: Qualifications

a. Education

The CODIS 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 CODIS 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 CODIS analyst shall have a minimum of six (6) months of experience of human DNA lab experience. This training entails the analysis of a range of samples routinely encountered in forensic databasing 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 CODIS 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: CODIS Quality Manager

2.1.5.1: Responsibility

The CODIS quality manager is responsible for implementing the quality assurance program for the CODIS 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: CODIS Technician

2.1.6.1: Responsibility

The database coordinator is responsible for processing convicted offender / arrestee samples that are delivered to the laboratory.

2.1.6.2: Job Function

a. Receives convicted offender / arrestee samples into the laboratory
b. Enters the convicted offender / arrestee samples into the State Convicted offender / arrestee Database System
c. Ships database collection kits to law enforcement personnel
d. Schedule training with law enforcement agencies
e. Facilitate communication between collection facilities
f. Prepares samples for DNA analysis

2.1.6.3: Training

The CODIS technician shall complete the CODIS training program with individuals, agencies, or other laboratories that have an established training program and considerable experience in DNA methods and casework. Additionally the technician shall successfully complete a competency test and proficiency test before beginning independent DNA analysis.

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, and CODIS analysis. The DNA technical leader, CODIS Administrator and Casework Supervisor 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: CODIS Quality Manager

a. Can reject any chemical, reagent, supply or material which fails to meet the specifications set forth in the QC 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 CODIS Administrator and/or Technical Leader will initial to signify approval.

2.3.3: CODIS Administrator

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

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

2.3.4: CODIS 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

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.

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.

Database Security

To ensure the security of the DNA database the CODIS Server must remained behind locked doors at all times unless in use by the CODIS Administrator or designee.
All Analysts that access the CODIS database must be DNA analysts and have an FBI background check as per the NDIS guidelines. Each computer is password protected with individual logons. Logons and passwords must not be shared. No analysts, except the CODIS Administrator, should be logon to more than one CODIS computer concurrently.

DNA Laboratory Set-up

The CODIS 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.

DNA Pre-PCR Laboratory

The DNA Pre-PCR area consists of sample handling, DNA extraction and isolation, and preparation of samples for 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 that the small aliquots are retained until typing of the set of samples for which the aliquots were used is completed.
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.

CODIS Post-PCR Laboratory

The CODIS Post-PCR area consists of 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. Store plates of amplified DNA in the work area until all reviews are completed.

SECTION 4: CONVICTED OFFENDER / ARRESTEE SAMPLE CONTROL

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

NOTE: Convicted offender / arrestee samples are handled differently than casework evidence due to the fact that offender samples are not considered evidence at the Arkansas State Crime Laboratory they are considered reference materials.

4.1: Convicted Offender / Arrestee Sample Handling Procedures

Convicted offender / arrestee samples enter the Arkansas State Crime Laboratory through the Evidence Receiving Section of the laboratory. The samples are then sent to the CODIS section. A CODIS Technician assigns a unique number to each convicted offender / arrestee sample. The sample is stored in a secure area before and after analysis.

All samples are worked in chronological order according to the unique identifier number unless directed by the CODIS Administrator or designee. All CODIS hit confirmations will be expedited in the work flow process.

All samples are collected, received, handled, sampled and stored so as to preserve the identity, integrity, condition and security of the sample.

4.2: Chain of Custody

A clear, well-documented chain of custody must be maintained from the time the convicted offender / arrestee sample is first received by the CODIS unit.

4.3: Transferring

When CODIS samples are transferred between CODIS employees, the sample(s) must be scanned out to the employee receiving the sample(s).

4.4: Release of Information

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

4.5: Disposition

All sample remaining after analysis will be retained by the CODIS section. The CODIS section will not store amplified products after sample has been uploaded to NDIS.

4.6: Destruction of Evidence

The CODIS Section destroys no samples.

4.7: Sample Handling and Storage

The following written policy ensures that samples will be handled, processed and preserved so as to protect against loss, contamination or deleterious change. Testing of CODIS 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 CODIS Section.

**See NRC 1996 recommendations*

4.7.1: Sample Labeling

Each working sample must be labeled with a unique identifier. The CODIS Technician of the CODIS section generates this unique identifier. For convicted offender samples this number is designated by the year, an "-0-" and numerical order of cases submitted to the laboratory (ex: 2009-0-12345). For arrestee samples this number is designated by the year, an

"-1-" and numerical order of cases submitted to the laboratory (ex: 2009-1-12345). Other identifiers may be utilized if appropriate for the specific case. The numerical order of cases submitted will restart at the beginning of the year.

NOTE: All samples prior to January 1, 2007 were designated by the year and numeric order of cases submitted. Ex: 2006-12345.

4.7.2: Convicted offender / arrestee Processing

The convicted offender / arrestee samples accepted for DNA are tracked during analysis and accounted for on internal forms included in each case folder. The examination worksheets include the following forms:

1. Plate Map
2. Identifiler Master Mix
3. CODIS Database Failure Review Sheet*
4. CODIS Database Review

These forms should be numbered, initialed and dated by the analyst.
*The CODIS Database Failure Review Sheet is not present in each case folder

1. Prepare the work area. The bench space must be clean and free of clutter.

2. 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.

3. The CODIS Analyst fills in each form completely with appropriate information, sample numbers and lot numbers. Once samples are complete and imported into the CODIS system, these forms and the import reconciliation forms are stored together in a binder.

4.7.3: Long Term Storage

Upon completion of the testing, the CODIS Analyst has the ultimate responsibility for long-term storage of the samples. All samples are returned to a CODIS Technician for long term storage. This is tracked by the chain of custody which is located in the DNA Database program on the computer. All CODIS samples are stored in the Evidence Section of the Arkansas State Crime Laboratory.

4.7.4: Request for Buccal Collection Kits

All requests for database kits should be made in writing on the agency's letterhead. All requests will be documented within the CODIS Section. All requested kits, if available, will be sent by the Arkansas State Crime Laboratory to the requesting agency.

4.7.5: Expungements

It is recognized that occasionally a profile that was previously entered into CODIS will need to be expunged. The following process will allow for expungements:

Removal and destruction of the DNA record and DNA sample:

Any person whose DNA record is included in the State DNA Data Base and whose DNA sample is stored in the State DNA Data Bank as authorized by Arkansas Law may apply to the State Crime Laboratory for removal and destruction of the DNA record and DNA sample if the arrest that led to the inclusion of the DNA record and DNA sample:

(1) Resulted in a charge that has been resolved by:
(A) An acquittal;
(B) A dismissal;
(C) A nolle prosequi;
(D) A successful completion of a pre-prosecution diversion program or a conditional discharge; or
(E) A conviction of a Class B misdemeanor or Class C misdemeanor; or has not resulted in a charge within one (1) year of the date of the arrest.

The State Crime Laboratory shall remove and destroy a person's DNA record and DNA sample by purging the DNA record and other identifiable information from the State DNA Data Base and the DNA sample stored in the State DNA Data Bank when the person provides the State Crime Laboratory with:

(1) A court order for removal and destruction of the DNA record and DNA sample; and (2) Either of the following:
(A) A certified copy of an order of acquittal;
(B) An order of dismissal;
(C) An order nolle prosequi;
(D) Documentation reflecting a successful completion of a pre-prosecution diversion program or a conditional discharge;
(E) A judgment of conviction of a Class B misdemeanor or Class C misdemeanor
(F) A court order stating that a charge arising out of the person's arrest has not been filed within one (1) year of the date of the arrest.

The State Crime Laboratory shall not remove or destroy a person's DNA record or DNA sample if the person had a prior felony or Class A misdemeanor conviction or a pending charge for which collection of a DNA sample is authorized under Arkansas law.

