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# DNA INDEXING
Last Review Date: September 13, 2024

# FOREWORD

The DNA Indexing Procedures Manual contains policies and procedures for the receipt, processing, storage, and analysis of offender samples and the compilation of files and data for the comparison of DNA results from offenders and probative forensic DNA profiles. The authority for the DNA Indexing Program is by statute (730 ILCS 5/5-4-3 of the United Code of Corrections).

The DNA Indexing Laboratory does not accept or analyze casework reference or standard samples. None of the samples analyzed by the DNA Indexing laboratory are considered evidence and are not subject to the rules and procedures for handling evidence.

The DNA Indexing Laboratory does not issue formal reports of analysis; the laboratory only issues letters of verification. The Report Wording Section is included as a standard Forensic Sciences Command Procedure Manual format.

This manual is written with the understanding that minor variations that do not significantly alter the described procedure may be used. Non-routine procedures not specifically stated in this manual may be used only with the prior approval of the appropriate technical leader.

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## I. CLEAN TECHNIQUE

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## INTRODUCTION

All laboratory personnel must follow Clean Technique. The purpose of clean technique is to prevent unwanted DNA from entering a sample and to protect laboratory personnel from contact with potentially biohazardous materials. The first line supervisor through the laboratory director is responsible for ensuring this policy is followed. There are two main sources of unwanted DNA. The first is extraneous DNA. This is a DNA profile in an offender sample or blank that may have originated from a vendor or a laboratory employee. The second is cross contamination, DNA transferred between samples. There are many sources of cross contamination and extraneous DNA such as: aerosols, liquids or dry flakes/dust, unclean tools, unclean gloves, and contaminating materials on lab coats. Proper precautions must be taken to ensure that cross contamination and the introduction of extraneous DNA does not occur. These precautions must include a clean, disposable lab coat and gloves. Additionally, a mask must be worn during sample check-in.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices

Warning: Treat all reagents/samples as potential biohazards.

Refer to safety considerations under the individual sections of the DNA Indexing Procedures Manual.

## PREPARATION

Equivalent preparations may be purchased commercially, if available.

| | |
|---|---|
| 10% Bleach Solution | |
| Commercial Bleach | 10 ml |
| Water | 90 ml |
| | |
| 70% Ethanol | |
| Denatured Ethanol | 70 ml |
| ddi Water or equivalent | 30 ml |

## INSTRUMENT SPECIFICATIONS

Standard Laboratory Instrumentation.

## MINIMUM STANDARDS AND CONTROLS

Refer to the minimum standards and controls located in the individual sections of DNA Indexing Procedures Manual.

## PROCEDURE

### Initial Steps Prior to Starting Analysis:

1. Disposable gloves must be worn whenever an individual is in the laboratory and might touch equipment. Gloves must be changed between procedures or when visibly contaminated.
2. Conversations between laboratory personnel should be avoided when samples are handled.
3. The temperature of the DNA Laboratory must be monitored and kept at a comfortable level.
4. Decontaminate surfaces where samples are processed with a 10% bleach solution. Ensure the surface is dry before handling samples. Do not store bleach solutions in open containers.
5. Decontaminate all instruments that will be used to process samples (e.g., forceps, scissors, and pipettes) by rinsing with a 10% bleach solution.
6. Place dried samples in clean containers or on clean surfaces for processing. Extract samples by phenol method in a fume hood.
7. For manual extractions, use a 10% bleach solution to decontaminate instruments between samples. Instruments may be rinsed with distilled water after bleaching. Use a new Kimwipe to dry the instrument between uses.
8. For all extraction methods, process a reagent blank.
9. When preparing reagents, one large stock solution will be prepared and dispensed or aliquoted before use.
10. When finished, clean equipment and work areas with the appropriate cleaning solution and when a sample spill occurs.

**Sample Processing Through DNA Extractions:**

1. When processing long-term storage swabs, only one envelope will be open at a time.
2. Process the blanks last for manual extractions.
3. Use clean instruments and fresh glassine paper for each dried stain sample processed by the manual extraction method.
4. Manual extraction: Manually process samples individually. Open only one tube at a time. Close the appropriately labeled tube immediately after a sample has been placed into it. (Do not process samples in a manner that would allow cuttings, flakes, or aerosols of biological material to fall into tubes destined to hold another sample).
5. Aerosol resistant tips (ART) must be used for all manual methods. Place the tip on the pipette immediately before use. When adding aliquoted reagents and pre-aliquoted reagents to each sample (such as proteinase K), a new ART tip must be used.
6. All stock reagents should be closed when processing stains for manual extraction.
7. Process the blanks identically to all the other samples.
8. In order to prevent any liquid from getting on the lids of tubes, briefly spin the tube prior to opening.

**Amplification Set-up:**

1. All amplifications must be set up in a designated area.
2. For all amplifications, process an amplification negative.
3. Pipettes dedicated for amplification set-up and located in the amplification area must be used.
4. The designated amplification area must be bleached before and after the samples are prepared.
5. Plates transferred to the post-amplification room will be discarded in that room and will not be returned to the pre-amplification areas of the laboratory.

**Post-amplification Room:**

1. All surfaces and applicable instruments must be thoroughly cleaned with 10% bleach solution. Capillary electrophoresis instrument parts must be cleaned as directed by the instrument's software wizards. Do not bleach thermal cycler blocks or instrument display screens.
2. When processing samples, disposable lab coats must be worn in the post-PCR rooms and must be discarded in post-PCR area biohazard waste containers.
3. The sample preparation hood and pipettes must be bleached before and after the samples are prepared.

4. After working with amplified DNA, an analyst or technician must not work with any other non-amplified samples that day.

## REPORT WORDING

Not Applicable.

## REFERENCES

Not Applicable.

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## II. DEFINITIONS

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**Administrative Removal**
The removal of a sample upon official notification that it is not eligible for inclusion into CODIS. Administrative removals fall under the "Legal Expungement" category in STACS-DB.

**Allele**
DNA fragments of variable length and/or sequence.

**Alternate CODIS Administrator/Manager**
The alternate CODIS administrator (or equivalent role, position, or title as designated by the Laboratory Director) is an employee of the laboratory responsible for assuming the duties of the CODIS Administrator/Manager in the event the CODIS Administrator/Manager is absent or unable to perform those duties.

**Arrestee (Specimen Category)**
The known sample from a person who has been arrested and in accordance with statute (730 ILCS 5/5-4-3) is required to provide a DNA sample for analysis and entry into the state DNA database. The term "arrestee" includes persons who have been charged in a formal criminal instrument, such as an indictment or an information. The DNA record for the specimen category is stored in the Arrestee Index.

**Arrestee Index**
An Arrestee Index consists of DNA records of persons who have been arrested or indicted or charged with a crime and are required by statute (730 ILCS 5/5-4-3) to provide DNA samples.

**Benchwork Match**
The match of profiles from forensic samples or standard samples from different cases made by the analyst prior to the database search detecting the match.

**Biological Child (Specimen Category)**
The known reference sample voluntarily provided by an adult child or provided with the parental/guardian consent for a minor child of a reported missing person. The DNA record for this specimen category is stored in the Relatives of Missing Person Index and the Pedigree Tree Index.

**Biological Father (Specimen Category)**
The known reference sample voluntarily provided by the biological father of a reported missing person. The DNA record for this specimen category is stored in the Relatives of Missing Person Index and the Pedigree Tree Index.

**Biological Mother (Specimen Category)**
The known reference sample voluntarily provided by the biological mother of a reported missing person. The DNA record for this specimen category is stored in the Relatives of Missing Person Index and the Pedigree Tree Index.

**Biological Sibling (Specimen Category)**
The known reference sample voluntarily provided by the full or half biological adult sibling or provided with the parental/guardian consent of a full or half biological minor sibling of a reported missing person. The DNA record for this specimen category is stored in the Relatives of Missing Person Index and the Pedigree Tree Index.

**Candidate Match**
A possible match between two or more DNA profiles discovered by CODIS software.

**Case Analyst**
The analyst qualified to perform DNA analysis on a forensic case.

**CODIS**
Combined DNA Index System administered by the FBI. CODIS houses DNA profiles from convicted offenders, forensic specimens, population samples, and other specimen types.

**CODIS Administrator**
An employee of the laboratory and CODIS user who is responsible for administration and security of the laboratory's CODIS at a laboratory performing DNA analysis on forensic and casework reference samples or a laboratory that owns the database and/or known samples.

**CODIS Contract User**
An employee of a vendor laboratory who meets the requirements of a qualified DNA analyst and is responsible for producing DNA profiles stored in NDIS, but is not authorized to read, add, modify, or delete DNA records in CODIS. A CODIS Contract user does not fulfill the NDIS requirements for DNA data review and acceptance.

**CODIS Core Loci**
The loci specified by the Federal Bureau of Investigation for PCR DNA records that are required for inclusion in the National DNA Index System. Effective January 1, 2017, the CODIS Core Loci required for inclusion in the National DNA Index System are: CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, and D22S1045. See also Original CODIS Core Loci.

**CODIS IT User**
A government employee of the NDIS participating laboratory or an IT contractor working on-site at the NDIS participating laboratory or its supervisory criminal justice agency, who has login access to the CODIS (i.e., State or Local) system for computer hardware/software and telecommunications maintenance purposes and who shall be processed as a CODIS IT user (and not as a CODIS Contract user). CODIS IT users are not authorized to add, modify, or delete DNA records in CODIS.

**CODIS User**
A government employee of the laboratory who: (1) has login access to the CODIS system and is authorized to read, add, modify, or delete DNA records in CODIS; or (2) is a qualified DNA analyst responsible for producing DNA profiles stored in NDIS.

**Compromised Sample (Specimen Category)**
Unsourced DNA profiles that are found during analysis at the Indexing Laboratory. These are searched against other Indexing profiles to try and find the source.

**Compromised Sample Index**
The Index that houses the profiles from the Compromised Sample Specimen Category.

**Criminal Parentage (Specimen Category)**
DNA profiles from offspring in which the known parent's alleles have been removed to leave only the other parents potential alleles for searching.

**Criminal Parentage Index**
The Index that houses the profiles from the Criminal Parentage Category.

**Convicted Offender**
Convicted offender is an individual required by statute (730 ILCS 5/5-4-3) to submit a standard sample for DNA databasing.

**Convicted Offender (Specimen Category)**
The known sample from a person who has been convicted in accordance with statute (730 ILCS 5/5-4-3) is required to provide a DNA sample for analysis and entry into the state DNA database. The DNA record for this specimen category is stored in the Offender Index.

**Deduced Missing Person (Specimen Category)**
The DNA profile of a reported missing person that has been generated by examining intimate items purported to belong to the missing person such as a toothbrush, and compared to close biological relatives, if possible. Considered a reference sample, this DNA record is stored in the Missing Person Index.

**Designated State Official (DSO)**
The DSO is the person selected by a state to make decisions and to contact on behalf of that state. The DSO is responsible for the participation of local laboratories in that state in NDIS and shall ensure that all laboratories adhere to and comply with the terms and conditions of the Memorandum of Understanding (MOU) between the FBI and the state.

**DNA Index**
A collection of DNA profiles from one or more specimen categories to be maintained. DNA Indexes are searched in CODIS against other designated DNA Indexes.

**DNA Index of Special Concern**
A DNA Index of Special Concern consists of forensic unknown DNA records designated by the NDIS participating laboratory and developed from unsolved homicide, rape/sexual assault, kidnapping and terrorism cases, which will be searched against rapidly enrolled arrestee DNA records.

**DNA Record**
A database record that includes the DNA profile as well as data required to manage and operate SDIS, i.e., the Specimen Identification Number; and DNA personnel associated with the DNA profile entry.

**Familial Search**
A DNA familial search is a deliberate search, conducted with CODIS software, to determine if a close biological relative of an offender in the Illinois DNA Index could be the source of the DNA profile from an unsolved criminal case.

**Forensic Index**
A Forensic Index consists of DNA records originating from and associated with an evidence sample from a single source (or a fully deduced profile originating from a mixture) that is found at a crime scene. The Forensic Index contains Forensic Unknowns.

**Forensic Limited (Specimen Category)**
A specimen category in the CODIS software that is stored in the Forensic Limited Index and originates from a single source (or a fully deduced profile originating from a mixture) Forensic Sample attributable to the putative perpetrator with either locus or allelic dropout at any of the 13 core CODIS loci. Profiles from this category have a low Moderate Match Estimate (MME) and are expected to generate a large number of adventitious candidate matches with a one-mismatch search configuration. Samples in this category are not eligible for upload to NDIS.

**Forensic Limited Index**
A Forensic Limited Index consists of DNA records from forensic samples that do not contain results for all 13 core CODIS loci and/or that may indicate a possibility of allelic dropout. These samples also have a low MME.

**Forensic Mixture (Specimen Category)**
A specimen category in the CODIS software that is stored in the Forensic Mixture Index and originates from a forensic sample (biological sample found at the scene of a crime) that contains DNA contributed from more than one source attributable to a putative perpetrator(s).

**Forensic Mixture Index**
A Forensic Mixture Index consists of DNA records from forensic samples that contain DNA contributed from more than one source. The Forensic Mixture Index contains Forensic Mixture DNA records.

**Forensic Partial (Specimen Category)**
A specimen category in the CODIS software that is stored in the Forensic Partial Index and originates from a single source (or a fully deduced profile originating from a mixture) Forensic Sample attributable to the putative perpetrator with either locus or allelic dropout at any of the 13 Original CODIS core loci.

**Forensic Partial Index**
A Forensic Partial Index consists of DNA records from forensic samples that do not contain results for all 13 Original CODIS core loci and/or that may indicate a possibility of allelic dropout.

**Forensic Sample**
A biological sample originating from and/or associated with a crime scene and whose source is attributable to a putative perpetrator. These are not reference samples from known individuals, such as from victims, suspects, offenders, etc.

**Forensic Targeted (Specimen Category)**
A DNA profile developed from a specimen that was generated from a crime scene that is attributable to a putative perpetrator. The forensic targeted specimen category allows for specimens that meet the accepted MRE threshold at SDIS and NDIS. The specimens are searched with configurations set for floating moderate and mixture profiles.

**Forensic Targeted Index**
The Index that houses the profiles from the Forensic Targeted Specimen Category.

**Forensic Unknown (Specimen Category)**
A specimen category in the CODIS software that is stored in the Forensic Index and originates from a single source (or a fully deduced profile originating from a mixture) Forensic Sample attributable to the putative perpetrator that has full results at the 13 Original CODIS Core Loci.

**Hit**
A confirmed match that aids an investigation and one or more of the case(s) involved in the match are unsolved.

**Indexing Analyst**
An analyst qualified to perform DNA analysis on indexing samples.

**Interpretable Allele**
A DNA fragment specified by a PCR analysis that meets ISP DNA Indexing interpretation guidelines.

**Juvenile (Specimen Category)**
The known sample from a juvenile who has been convicted in accordance with statute (730 ILCS 5/5-4-3) and is required to provide a DNA sample for analysis and entry into the state DNA database. The DNA record for this specimen category is stored in the Offender Index.

**Legal (Specimen Category)**
The known reference sample from a person whose DNA sample is collected under applicable legal authorities (State Law), provided that DNA samples that are voluntarily submitted solely for elimination purposes shall not qualify as a Legal specimen. This would include, but is not limited to, victims of homicidal means collected under statute (55 ILCS 5/3-3013). The DNA record for this specimen category is stored in the Legal Index.

**Legal Index**
A Legal Index consists of DNA records of persons whose DNA samples are collected under applicable legal authorities (State Law).

**LIMS**
Laboratory Information Management System (LIMS) is the laboratory software used to effectively manage samples and associated data. LIMS automates workflows, integrates instruments, and manages samples and associated information.

**Manipulation Blank**
A control consisting of a sterile swab and all reagents used in the manual extraction process. This control is manipulated in the same manner as the samples being extracted at that time. This is used to detect DNA contamination.

**Maternal Relative (Specimen Category)**
The known reference sample voluntarily provide by a maternal biological relative who is not a mother, child or sibling of a reported missing person. The DNA record for this specimen category is stored in the Relatives of Missing Person Index and the Pedigree Tree Index.

**Missing Person (Specimen Category)**
The known reference sample from an individual that is missing. The source of the DNA has been verified as originating from the missing person and is stored in the Missing Person Index.

**Missing Person Index**
A Missing Person Index consists of DNA records from missing persons and deduced missing persons.

**Monitor**
A periodic check conducted on instrumentation to ensure proper performance.

**Multi-allelic Offender (Specimen Category)**
An offender (arrestee, convicted offender, detainee or Legal Index specimen) DNA record having three or more alleles at two or more loci.

**Multi-allelic Offender Index**
A Multi-allelic Offender Index consists of DNA records from offenders (arrestees, convicted offenders, detainees or Legal Index specimens) having three or more alleles at two or more loci.

**National DNA Index System (NDIS)**
NDIS is the storage area that contains selected data from each participating SDIS laboratory and various federal laboratories.

**Negative Amplification Control**
A negative amplification control is used to detect DNA contamination of the amplification reagents. This control consists of only amplification reagents without the addition of template DNA.

**Offender Index**
A Convicted Offender Index consists of DNA records from offenders and juveniles convicted in accordance with statute (730 ILCS 5/5-4-3) and are required to provide a DNA sample.

**Original CODIS Core Loci**
The following 13 CODIS Core Loci were required for inclusion in the National DNA Index from October 13, 1998 until December 31, 2016: CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11.

**Other (Specimen Category)**
DNA profiles produced from biological samples that are not eligible for upload to NDIS, but have been approved for upload to SDIS. These samples do not fit into the other non-NDIS categories such as staff or suspects. These samples include, but are not limited to samples in which an elimination standard has not been requested.

**Other Index**
The Other Index consists of DNA records that do not qualify for upload to NDIS, but may be uploaded to SDIS and do not qualify for entry into other non-NDIS categories such as the Staff or Suspect Indexes.

**Paternal Relatives (Specimen Category)**
The known reference sample voluntarily provided by a paternal biological relative who is not a father, child or sibling of a reported missing person. The DNA record for this specimen category is stored in the Relatives of Missing Person Index and the Pedigree Tree Index.

**Pedigree Tree**
A Pedigree Tree contains genetic information from two or more biological relatives of missing persons (may include spouses, where applicable). A Single Typed Node Pedigree contains the genetic information from only one biological relative of the missing person. Pedigree Trees are also used for Familial Searching.

**Pedigree Tree Index**
A Pedigree Tree Index consists of DNA records of biological relatives and spouses of missing persons that are associated with a Pedigree Tree.

**QC (Specimen Category)**
DNA profiles from known positive controls that are searched for possible crossover into other samples**.**

**QC Index**
The Index that houses the profiles from the QC Category.

**Reagent Blank**

A control consisting of all reagents used in the test process without any sample. This is used to detect DNA contamination of the analytical reagents.

**Relatives of Missing Person Index**
A Relatives of Missing Person Index consists of DNA records from the biological relatives of individuals reported missing.

**Sample Tracking and Control System (STACS-DB)**
The sample tracking system used by the Indexing Laboratory.

**Specimen Category**
DNA profiles in CODIS which originated from a similar type of source (Arrestee, Legal, etc.) and/or further broken down as to the type of profile entered (Forensic Mixture, Forensic Partial, etc.). Specimen categories are stored in DNA Indexes.

**Spouse (Specimen Category)**
The known reference sample voluntarily provided by a presumptive parent of a common child. The DNA record for this specimen category is stored in the Spouse Index and the Pedigree Tree Index.

**Spouse Index**
A Spouse Index consists of the DNA records of a presumptive parent of a common child of a missing person.

**Staff (Specimen Category)**
The known reference samples that have been collected in accordance with Command Directive TCH-21. This includes, but is not limited to, DNA analysts.

**Staff Index**
The Staff Index consists of the DNA records collected in accordance with Command Directive TCH-21.

**State DNA Index System (SDIS)**
SDIS is a storage area for selected DNA records generated by CODIS eligible facilities within a state that are available for searches at the state level. NDIS eligible profiles are transmitted from SDIS to NDIS.

**Suspect, Known (Specimen Category)**
A DNA profile generated from a suspect's known standard in a forensic case.

**Suspect Index**
A Suspect Index consists of DNA profiles originating from suspect's standards in forensic cases.

**Unidentified Human (Remains) Index**
An Unidentified Human (Remains) Index consists of DNA records from recovered living persons (e.g., children and others who can't or refuse to identify themselves),and recovered dead persons (including their body parts and tissues) whose identities are not known.

**Unidentified Person (Specimen Category)**
The DNA profile developed from the recovered deceased (including body parts and tissue) or an individual who is unidentified (e.g., children and others who can't or refuse to identify themselves). The DNA record for this specimen category is stored in the Unidentified Human (Remains) Index.

**Verification**
A quality control procedure where the sample's administrative information and analytical results are reviewed and confirmed prior to release of the offender's information.

**Voluntary (Specimen Category)**
A DNA profile developed from an individual who has voluntarily submitted a sample for inclusion in the State Database in accordance with Illinois Administrative Code Title 20 Part 1285.40.

**Voluntary Index**
The Voluntary Index consists of DNA profiles collected in accordance with Illinois Administrative Code Title 20 Part 1285.40.

**Work Product**
Material generated in analysis that may be discarded upon completion of analysis. Work product includes but is not limited to:

- Extracted DNA from items for which there is sufficient sample to repeat the analysis
- Amplified DNA

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## III. SAMPLE RECEIPT
## A. KIT SUBMISSION

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## INTRODUCTION

This procedure describes how to receive DNA Indexing Kits.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all reagents/samples as potential biohazards.

## PREPARATIONS

Refer to the Clean Technique section.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

When a kit is received in the laboratory:

1. Date each kit or batch of kits upon receipt unless the kit will be processed on the same day.
2. Liquid blood kits should be placed in the refrigerator until they can be processed.

## REPORT WORDING

Not Applicable.

## REFERENCES

Not Applicable.

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

# B. KIT PROCESSING

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## INTRODUCTION

This section describes procedures for kit processing and sample preparation. This procedure requires the use of the STACS-DB Kit Receipt, Sample Check-In, and Storage Subsystem modules. Laboratory personnel performing sample check-in must wear a mask.

Each kit must have the manufacturer's barcode number on the sample receipt. Kits without this number on the receipt will be rejected.

STACS-DB barcodes for the receipt have the format 'Iyy-nnnnnn' where 'I' indicates DNA Indexing section, 'yy' indicates the last two digits of the year the sample was received, and 'nnnnnn' indicates a sequential number beginning with '000001' for the first sample checked-in each year. Barcodes identifying swabs have a sequential letter appended to the I-number.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all samples as potential biohazards.

## PREPARATIONS

Refer to the Clean Technique section.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

### Swab Kit:

1. Check the outer packaging for an intact seal.
2. Remove the swab envelope and receipt from the outer packaging.
3. Check the swab envelope for an intact seal. If neither the outside packaging seal nor the swab envelope seal are intact, indicate on the receipt and reject the submission.

4. Initial and date the receipt with the date the kit arrived in the laboratory. A date stamp is acceptable.
5. Ensure the receipt is filled out with the required information. If necessary, reject the submission and indicate the reason for rejection on the receipt. A reject stamp is acceptable.
6. Scan the manufacturer's swab envelope barcode and receipt barcodes into the STACS-DB Kit Receipt Module.
   A. STACS-DB will alert the user if the barcodes do not match.
   B. If necessary, indicate on the receipt that the submission is rejected for mismatched barcodes.
7. Kits are acceptable if there is no name on the envelope but the manufacturer's barcodes match.
8. Staple pertinent paperwork to the sample receipt (i.e. court orders).
9. Any written additions to the sample receipt must be dated, initialed, and include the source of the information.

The following steps require the use of the STACS-DB Sample Check-In module.

1. Scan storage locations for the swabs when the Check-In Module is initially opened or when storage locations need changed.
2. Scan the 2D barcode on the swab tube.
3. Scan the manufacturer's barcode.
4. Separate the sample receipt from the swab envelope. If the kit has been rejected, enter "No," in the "Kit Acceptable" field and select the appropriate option under the "Unacceptable Reason" drop down menu.
5. Open the swab envelope and count the number of swabs.
   A. Kits must contain at least 4-6 swabs. If not, reject and select appropriate reason and number of swabs.
6. After the barcodes are printed, verify them by scanning them into the "Barcode Verification" screen.
7. Insert one swab into the "A" tube. Break the swab stick so that the tube can be capped.
8. Place the remaining swabs in the envelope. Break the sticks so that the flap can be closed. Tape the envelope closed.
9. Place the STACS-DB barcode, without the appended letter, on the sample receipt. Place the barcode ending in "A" on the swab tube.
10. Place the remaining barcode label(s) on the coin envelope.

**Blood Kit:**

1. Attach a unique barcode label to the kit. Blood kits were not barcoded by the manufacturer. This label serves as the manufacturer's barcode.
2. Scan the barcode into the STACS-DB Kit Receipt Module.
3. Choose "Blood Stain" as sample Nature.
4. Scan the barcode into the STACS-DB Sample Check-In module. Three STACS-DB barcodes will be printed.
5. Attach one label to the blood tube, one to the receipt, and save the third for the stain card.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual.

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## C. PERSONAL DATA ENTRY

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## INTRODUCTION

This procedure describes the conditions and actions involved in entering personal information into STACS-DB. It requires the use of the STACS-DB Submission Worklist module or Submission module.

## SAFETY CONSIDERATIONS

Not Applicable

## PREPARATIONS

Not Applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

1. Scan the STACS-DB barcode.
2. Enter the personal information from the sample receipt.
3. Initial and date any additions or changes to the receipt and indicate the source.
4. Place the receipt in the appropriate location for filing or follow-up.
5. If the sample has been rejected for any reason, refer to the Managing Potential Rejects List procedure.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual

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## D. SAMPLE RECOLLECTION

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## INTRODUCTION

This procedure describes the conditions and actions involved in requesting a sample recollection. It requires the use of the STACS-DB Sample Check-In module, the Submission module, and the Potential Rejects module.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all samples as potential biohazards

## PREPARATIONS

Refer to the Clean Technique section.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

Request that the submitting agency collect a new sample under the following conditions:

1. The collection information is not listed.
2. The sample is received in a condition that is unsuitable**.**
2. The names are inconsistent.
3. The sample is insufficient. The kit must contain 4-6 swabs.
4. More than four swabs were received in the kit if the manufacturer's barcode is <1000000, or more than 6 swabs in the kit if the barcode is >1000000 and the collecting agency cannot confirm they are all from the sample donor.
5. The sample was submitted without an original inked print or the Latent Fingerprint Section has determined the print is unsuitable.
6. The barcode numbers on the receipt and sample envelope do not match or the manufacturer's barcode is missing on the receipt.
7. The first two swabs analyzed appear to contain an insufficient quantity of DNA.
8. Quality issues are identified during the processing of the sample.

Request a sample recollection from the submitting agency as soon as possible after the sample is received. Refer to the Manage Potential Rejects List procedure.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual.

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## E. SAMPLE DUPLICATES

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Approved by:

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DNA Indexing Technical Leader

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## INTRODUCTION

Duplicate samples are submitted by supervising agencies. This procedure describes how to identify and process duplicate samples and requires the use of STACS-DB Submission or Submission Worklist and Potential Duplicate modules. For the procedure regarding Offender to Offender matches, refer to section "Indexing VII-C".

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all samples as potential biohazards.

## PREPARATIONS

Not Applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

**Verbal Identification of Possible Duplicates**:

Follow the procedure outlined below when an agency contacts the laboratory to determine if an offender has already been collected and the sample has been received by the DNA Indexing Laboratory.

1. If all of the information listed below matches the original collection information, and the sample is not voluntary, the sample does not need to be recollected.
   A. First and last names
   B. Date of birth
   C. SID, inmate numbers, and/or previous collection information. Inmate numbers, IDOC, and IDOJJ are considered separate pieces of information.
   D. All additional personal information that is provided must match.

E. If the original eligibility status is marked voluntary, the sample must be re-collected.
F. If the original sample was marked rejected, request a re-collect.

2. If any of the information above does not match, instruct the agency to collect a sample from the offender.

**Identification of Received Duplicates:**

1. A duplicate check is performed by the STACS-DB Submission Worklist module or Submission module.
2. If the sample is flagged as a potential duplicate, click ok.

**Review:**

The laboratory manager or designee will work with agencies to minimize duplicates.

**Analysis:**

Duplicate samples will be routinely analyzed for quality assurance purposes.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual.

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# DNA INDEXING
# PROCEDURES MANUAL

## F. MANAGE MISSING INFORMATION LIST

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## INTRODUCTION

This procedure describes the management of the Missing Information List and requires the use of STACS-DB Submission and Missing Information modules. Submissions on the list may be analyzed. The results may be imported into CODIS on a case-by-case basis.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all samples as potential biohazards.

## PREPARATIONS

Not applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

1. A missing information check is performed by STACS-DB. If information is missing, reject the sample. This will send the sample to the Potential Rejects Module, and the sample is managed from there.
2. Manage the Missing Information List for unresolved samples.
3. Samples should be processed while awaiting personal data.
4. Send missing information request to the submitting agency.
5. Check LEADS or appropriate websites for missing information.
6. Contact the submitting agency for submissions on the list longer than one month.
7. Document changes with the source of information and the date and initials of the person making the change.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual.