An Expungement Request form should be completed with each Expungement process. When the State Crime Laboratory removes and destroys a person's DNA record and the State Crime Laboratory shall request that the person's DNA record be expunged from the National DNA Index System.

4.7.5.1 Expungement Responsibilities from NDIS

Federal law requires that states participating in NDIS expunge the DNA records of persons whose qualifying convictions had been overturned.

The Federal DNA Identification Act of 2001 requires states that participate in NDIS promptly expunge DNA profiles if the state receives the following from the responsible agency or official:

- A certified copy of a final court order establishing that the specific qualifying offense has been overturned
    - A court order is not considered "final" for these purposes if time remains for an appeal or application for discretionary review with respect to the order (Federal DNA Identification Act).

For states uploading the DNA data of arrestees, indicted persons or similar legal specimens, amendments made by the DNA Fingerprint Act of 2005 require expungements in the event of the charge is dismissed or results in an acquittal or no charge was filed within the applicable time period.

NDIS participating states are required to expunge from NDIS the DNA profile of a person included in NDIS by that State if:

- the person has not been convicted of an offense on the basis of which that analysis was or could have been included in the index and
- the responsible agency or official of that State receives, for each charge against the person on the basis of which that analysis was or could have been included, a certified copy of a final court order establishing that such charge has been dismissed or has resulted in an acquittal or that no charge was filed within the applicable time period."

4.7.6 Administrative Removals

Administrative removal may be warranted in such occasions: (1) and individual did not meet a qualifying offense, (2) the collection agency notifies the Arkansas State Crime Laboratory, (3) there was a procedural deficiency in the collection of the DNA sample that cannot be resolved, or any other reason deemed necessary by the CODIS Administrator or Technical Leader.

To complete and Administrative Removal a 'Deleted/Amended Specimen Request' Form must be completed along with the supporting paperwork necessary.

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, 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 CODIS Section:

Commercial Kits:

Identifiler DNA Kits	Applied Biosystems
Minifiler DNA Kits	Applied Biosystems
Profiler Plus Kits	Applied Biosystems

Miscellaneous Items:

9947A	Promega
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. All commercial reagents will be labeled with the identity of the reagent and the expiration date if applicaple.

6.1.1.2: Supply and Materials Inventory

Upon receipt of all materials, reagents, chemicals and supplies, the packing slip will be checked for agreement with the items received when available. The packing slips include information on supplies, i.e. catalog number, lot number, date received, manufacturer's expiration data, and initials of receiver. Reagents and supplies, which have passed their expiration date, will not be used on CODIS samples. They may, however, be designated for use on non-critical samples, i.e. training samples, etc.

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 is 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 CODIS Section. Quality control test results will be recorded in the Quality Control of Critical Reagents Binder.

6.1.1.7.1: Identifiler, Minifiler, and Profiler Plus 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.

6.1.1.7.2: 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 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

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, CODIS Administrator and/or Laboratory Director will be consulted to analyze the samples and reagent blank. If after analysis the source of the contaminating DNA does not appear to be in the samples, then the contamination will be noted in the report. If the extraneous DNA is present in both the reagent blank and associated sample, then the sample will be reported as inconclusive.

6.1.2.2: Positive Control

The positive control contains DNA from a known source with a known DNA profile. The positive control will be amplified and analyzed concurrently in the same instrument with the same samples and same PCR kit.

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.

NOTE: Internal Positive Control: A NIST traceable internal positive control may be run along side the 9947a. If the control genotypes are correct, the amplification is considered correct and the samples can be used.

6.1.2.3: Negative Control (AMP-)

The negative control (amplification blank) contains all the reagents for the amplification mix but no DNA. The negative control will be amplified and analyzed concurrently in the same instrument with the same samples and same PCR kit.

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

6.1.2.4: 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.

Allelic Ladder

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) 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 CODIS analyst is found to be the source of the contamination, the CODIS Administrator will be notified and take the necessary corrective actions. If the contamination is from outside the CODIS section, the appropriate supervisor will be notified to address the contamination source.

6.2: Standard Operating Procedures

6.2.1: Convicted offender / arrestee Samples Processing (CODIS Technician)

6.2.2.1: Intake of Convicted offender / arrestee Samples (Blood Samples)

A. Database envelopes are collected from Evidence Receiving

B. Envelopes are opened in a clean area and kits are placed in bundles. Gloves will be worn during the processing of any biological sample.

C. All convicted offender / arrestee data on the database card is carefully entered into the Arkansas State Crime Laboratory DNA database program. Any missing offender information can be searched using ACIC (see ACIC Access 6.2.2.1). The following is the process to enter a convicted offender / arrestee in the in-house database program:

1. Click on the "DNA Database" icon located on the desktop
2. Click on "File"
3. Click on "Add a New Offender"
4. Enter in all appropriate data

5. If the entered data is a duplicate, a screen will appear to verify the information
6. If the sample is a duplicate, print a barcode label, attach it to the database card and file with original
7. If there is not a duplicate offender found, click on "Add/Print" and print out a new barcode
8. Five barcode labels are generated and placed with each kit
9. Barcode labels are placed in the following locations:
    a. Outside of coin envelope
    b. Inside of coin envelope (loose)
    c. Database card/upper left corner on fingerprint side
    d. Database card/inside on "Place Barcode Here"
    e. On cut sample

D. Each blood sample is halved. One half of the blood stain card is placed in a coin envelope to be analyzed and the other half is to be retained for confirmation purposes.

E. The database cards (the half with the offender's information) are boxed numerically for storage.

F. The coin envelopes are filed in a secure storage cabinet to be analyzed.

G. Coin envelopes are scanned out to the analyst or technician for punching.

H. After punching, the coin envelopes are scanned back in and filed with the original database cards.

NOTE: If inadequate sample amount or inadequate information is given on the database card, a phone call is made to the submitting officer or his/her supervisor.

6.2.2.2: Intake of Convicted offender / Arrestee Samples (Buccal Samples)

A. Database envelopes are collected from Evidence Receiving

B. Envelopes are opened in a clean area and kits are placed in bundles of 10 for counting purposes. Gloves will be worn during the processing of any biological sample.

C. All convicted offender / arrestee data on the database card is carefully entered into the Arkansas State Crime Laboratory DNA database program. Any missing offender information can be searched using ACIC (see ACIC Access 6.6.2.1). The following is

1. Click on the "DNA Database" icon located on the desktop
2. Click on "File"
3. Click on "Add a New Offender"
4. Enter in all appropriate data
5. If the entered data is a duplicate, a screen will appear to verify the information
6. If the sample is a duplicate, print a barcode label, attach it to the database card and file with original
7. If there is not a duplicate offender found, click on "Add/Print" and print out a new barcode.
8. Five barcode labels are generated and placed with each kit
9. Barcode labels are placed on the outside of the envelope on in the specified area on the 'Specimen Identification Card'.

NOTE: Extra barcodes are placed with the envelopes.

D. Barcodes are placed on the DNA Collectors in the DNA clean room.

E. DNA Collectors are punched and placed in Long Term Storage.

NOTE: If inadequate sample amount or inadequate information is given on the database card, a phone call is made to the submitting officer or his/her supervisor.

6.2.2.1: ACIC Access

Prior to obtaining access to the Arkansas Criminal Information Center (ACIC) an individual must attend a training class and be issued a unique CSN (Central System Number) and certification. The training gives options to access different data depending on the "known" information available.

To access Criminal History of an offender:

A. LOGON
B. After confirmation of "Connection Successful" and "LOGON Accepted" information can be obtained
C. F4: Query Name; can be accessed when only the name is known
D. F5: The agency information will be automatically filled in. Under "PERSON DATA" enter all available information for the offender
E. F6: REQUESTING OFFICER (OFC) is the name of the individual requesting the data (last name, first initial). Fill in the "OPERATOR DATA" (OPR) the same as OFC data.
F. Use the "+" key to enter data (not "enter" key)
G. F7: Queries with only the SID (State ID) number

H. F2: LOGOFF

NOTE: Most information is listed under the F5 option

6.2.2.2: BSD Punch

1. Making Plates:

   a. Open BSD Duet 600 software. Icon for program is on computer desktop.
   b. Enter username and password. Click: 'Continue'
   c. Click: 'Edit Test Sequences'
   d. Click: 'Create a new test'
   e. Select appropriate punching file then Click: 'Open'
   f. Each well (96 total) will be listed as a ***Sample.*** Double click the wells you want to change. Mark all cells that are not going to be used as ***Unused.*** All wells that will contain + and – controls need to be labeled ***Liquid Control.***
   g. After labeling all the cells, Click 'Test' in the toolbar. Click 'Test Configuration'. Change direction to 'Vertical'. A template plate can be used instead of creating a new plate format.
   h. Click 'File'. Click 'Save.' Name plate and Click 'Save'.
   i. After test is saved, close Test window.

2. Filling Plates:

   a. Click Distribute Spots button on main BSD menu.
   b. Place a 96 well plate in the BSD Punch.
   c. Wait for spot detector to self adjust. Click 'Continue' when button appears.
   d. Click 'Continue' at next screen.
   e. Select appropriate plate in left hand list. Only the plate you want should be check marked.
   f. Above Continue button; the Samples, Standards and Controls should be checked. Cleaning should not have a checkmark.
   g. Click 'Continue'
   h. Starting sample is #1. Click 'Continue'.
   i. Enter plate name. Click 'Continue with manually entered barcode or scan the plate barcode'.
   j. At next screen Click 'Continue'.
   k. Start scanning the sample barcodes and punching the appropriate samples.
   l. If you have to enter a barcode manually, after typing in the barcode Click 'Continue with manually entered barcode'.

m. All CODIS hit verifications must contain the letters CH at the end of the barcode numbers.
n. After last spot is punched, Click 'All Spots Present' on pop up screen. Click 'All Spots Present' on second pop up screen.
o. Click: 'Print plate maps and end'.

6.2.2.3: Transferring Plate Setup to 3130xl

1. Set-up data for 3130xl:

a. Put Travel Drive into USB Slot.
b. Open FP2 from desktop.
c. Select INPUT FOLDER.
d. Make sure box 1,1 is highlighted.
e. Select "Import DATA" from the Import External Data menu from the DATA menu.
f. When prompted for the data file select "BSD Output Files" from the Desktop.
g. Change Type of Files to ALL FILES at the bottom of the window.
h. Scroll down and Select the last file in the list that has a file size of 5 or 6 KB.
i. Make sure delimited is check and press NEXT.
j. Check Tab and Comma then click FINISH and OK.
k. Press Control-J
l. Press Control-H
m. Go to the Output tab on the bottom
n. Select Save-As and select Travel Drive, TEXT TAB Delimited – NAME PLATE (i.e. 09-##)
o. Press YES, then YES
p. Select Save-As – EXCEL WORKBOOK- NAME PLATE (i.e. 2009-##)

2. Transferring to 3130xl:

a. Put Travel Drive into USB Slot.
b. Select Plate Manager.
c. Press IMPORT.
d. Select txt file on Travel Drive.
e. Click OK
f. Make sure file imported correctly.

6.2.2: Extraction Protocols

6.2.2.1: Reagents

6.2.2.1.1: Tris EDTA Buffer (TE) (1L)

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

6.2.2.1.2: 0.1 M Tris - HCL, pH 7.3 (250 mL)

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 3.03 g of Tris base in 250 ml of DI water. Adjust to pH 7.3 (+/- 0.1) at room temperature by adding approximately 45 ml of concentrated HCL.
Expiration Date: 2 Weeks

6.2.2.1.3: 0.01 N NaOH (250 mL)

Dissolve 0.1 g of Sodium Hydroxide (NaOH) in 250 mL of DI water.
Expiration Date: 2 Weeks

6.2.2.2: Manual Washing of the FTA Blood Punches

1. Wash each 1.2 mm punch three times with FTA Purification Reagent.
2. Soak samples between wash for approximately 5 minutes.
3. Rinse samples two times with TE Buffer with approximately 5 minute soak period.
4. Let samples dry in oven.
5. Samples are now ready for amplification.

6.2.2.3: Manual Washing of the Buccal Punches

1. Place 52 µl of .01 M NAOH in each well with the 2.0 mm.
2. Incubate samples at 65°C for 10 minutes.
3. Add 10 µl of .1 M Tris HCl (pH 7.3).
4. Mix.
5. Let stand for 5 minutes.
6. Samples are now ready for amplification.

6.2.2.4: Automated Washing of the Buccal Punches

1. Turn on Robot and Heat Block
2. Select RUN on the Computer
3. Select METHOD from the Menu
4. Select OPEN from the method menu
5. Select the method you want, corresponding the the number of plates you have to wash.
6. Press START
7. Ensure the setup on screen matches the Robotic platform
8. Ensure the temperature on the heat block is 95°C
9. Click ACCEPT ALL
10. Once the run is complete exit progam
11. Turn off robot and heat block

6.2.2.5: Controls

Reagent Blank (RB)

The reagent blank tests for possible contamination of the sample preparation, reagents, and/or supplies by an external DNA source. 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.

6.2.3: Amplification Protocols

6.2.3.1: AmpFℓSTR® Identifiler ™ PCR Amplification Kit

6.2.3.1.1: 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 fluorescent dye during oligonucleotide synthesis (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

6.2.3.1.2: Amplification Setup

Samples amplified using AmpFℓSTR Identifiler chemistries.

Create a master mix of PCR reagents by combining the reagents. According to the Master Mix sheets appropriate for the samples being amplified.

Dispense the amount of Master Mix and samples specified on the Master Mix sheet 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	24*cycles
59°C	1min	24*cycles
72°C	1min	24*cycles
60°C	60min	
4°C	forever	

*NOTE: 24 cycles are used for 1.2mm FTA punched cards which are manually washed. 25 cycles can be used for 1.2mm FTA punched card when 24 cycles fails to produce a complete profile twice. 28 cycles are used for samples which are extracted by alternative methods.

6.2.3.1.3: 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.

6.2.3.1.4: 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.

6.2.3.1.5: Analysis of Raw Data / GeneMapper ID

GeneMapper ID analysis software is used to analyze the raw data collected by the 3130xl 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. All convicted offender / arrestee data is analyzed using GeneMapper ID

2. Open GeneMapper ID

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

4. All sample files should be located in My Computer→Local Disk (H:)→CODIS Run data

5. Find correct sample files

6. Click: Add to List

7. Click: Add or Add & Analyze

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

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

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

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

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

13. Re-Run all profiles that are incomplete for the CODIS core 13 loci and all profiles that experience out-of-bin microvariants or tri-allelic patterns. These samples can be tracked on the CODIS Database Failure Review Sheet.

14. Save the GeneMapper project for review and import into CODIS

6.2.3.1.6: Controls

6.2.3.1.6.1: 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-injected or re-amplified. If the typing results remain above threshold after re-injection or 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 CODIS Administrator, DNA technical leader and/or Laboratory Director will be consulted to analyze the samples and reagent blank. If after analysis the source of the contaminating DNA

does not appear to be in the samples. If the extraneous DNA is present in both the reagent blank and associated sample, then the sample will be reported as inconclusive.