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## G. MANAGING POTENTIAL REJECTS LIST

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## INTRODUCTION

This procedure describes the management of the Potential Rejects List and requires the use of STACS-DB Potential Rejects and Potential Duplicates modules. Submissions are placed on the list at 1) Sample Check-In and may not be analyzed until the submission is "Activated" or "Sent to Lab" or 2) Submission entry or edit and they may or may not be available for analysis. Their analysis availability will not be changed by being placed on the list.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices

## PREPARATIONS

Not Applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

1. The Potential Rejects List will be managed for unresolved samples.
   A. Potential Rejects with the exceptions of Check Names and Missing Information (including, but not limited to samples rejected due to insufficient quantity and samples rejected due to quality issues)
      1) In STACS-DB, change the eligibility status to indicate a re-collect.
         i) "Recollect, CODIS OK" means the sample may be activated and can be uploaded to CODIS.
         ii) "Recollect, No CODIS" means the sample may be activated but cannot be uploaded to CODIS without approval from the technical leader or the Assistant Laboratory Director.
      2) Issue letters for pending reject samples using the STACS-DB Potential Rejects Module.
      3) Send the letter to the agency.
      4) Follow-up for recollection regularly.

5) Put rejected samples into process by selecting samples and clicking "Send to Lab".
   i) If a sample is rejected because of too many swabs:
      (a) All swabs must be analyzed.
         (i) Refer to automated DNA isolation from swabs for situations when no genomic peaks are obtained.
6) A list of recollection requests will be maintained electronically.

B. Check Names:
1. Select the sample and click "Send to Lab". This will put the sample into process while the name discrepancy is being resolved.
2. Contact the submitting agency to obtain the necessary information to clarify the name discrepancies.
3. When the information is received:
   i) Document changes and the source of information with the date and initials of the person making the change.
   ii) Unreject the sample.

C. Missing Information:
1. Select the sample and click "Send to Lab". This will put the sample into process while awaiting the missing personal data.
2. Contact the submitting agency to obtain the required missing information or look up via appropriate County websites/LEADS.
3. When the information is received:
   i) Document changes and the source of information with the date and initials of the person making the change.
   ii) Unreject the sample.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual.

# ILLINOIS STATE POLICE

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# PROCEDURES MANUAL

## IV. COMMODITY RECEIPT AND TRACKING

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## INTRODUCTION

This procedure describes the receipt and tracking of commodities and requires the use of STACS-DB Receiving, Storage Subsystem, and Inventory modules. The Laboratory uses STACS-DB to track the QC status of reagents and the inventory status of reagents and commodities.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all samples as potential biohazards.

## PREPARATIONS

Not applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

**Receiving:**

1. Verify that the packing list is accurate.
2. Date and sign the packing list.
3. Place the packing list in the appropriate location.
4. Using the STACS-DB Receiving Module:
   - A. Choose Manufacturer/Supplier from the drop down list and then choose the item being received from the Material/Chemical/ Reagent drop down.
   - B. Enter lot numbers if available.
   - C. For reagents requiring release, use 01/01/2099 as the expiration date unless an expiration date is provided by the manufacturer. The expiration date will be one year from date released.
   - D. For non-reagents with no expiration date marked, set the expiration date so far in the future that the item will not expire before use.
   - E. Enter the Number of Units received. This is the number of barcode labels that will be printed.
   - F. Label each item with a barcode.

G. Store the item(s).

**Chemical Releasing:**

Chemicals with the expiration date of 01/01/2099 require releasing in STACS-DB before use. Chemicals requiring releasing have no manufacturer's expiration date on them and therefore expire one year from date in use. A new barcode with the one year expiration date will be printed through STACS-DB when the item is put into use. This process requires the use of the STACS-DB Chemical Releasing Module.

1. Use the STACS-DB Chemical Releasing Module to release a reagent.
2. Enter an expiration date of one year from the date put into use.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual.

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## V. SAMPLE FILES

## A. PERSONAL IDENTIFICATION (PID) FILES

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## INTRODUCTION

This procedure describes the contents of personal identification (PID) files.

## SAFETY CONSIDERATIONS

Not Applicable.

## PREPARATIONS

Not applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

A PID file is a file that contains the name of the offender and information about the sample. The file will contain, at a minimum, the following for each sample:

1. The original sample receipt and retained associated paperwork.
2. Documentation of conversations and contacts (May be paper or electronic files).
3. The computer card (if RFLP analysis was performed).

## REPORT WORDING

Not Applicable.

## REFERENCES

Not Applicable.

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## B. LABORATORY ANALYSIS FILES

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## INTRODUCTION

This procedure describes the analytical files maintained for offender samples.

## SAFETY CONSIDERATIONS

Not Applicable.

## PREPARATIONS

Not applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

Manual Reviews

1. Plate numbers are assigned by STACS-DB. Extraction plates have an 'EXT' prefix and amplification plates have an 'AMP' or YMP prefix. STACS-DB plate numbers are associated with a date where the format is AAA-yymmdd-nn. 'AAA' is the prefix indicating the plate type, 'yy' is the year, 'mm' is the month, 'dd' is the date of creation, and 'nn' is a sequential number of all plates created on that date.
2. Hard copy files will contain, at a minimum:
   A. Coversheet
   B. Review Table: The first and second-readers review, date, and initial the table. The first page of the review table may serve as the coversheet.
3. Electronic files will contain, at a minimum:
   A. The first and second-readers review and initial each sample electronically in GeneMapper.

4. Laboratory analysis files will be retained and organized by the extraction plate name.
5. Manual processing outside of STACS-DB should be done only when absolutely necessary. All documents generated during the analysis will be retained.
6. STACS-DB will maintain the sample information and analysis history. Use STACS-DB to track both manual and robotic processing. Analysis histories for some samples analyzed prior to January 23, 2005, were maintained in the program "PlateTracker". This information is accessible through the STACS-DB "Historical Sample Processing" report.
7. All data collected from the Genetic Analyzers and generated during data analysis will be organized by amplification plate name.
8. Network server files will be backed up nightly from Monday through Friday. Weekend backups may be made depending on anticipated weekend usage.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual.

# ILLINOIS STATE POLICE

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## C. SAMPLE VERIFICATION FILES

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## INTRODUCTION

This procedure describes sample verification files.

## SAFETY CONSIDERATIONS

Not Applicable.

## PREPARATIONS

Not applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

1. Sample verification documents will be maintained separately from the PID and data review tables.
2. Hard copy hit verification files will contain at a minimum:
   - A. Sample verification form.
   - B. Documentation produced outside of STACS-DB, except electropherograms. These are stored electronically.
     - 1.) Electropherograms from outsourced data may be included in the sample verification file.
   - C. Hit Confirmation Report from the Hit Confirmation Module in STACS-DB or the QA/QC Report from the QA/QC Runs Module in STACS-DB.
   - D. Verification Request for NDIS hits.
   - E. Photocopy of the sample receipt.
   - F. CODIS Match Report.
   - G. Verification letter.
   - H. Verification File Checklist.
3. Electronic hit verification files will contain at a minimum:
   - A. Verification request for NDIS hits.
   - B. Scanned copy of the sample receipt.
   - C. CODIS Match Report.
   - D. Verification letter.

4. Hardy copy hit verification files that include sample duplicates or twins will be organized as follows:
   A. Label the outside of the hit verification file and the pages with the sample dispositioned as the Offender Hit..
   B. The file must include:
      1.) Sample verification form.
      2.) Documentation produced outside of STACS-DB, except electropherograms. These are stored electronically.
         a. Electropherograms from outsourced data may be included in the sample verification file.
      3.) Hit Confirmation Report from the Hit Confirmation Module in STACS-DB or the QA/QC Report from the QA/QC Runs Module in STACS-DB or the duplicate offender match report from CODIS.
      4.) Verification Request for NDIS hits.
      5.) Photocopies of all sample receipts.
      6.) CODIS Match Reports.
         a. Documenting each sample matching the case sample.
         b. Documenting each sample matching each other.
      7.) Verification letter.
      8.) Verification File Checklist.
5. Electronic hit verification files that include sample duplicates or twins will include:
   A. Verification Request for NDIS hits.
   B. Scanned copy of the sample receipts.
   C. CODIS Match Reports.
      1.) Documenting each sample matching the case sample.
      2.) Documenting each sample matching each other.
   D. Verification letter.
6. Hard copy or electronic non-hit sample verification files will contain at a minimum the items listed for a hit verification except the CODIS match report. The verification letter must be included if produced.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual

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# DNA INDEXING PROCEDURES MANUAL

## VI. SAMPLE ANALYSIS

## A. LIQUID BLOOD SAMPLE PREPARATION FOR STORAGE

---

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## INTRODUCTION

Liquid blood samples may be processed manually. This requires the use of the STACS-DB Storage Subsystem module.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices.
Warning: Treat all reagents/samples as potential hazards.

## PREPARATION

Refer to the Clean Technique section.

## INSTRUMENT SPECIFICATIONS

Standard Laboratory Instrumentation.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

1. Attach a STACS-DB label to the stain card. Write the sample number, date and initials on the card.
2. Confirm that the information on the tube matches the information on the receipt.
3. Mix liquid sample thoroughly.
4. Make 5 blood spots on the stain card.
5. Allow to dry thoroughly.
6. Store the stain card.
7. Discard the original blood tube as biohazard waste.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual.

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## B. MANUAL ISOLATION OF DNA

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## INTRODUCTION

Sample extractions may be conducted manually from liquid blood, bloodstains, and swabs. This procedure requires the use of the STACS-DB Extraction module.

Record reagent barcodes, commodity barcodes, sample numbers, instrument barcodes, activity dates, and analyst initials when analysis is performed outside of STACS-DB. Ensure all consumed reagents and commodities are properly discarded in STACS-DB.

## SAFETY CONSIDERATIONS

1. Observe Standard Laboratory Practices.
2. Warning: Treat all reagents/samples as potential biohazards.
3. Warning: The following are considered hazardous reagents:
   A. Phenol-Chloroform-Isoamyl Alcohol is a toxic and corrosive solution that is harmful or fatal if ingested, inhaled, or absorbed through the skin. Furthermore, this solution can cause irritation and/or damage to the eyes and is a suspected carcinogen. Accidental skin or eye contact will be treated according to routine laboratory safety procedures. If ingested, immediately seek medical attention and DO NOT induce vomiting. Special protection: use of a local exhaust hood is required.

## PREPARATION

Refer to the Clean Technique section.

1. DNA Isolation Reagents for Stains:
   A. 390 mM DTT

| | |
|---|---|
| Dithiothreitol | 620 mg |
| ddi $H_2O$ or equivalent | 10 mL |

Aliquot into convenient size volumes and store frozen.
Remix after thawing before use.

   B. Proteinase K (20 mg/mL)

| | |
|---|---|
| Proteinase K | 50 mg |
| ddi $H_2O$ or equivalent | 2.5 mL |

Aliquot immediately into convenient size volumes and freeze.
Remix after thawing before use.

C. Stain Extraction Buffer (SEB)

| | |
|---|---|
| 1.0 M Tris, pH 8.0 | 10 mL |
| 500 mM EDTA, pH 8.0 | 20 mL |
| NaCl | 5.84 g |
| 20% SDS | 100 mL |

Add ddi $H_2O$ (or equivalent) to 1.0 liter. Store at room temperature.

## INSTRUMENT SPECIFICATIONS

Standard Laboratory Instrumentation

## MINIMUM STANDARDS & CONTROLS

A manipulation blank must be processed with each set of samples for every extraction protocol followed. Process the manipulation blank last with each set of samples. The purpose of this control is to ensure that contamination has not occurred due to the manipulation of the sample or the reagents used in the procedure. Whenever an additional manipulation is done on a sample, (i.e., re-concentration of the DNA) the same manipulation must be done to the manipulation blank.

Manual Isolation of DNA may be performed on samples that have one swab remaining. Isolation of DNA should be performed on half the remaining swab. Notify the DNA Indexing Technical Leader before performing analysis on the remaining portion of a sample.

For samples that have only extracted DNA remaining, the sample extract and the associated blank must be labeled, frozen, and preserved for future analysis. Labeling for the sample should include the sample number and STACS-DB plate name. The associated blank must be labeled to identify the samples it is associated with. The plate should be stored appropriately in STACS-DB.

## PROCEDURE

1. Select the samples for analysis from the Manual Plate source worklist in the STACS-DB Extraction module.
2. Scan all reagents and equipment, i.e., pipettes, into STACS-DB.
3. Organic extraction of stains

A. Add the sample to the microfuge tube. Also, process a manipulation blank at this time. Refer to the Clean Technique section and the Minimum Standards and Controls above.
B. To the tube add: 400 µL Stain Extraction Buffer, 10 µL Proteinase K (20 mg/mL), and 5 µL 390 mM DTT.
C. Mix and spin briefly to force the cutting into the liquid.
D. Incubate bloodstains or buccal swabs for at least two hours at 56°C.
E. Place the cuttings in the basket of the microcentrifuge tube. Remove the stain extraction buffer by spinning for 5 minutes at 10,000 x g.
F. Add 500 µL phenol/chloroform/isoamyl alcohol. This step must be done in the fume hood.
G. Vortex for approximately one minute to achieve a milky emulsion in the tube.
H. Spin the tube for two minutes at maximum speed. Note: Do not use microcentrifuge tubes provided for use with Microcon filters for organic extractions.
I. At the analyst's discretion, the aqueous and interface may be re-extracted in a new tube. Discard the old tube containing the phenol into the appropriate waste container in the fume hood.
J. Transfer the aqueous phase from the phenol/chloroform/isoamyl mixture in a Microcon 100 tube.
K. Spin at approximately 500 x g for 8 to 10 minutes. Centrifuge for additional time if liquid remains on the filter. It may be necessary to increase speed to approximately 1500 x g for some samples.
L. Add 50-100 µL of elution buffer and centrifuge as before to wash residual extraction components from the DNA. Examine the filter unit to verify that no tearing or cracking has occurred. Do not use microcentrifuge tubes provided for use with Microcon filters for organic extractions or high-speed centrifugation. They are not designed to withstand these conditions.
M. Add the appropriate volume of elution buffer (30 to 100 µL) (depending on anticipated DNA recovery), invert filter, vortex 15 seconds, and spin out liquid.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual

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## C. YIELD GEL QUANTITATION OF DNA

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## INTRODUCTION

The purpose of quantitation for offender sample analysis is to estimate the amount of DNA present in the sample. A yield gel is used for quantitation of DNA. Quantitation is only required for manually extracted offender samples.