6.2.3.1.6.2: 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.

6.2.3.1.6.3: 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.

6.2.3.1.6.4: 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.

6.2.3.1.7: Interpretation Guidelines

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

- Conclusions for CODIS samples 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 is to amplified STR data and determine the DNA profiles for NDIS.

- 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.

6.2.3.1.7.1: 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.

6.2.3.1.7.2: 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.

6.2.3.1.7.3: 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.

6.2.3.1.7.4: 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 CODIS Administrator or Technical Leader may need to be notified to determine how to report the locus).

6.2.3.1.7.5: 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.

6.2.3.1.7.5.1: 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 should be noted and may be re-loaded.

6.2.3.1.7.5.2: 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.

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

6.2.3.1.7.5.3: 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.

6.2.3.1.7.5.4: 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.

6.2.3.1.7.5.5: 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.

6.2.3.1.8: STR Profile Interpretation

Amplified products from convicted offender / arrestee 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 called in the CODIS section must meet a minimum RFU threshold of 100.

In general, a single source profile at each locus will appear as a single peak or a double peak. On rare occasions, a tri-allelic pattern may be detected. The observation of tri-allelic patterns does not preclude that locus from interpretation. However, a tri-allelic pattern must be confirmed by, at minimum, re-injecting the sample.

Inconclusive Allele Calls: In those cases where peaks are not present or are below the minimum 100 RFU threshold for D2S1338 and D19S433, allele calls for that sample at that locus may be designated as inconclusive "INC". If any of the CODIS core loci have alleles that are not present or are below the RFU threshold, the sample must be re-amplified to gain a complete profile at the 13 core loci.

6.2.3.2: AmpFℓSTR® Minifiler ™ PCR Amplification Kit

The use of Minifiler in CODIS is restricted to samples that have exhibited peaks that fall between the following loci with Identifiler; D8S1179 / D21S11, and D16S539 / D2S1338. It will be used to confirm which loci the peak belongs to.

6.2.3.2.1: 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	Label	Chromosomal	Alleles Included in Identifiler	Control

Locus		Location	Allelic Ladder	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

6.2.3.2.2: 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	*cycles
59°C	2min	*cycles
72°C	1min	*cycles
60°C	45min	
4°C	forever	

*NOTE: 25 cycles are used for 1.2mm FTA punched cards which are manually washed. 28 cycles are used for 2.0mm Buccal samples.

6.2.3.2.3: Sample Setup for the 3130xl Instrument

The same Sample Setup for the 3130xl is used in Identifiler as are used in Minifiler please refer to the previous section of Sample Setup for the 3130xl Instrument.

6.2.3.2.4: 3130xl Instrument Setup

The same Instrument Setup is used in Identifiler as are used in Minifiler please refer to the previous section of 3130xl Instrument Setup.

6.2.3.2.5: 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).

6.2.3.2.6: Controls

The same controls and Interpretational Guidelines are used in Identifiler as are used in Minifiler please refer to the previous section of Controls and Interpretational Guidelines.

6.2.3.2.7:

6.2.3.2.8: Note:007 is the positive control that is supplied with the AmpFℓSTR® Minifiler™ PCR Amplification Kit. However, 9947a supplied with the Identifiler kit can be used in place of the 007 as a positive control for Minifiler.

Stutter Ratios for Minifiler:

LOCUS	**STUTTER RATIOS APPLIED**
D13S317	0.06
D16S539	0.06
D2S1338	0.09
D18S51	0.16
AMEL	0.0
FGA	0.13
D21S11	0.06
D7S820	0.05
CSF1PO	0.05

6.2.3.3: AmpFℓSTR® Profiler Plus ™ PCR Amplification Kit

The use of Profiler Plus in CODIS is restricted to samples that have exhibited peaks that fall between loci with Identifiler. Any loci gap except D8S1179 / D21S11, and D16S539 / D2S1338, which will use Minifiler. It will be used to confirm which loci the peak belongs to

6.2.3.3.1: Background

The AmpFℓSTR® Profiler Plus™ PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies 10 STR loci in al single PCR reaction. The kit amplified the loci in the

The following table shows the loci amplified by the Profiler Plus kit and the corresponding dyes used. The AmpFℓSTR Profiler Plus Kit Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder and the genotype of the Control DNA 9947a are listed in the table.

Table 2. The AmpFℓSTR® Profiler Plus™ PCR Amplification System

STR Locus	Label	Chromosomal Location	Alleles Included in Identifiler Allelic Ladder	Control 9947a
D18S51	JOE	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	JOE	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
D3S1358	5-FAM	3p	12, 13, 14, 15, 16, 17, 18, 19	14, 15
FGA	5-FAM	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
D8S1179	JOE	8	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	13, 13
VWA	5-FAM	12p12-pter	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24	17, 18
Ameogenin	JOE	X:p22.1-22.3 Y: p11.2	X, Y	X
D7S820	NED	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15	10, 11
D13S317	NED	13q22-q31	8, 9, 10, 11, 12, 13,	11, 11

			14, 15	
D5S818	NED	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16	11, 11

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 three dyes used in the AmpFℓSTR® Profiler Plus™ PCR Amplification Kit to label samples are 5-FAM™, VIC®, NED™ dyes. The fourth dye, ROX®, is used to label the GeneScan™-500 Size Standard.

6.2.3.3.2: Amplification Setup

Samples amplified using AmpFℓSTR Profiler Plus chemistries.

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

AmpFℓSTR PCR Reaction Mix	21.0µL
AmpliTaq Gold DNA Polymerase	1.0µL
AmpFℓSTR Primer Mix	11.0µL

Dispense 30 µL of master mix and 20 µL of sample into a 96-opti well plate. It is recommended that 10 µL of 9947a and 10 µL of TE buffer is used for the positive control. However, the amount of input 9947a can vary upon analyst discretion and injection conditions. 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 Profiler Plus:

95°C	11min	
94°C	1min	*cycles
59°C	1min	*cycles
72°C	1min	*cycles
60°C	45min	
4°C	forever	

*NOTE: 25 cycles are used for 1.2mm FTA punched cards which are manually washed. 28 cycles are used for 2.0mm Buccal samples.

6.2.3.3.3: 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 ROX 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.

6.2.3.3.4: 3130xl Instrument Setup

The same 3130xl Instrument Setup is used in Identifiler as are used in Profiler Plus please refer to the previous section of 3130xl Instrument Setup.

6.2.3.3.5: Analysis of Raw Data / GeneMapper ID

15. All data is analyzed using GeneMapper ID
16. Open GeneMapper ID
17. Add samples to project (File→Add Sample to Project)
18. Find correct sample files
19. Click: Add to List
20. Click: Add or Add & Analyze
21. By using the pull down menus ensure the Sample type, Analysis Method and Specimen Category are correct.
22. Click on the green arrow or Analysis→Analyze to begin the process.
23. Check the Size Match Editor and Display plots for each sample analyzed.
24. If needed, the raw data can be accessed to confirm calls.
25. Describe the reason for each artifact deleted (ex; pull-up, spike, etc…).
26. 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'.

6.2.3.3.6: Controls

The same Controls and Interpretational Guidelines are used in Identifiler as are used in Profiler Plus please

refer to the previous section of Controls and Interpretational Guidelines.

NOTE: The following is the Stutter Ratios for Profiler Plus:

6.2.3.4 Re-Runs

All samples that have been labeled as re-run will be reprocessed. The sample will be marked and verified for re-run. . Recently entered sampled into SDIS will be compared to all sampled into the DNA Database at the point of sample data entry.