Record reagent barcodes, commodity barcodes, sample numbers, instrument barcodes, activity dates, and analyst initials when analysis is performed outside of STACS-DB. Ensure all consumed reagents and commodities are properly discarded in STACS-DB.

## SAFETY CONSIDERATIONS

1. Observe Standard Laboratory Practice.
2. Warning: Treat all reagents/samples as potential biohazards.
3. Warning: Hazardous reagents:
   A. Ethidium Bromide is a hazardous chemical that may be harmful if ingested and is irritating if exposed to mucous membranes and upper respiratory tract, eyes and skin. It is a mutagen and moderately toxic. Proper personal protective equipment including gloves should be used when making and working with solutions that contain this dye. In addition, yield gels must be run in a dedicated area of the laboratory.

## PREPARATIONS

Refer to the Clean Technique section.

1. DNA Quantitation Reagents:
   A. 1 % Agarose with Ethidium Bromide
      1) Agarose 2.0 g
      2) 1 X TAE 200 ml
      3) Dissolve agarose by boiling in microwave oven.
      4) Add ethidium bromide solution 10 µl.
   B. Ethidium Bromide Solution: prepared or purchased equivalent.
      1) Ethidium bromide 5 mg
      2) ddi H2O 1.0 ml
      3) Protect from light. Mutagenic substance. Wear gloves.
   C. Loading Buffer
      1) Glycerol 5 ml
      2) Bromophenol blue 0.02 g
      3) 10 x TAE 1.0 ml

| | | | |
|---|---|---|---|
| | 4) | ddi H2O | 4.5 ml |

D. 1 x TAE

| | | |
|---|---|---|
| 1) | 10 x TAE | 100 ml |
| 2) | ddi H2O | 900 ml |

E. Yield Gel Standards

1) Lambda phage DNA at 250 µg/ml = stock

2) Carry out serial doubling dilutions of the stock with TE to obtain the solutions shown below:

a. 0.125 ml stock+ 0.875 ml TE = 31.3 µg/ml
b. 0.5 ml 31.3 µg/ml + 0.5 ml TE = l5.6 µg/ml
c. 0.5 ml 15.6 µg/ml + 0.5 ml TE = 7.8 µg/ml
d. 0.5 ml 7.8 µg/ml + 0.5 ml TE = 3.9 µg/ml
e. 0.5 ml 3.9 µg/ml + 0.5 ml TE = 1.9 µg/ml
f. 0.5 ml l.9 µg/ml + 0.5 ml TE = 0.9 µg/ml

3) Combine 0.5 ml aliquots of the diluted standards with 0.25 ml loading buffer to produce the following standard solutions: 125 ng/6 µl, 63 ng/6 µl, 31 ng/6 µl, 15 ng/6 µl, 8 ng/6 µl, 4 ng/6 µl.

## INSTRUMENT SPECIFICATIONS

Standard Laboratory Instrumentation

## MINIMUM STANDARDS & CONTROLS

Yield gel standards are made from dilutions of lambda phage DNA. There are 6 standards of known quantity: 125, 63, 31, 15, 8, and 4 ng. The purpose of these standards is to estimate the amount of DNA present in the samples. Results are interpreted by comparing the signal intensity of the DNA samples to the signal intensity obtained for the DNA standards.

## PROCEDURE

1. Prepare a minigel with 1 % agarose with ethidium bromide. Insert one or two combs, depending on how many lanes are needed. When the gel is set, add IX TAE and remove the combs.

2. Combine 4 µl of each sample with 2 µl loading buffer. In the first six wells load 6 µl of each yield gel standard. Standards must include the quantities 4 through 125 ng.
3. Place the samples mixed with loading buffer (6 µl total volume) into the remaining wells.
4. Electrophorese at 200 volts for approximately 8 minutes.
5. After electrophoresis is complete, remove the gel from the tank. Examine the gel using an ultraviolet light transilluminator. DO NOT EXPOSE YOURSELF TO THE UV LIGHT FOR AN EXCESSIVE AMOUNT OF TIME. ALWAYS WEAR UV PROTECTION WHEN WORKING WITH THE TRANSILLUMINATOR.
6. From the gel, assess the quantity of DNA in test specimens by comparing with the DNA standards. The standards and high molecular weight DNA will migrate as a band only a short distance. A smear from the origin toward the dye front indicates that the DNA has been fragmented.
7. Save a photograph of the yield gel under its corresponding plate name to H: Yield gel photos.
8. Quantitation estimates of DNA must be made by interpolation between two standards of known quantity. Values above the highest visible standard must be designated as greater than (>) the highest visible standard. Such samples must be diluted and rerun. Values below the lowest visible standard must be designated as less than (<) the lowest visible standard.
9. Dilute the extracted DNA solution for amplification.
   A. The following dilutions of DNA will produce a final DNA concentration of 0.5 ng/ul:

| Yield Gel Results | µl Extracted DNA | µl Water |
|---|---|---|
| 125 | 2 | 123 |
| 64 | 4 | 124 |
| 32 | 8 | 120 |
| 16 | 16 | 112 |
| 8 | 16 | 48 |
| 4 | 16 | 16 |

B. Record the yield gel results on the yield gel worksheet.

## REPORT WORDING

Not Applicable.

## REFERENCES

ISP Validation Studies (listed by number):

10. Validation Study I.
2. Quantitation of DNA by Spectrophotometry versus Yield Gels.

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## E. MANUAL AMPLIFICATION OF STRS USING POWERPLEX Y23 KIT

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## INTRODUCTION

This section describes the amplification process of offender DNA samples using the Promega PowerPlex Y23 kit. Amplification reaction volume of 25 µL must be used.

Record reagent barcodes, commodity barcodes, sample numbers, instrument barcodes, activity dates, and analyst initials when analysis is performed outside of STACS-DB. Ensure all consumed reagents and commodities are properly discarded in STACS-DB.

This procedure requires the use of STACS-DB Extraction, Master Mix Addition, and Amplification Modules.

## SAFETY CONSIDERATIONS

Observe standard laboratory practices.
Warning: Treat all reagents/samples as potential biohazards.

## PREPARATIONS

Refer to the Clean Technique section.

Diluted Positive Amplification Control Preparation:

1) Mix 25 µL of the concentrated control with 175ul ddi water (or equivalent). This dilution may be adjusted depending on the quality of the data, and other dilutions may be used with the approval of Technical Leader.
2) Store in the refrigerator.

Amplification Master Mix Preparation:

Calculate the volume of each reagent required.

| Component | Volume (µL) per sample (25 µL) |
|---|---|
| *ddi Water or equivalent | 15.5 |
| PowerPlex Y23 5X Master Mix | 5.0 |
| PowerPlex Y23 10X Primer Pair Mix | 2.5 |
| Total | 23.0 |

Mix and spin briefly.

*This volume of ddi water (or equivalent) is used when amplifying 2 µL of extracted DNA for a 25 µL reaction volume. Other volumes of extracted DNA require the amount of ddi water to be adjusted so that the total volume of water and DNA equals 17.5 µL.

**Thermal Cycler Parameters**

Denature:
96°C for 2 minutes
27 cycles at:
94°C for 10 seconds
61°C for 1 minute
72°C for 30 seconds

Ramp Rate 9700 Simulation

60°C for 20 minutes
4°C Soak

## INSTRUMENT SPECIFICATIONS

Applied Biosystems ProFlex thermal cycler
Centrifuge

## MINIMUM STANDARDS AND CONTROLS

1. Negative Amplification Control and Blank
   A. Each plate must contain an extraction blank.

B. Each plate must contain a negative amplification control.

2. Positive Amplification Control
   A. Each plate must contain a positive amplification control.

**PROCEDURE**

1. Select the appropriate plate from the STACS-DB Master Mix Addition module. Enter the required information.
2. Write the barcode number on the amplification plate.
3. Attach the STACS-DB plate barcode to the lip of the amplification plate.
4. Pipette the correct volume of amplification master mix into the appropriate wells. The volume may be adjusted for different volumes of DNA sample.
5. Pipette the extracted DNA into the appropriate well. Adjust the volume as necessary.
6. Pipette the correct volume of the diluted positive control into the designated well.
7. Pipette the correct volume of water into the negative amplification control well.
8. Seal the plate. Spin briefly.
9. Document any non-routine manipulations or events by entering the information into the comments field in STACS-DB.
10. Enter the required information into the STACS-DB Amplification module.
11. Place the plate into the thermal cycler. Select the appropriate program file and start amplification.
12. Store the amplification plate or begin sample preparation for CE analysis.
13. Discard all barcoded commodities using the STACS-DB Storage Subsystem.

Re-amplification of samples:

1. Retrieve extraction plates containing samples requiring re-amplification.
2. Select samples from STACS-DB Extraction Rework Consolidation module.
3. Referring to the "pickSample" files created by STACS-DB, select the samples to be reamplified and place in the proper well of a newly created "extraction plate".
4. Verify the position of the samples on the new "extraction plate" using the plate scanner. Import the file into STACS-DB for comparison.
5. For samples being diluted and reamplified:
   a. Once the samples have been consolidated on to the new "extraction plate", add the appropriate amount of ddi water to the samples that require dilution.
6. For samples requiring concentration, refer to the Concentration of DNA procedure.
7. Use the newly produced "extraction plate" in the procedure above.

## REPORT WORDING

Not Applicable.

## REFERENCES

PowerPlex® Y23 System Technical Manual 2012
PowerPlex® Y23 Validation Study, Illinois State Police DNA Indexing Laboratory
PowerPlex® Y23 Validation Study, Illinois State Police Research and Development Laboratory.
ISP DNA Indexing 3500 Validation

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## F. CONCENTRATION OF DNA

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## INTRODUCTION

This procedure may be used when complete profiles are not obtained due to low DNA concentrations.

## SAFETY CONSIDERATIONS

1. Observe Standard Laboratory Practices.
2. Warning: Treat all reagents and samples as potential biohazards.

## PREPARATION

Refer to the Clean Technique section.

## INSTRUMENT SPECIFICATIONS

Standard Laboratory Instrumentation

## MINIMUM STANDARDS & CONTROLS

The manipulation blank for each sample being concentrated must be included. Process the manipulation blank last with each set of samples. The purpose of this control is to ensure that contamination has not occurred due to the manipulation of the sample or the reagents used in the procedure.

## PROCEDURE

1. Identify the samples for concentration from the STACS-DB Extraction Rework Consolidation module.
2. Transfer the extracted DNA from the extraction plate to a Microcon 100 tube.
3. Spin at approximately 500 x g for 8 to 10 minutes. Centrifuge for additional time if liquid remains on the filter. It may be necessary to increase speed to approximately 1500 x g for some samples.
4. Optional step. Add 50-100 µl of elution buffer and centrifuge as before to wash residual extraction components from the DNA. Examine the filter unit to verify that no tearing or cracking has occurred.
5. Add the appropriate volume of elution buffer (20 to 100 µl) (depending on how much concentration is needed), invert filter, vortex 15 seconds, and spin out liquid. The blank should be reconstituted in the smallest volume used for its associated samples. Return the concentrated sample to its appropriate well in the extraction plate.
6. The sample is now ready for quantitation or may be amplified

without quantitation, as necessary.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual.

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# PROCEDURES MANUAL

## H. SAMPLE PREPARATION FOR CE ANALYSIS PPY23

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## INTRODUCTION

This procedure requires the STACS-DB Electrophoresis Plate Prep and Electrophoresis Plate Denature modules.

Record reagent barcodes, commodity barcodes, sample numbers, instrument barcodes, activity dates, and analyst initials when analysis is performed outside of STACS-DB. Ensure all consumed reagents and commodities are properly discarded in STACS-DB.

## SAFETY CONSIDERATIONS

1. Observe Standard Laboratory Practice.
2. Warning: Treat all reagents/samples as potential biohazards.
3. Warning: Hazardous reagents:
   A. Formamide is an irritant, a suspected teratogen, and causes irritation to the eyes, skin, and mucous membranes. Do not inhale or ingest. Wear appropriate personal protective equipment and handle in a fume hood to prevent exposure. Dispose of properly.

## PREPARATIONS

Refer to the Clean Technique section.

### Deionized Formamide

Aliquot into convenient volumes and store frozen. Frozen aliquots can be stored for a maximum of one year.

PowerPlex Y23

| Component | Volume (µl) per sample |
|---|---|
| PowerPlex Y23 5-Dye Internal Lane Standard | 0.5 |
| Deionized Formamide | 9.5 |
| Total | 10 |

**Thermal cycler**

Denature:

$95^{O}$C for 3 minutes

Cool:

$4^{O}$C for 3 minutes or greater

## INSTRUMENT SPECIFICATIONS

Standard Laboratory Instrumentation
Applied Biosystems ProFlex thermal cycler

## MINIMUM STANDARDS AND CONTROLS

1. Positive Control
2. Negative Controls

## PROCEDURE

1. Enter the required information into the STACS-DB Electrophoresis Plate Prep module.
2. Pipette 10 µl of internal lane standard/formamide solution into the appropriate electrophoresis plate wells.
3. Pipette 1 µl of amplified product into the electrophoresis plate. The layout of the electrophoresis plate must be identical to the amplification plate.
4. Pipette 1 µl of ladder into the appropriate well of the electrophoresis plate.
5. Seal the plate and spin briefly.
6. Enter the required information into the STACS-DB Electrophoresis Plate Denature module.
7. Place the plate into the thermal cycler. Select the appropriate program file and start denaturation.

## REPORT WORDING

Not Applicable.

## REFERENCES

ISP DNA Indexing Validation Studies
STACS-DB User's Manual
ProFlex Thermal Cycler User's Manual
Promega PowerPlex Y23 Technical Manual

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## I. ANALYSIS OF STRS WITH A GENETIC ANALYZER

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## INTRODUCTION

Amplified STR fragments are separated by capillary electrophoresis. Fragment sizes are described by allele designations allowing for valid comparisons of profile type between different analysis methods. This procedure requires the use of STACS-DB Post PCR and Storage Subsystem modules.

Record reagent barcodes, commodity barcodes, sample numbers, instrument barcodes, activity dates, and analyst initials when analysis is performed outside of STACS-DB. Ensure all consumed reagents and commodities are properly discarded in STACS-DB.

## SAFETY CONSIDERATIONS

1. Standard Laboratory Practices
2. Warning: Treat all samples as potential biohazards
3. Warning: Hazardous Reagents

   Formamide causes irritation to the eyes, skin, and mucous membranes and is a suspected teratogen. Do not inhale or ingest. Wear protective eyewear, appropriate gloves, and laboratory coat. Dispose of properly.