6.2.4: CODIS Data Import and Searching

6.2.4.1: Import STR Data

All STR data created by the Arkansas State Crime Laboratory or a contract company which is NDIS acceptable (see NDIS Acceptance Form) will be entered and searched in CODIS.

Profiles can be manually entered or entered using the import program into the system. Any profiles entered into CODIS by the import program must be in the Common Message Format (CMF). Each CMF file must have a unique file name to ensure that the correct file is entered into CODIS. The Arkansas State Crime Laboratory maintains several indexes of data in CODIS.

6.2.4.1.1: Creating a CMF File

1. File → Export table from CODIS
2. Save the file on desktop or a thumb drive
3. Fill in Source and Destination as "AR06035Y"
4. Click Export

6.2.4.1.2: Importing a CMF File

1. Open specimen manager
2. Click "Import"
3. Locate and highlight files ready for import
4. Click "Open"
5. Assign Read, when prompted and Click "OK"
6. Ensure that correct number of files are imported
7. Open DNA Comm
8. Under the "Import Files" tab, click on each file imported to either verify or execute the file

9. Under "Import Reports" tab, click each file to create a reconciliation report and print.
10. Ensure all loci/samples have successfully been imported.
11. All reconciliation reports must be filed.

6.2.4.2: Searching the CODIS Indexes

6.2.4.2.1: Keyboard Searches

1. All profiles from the casework section will be keyboard searched and a confirmation sheet placed in the case file prior to entry to SDIS.

2. Keyboard searches will be searched against the following indexes:

   a. Deceased Victim's Knowns
   b. Incomplete Forensic Mix STR
   c. Incomplete Forensic STR
   d. Incomplete Offender STR
   e. Offender STR
   f. Staff and Suspect
   g. Incomplete Profiles

NOTE: No profile will be searched in the CODIS system until a technical review is preformed on the sample in question.

3. If a match occurs against a convicted offender / arrestee, a Database Coordinator should be consulted to begin the confirmation process. A "CODIS Hit Verification" form should be used. (See Section 12.0 for Verifying a CODIS Hit).

4. If a match occurs against another casework sample, the match should be investigated.

5. To perform a keyboard search:

   a. From the Target Profile window, type the Lab ID field (AR060035Y)
   b. From the Target Profile Window, type the Specimen ID field.
   c. Enter the allelic values for the loci

d. Conduct the Search and print out the match results to be placed into the case file
e. If a match is made, the analyst must set the disposition
f. Save all matches to Match Manager.

6.2.4.2.2: AutoSearches

1. Periodically AutoSearches are performed. Many indexes are searched against each other. For a complete list of indexes searched, please see the AutoSearcher program.
2. All convicted offender / arrestee samples must be confirmed.
3. All hits must be investigated to determine the disposition of the match

6.2.4.2.3: NDIS Searches

1. Once a week, a search is performed at the NDIS level. The matches are routinely checked.
2. Convicted offender / arrestees are verified and confirmations are sent once requested by the agency with the hit. The confirmations are sent to other NDIS hits on "CODIS DNA Match Data Response" forms.
3. Matches with ASCL cases and other NDIS agencies convicted offender / arrestees are requested for verification by using "CODIS Match Data Requested". Once the confirmation is received a CODIS hit letter is sent to the investigating agency.

6.2.4.2.4: CODIS HITS

Every convicted offender / arrestee match must be confirmed before a "CODIS Hit Letter" is sent to the agency. The CODIS Administrator or designee will send the letter to the agency.

1. Inform a Database Coordinator or the CODIS Administrator of any hit that should be confirmed. Hits will also be determined by routine Autosearches.
2. A "CODIS Hit Verification" form should be completed
3. The Database Card or DNA Collector is taken from secure storage
4. The sample is processed (See Section 6)

5. Every CODIS Hit should contain the letters 'CH' at the end of the unique identifier number.
6. The fingerprint is confirmed in the Latent Print Section
7. After confirmation and review a "CODIS Hit Letter" is sent to the investigating agency or a "CODIS DNA Match Data Response" is sent to the CODIS Administrator of the other matching laboratory.

NOTE: A minimum of 8 core loci have to be obtained for a CODIS confirmation.

6.2.4.2.5: Familial Searching

When a DNA profile is obtained from evidence (forensic unknown) it is routinely searched in the Arkansas CODIS Database. If a potential familial match is determined the match will be reviewed by the following criteria: a complete profile shares at minimum13 STR alleles; an incomplete profile will be examined on a case-by-case basis by the CODIS Administrator or designee. Any potential familial match with a potentially related profile, the name of the offender may be released to the investigating agency if the protocol outlined below has been followed and all of the following conditions are met:

1. The crime scene (evidence) profile is a single-source profile or an interruptible mixture.
2. The case must be a sexual assault or homicide. Any other case must have prior approval by the Laboratory Directory and CODIS Administrator.
3. All complete profiles must be searched at NDIS prior to review of the potential familial match.
4. The case is unsolved and all investigative leads have been exhausted (conversation sheet must document that an inquiry has been made about the case with the submitting officer).
5. No other probative evidence yielding biological fluids are available for DNA typing.
6. Y-STR typing of the same crime scene (evidence) that resulted in the potential familial match must be concordant with the 'matching' Y-STR profile.

If all of the above conditions are met, the CODIS Administrator, DNA Supervisor and DNA Casework analyst assigned to the case must review all the information before a decision is reached to release the name of the individual.

Any predetermined case that matches the above criteria may go into a Batch Target file if approved by the CODIS Administrator. This file will be periodically searched with modified search parameters and all matches reviewed for familial match eligibility.

6.2.4.3: Match Dispositions

6.2.4.3.1: Candidate Match

Candidate Match is defined as a possible match between two or more DNA profiles discovered by CODIS (AutoSearcher, Searcher, Batch Search, or Remote Searcher.) Candidate Matches must be confirmed or refuted by qualified DNA analysts. If profiles from multiple laboratories are included in a Candidate Match, a qualified DNA analyst from each lab must participate in the confirmation process. Candidate Match dispositions will be overwritten by newer match results.

6.2.4.3.2: Waiting for More Loci

Waiting for More Loci is an intermediate step indicating that the match is undergoing confirmation by at least one qualified DNA analyst. The qualified DNA Analyst confirming the Candidate Match has determined that one (or more) additional loci must be analyzed before the match can be confirmed or refuted. The Candidate Match enters the Waiting for More Loci state until the loci are completed. Waiting for More Loci dispositions will be overwritten by newer match results.

6.2.4.3.3: Pending

Pending indicates that the Candidate Match is being confirmed by a qualified DNA analyst. The Candidate Match enters the Pending step until the qualified DNA analyst completes the conformation process and either declares or refutes the Pending match.

6.2.4.3.4: Offender Hit

Offender Hit occurs when one or more forensic samples are linked to a convicted offender / arrestee sample. Offender Hits are sometimes called “Case-to-Offender” hits.

6.2.4.3.5: Forensic Hit

Forensic Hit occurs when two or more forensic samples are linked at LDIS, SDIS or NDIS. Forensic hits are sometimes called "Case-to Case" hits.

6.2.4.3.6: Conviction Match

Conviction Match occurs when CODIS matches a DNA profile developed from crime scene evidence to a DNA profile from a convicted offender / arrestee, but the crime from which the evidence was collected has already been solved and linked with the offender.

- For intrastate matches, a Conviction Match is usually between the convicted offender / arrestee's DNA profile and the evidence used to convict him/her
- For interstate matches, a Conviction Match usually indicates that the perpetrator has been convicted of a different crime in another state.

In some instances, a Conviction Match can be determined directly from reviewing the Candidate Match; the Pending and Waiting for More Loci steps can be skipped. In a sense, Conviction Matches are a form of blind external testing; in other words, the offender ought to match the evidence for which she/he was convicted.

6.2.4.3.7: Benchwork Match

Benchwork Match is like a Conviction Match, except it applies only to the Forensic index. Benchwork Matches occur when profiles from several cases link external to CODIS (i.e. the example links the cases by matching DNA profiles on the workbench) are also matched by CODIS.

6.2.4.3.8: Offender Duplicate

Offender Duplicate indicates the two convicted offender / arrestee profiles match. Offender Duplicates occur when the Offender index is searched against itself. While an Offender duplicate match does not provide probative information, it is a form of quality control testing.

6.2.4.3.9: Investigative Information

Investigative information is a cross between a No Match and a Warm Match. Consider the following scenario: a

police officer develops a suspect in a violent crime and has the suspect's profile searched in CODIS. The suspect's profile does not match any other profiles in CODIS. Although the search is a No Match, it does provide probative information. Based on the suspect being excluded by the CODIS search, the investigating agency can re-deploy resources to other suspects/leads.

6.2.4.3.10: No Match

During the confirmation process a qualified DNA analyst determines that a match is dispositioned as Candidate, Pending or Waiting for More Loci is not a confirmed DNA match.

6.2.4.3.11: Twins

Twins disposition occurs when individuals have the same DNA profile because they are identical twins.

6.2.4.3.12: User Defined #1

Used Defined #1 disposition is used when the sample matches itself, or another sample(s) within the case. This is also a disposition for all miscellaneous matches that are not considered true or valuable matches.

6.2.4.3.13: User Defined #2

User Defined #2 is reserved for all matches that occur because of contamination reasons.

6.2.4.3.14: User Defined #3

User Defined #3 is used when a sample from the deceased victim's index matches a convicted offender.

6.2.4.4: Indexes

6.2.4.4.1: Convicted Offender Index

This index contains profiles from individuals convicted of felonies, misdemeanor sex offenses and violent offenders as according to Arkansas Law (Act 1470 of 2003). It also contains qualifying juvenile offenses according to Arkansas

Law (Act 1780 of 2001). This index is uploaded to NDIS. For specimens that contain out-of-bin microvariants or tri-allelic patterns, the remaining loci may be entered into CODIS pending confirmation.

6.2.4.4.2: Arrestee Index

This index contains profiles from individuals arrested of capital murder, murder in the first, kidnapping, first and second degree sexual assault according to Arkansas Law (Act 974 of 2009). This index is uploaded to NDIS. For specimens that contain out-of-bin microvariants or tri-allelic patterns, the remaining loci may be entered into CODIS pending confirmation.

6.2.4.4.3: Forensic Unknown Index

This index contains profiles from crime scene evidence deemed appropriate for entry into CODIS. This index is uploaded to NDIS. The primary purpose of entering a forensic casework profile into the database is to identify the possible perpetrator of that particular crime for which the DNA analysis was conducted. This should be kept in mind when considering whether a profile is probative and should be entered into CODIS. Forensic 'Unknown' samples for which there is no suspect or the suspect has been eliminated should be entered in as the case number, the item number followed by a question mark (ex. 2006-lit-12345Q1?). 'Unknown' samples which include a submitted suspect should be entered in as the case number, the item number, followed by a CFM (Case File Match) (ex. 2006-lit-12345Q1CFM) CODIS entries should be documented on the "CODIS Entry Sheet". The source ID on case work samples should be marked as either "Yes" or "No" depending if the source has been identified through DNA testing. If a Forensic Unknown profile is incomplete, it can only be entered into the system if it contains ten or more of the core loci.

6.2.4.4.4: Forensic Mixture Index

This index contains a profile from crime scene evidence which has multiple contributors. Mixtures are only deemed appropriate for CODIS if they do not violate the 4X4 Rule. This index is uploaded to NDIS. The 4X4 rule states that

forensic mixture DNA profiles submitted to NDIS shall have up to 4 alleles at a maximum of 4 core loci and the remaining 9 core loci shall have no more than 2 alleles at each locus. Analyst discretion will be used to determine what alleles will be entered into CODIS. The victim's profile will be subtracted from the mixture, leaving the profile that is determined to be the Most Likely Profile (MLP) to have come from the suspect. The profile will be entered into CODIS as the case number, the item number followed by MLP (ex. 2006-lit-12345Q1MLP). The MLP is determined by placing the victim's and evidence profile on the "CODIS Entry Sheet". Another qualified analyst must review the mixture and the MLP determination prior to entering the sample into CODIS.

When three alleles are present and the victim is heterozygous at that locus, the analyst must determine the obligate allele. The following is an example:

Victim = 12, 17 Evidence = 12, 17, 18

The analyst would search and enter this locus as 12, 17, 18+ (+) indicated the obligate allele.

6.2.4.4.5: Deceased Individuals Index

This index contains samples from all deceased individuals which are submitted to the DNA Section by the Medical Examiner's Section. This index is not uploaded to NDIS. The profile will be entered into CODIS as the case number, K#, and V (ex. 2006-li-12345K1V). The source identified field should be marked as "Yes".

6.2.4.4.6: Unidentified Human Remains Index

This index contains profiles from living persons of unknown identity and profiles from recovered dead persons whose identities are not known. This index is uploaded to NDIS.

6.2.4.4.7: Missing Persons Index

This index contains profiles of Known samples of missing persons and profiles obtained by examining intimate items

purported to belong to a reported missing person, such as a tooth brush.

6.2.4.4.8: Staff Index

This index contains profiles of all Arkansas State Crime Laboratory staff members hired since July 18, 2005 and all staff members who worked at the lab prior to that date who volunteered their samples. Each member of the staff is given a unique number that is only known to the CODIS Administrator.

6.2.4.4.9: Suspect Knowns Index

This index contains profiles of suspects submitted in DNA cases. The suspect "Knowns" do not need to be entered on Case File Matches (CFM), only the evidence sample profile should be entered. The profile will be entered into CODIS as the case number and K# (ex. 2006-lit-12345K#).

6.2.4.4.10: Incomplete Forensic Unknown Index

This index contains profiles from crime scene evidence deemed appropriate for entry into CODIS that contain less than 10 loci. This index is not uploaded to NDIS. The primary purpose of entering a forensic casework profile into the database is to identify the possible perpetrator of that particular crime for which the DNA analysis was conducted. This should be kept in mind when considering whether a profile is probative and should be entered into CODIS. Forensic 'Unknown' samples for which there is no suspect or the suspect has been eliminated should be entered in as the case number, the item number followed by a question mark (ex. 2006-lit-12345Q1?). 'Unknown' samples which include a submitted suspect should be entered in as the case number, the item number, followed by a CFM (Case File Match) (ex. 2006-lit-12345Q1CFM) CODIS entries should be documented on the "CODIS Entry Sheet". The source ID on case work samples should be marked as either "Yes" or "No" depending if the source has been identified through DNA testing.