   Genetic Analyzer Performance Optimizing Polymer 4 (POP 4): POP 4 may cause irritation to the respiratory tract, skin, and eyes. Avoid contact with skin, eyes, and clothing. Do not inhale or ingest. Wear protective eyewear, appropriate gloves, and laboratory coat.

   Anode Buffer Container: May cause irritation to the respiratory tract, skin, and eyes. Do not inhale or ingest. Use adequate ventilation. Wear protective eyewear, appropriate gloves, and laboratory coat.

   Cathode Buffer Container: May cause irritation to the respiratory tract, skin, and eyes. Do not inhale or ingest. Use adequate ventilation. Wear protective eyewear, appropriate gloves, and laboratory coat.

4. Electrical Shock Hazard: The AB Genetic Analyzer contains a high voltage power supply. Handle with caution. Arcing may result from incomplete drying of instrument components.

5. Laser Hazard: The AB Genetic Analyzer contains a laser. Operate only with doors closed. The instrument must be serviced by qualified personnel only.

## PREPARATIONS

Refer to the Clean Technique section.

3500xl Data Collection Module Parameters
PowerPlex Y23/PowerPlex Fusion

| | |
|---|---|
| Run Temperature | 60°C |
| Injection Voltage | 1.2 kV |
| Run Voltage | 15.0 kV |
| Injection Time | 24 seconds |
| Run Time | 1210 seconds |

### Computer Defragmentation of the Genetic Analyzer

1. Defragmentation is auto scheduled.
2. The current log for defragmentation can be found on the instrument computer.

### Changing the Capillary on the Genetic Analyzer

1. Use the Change Capillary Wizard.
2. Capillary usage auto updates on the 3500xl with the RFID tag.

### Routine Maintenance of the 3500xl Genetic Analyzer

The polymer must be allowed to reach room temperature before loading onto the CE.

Before each run, perform a visual check of the instrument to ensure that all components are properly assembled, clean, and undamaged. In addition, check that the levels of polymer, buffer, and water are sufficient for runs.

Clean the instrument and supply fresh buffer and polymer approximately every 14 days or more often as needed.

1. Under Wizards perform the Wash the Pump Chambers and Channels task with Conditioning Reagent.
2. Flush the water trap.
3. Install a new polymer pouch, anode buffer container, and cathode buffer container.
4. Add septa to cathode buffer container.
5. If the capillary array was removed or replaced, both a spatial calibration and spectral must be completed successfully before performing a run.

Inspect block components for leaks and make sure no air bubbles are present before starting a run. Record the 3500xl Genetic Analyzer maintenance.

## INSTRUMENT SPECIFICATIONS

AB 3500xl Genetic Analyzer
AB 3500xl Genetic Analyzer Data Collection Software

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

### 3500xl Data Collection

1. Enter the required information into the STACS-DB Post PCR module.
2. Create the sample sheet.
3. Confirm that the correct plate record was selected for import.
4. Confirm the following required fields are correct:
   A. Assay
   B. File Name Conventions
   C. Results
5. Highlight the plate record and link it to the appropriate plate.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual
PowerPlex Y23 Technical Manual
PowerPlex Fusion Technical Manual
AB 3500xl Genetic Analyzer Maintenance, Troubleshooting, and Reference Guide
ISP DNA Indexing 3500 Validation

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## K. MANUAL DATA ANALYSIS POWERPLEX Y23

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## INTRODUCTION

Manual data analysis must be performed by a qualified analyst. A technical review by a second qualified analyst must be conducted before a sample profile is eligible for CODIS. This procedure requires the STACS-DB Data Analysis module.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices for using a keyboard and mouse.

## PREPARATIONS

PowerPlex Y23
- Applied Biosystems® 3500xl Series Data Collection 4.0
- Current Version of GeneMapper
- GeneMapper Settings for PowerPlex Y23
- Sizing Range
  - Full Range
- Peak Detection- Peak Amplitude Threshold
  - 50 for sample dyes and 150 for ILS
- Size Calling Method
  - Local Southern Method

## INSTRUMENT SPECIFICATIONS

IBM-compatible computers

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

PowerPlex Y23

1. Save data to appropriate network location.
2. Perform GeneMapper analysis for PowerPlex Y23 through STACS-DB. Include the most informative injection of each sample. This means that a project may include profiles from different injections. Remove unused instances of samples from project. Save the project to the appropriate network locations.
3. View each electropherogram on the computer screen. Check the raw data of all blank and negative controls for primer dimer peaks and document

review in the GeneMapper project. Enter initials in the GeneMapper project to indicate the review of all the samples in the project.

4. Leave samples requiring reanalysis in the project. Remove alleles from samples requiring rework. Designate reworks points for samples requiring additional analysis.
5. Repeat steps 3 and 4 with a second analysis and ensure documentation of the check for primer-dimer peaks. This constitutes a technical review of the data and an administrative review of the project.
6. Create a STACS-DB Table.
7. If the sample shows reproducibly low DNA quantity, refer to the Sample Recollection procedure and place the sample on the recollection list.
8. Upload alleles obtained for exclusionary purposes.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual
GeneMapper User's Guide

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## M. MANUAL INTERPRETATION OF Y-STRS

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## INTRODUCTION

This section defines the manual interpretation rules for Y-STRs.

## SAFETY CONSIDERATIONS

Not Applicable.

## PREPARATION

Not Applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

1. Definitions
   - A. Blank control: verifies that all reagents were not contaminated.
   - B. Injection: electrophoresis data collected simultaneously from one capillary array.
   - C. Known orientation control: verifies the correct positioning of the analytical plate.
   - D. Negative amplification control: verifies that the amplification reagents were not contaminated.
   - E. Positive amplification control: verifies correct amplification conditions.
   - F. Positive interpretation control: verifies that the ladder typed correctly using the control included with the amplification kit or the known orientation control at all loci.
   - G. Project: group of runs contained in one .ser file.
   - H. Run: group of injections with the same analytical conditions contained in the same data collection.
2. The negative amplification control and blank must not exhibit reproducible alleles greater than the minimum interpretation threshold.
   - A. Document that all have primer peaks.
   - B. All samples associated with a contaminated blank must be reworked.
3. For each amplification, the positive amplification control must pass interpretation thresholds and guidelines; and must type correctly at all loci.

4. The orientation control must be located in the correct position; must match the expected profile at a minimum of 6 Y-STR loci for and be consistent at all labeled alleles. Exceptions must be approved by the DNA Indexing Technical Leader.
5. An orientation control is not required on plates containing previously analyzed samples or plates where the orientation is evident; for example, plates with only two columns of samples.
6. A single ladder must be included in each project. If a ladder fails, one can be imported. Controls cannot be imported from other projects.
7. Samples must be of the same or lesser injection time and voltage as the passing blank and amplification negative.

## PROCEDURE

### General considerations for interpreting data:

1. Interpretations will be made based on the entire DNA profile for a sample.
2. Raw data may aid in interpretation.
3. It may be necessary to interpret data across more than one electropherogram to confirm the detected allele types. This will be considered sample by sample. All labeled alleles on all electropherograms must agree. The DNA Indexing Technical Leader must review the interpretation when a discrepancy exists.
4. A single ladder must be included in each project. If a ladder fails, one can be imported from another project. Controls cannot be imported from other projects.
5. Notify the Indexing Technical Leader if possible contamination is identified. Repeat the analysis from extraction or as directed by the DNA Indexing Technical Leader.

### Assessment of successful amplification and electrophoresis:

1. Filters:
   - A. PowerPlex Y23
     - 1) Stutter
       - a) 20% except at DYS481, which is 29.84%
     - 2) Fractional
       - a) 20% global
2. Minimum interpretation thresholds for peak heights of samples and controls:
   - 1) Alleles: 125 RFUs
   - 2) Ladders: 50 RFUs
   - 3) ILS 150 RFU
3. There are no set maximum interpretation levels. However, peak heights should be such that the matrix is able to accommodate spectral overlap.

Fluorescent pull-up may be allowed if it does not interfere with interpretation.

4. The internal sizing standard will be examined for each sample and control to ensure that all peaks in the analytical range were sized correctly. Ensure that extraneous peaks have not interfered with sizing. The profile may be acceptable when some of the larger sizing standard peaks are missing.
5. Any allelic ladder used for interpretation must have a corresponding positive interpretation control that types correctly in that run. The allelic ladder used for genotyping will be examined to ensure that it will correctly assign alleles.
    A. In order to verify that the ladder typed the samples correctly:
        1. The run requires a positive interpretation control that passes the interpretation guidelines.
        2. If the run positive interpretation control fails and the original run ladder is used for data analysis, a positive interpretation control may be used from another run that originates from the same analysis project as the samples.
            a) Document what control is being used to verify the ladder.
            b) Samples from both runs may be used.
    B. If all positive interpretation controls fail the analysis in one project, none of the samples in that project can be used. Notify the technical leader if the problem persists.

**Artifacts and other situations to be interpreted with caution:**

1. Artifacts: Examine each sample for artifacts. Labeled artifacts that do not interfere with interpretation must be noted. Rework the sample if artifacts interfere with interpretation. The analyst may choose to rework the sample from extraction, amplification, or CE plate preparation.
    A. Incomplete A-nucleotide addition results in a peak located one base pair smaller than a major peak.
    B. Fluorescent pull-up is defined as any small peaks present in one or more dyes which echo the presence of a relatively large peak. The size of these peaks must be within +/-0.5 base pairs of the large peak.
    C. Stutter peaks are often observed one repeat unit below the major allele. Occasionally, stutter may be found two repeat units smaller or one repeat unit larger than the major peak. Stutter peaks should typically fall below the 20% filter range, but peaks above may be interpreted with caution at the analyst's discretion.
    D. The PowerPlex Y23 Technical Manuals note several artifacts that are specific to each amplification system.
2. Off Ladder Alleles (OLAs): STACS-DB maintains a list of alleles acceptable to upload without re-amplification. This list contains alleles contained in the ladder; named by Promega; named by the National Institute

of Science and Technology (NIST); named by the Y chromosome Haplotype Reference Database (YHRD); or previously verified by ISP. Alleles not on this list, and inside the ladder range, will require a second amplification to confirm the OLA. When confirmed, enter the new OLA into STACS-DB.

3. Y-STR Duplications:
   A. Loci other than DYS385a/b with more than 1 allele require a second amplification.
   B. The PowerPlex Y23 loci DYS570 and DYS576 have been identified as rapidly mutating Y-STRs.

It is recognized that the above rules cannot cover all situations. The DNA Indexing Technical Leader must be consulted in unique situations or if there is any question as to the quality of data. Any deviation from the procedures manual must be approved by the DNA Indexing Technical Leader.

## REPORT WORDING

Not Applicable.

## REFERENCES

ISP DNA Indexing Evaluation of 10 µl Reactions
ISP DNA Indexing PowerPlex Y23 Validation
Promega PowerPlex Y23 Technical Manual
GeneMapper User's Guide
ISP DNA Indexing 3500 Validation

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## N. CODIS DATA IMPORT

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## INTRODUCTION

Data import must be performed by an analyst qualified for data interpretation. This procedure requires the STACS-DB CODIS Upload module, STACS-DB CODIS Confirmation module, and CODIS software.

## SAFETY CONSIDERATIONS

Not Applicable.

## PREPARATION

Not Applicable.

## INSTRUMENT SPECIFICATIONS

IBM-compatible computers with CODIS software installed.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

Autosomal STRs

1. Select the sample profile in STACS-DB CODIS Upload. Generally, all selected samples should be assigned to the same analyst.
2. Create the file.
3. Use CODIS Specimen Manager and import the file.
4. Process the import file with CODIS Message Center.
5. Verify that samples were successfully imported into CODIS via the STACS-DB CODIS Confirmation module and the CODIS Import Reconciliation Report.

Y-STRs

1. Select all the Y-STR loci to be uploaded for each sample in the STACS-DB Hit Confirmation Module.

## REPORT WORDING

Not Applicable.

## REFERENCES

CODIS Training Manual
NDIS Procedures Manual
STACS-DB User's Manual

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## O. SAMPLE PRESERVATION

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## INTRODUCTION

This procedure describes preservation requirements for offender samples and aliquots. Original samples will be preserved indefinitely. This procedure requires the use of the STACS-DB Storage Subsystem.

All samples will be stored in a secure location. Refrigerator storage will be between 1-8°C. Freezer storage will be at or below -2°C. Room temperature storage will be approximately 25°C. It will fluctuate within the limits of the building's heating and cooling system. Refrigerator and freezer temperatures will be monitored.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all samples as potential biohazards.

## PREPARATIONS

Refer to the Clean Technique section.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

1. Open the STACS-DB Storage Subsystem module.
2. Enter the storage location.
3. Enter the sample number

**Storage Conditions:**
Short-term Storage
1. Store samples awaiting extraction at room temperature or in a freezer.
2. Store extracted DNA in a refrigerator or freezer.
3. Store amplified DNA in a freezer.
4. Store unopened kits at room temperature.

Long-term Storage

1. Store buccal swabs enclosed in tubes or plastic in a freezer.
2. Store buccal swabs in coin envelopes at room temperature.
3. Store aliquots of whole blood samples and corresponding stains in a freezer.
4. Preserve extracted DNA if no original sample remains. Store extracted DNA in a freezer. Otherwise, discard extracted DNA after analysis.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual

# ILLINOIS STATE POLICE

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# P. CONTRACTUAL ANALYSIS

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## INTRODUCTION

This section describes the policies and procedures for contractual analysis of offender samples. This procedure requires the use of the STACS-DB Outsourced Batch Management and Outsourced Batch Receiving modules.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all samples as potential biohazards.

## PREPARATIONS

Refer to the Clean Technique section.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

As specified by the contract.

## PROCEDURE

1. Contracting laboratories will adhere to the specifications outlined in the current version of the following:
   - A. Quality Assurance Standards for DNA Databasing Laboratories, FBI, Washington, D.C.
   - B. ISO 17025 Certified.
   - C. Current contract specifications to include a visit to the Laboratory.
2. Laboratory procedure. Modifications and additions to the procedure may be made as necessary in accordance with the appropriate contract.
   - A. Select samples to be outsourced in STACS-DB using the Outsourced Batch Management module.
   - B. Prepare a manifest of sample numbers for documentation. Add a percentage of previously analyzed samples to serve as Quality Control Samples. The exact percentage is based on the current contract.
     - 1) Include the date sent and ISP employee's name on each page.
     - 2) Include on each page a place to record the date the samples are received by the contract laboratory and the signature of the person who received the samples.

C. Send samples via overnight carrier to the contractor. Samples must be shipped with tracking receipt. Include the manifest for their signature upon receipt. Instruct the contractor to return the signed manifest to the laboratory, documenting sample receipt. Retain the signed manifest.