6.2.4.4.11: Incomplete Forensic Mixture Index

This index contains a profile from crime scene evidence which has multiple contributors that contain less than 10

loci. Mixtures are only deemed appropriate for CODIS if they do not violate the 4X4 Rule. This index is not uploaded to NDIS. The 4X4 rule states that forensic mixture DNA profiles submitted to NDIS shall have up to 4 alleles at a maximum of 4 core loci and the remaining 9 core loci shall have no more than 2 alleles at each locus. Analyst discretion will be used to determine what alleles will be entered into CODIS. The victim's profile will be subtracted from the mixture, leaving the profile that is determined to be the Most Likely Profile (MLP) to have come from the suspect. The profile will be entered into CODIS as the case number, the item number followed by MLP (ex. 2006-lit-12345Q1MLP). The MLP is determined by placing the victim's and evidence profile on the "CODIS Entry Sheet". Another qualified analyst must review the mixture and the MLP determination prior to entering the sample into CODIS.

When three alleles are present and the victim is heterozygous at that locus, the analyst must determine the obligate allele. The following is an example:

Victim = 12, 17 Evidence = 12, 17, 18

The analyst would search and enter this locus as 12, 17, 18+ (+) indicated the obligate allele.

NOTE: The CODIS Administrator will resolve all discrepancies on match dispositions, CODIS index and STR entries.

6.2.5: FSS-i^3 Sample Analysis

6.2.5.1: Process RAW Data

1. Open GeneMapper ID.
2. Add samples to project (File→Add Sample to Project).
3. Find correct sample files.
4. Click: Add to List.
5. Click: Add.
6. By using the pull down menus ensure the Sample type is set to allelic ladder for the ladders, Analysis Method is set to RAW panel is set to Identifiler_FSSi3_v1.0.

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

8. Click on the "Genotypes" tab.

9. Select "Export Table" from the file menu.

10. Save table as the same file name as the project.

11. Minimize GeneMapper ID.

6.2.5.2: Import and Analyze Data with FSS-i^3

1. Open FSS-i^3.

2. Select Batch Import (top-left).

3. Select the GeneMapper ID Table file that was saved above.

4. Check to see if Ladders passed
   *Only one ladder has to pass in order to continue with analysis.

5. Click "Designate Alleles" (bottom-right)

6. Click "Apply Rules" then Click Yes in center box.

6.2.5.3: Analysis Guidelines

FSS-i^3 will analyze the data against a set of rules that is described below. If the data exhibits peaks that are outside the 'acceptable' range for the rules, then FSS-i^3 will mark the sample for review and designate what rule caused the need for review.

6.2.5.3.1: Rule Set

1. Amelo
   - Marks the sample if there is no X allele is present

2. Signal:noise
   - Marks the sample if there are no designated alleles

3. Extra Allele

- Marks the sample if more than 2 alleles are designated in a loci

4. Extra Peak
   - Marks the sample if there is a peak over 100 rfus that does not fall in an allele bin

5. High Signal
   - Marks a samples if there is signal above 9000 rfus

6. Low Heterozygote
   - Marks a sample if there are two alleles and one is below 150 rfus

7. Low Homozygote
   - Marks a sample if there is only one allele and it is below 250 rfus

8. minusA
   - Marks a sample if there is a split peak separated by one basepair

9. Off Ladder
   - Marks a sample if an allele is more than 0.75 basepairs from the expected size

10. Peak Morph
    - Marks a sample if the peak morphology (base:height ratio) is more than 18%

11. Pre Amp
    - Marks a sample if there is a peak imbalance of more than 50%

12. Primer Dimer
    - Marks a sample if there are peaks that are over 1000 rfus in the 30 to 100 basepairs region

13. Pull Up
    - Marks a sample if a peak matches with a peak in another dye within .5 basepairs and is less than 30% of the peak

14. Rares
    - Marks a sample if the allele name has a OL in the name, it signifies that it is a microvariant

15. Uncorrelated
    - Marks a sample if the sample show more than .4 basepair deviation from the ladder

16. Degradation
    - Marks a sample if the samples shows a 75 percent decrease in the area of alleles from the left side (small amplicons) to the right side (large amplicons)

6.2.5.3.2: Common Problems and the Associated Rules

1. Above-Below Ladder: The CrossOver rule fired in the first locus that is associated with the allele.
2. Contamination: The Negative rule fired in all the loci that showed a signal above threshold in these samples labeled as reagent blanks.
3. Locus Peak imbalance: The PreAmp AB rule fired in the loci that showed an imbalance in peaks.
4. Micro-Variants: The Rare rule fired in all the loci that showed an OL designation for an allele
5. Missing Locus: The Noise rule fired in all loci that didn't have any designated alleles.
6. Mixture: The profile results show a designation of mixture.
7. Pull-up: The Pull Up rule fired on all loci that showed peaks that corresponded to a peak with a high RFU value in another channel within 0.5 bp.
8. Shoulders: The Split Peak rule fired in all loci that showed a shoulder to a peak.
9. Spikes: The Extra Peak rule fired in several of the channels when a spike was present.
10. Stutter: The Stutter rule fired in all loci that exhibited stutter above the level set in the rules.
11. Tri-Allelic: The Extra Allele rule fired in all loci that contained a tri-allelic pattern.

6.2.5.3.3: Analyzing Data in FSS-i^3

See section 6 of the FSS-i^3 manual for instructions on analyzing batch data and modifying allele calls.

6.2.5.3.4: Prepare data for CODIS Import in FSS-i^3

After last rule is reviewed click the Output box (bottom-right). Highlight CODISEXPORT and click ok. Select "Export Tool" from the Tools menu. Select the output file that was created above. Select Export Format; CMF 1.0. Input Lab number

(AR060035Y) and Click export file. Enter your CODIS ID. Copy .dat file from the D drive and import into CODIS following the SOP.

6.3: Reports

No reports are required for the CODIS Section. A CODIS hit letter informs the agency about a hit. The letter is produced by the CODIS Administrator or her Designee. All CODIS hit documentation is stored in the CODIS section. A copy of official hit letter is also stored in JusticeTrax in the case file(s).

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	Heat Blocks
pH Meter	3130xls
BioMek 2000	

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 and maintained on the 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 on the S:
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 CODIS Quality Manager must inform the Technical Leader and CODIS Administrator 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 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. Schedule for maintenance is found in the DNA drive on the computer.

7.5.1: 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.

7.5.2: Annually

- Pipettes – performance checks and calibrated by an outside company.
- Drift-con- Thermal cycler calibration system must be sent out annually for calibration.
- BioMek 2000- all systems must calibrated and checked by laboratory personnel.
- 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.
- Vacuum Pumps - clean and flush by laboratory personnel.
- Centrifuges - cleaning by laboratory personnel with 10% bleach solution.
- Bio-Mek 2000- Check the pipette spacing

7.5.3: 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).

7.5.4: 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.
- FSS-I^3-performance check by laboratory personnel

- Balances are performance-checked.

7.5.5: Bi-Monthly

- 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.

7.5.6: Monthly

- Swipe tests: Cotton swabs and/or FTA paper are moistened with distilled water and rubbed on the analyst's bench top. All documentation is kept in the in the DNA drive on the computer.
- The 3130xl and computer restarted.
- Burn DVD's with data and back-up the oracle database and clear database.
- BoMek 2000 cleaned with bleach and/or DI water (when in use).

7.5.7: 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.
- BioMek 2000 Position Calibrated
    - Turn on the BioMek and select DIAGNOSTIC on the Computer
    - Wait for the Green Light on the BioMek Communication Software
    - Select ALIGN from the Menu
    - Select Position Calibrate from the Align Menu
    - Once Completed select OK, OK then Close the Diagnostic Program
-

7.5.8: 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.

7.5.9: 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 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 offered from approved ASCLAD-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 analysis.

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 or designee.

The Technical Leader or designee 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 Custodian.
*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, or designee 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 CODIS Administrator 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 CODIS Administrator to assure that discrepancies are acknowledged and that any corrective action is documented.