D. Receive data from the contractor. The format will be specified in the contract.

E. At least one qualified ISP DNA analyst will perform a technical review of the contractor's sample results. All DNA typing results must be reviewed before entry into CODIS.

1) Check blinds and known QC samples for consistent results. The contractor and/or ISP will re-analyze any QC samples with inconsistent results.

a. Continued and/or multiple inconsistencies may result in contract termination.

2) Verify that the Positive and Negative Controls are correct.

3) Assess electropherograms and any relevant analytical data necessary for interpretation.

4) Request reanalysis of any sample with ambiguous results.

5) Transfer sample data to STACS-DB using the Outsourced Batch Receiving module.

6) Retain all contractor data.

F. The contract laboratory must return all remaining samples and extracted DNA unless directed otherwise. Confirm that all samples/extracts have been returned.

G. The contractor shall destroy remaining amplified product after the sample analysis results have been accepted. The contractor must certify in writing which samples have been destroyed. The contractor may not use samples, remaining amplified product or data for any purpose.

## REPORT WORDING

Not Applicable.

## REFERENCES

Current outsourcing contract
STACS-DB User's Manual

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# DNA INDEXING PROCEDURES MANUAL

## Q. RANDOM REANALYSIS FOR CONTRACTUAL ANALYSIS

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## INTRODUCTION

This section describes the procedures for random reanalysis of outsourced samples. This procedure requires the use of the STACS-DB Outsource Batch Receiving module.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all samples as potential biohazards.

## PREPARATIONS

Refer to the Clean Technique section.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

As specified by the contract.

## PROCEDURE

### STACS-DB Manual Method

Reanalysis occurs before return of outsourced sample results. This method uses CODIS to check the concordance between outsourced and reanalyzed sample profiles. After comparison with the outsourced profiles, the reanalyzed samples must be removed from CODIS for the sample data to be entered as outsourced profiles.

1. Identify plate or samples for reanalysis.
2. Perform sample analysis according to the current ISP DNA Indexing procedures. Analyze and interpret data. Modifications to interpretation rules are:
   A. A minimum of six autosomal CODIS loci that meet the RFU and peak height ratio thresholds is required.
   B. Tri-alleles do not need to be re-amplified.
   C. Additional peaks below the RFU and peak height ratio thresholds do not need to be repeated and may be used for exclusionary purposes.
   D. No second read is necessary.
3. Create a CODIS import file. Ensure that the specimen category is “QC” so that the specimens are not searched or uploaded.
4. Import the reanalysis data into CODIS.

5. Import the outsourced results into CODIS using the STACS-DB Outsource Batch Receiving module. This may happen over an extended period of time.
6. Check the CODIS Message Center Import Report for non-concordant samples.
   A. Verify that the differences are not problematic.
   B. Report any problem samples to the Indexing Technical Leader.
7. Remove the reanalysis samples listed in the import report from CODIS.
8. Import the outsourced profiles again.

**STACS-DB System Method**

Reanalysis occurs after return of outsourced swabs. This method uses STACS-DB to verify concordance.

1. Ensure that the proper reanalysis percentage has been entered into STACS-DB by the supervisor.
2. Import outsourced results into the STACS-DB Outsource Batch Receiving module.
3. STACS-DB will randomly identify samples for reanalysis.
4. Conduct analysis on selected samples using STACS-DB.
5. Perform data analysis and interpretation. STACS-DB monitors concordance. Modifications to interpretation rules are:
   A. A minimum of six autosomal CODIS loci that meet the RFU and peak height ratio thresholds is required.
   B. Tri-alleles do not need to be re-amplified.
   C. Additional peaks below the RFU and peak height ratio thresholds do not need to be repeated and may be used for exclusionary purposes.
   D. No second read is necessary.

Notify the Indexing Technical Leader of problematic discrepancies, such as mismatches not related to primer differences, contaminated samples, etc.

## REPORT WORDING

Not Applicable.

## REFERENCES

Current outsourcing contract
STACS-DB User's Manual

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## R. LABORATORY PERSONNEL DNA DATABASE

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Approved by:

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DNA Indexing Technical Leader

Brenda Danosky
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## INTRODUCTION

In accordance with Command Directives TCH 21, buccal samples from laboratory personnel will be analyzed at the DNA Indexing Laboratory. This procedure describes how these samples will be processed. Indexing laboratory personnel performing sample check-in must wear a mask.

The State CODIS Administrator maintains the information related to the laboratory personnel DNA database.

The numbering system used by the State CODIS Administrator for the Laboratory Personnel database will have the format "SD" and six numeric placeholders. The "SD" indicates Staff Database. The State CODIS Administrator will not begin with the number SD000001 and the numbers should be randomized when possible in order to further improve anonymity.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all samples as potential biohazards

## PREPARATIONS

Refer to the Clean Technique section.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

1. Samples will be received with TCH Appendix 11 attached to the outer package.
2. The State CODIS Administrator will receive the samples and attach a unique number to TCH Appendix 11 and the sealed kit. Only the State CODIS Administrator and the Alternate State CODIS Administrator

will have access to the name of the laboratory personnel associated with the sample number.

3. TCH Appendix 11 will be removed from the kit and secured by the State CODIS Administrator.
4. A copy of each TCH Appendix 11 will be scanned into a secure location. Access to this location will be limited to the State CODIS Administrator and the Alternate State CODIS Administrator. After at least two backups have been completed, the original/paper copy of TCH Appendix 11 will be destroyed by the State CODIS Administrator.
5. Samples will be tracked using anonymous unique identifiers assigned by the State CODIS Administrator.
6. Samples will be processed outside of STACS-DB. The "A" swab will be placed in the extraction tube. The remaining swabs will be placed in a taped coin envelope and stored in the defined location for Laboratory Personnel samples.
7. All samples will be run in the current autosomal STR kit and Y-STR kit.
8. Samples will be diluted, concentrated, or re-extracted as required to obtain a full profile.
9. Once a full profile is obtained using the autosomal and Y-STR kit, a sample verification run will be performed using an additional swab. This verification run will be performed with the current autosomal STR kit only. Following this verification run, the sample will be uploaded to CODIS.
10. CMF files will be exported for profile upload to the Staff Category and Staff Index of CODIS. The profiles will not be uploaded to the National Index.
11. For the purpose of sample destruction, the State CODIS Administrator will follow the policy outlined in the Command Directives TCH 21. Once notified of an employee's departure from ISP employment, the State CODIS Administrator will pull the remaining swabs of the employee for destruction. The Alternate State CODIS Administrator will verify the barcode numbers on the swabs pulled by the State CODIS Administrator. The State CODIS Administrator will then destroy the remaining swabs and provide notification to the FB/DNA Program Manager.

## REPORT WORDING

Not Applicable.

## REFERENCES

None.

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## S. DNA FAMILIAL SEARCH

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## INTRODUCTION

In accordance with Command Directive TCH-27, the DNA Indexing Laboratory will conduct Familial Searches. A familial search is a non-routine, deliberate search of the DNA database to identify a potentially close biological relative of an unknown forensic profile from an unsolved criminal case. Familial searches will be conducted on profiles that have been approved by the FSC Familial Search Committee. This procedure details how familial searches will be conducted and requires the use of multiple CODIS programs and STACS-DB modules.

## SAFETY CONSIDERATIONS

1. Observe Standard Laboratory Practices
2. Warning: Treat all reagents/samples as potential biohazards.
3. Refer to safety considerations under the individual sections of the DNA Indexing Procedures Manual.

## PREPARATIONS

1. Refer to the Clean Technique section.
2. Refer to the appropriate extraction and amplification sections.

## INSTRUMENT SPECIFICATIONS

Refer to the appropriate extraction and amplification sections.

## MINIMUM STANDARDS & CONTROLS

Refer to the appropriate extraction and amplification sections.
Refer to the Interpretation of STRs section.

## PROCEDURE

1. CODIS
   A. Pedigree Manager Module-The Indexing laboratory will create two Pedigree Trees.
      1) Two separate pedigree trees will be created to represent a Parent/Offspring relationship or a Full Sibling relationship.
      2) The search profile will be associated to each pedigree tree as a known relative.
      3) Save each pedigree tree in Pedigree Manager.
         Naming Convention:

PO-Search Profile Name
SIB-Search Profile Name

B. Searcher Module-Familial searches in CODIS should only be conducted by the State CODIS Administrator or the Alternate State CODIS Administrator.
   1) Search the Parent/Offspring pedigree tree.
   2) Search the Full Sibling pedigree tree.
   3) The candidates for each search will be ranked from highest to lowest based on the Joint Pedigree Likelihood Ratio (JPLR).
   4) Export results as a text file.
      Parent/Offspring- Export top 91 ranked candidates.
      Sibling- Export top 182 ranked candidates.
   5) Dispositions
      A) Dispo potential first degree relative as "Pending local disposition."
      B) All other returns dispoed as Investigative Information.

2. STACS
   A. Post Familial Search Module
      1) Click New to create a new familial search.
      2) The search ID will be the search profile name.
      3) Fill in Agency name, case type, and crime type.
      4) Import the Parent/Offspring results from CODIS.
      5) Import the Full Sibling results from CODIS.
         1. All candidates will be attempted to be verified in PowerPlex Fusion.
         2. Male candidates will be attempted to be amplified in PowerPlex Y23.
      6) After laboratory processing is complete and additional loci uploaded to CODIS, the candidates will be forwarded back to the casework lab for evaluation.
         1. Candidates that can be excluded will be dispoed as no match.
         2. Candidates that cannot be excluded will be dispoed as State Defined #3.
         3. The biographical information of all candidates that cannot be eliminated as potential relatives will be forwarded to State Terrorism and Intelligence Center (STIC) for further investigation.

**REPORT WORDING**
Not Applicable.

**REFERENCES**

DNA Indexing Laboratory Familial Search Validation
Familial Searching Manual for CODIS 8.0
STACS-DB Users' Manual

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## T. MANUAL AMPLIFICATION OF STRs POWERPLEX FUSION

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## INTRODUCTION

This section describes the direct amplification process of offender DNA samples using the Promega's PowerPlex Fusion kit. An amplification reaction volume of 12.5 µl must be used. Ensure all consumed reagents and commodities are properly discarded in STACS-DB.

This procedure requires the use of STACS-DB Extraction, Extraction Rework Consolidation, Master Mix Addition, and Amplification Modules.

## SAFETY CONSIDERATIONS

Observe standard laboratory practices.
Warning: Treat all reagents/samples as potential biohazards.

## PREPARATIONS

Refer to the Clean Technique section.
Thaw Swab Solution overnight in refrigerator.

Diluted Positive Amplification Control Preparation:

1) Mix 25ul of the concentrated control with 375 ul ddi water (or equivalent). This dilution may be adjusted depending on the quality of the data, and other dilutions may be used with the approval of the Technical Leader.

Amplification Master Mix Preparation:
Calculate the volume of each reagent required.

| Reaction Component | Volume per Reaction |
|---|---|
| Fusion 5X Master Mix | 2.5 ul |
| Fusion 5X Primer Pair | 2.5 ul |
| ddi Water or equivalent | 5.5 ul |
| Template DNA | 2.0 ul |
| **Total Reaction Vol.** | **12.5 ul** |

Mix and spin briefly.

*This volume of ddi water (or equivalent) is used when amplifying 2µl of extracted DNA for a 12.5µl reaction volume. Other volumes of extracted DNA require the amount of ddi water (or equivalent) to be adjusted so that the total volume of water and DNA equals 7.5µl

**Thermal Cycler Parameters**

Initial Incubation:
96°C for 1 minute

27 cycles at:
94°C for 10 seconds
59°C for 1 minute
72°C for 30 seconds

Ramp Rate 9700 Simulation

Final Extension
60°C for 20 minutes

Final Hold
4°C

## INSTRUMENT SPECIFICATIONS

Applied Biosystems ProFlex Thermal Cycler
Centrifuge

## MINIMUM STANDARDS AND CONTROLS

1. PowerPlex Fusion PCR Amplification Kit
2. Negative Amplification Control and Blank:
   A. Each plate must contain an extraction blank.
   B. Each plate must contain a negative amplification control.
   C. All blanks and negatives must have primer-dimer peaks.
   D. All samples associated with contaminated blanks and negatives must be reworked.
3. Positive Amplification Control:
   A. Each plate must contain a positive amplification control.

## PROCEDURE

Swab Solution Incubation

Recently collected swab samples
1. Add 900µl of Swab Solution to each sample.
2. Cap samples and incubate for 60 minutes at 90°C.
3. Dilute samples with DNA Grade Water with the appropriate dilution.

Swab hit verifications and problematic samples

1. Add 450µl of Swab Solution to each sample.
2. Cap samples and incubate for 60 minutes at 90°C.

Blood samples

1. Pipet 30µl of sample into tube
2. Add 900µl of Swab Solution to each sample.
3. Cap samples and incubate for 60 minutes at 90°C.

Samples may be manually extracted at analyst discretion regardless of how many swabs remain. If only one swab from the sample remains, DNA IQ or a Manual Isolation is required on half of the swab. Exceptions are described below.

A. New submission and a complete profile has not been obtained
   1. If only one swab remains and any genomic peaks were observed from any analysis, DNA IQ or Manual Isolation is required on half of the swab.
   2. If only one swab remains and no genomic peaks were observed from any analysis, the final swab may be extracted using Swab Solution.

B. Hit/QC verification
   1. If only one swab remains, DNA IQ or Manual Isolation is required on half of the swab.
   2. If more than one swab remains, the swab may be extracted with Swab Solution, however, Manual or DNA IQ isolation should be considered if a verification was already attempted and failed.

C. Too many swabs were submitted and the agency cannot confirm that all the swabs were collected from the same sample donor. All swabs must be analyzed.
   1. If a complete profile was obtained from a previous analysis, additional swabs may be extracted using Swab Solution.
   2. If only one swab remains and no genomic peaks were observed from any analysis, the final swab may be extracted using Swab Solution.
   3. If only one swab remains and any genomic peaks were observed from any analysis, Manual or DNA IQ Isolation is required on half of the swab.

Amplification

1. Pipette the correct volume of amplification master mix into the appropriate wells. The volume may be adjusted for different volumes of DNA solution.
2. Pipette the extracted DNA into the appropriate well. Adjust the volume as necessary. The amount of extraction blank amplified should be equivalent to the highest volume of sample amplified.

3. Pipette the correct volume of positive control into the designated well.
4. Pipette the correct volume of water into the designated well.
5. Seal the plate. Spin briefly

Re-amplification of samples:

1. Retrieve extraction plates containing samples requiring re-amplification.
2. Select samples from STACS-DB Extraction Rework Consolidation module.
3. Referring to the "pickSample" files created by STACS-DB, select the samples to be reamplified and place in the proper well of a newly created "extraction plate".
4. Verify the position of the samples on the new "extraction plate" using the plate scanner. Import the file into STACS-DB for comparison.
5. For samples being diluted and reamplified:
   a. Once the samples have been consolidated on to the new "extraction plate", add the appropriate amount of ddi water to the samples that require dilution.
6. For samples requiring concentration, refer to the Concentration of DNA procedure.
7. Use the newly produced "extraction plate" in the procedure above.

## REPORT WORDING

Not Applicable.

## REFERENCES

Direct Amplification Validation Study, Illinois State Police DNA Indexing Laboratory
Promega Corporation PowerPlex Fusion System Technical Manual, TMD039
Promega Corporation Swab Solution Kit Technical Manual, TMD037
ISP DNA Indexing 3500 Validation

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## U. SAMPLE PREPARATION FOR CE ANALYSIS POWERPLEX FUSION

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## INTRODUCTION

This procedure requires the STACS-DB Electrophoresis Plate Prep, Electrophoresis Plate Denature, and Storage Subsystem modules.

Record reagent barcodes, commodity barcodes, sample numbers, instrument barcodes, activity dates, and analyst initials when analysis is performed outside of STACS-DB. Ensure all consumed reagents and commodities are properly discarded in STACS-DB.

## SAFETY CONSIDERATIONS

1. Observe Standard Laboratory Practice.
2. Warning: Treat all reagents/samples as potential biohazards.
3. Warning: Hazardous reagents:
   A. Formamide is an irritant, a suspected teratogen, and causes irritation to the eyes, skin, and mucous membranes. Do not inhale or ingest. Wear appropriate personal protective equipment and handle in a fume hood to prevent exposure. Dispose of properly.

## PREPARATIONS

Refer to the Clean Technique section.

### Deionized Formamide

Aliquot into convenient volumes and store frozen. Frozen aliquots can be stored for a maximum of one year.

PowerPlex Fusion

| Component | Volume (µl) per sample |
|---|---|
| WEN ILS 500 | 0.5 |
| Deionized Formamide | 9.5 |
| Total | 10.0 |

**Thermal cycler**

Denature:
95$^{O}$C for 3 minutes
Cool:
4$^{O}$C for 3 minutes or greater

## INSTRUMENT SPECIFICATIONS

Standard Laboratory Instrumentation
Applied Biosystems ProFlex thermal cycler

## MINIMUM STANDARDS AND CONTROLS

1. Positive Control
2. Negative Controls

## PROCEDURE

1. Enter the required information into the STACS-DB Electrophoresis Plate Prep module.
2. Pipette 10 µl of internal lane standard/formamide solution into the appropriate electrophoresis plate wells.
3. Pipette 1 µl of amplified product into the electrophoresis plate. The layout of the electrophoresis plate must be identical to the amplification plate.
4. Pipette 1 µl of ladder into the appropriate well of the electrophoresis plate.
5. Seal the plate and spin briefly.
6. Enter the required information into the STACS-DB Electrophoresis Plate Denature module.
7. Place the plate into the thermal cycler. Select the appropriate program file and start denaturation.

## REPORT WORDING

Not Applicable.

## REFERENCES

ISP DNA Indexing Validation Studies
STACS-DB User's Manual
ProFlex Thermal Cycler User's Manual
PowerPlex Fusion Technical Manual

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## V. MANUAL DATA ANALYSIS POWERPLEX FUSION

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## INTRODUCTION

Manual data analysis must be performed by a qualified analyst. A technical review by a second qualified analyst must be conducted before a sample profile is eligible for CODIS. This procedure requires the STACS-DB Data Analysis module.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices for using a keyboard and mouse.

## PREPARATIONS

PowerPlex Fusion
Applied Biosystems® 3500xl Series Data Collection 4.0
Current Version of GeneMapper
GeneMapper Settings for Fusion
Sizing Range
    Full Range
Peak Detection-Peak Amplitude Threshold
    50 for all dyes
    150 for ILS
Size Calling Method
    Local Southern

## INSTRUMENT SPECIFICATIONS

IBM-compatible computers

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

PowerPlex Fusion

1. Save data to appropriate network location.
2. Perform GeneMapper analysis through STACS-DB. Include the most informative injection of each sample. This means that a project may include profiles from different injections. Remove unused instances of samples from project. Save the project to the appropriate network locations.
3. View each electropherogram on the computer screen. Check the raw data of all blank and negative controls for primer dimer peaks and document

review in the GeneMapper project. Enter initials in the GeneMapper project to indicate review of all of the samples in the project.

4. Leave samples requiring reanalysis in the project. Remove alleles from samples requiring rework. Designate rework points for samples requiring additional analysis.
5. Repeat steps 3 and 4 with a second analysis and ensure documentation of the check for primer-dimer peaks. This constitutes a technical review of the data and an administrative review of the project.
6. Create a STACS-DB Table.
7. If the sample shows reproducibly low DNA quantity, refer to the Sample Recollection procedure and place the sample on the recollection list.

## REPORT WORDING

Not Applicable.

## REFERENCES

STACS-DB User's Manual
GeneMapper User's Guide

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## W. MANUAL INTERPRETATION OF STRS POWERPLEX FUSION

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## INTRODUCTION

This section defines the manual interpretation rules for offender sample profiles.

## SAFETY CONSIDERATIONS

Not Applicable.

## PREPARATION

Not Applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

1. Definitions
   - A. Blank control: verifies that all reagents were not contaminated.
   - B. Injection: electrophoresis data collected simultaneously from one capillary array.
   - C. Known orientation control: verifies the correct positioning of the analytical plate.
   - D. Negative amplification control: verifies that the amplification reagents were not contaminated.
   - E. Positive amplification control: verifies correct amplification conditions.
   - F. Positive interpretation control: verifies that the ladder typed correctly using the control included with the amplification kit or the known orientation control at all loci.
   - G. Project: group of runs contained in one.ser file.
   - H. Run: group of injections with the same analytical conditions contained in the same data collection.
2. The negative amplification control and blank must not exhibit reproducible alleles greater than the minimum interpretation threshold.
   - A. Document that all have primer peaks.
   - B. All samples associated with a contaminated blank must be reworked.
3. For each amplification, the positive amplification control must pass interpretation thresholds and guidelines; and must type correctly at all loci.

4. The orientation control must be located in the correct position; must match the expected profile at a minimum of 6 autosomal loci and be consistent at all labeled alleles. Exceptions must be approved by the DNA Indexing Technical Leader.
5. An orientation control is not required on plates containing previously analyzed samples or plates where the orientation is evident; for example, plates with only two columns of samples.
6. A single ladder must be included in each project. If a ladder fails, one can be imported from another project. Controls cannot be imported from other projects.

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## PROCEDURE

**General considerations for interpreting data:**

1. Interpretations will be made based on the entire DNA profile for a sample.
2. Raw data may aid in interpretation.
3. It may be necessary to interpret data across more than one electropherogram to confirm the detected allele types. This will be considered sample by sample. All labeled alleles on all electropherograms must agree. The DNA Indexing Technical Leader must review the interpretation when a discrepancy exists.
4. A single ladder must be included in each project. If a ladder fails, one can be imported from another project. Controls cannot be imported from other projects.
5. Notify the Indexing Technical Leader if possible contamination is identified. Repeat the analysis from extraction or as directed by the DNA Indexing Technical Leader.

**Assessment of successful amplification and electrophoresis:**

1. Filters:
   - A. STRs
     - 1) 20% global fractional filter
2. Minimum interpretation thresholds for peak heights of samples and controls:
   - A. PowerPlex Fusion
     - 1) Heterozygotes: 125 RFUs
     - 2) Homozygotes: 390 RFUs
     - 3) DYS391: 125 RFUs
     - 4) Ladders: 50 RFUs
     - 5) ILS: 150 RFUS
3. There are no set maximum interpretation levels. However, peak heights should be such that the matrix is able to accommodate spectral overlap. Fluorescent pull-up may be allowed if it does not interfere with interpretation.
4. The internal sizing standard will be examined for each sample and control to ensure that all peaks in the analytical range were sized correctly. Ensure that extraneous peaks have not interfered with sizing.
5. Any allelic ladder used for interpretation must have a corresponding positive interpretation control that types correctly in that project. The allelic ladder used for genotyping will be examined to ensure that it will correctly assign alleles.
   - A. In order to verify that the ladder typed the samples correctly:

1. The run requires a positive interpretation control that passes the interpretation guidelines.
2. If the run positive interpretation control fails and the original run ladder is used for data analysis, a positive interpretation control may be used from another run that originates from the same analysis project as the samples.
    a) Document what control is being used to verify the ladder.
    b) Samples from both runs may be used.

B. If all positive interpretation controls fail the analysis in one project, none of the samples in that project can be used. Notify the technical leader if the problem persists.

**Artifacts and other situations to be interpreted with caution:**

1. Artifacts: Examine each sample for artifacts. Labeled artifacts that do not interfere with interpretation must be noted. Rework the sample if artifacts interfere with interpretation. The analyst may choose to rework the sample from extraction, amplification, or CE plate preparation.
    A. Incomplete A-nucleotide addition results in a peak located one base pair smaller than a major peak.
    B. Fluorescent pull-up is defined as any small peaks present in one or more dyes which echo the presence of a relatively large peak. The size of these peaks must be within +/-0.5 base pairs of the large peak.
    C. Stutter peaks are often observed one repeat unit below the major allele. Occasionally, stutter may be found two repeat units smaller or one repeat unit larger than the major peak. Stutter peaks should typically fall below the 20% filter range, but peaks above may be interpreted with caution at the analyst's discretion.
2. Off Ladder Alleles (OLAs): STACS-DB maintains a list of alleles acceptable to upload without re-amplification. This list contains alleles contained in the ladder; named by the National Institute of Science and Technology (NIST); or previously verified by ISP. Alleles not on this list will require a second amplification to confirm the OLA. When confirmed, enter the new OLA into STACS-DB.
3. Trialleles: Reamplify samples with three or more alleles per locus. Upload reproducible alleles.
4. Peak Height Ratios (PHR): Peaks that are 125 RFU or greater and produce a peak height ratio less than 30% must be examined with caution. Peaks that are less than 125 RFU and produce a peak height ratio less than 30% may be unlabeled without re-amplification if the other peak is at or above the 390 homozygote threshold. There are no PHR limits for Amelogenin.
5. Unlabeled Peaks Between Loci: Occasionally, a peak will fall between the marker ranges and will need to be manually labeled and assigned to a locus or loci.
    A. If the sample can be interpreted based on peak heights, enter the profile as follows:
        1. If there are four alleles of similar height with one locus being a heterozygote, assign the unlabeled allele to the single allele locus.

2. If there are three alleles with one locus having a peak height characteristic of a homozygote, assign the unlabeled peak to the other locus.

B. If the sample cannot be interpreted based on peak heights, enter the three possible profiles as follows:
   1. Use the alleles called by GeneMapper for the original sample number and STACS-DB.
   2. Assign the unlabeled peak to the left locus and enter the profile into CODIS with the original sample number plus an "A" (i.e. IYY-######A).
   3. Assign the unlabeled peak to the right locus and enter the profile into CODIS with the original sample number plus a "B" (i.e. IYY-######B)

C. Samples that contain more than one unlabeled peak located outside the ladder must be reviewed and approved by the DNA Indexing Technical Leader.

D. Notify the DNA Indexing Technical Leader if no genomic peaks have been detected from the initial swabs and a full profile is obtained from the last swab.

6. It is recognized that the above rules cannot cover all situations. The DNA Indexing Technical Leader must be consulted in unique situations or if there is any question as to the quality of data. Any deviation from the procedures manual must be approved by the DNA Indexing Technical Leader.

## REPORT WORDING

Not Applicable.

## REFERENCES

ISP DNA Indexing Direct Amplification Validation
PowerPlex Fusion Technical Manual
GeneMapper User's Guide
ISP DNA Indexing 3500 Validation

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## X. DNA IQ™ EXTRACTION: MAXPREP LIQUID HANDLER AND MAXWELL FSC

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**Protocol**: DNA Analysis

**Method:** DNA Isolation

**Procedure:** DNA IQ™ Extraction: Maxprep Liquid Handler and Maxwell FSC

## INTRODUCTION

DNA Extraction techniques isolate DNA from nucleated cells contained in biological samples. The Promega DNA IQ™ System paramagnetic resin purifies DNA while eliminating inhibitors. Below is the procedure for the processing of samples using the Promega DNA IQ System, the Maxprep Liquid Handler, and Maxwell FSC instrumentation.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices.
Reference SDS for all commodities prior to use.
Warning: Treat all reagents/samples as potential biohazards.
Caution/Health Hazard: Do not mix DNA IQ™ Lysis Buffer and bleach.

## INSTRUMENTATION

Standard laboratory instrumentation
Promega Corporation Maxwell® FSC and Maxprep Liquid Handler

## MINIMUM STANDARDS AND CONTROLS

Refer to the Clean Technique section.

The maximum number of samples for any extraction set is defined by the number of instruments that can be run concurrently. Each instrument run must contain at least one reagent blank.

Consecutive runs on the same instrument are considered separate extraction batches, which require their own reagent blank(s).

Each batch of samples must include an extraction blank. A manipulation blank must be processed with each set of samples for every extraction protocol followed. Process the manipulation blank last with each set of samples. The purpose of this control is to ensure that contamination has not occurred due to the manipulation of the sample or the reagents used in the procedure. Whenever an additional manipulation is done on a sample, (i.e., reconcentration of the DNA) the same manipulation must be done to the manipulation blank. Notify the DNA Indexing Technical Leader before performing analysis on the remaining portion of a sample.

## PREPARATIONS

18 mg/mL Proteinase K Solution

1. Add 556 µL of Nuclease Free Water (from Casework Extraction Kit) to the tube of lyophilized Proteinase K (from Casework Extraction Kit) and gently invert to dissolve.
2. Store Proteinase K solution at -10 to -30°C. Prior to use, thaw and remix.

## CRITICAL REAGENTS

None

## PRODUCT COMPONENTS AND STORAGE CONDITIONS

Maxwell FSC Cartridges 15-30°C

Maxwell Plunger Pack 15-30°C

Elution Tubes 15-30°C

Elution Buffer 15-30°C

Lysis Buffer 15-30°C

Casework Extraction Buffer 15-30°C

Proteinase K -30°C to -10°C

Nuclease Free Water below 30°C

1-Thioglycerol 2-10°C

## PROCEDURE

1. Perform required maintenance for the Maxprep Liquid Handler and Maxwell FSC and record in STACS.
2. Create the plate in STACS extraction module.
3. Scan reagents and equipment into STACS-DB.