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 CODIS Administrator 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.

8.7.2: 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 the import of data into SDIS, all CODIS samples are subject to technical reviews. All CODIS hit documents are subject to an administrative review.

9.1: Reviews

9.1.1: Technical Review

All convicted offender samples must have a 100% review of the electronic data before entered into the CODIS system. The "CODIS Database Review" must be completed and all discrepancies must be alleviated before any sample can be entered into CODIS.

9.1.1.1: The Case File

After processing all Convicted Offender / Arrestee Samples, the file should contain a Plate Map, a Master Mix Work Sheet and a Review Sheet. Each worksheet must contain the analyst's and /or technician's initials, date and page number. They are scanned into the appropriate folder on the S:. The import reconciliation reports (if imported by CMF file) are filed along side the completed worksheets. The following is the order in which the worksheets must reside:

1. Review Sheet
2. Plate Map
3. Master Mix Sheet
4. CODIS Database Failure Review Sheet (if applicable)
5. Import Reconciliation Reports (if applicable)

Handwritten notes and observations must be in ink. Nothing in the handwritten information will be obliterated or erased. Any corrections will be made by a single line strikeout (so that what is stricken can still be read) and initials. Correction fluid or correction tape may not be used.

The unique CODIS plate number, handwritten examiner's initials, date and page number must be on each page of the examination documentation in the case record.

9.1.1.2: Sample Review

The laboratory will conduct a technical/peer 100% review of all case files and reports. This review can be conducted using the FSS-I^3 Expert System.

All CODIS samples will be technically reviewed. An examiner qualified under the QAS guidelines will execute the review.

The Technical Leader or CODIS Administrator should resolve discrepancies and concerns that are detected by the technical or

administrative review. The Technical Leader or CODIS Administrator will ensure that appropriate action has been taken before permitting any sample to be entered into the CODIS system.

Steps performed by the Technical Reviewer if Expert System is not used for review of data:

- Technical reviewer must examine all areas described on the CODIS Database Review Sheet.
- During the review process, the reviewer must do the following in addition to those items listed on the review form.
- All allele calls must be checked for accuracy and the absence of mixtures.
- All profiles are acceptable for upload to NDIS
- If discrepancies occur or clarification is needed during the technical review process, the reviewer must notify the CODIS analyst or the CODIS Administrator. The Technical Leader is responsible for any unresolved discrepancies between the analysts and reviewer.
- A review of all notes, all worksheets, and the electronic data (or electrophereograms) supporting the results.
- A review of all DNA types to verify that they are supported by the raw or analyzed data (electropherograms).
- A review of all controls, internal lane standards and allelic ladders with expected results
- A review to confirm that reworked samples have appropriate controls

If an Expert System is being used to conduct the review of data, the following must be technically reviewed by an examiner qualified under the QAS guidelines:

- The Technical Reviewer must ensure that all data has been placed through the Expert System.
- Ensure that all necessary analyses were performed according to established guidelines.
- Ensure that all analyses were documented according to the established guidelines.
- All necessary corrections have been completed.
- All appropriate examination documentation are completed and in proper order.
- All profiles not exported to the CMF file are either added to Re-run list or entered to SDIS via 'STR Data Entry' (i.e. microvariants, trialleles, and samples without the core loci).
- Verify that all samples on the plate will be entered into SDIS or placed on a re-run sheet to ensure a profile is obtained.

If no problems occur during the peer review process, the reviewer sends electronic file and worksheets to the CODIS Administrator or designee for import into CODIS. The analyst importing the samples into CODIS can make minor changes and sign the corrective action line on the "Database Review Form" before the samples are imported into CODIS. If an expert system is being used the analysts can import the data into CODIS.

Administrative Reviews

CODIS hit letters must have an administrative review of the official correspondence. The reviewer must document that he/she reviewed the hit on the 'CODIS Hit Verification' form. The clerical errors must be checked an documented at the bottom of each 'CODIS Hit Letter' with the initial of the individual of the performing the review. The review consists of the following:

- A review of individual's biographical data, qualifying offense, and DNA profile
- A review of accuracy of information
- A review of clerical errors

To ensure all sample have been entered into SDIS monthly all re-run sheets will be searched in 'Specimen Manager' to ensure the sample resides in the system. Also, a list of all Convicted Offenders/Arrestees can be printed and compared to the database to determine which samples have not had a profile entered into SDIS.

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 CODIS analysis. It is best practice to determine when possible to identify the discrepancy.

9.2.1: Authority and Accountability

The CODIS Administrator 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.

9.2.1.1: Administrative Error

Any significant discrepancy in a sample 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 CODIS 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 CODIS 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 CODIS 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 analysis or interpretation of convicted offender / arrestee 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 generator 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.

The Arkansas State Crime Laboratory can enter into CODIS outsourced data for other agencies. Data may only be entered into CODIS if the following is criteria are met:

- All requirements of Standard 17 from the QAS Document are fulfilled
- A letter from the laboratory the case originated stating:
  - NDIS eligibility
  - All potential court cost will be covered by the originating laboratory
  - ASCL has permission to enter the case into the CODIS system
  - A brief synopsis of the case
- Contact with ASCL to the originating state's CODIS Administrator should be made and documented.

A casefile in JusticeTrax may then be set up to electronically maintain the data. A review of all documents must occur prior to entering any data into CODIS. Once the data has been uploaded to CODIS a letter to the appropriate State CODIS Administrator (or applicable individual) should be mailed. All potential CODIS hit letter should be delivered to the appropriate CODIS State Administrator or applicable individual.

13.3: CODIS Hit Counting

The effectiveness of CODIS can be measured in the number of crimes the Hits solve. Thus an accurate measure of hit counting is important. There is a two track metrics involved in hit counting. The primary metric is the number of investigations aided by CODIS. The second metric is the number of hits made by CODIS. Counting the number of Hits gives laboratories credit for their investment in CODIS and indirectly shows the value CODIS adds to fighting crime. The best measurement of CODIS's value to society is the number of criminal investigations it assists.

Hit

A hit is a confirmed match between two or more DNA profiles discovered by CODIS software at a single instant in time. Hits may occur at any level in the CODIS hierarchy, LDIS, SDIS or NDIS. There are two categories of Hits.

- A forensic Hit (FH) occurs when two or more forensic samples are linked at LDIS, SDIS or NDIS. Forensic Hits are sometimes called case-to-case hits.
- An Offender Hit (OH) occurs when one or more forensic samples are linked to a convicted offender / arrestee sample at SDIS or NDIS. Offender Hits are sometimes called case-to-offender hits. Sometimes hits may be classified as both Offender Hits and Forensic Hits. For example, two unknown suspect cases match each other and a convicted offender / arrestee. When this occurs, always classify the hit as an Offender Hit.

Investigations Aided (IA)

Each CODIS hit typically assists one or more criminal investigations. For the purpose of hit counting, a criminal investigation is equated to a case, which equates to a submission to a laboratory. A hit can be considered an Investigation Aided if a case submitted to the Arkansas State Crime Laboratory was assisted by a CODIS hit. The following three rules must be followed to properly count Investigations aided and hits.

Rule #1: The level in the CODIS hierarchy (LDIS, SDIS, NDIS) at which hit occurs get credit for the hit. The following metrics track hit participation.

Rule #2: A single Hit may aid more than one investigation. For each case that is assisted by a CODIS hit, the laboratory that worked the case is credited with one "Investigation Aided". Additionally, laboratories receive participation credit for assisting investigations at the State and National levels.

Rule #3: An investigation may be aided only once. The number of investigations that CODIS has aided is counted, not the number of times CODIS has aided investigations.

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.