**Sample Preprocessing**

1. Place swab material up to entire swab head in CW spin basket in CW 1.5ml microfuge tube or 5µl of liquid blood in the bottom of a CW 1.5ml microfuge tube.
2. The extraction blank will consist of 300 µL of Extraction Mix and a sterile swab for swab samples. If processing blood samples only, the extraction negative does not need to contain a sterile swab. The extraction negative must be processed last in the batch of samples. A batch of samples is a maximum of 16 including controls.
3. Prepare Extraction Mix by combining 286 µL of Casework Extraction Buffer, 10 µL of Proteinase K Solution and 4 µL of 1-Thioglycerol per sample. **Note:** 1-Thioglycerol is viscous. Pipet slowly.
4. Dispense 300 µL of Extraction Mix to each sample.
5. Briefly vortex the samples. DO NOT SPIN SAMPLES.
6. Incubate samples at 56°C for 30 minutes.
7. Centrifuge for 2 minutes at maximum speed.
8. Make sure the liquid has spun through the basket. If basket fails, the liquid and substrate will need to be transferred to a new CW spin basket and CW 1.5 ml microfuge tube. Blood samples can just be quick spun.
9. If the liquid has spun through to the bottom of the tube, discard the basket and swab head.
10. The samples are now ready for processing on the Maxprep Liquid Handler.

**Maxprep Liquid Handler**

1. Load the preprocessed samples into the sample tube carrier on the Maxprep Liquid Handler. Open the tubes.
2. Place the number of Maxwell® FSC cartridges to be used into the deck tray and press down firmly to snap the cartridges into place. Hold the cartridge firmly and remove the seal.
3. Place the number of elution tubes required in the deck tray. Tubes should be opened before starting the run.
4. Select the Maxwell FSC DNA IQ FSC16 protocol. Follow the steps in the protocol for setting up the number of samples, elution volume, commodities and reagents. The elution volume is 50µl. For problem/low samples, the elution volume can be lowered to 30µl.

5. When the Maxprep run is complete, verify the sample lysates are in well 1 of the FSC cartridges, plungers are in well 8 of the cartridges, and elution buffer is in the elution tubes.

**Maxwell® FSC Setup**

1. Select Start.
2. Scan the barcode on the FSC tray. Click continue.
3. Highlight DNA IQ Casework. Click Proceed.
4. Confirm the samples numbers and positions when displayed on the screen.
5. The instrument door will open.
6. Load the tray into the instrument.
7. Click Start.
8. Upon completion, select Open Door.
9. Close the elution tubes immediately to prevent evaporation.
10. Remove the tray from the instrument.
11. Label the elution tubes with I# barcode label.
12. Transfer tubes to plate position from STACS in tube storage rack. Label the storage rack with the STACS plate barcode.
13. Discard the FSC cartridges and wipe components and interior of FSC instrument with 70% ethanol as specified in the operating manual.

Note: Small amounts of resin particles may be present in the elution tube. This will not affect downstream applications.

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## A. GENERAL ACCESS TO SAMPLES AND DATA

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Reviewed by:

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DNA Indexing Technical Leader

Brenda Danosky
FB/DNA Program Manager
Forensic Sciences Command

## INTRODUCTION

This section describes policies and procedures for allowing general access to samples and data. This procedure may require the use of STACS-DB modules for retrieval of information.

## SAFETY CONSIDERATIONS

Not Applicable.

## PREPARATIONS

Not Applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

1. Access to offender sample information or test results is permitted only as specified in 730 ILCS 5/5-4-3 and the Administrative Rules, federal law, the National DNA Index System (NDIS) Operational Procedures, and any applicable Memorandums of Understanding (MOU's) with the FBI. All other requests for information will be denied.
2. Offender samples will not be released to any agency or removed from the laboratory system unless directed by court order, by valid subpoena, by contract with an approved vendor for analysis, or under a memorandum of understanding.
3. The identity of any person/agency making a request for non-public information must be verified prior to release of information by return telephone call at an official telephone number or official facsimile. Requests made through CODIS/NDIS will meet this requirement (DNA Search Request by Search Request Form or STR Target File on the CODIS website).
4. Copies of court orders, subpoenas, and the documentation of responses to such, will be kept at the Indexing Laboratory.

## REPORT WORDING

Not Applicable.

## REFERENCES

730 ILCS 5/5-4-3
Title 20: Part 1285: Joint Committee on Administrative Rules
NDIS Procedures Manual

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## B. PARTICIPATION IN THE INDEX

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## INTRODUCTION

This section describes the policies and procedures for participation in the Index.

## SAFETY CONSIDERATIONS

Not Applicable.

## PREPARATIONS

Not Applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURE

1. Participation in the Illinois DNA Index will be available to Illinois Forensic Laboratories who have been approved by Illinois' Designated State Official (DSO) for CODIS, State CODIS Administrator, and also approved by the National DNA Index System (NDIS) Custodian. Full participation includes adding, deleting, and searching profiles.
2. Participation in the DNA Index will require adherence to published standards.
   A. Participation in the DNA Index requires adherence to the current NDIS Procedures including the FBI's "Quality Assurance Standards for Forensic DNA Testing Laboratories", the FBI's "Quality Assurance Standards for DNA Databasing Laboratories", the Memorandum of Understanding (MOU) between the FBI and Illinois State Police, the MOU between the State and local laboratories, and the MOU between the FBI and local laboratories.
3. Application for laboratory participation in the DNA Index must be made through the DSO and by a signed Memorandum of Understanding.
4. Suspension/Reinstatement: All documentation will be reviewed by the ISP DSO and State CODIS Administrator. The DSO will make recommendations on laboratory suspension or reinstatement to the Commander of the Forensic Sciences Command.
   A. LABORATORY:

1) Suspension of a laboratory may be initiated for one or more of the following circumstances:
   a. Loss of accreditation.
   b. Loss of capability to perform DNA analysis at its facility.
   c. Loss of DNA technical leader, no one in the laboratory who meets the Quality Assurance Standards qualifications and is available to serve in that position, and no contingency plan.
   d. Fewer than two full-time employees who are qualified DNA analysts.
   e. Failure to comply with external Quality Assurance Standards (QAS) audit requirement.

2) A copy of audit results or certificates of accreditation will be forwarded to the DSO to restore participation in the Index. Additional documentation and laboratory audits may be required.

B. CODIS USER: A significant analytical/interpretive error (failure) by a CODIS User may result in suspension of the CODIS User from participating in the Index until the cause of the problem is identified and corrected.

1) The State CODIS Administrator may remove the CODIS User's DNA data from the Index while the error is investigated if the Director of Quality Assurance and the appropriate DNA Technical Leader determine that the error is significant to halt casework.

2) The Director of Quality Assurance will inform the DSO when the CODIS User's data may be re-entered into CODIS and that the CODIS User is reinstated. Acceptance of the data will be documented by the appropriate Technical Leader and forwarded to the DSO.

3) Failure of a periodic FBI Security Check or Violation of the Criminal Code committed by the CODIS User may also be reasons for suspension.

C. NDIS requests: An NDIS request for suspension will be evaluated by the DSO and State CODIS Administrator with input from the appropriate technical leader.

D. All suspensions or reinstatements of laboratories or laboratory personnel will be reported to the FBI NDIS Custodian by the State CODIS Administrator.

5. Software and communication equipment installation for CODIS will be accomplished according to the directives of the FBI.

6. Requests to search the database with data generated by private laboratories will be considered under ISP Policy: TCH 20 - DNA Profiles Generated by Private Laboratories.
7. Batch Target Files will contain DNA profiles received from CODIS Laboratories through the CODIS Batch Target Files on the CJIS-WAN, those specified under ISP policy TCH 20, and those accepted under previous agreements or policies.

## REPORT WORDING

Not Applicable.

## REFERENCES

NDIS Procedures Manual.
FBI's "Quality Assurance Standards for Forensic DNA Testing Laboratories".
FBI's "Quality Assurance Standards for DNA Databasing Laboratories."
Memorandum of Understanding (MOU) between the FBI and Illinois State Police.
Memorandum of Understanding (MOU) between the State and local laboratories.
Memorandum of Understanding (MOU) between the FBI and the local laboratories.
ISP Policy TCH 20.

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## C. CODIS

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## INTRODUCTION

This procedure describes transferring profiles into and searching CODIS, managing hits, and interaction with the state and national systems. This procedure requires the use of multiple CODIS programs and STACS-DB modules.

## SAFETY CONSIDERATIONS

Not Applicable.

## PREPARATIONS

Not Applicable.

## INSTRUMENT SPECIFICATIONS

Not Applicable.

## MINIMUM STANDARDS AND CONTROLS

Not Applicable.

## PROCEDURES

### DNA Records Accepted at SDIS

In accordance with the DNA Identification Act of 1994 (as amended), Illinois State Law, and Legal Opinion, the following categories of DNA records may be stored and searched in the Illinois State DNA Index System:

1. Alleged Father
2. Alleged Mother
3. Arrestee
4. Biological Child
5. Biological Father
6. Biological Mother
7. Biological Sibling
8. Compromised Sample
9. Convicted Offender
10. Criminal Parentage
11. Deduced Missing Person
12. Forensic Unknown
13. Forensic Mixture

14. Forensic Partial
15. Forensic Limited
16. Forensic Targeted
17. Indexing QC
18. Juvenile
19. Legal
20. Maternal Relative
21. Missing Person
22. Multi-allelic Offender
23. Other
24. Paternal Relative
25. QC
26. Spouse
27. Staff
28. Suspect, Known
29. Unidentified Person
30. Voluntary
31. Additionally, the following rules govern the uploading of DNA records to SDIS:
    A. DNA profiles submitted to SDIS shall be interpretable (interpretable - any DNA data that could be used to make an exclusion).
    B. A laboratory submitting a DNA profile to the Forensic Index at SDIS that is derived from forensic evidence, shall only offer those alleles that are attributed to the putative perpetrator(s). Alleles derived from forensic profiles that are unambiguously attributed to a victim or individuals other than the perpetrator(s), such as, but not limited to a husband or boyfriend, shall not be offered to SDIS.
    C. The DNA results from any locus in which an ambiguity exists in the assignment of one or more alleles to the putative perpetrator(s) may be offered to SDIS. The mere observation of alleles that may be attributed to individuals other than the putative perpetrator, does not in itself, preclude offering DNA profiles to SDIS at that locus.
    D. Forensic unknown DNA profiles submitted to SDIS shall have up to 3 alleles at a maximum of 1 locus, and of the remaining loci shall have no more than 2 alleles at each locus.
    E. Forensic mixture and forensic partial DNA records submitted to NDIS shall be reviewed by the submitting laboratory to ensure the DNA records satisfy a statistical threshold for match rarity of approximately one in the size of the NDIS database.

A laboratory's failure to comply with these authorized categories of DNA records at SDIS may result in the suspension or termination of that laboratory's access to SDIS in accordance with the DNA Identification Act of 1994.

## DNA Indexes at SDIS

In accordance with the DNA Identification Act of 1994 (as amended), Illinois State Law, and Legal Opinion, acceptable DNA profiles and records will be stored and maintained in the following indexes and files at SDIS:

A. Arrestee Index
B. Compromised Sample
C. Criminal Parentage
D. DNA Index of Special Concern
E. Forensic Index
F. Forensic Mixture Index
G. Forensic Partial Index
H. Forensic Limited Index
I. Forensic Targeted Index
J. Legal Index
K. Missing Person Index
L. Multi-Allelic Offender
M. Offender Index
N. Other Index
O. Pedigree Tree Index
P. QC Index
Q. Relatives of Missing Person Index
R. Single Typed Node
S. Staff Index
T. Spouse Index
U. Suspect Index
V. Unidentified Human (Remains) Index
W. Voluntary Index

## State DNA Index System (SDIS)

1. CODIS will be configured to automatically execute uploads or the State CODIS Administrator or designee will process uploads weekly.
2. Forensic related searches will be performed daily using parameters established by the CODIS State CODIS Administrator. The local laboratory will assess all matches and the disposition will be communicated to the State CODIS Administrator or designee.
   A. Case to Offender matches
      1) Offender verifications will be performed upon notification.
3. Offender to offender searches will be performed every work day. Matches will be assessed by the Indexing Laboratory.

A. Verify that the signature and printed name match on each of the offender sample receipts.

B. Compare the offender's information and verify that the following information matches:
   1) First and last names
   2) Dates of birth
   3) All additional personal information that is provided
   4) If a SID# is provided, other sources may be used to verify information i.e. LEADS, DOC, etc.

C. If all of the above information is confirmed, change disposition to offender duplicate.

D. If samples have blatant discrepancies, a latent verification must be performed.
   1) Blatant discrepancies may include, but are not limited to:
      (1) Completely different first name or last name
      (2) Alternate spelling for first name or last name
      (3) Nicknames (i.e. Jon vs Jonathan)
      (4) Differences in the date of birth
      (5) Different SID numbers
   2) Samples with non-blatant discrepancies will be marked in CODIS with a user defined disposition in CODIS until resolved.

E. If the samples become involved in a hit verification, conviction match, or offender to offender match, and cannot be resolved, a latent verification must be performed.
   1) Photocopy both offender sample receipts.
   2) Retain the copies in the laboratory and request a latent verification by a qualified examiner.
   3) Update the latent sent date in STACS-DB.
   4) Upon return of the receipts, ensure that the print examiner indicated a comparison with date and initials.
   5) Update the latent received date in STACS-DB.
   6) Disposition the match in CODIS as appropriate.
   7) If the offender sample cannot be verified as a duplicate or potential identical twins after being sent to Latent Prints, inform the Indexing Technical Leader to determine the appropriate situation specific action.
      (1) Potential identical twins are indicated by the same last name, same dates of birth, and matching DNA profiles.

F. If the samples become involved in a conviction match and cannot be resolved after latents, a hit verification must be performed.

4. The state CODIS administrator or designee will enter faxed or other appropriate profiles to the Batch Target File for searching against SDIS.

5. Offender matches resulting from Emergency Upload and Search Requests (EUSR) will be communicated to the State CODIS Administrator or designee immediately.

**National DNA Index System (NDIS)**

6. The State CODIS Administrator will upload data as prescribed by NDIS. The State CODIS Administrator will submit manual keyboard searches of eligible samples to NDIS at the request of the local laboratory and with approval of the NDIS Custodian.
7. The State CODIS Administrator will remove profiles from SDIS uploads as directed by the NDIS Custodian.
8. NDIS forwards search results to the state and individual local CODIS laboratories. Local laboratories are responsible for reviewing candidate matches and reporting dispositions to the State CODIS Administrator within the month the candidate match is declared.
9. No DNA profiles shall be uploaded to NDIS in the event that the CODIS Administrator position is unoccupied.

## REPORT WORDING

Not Applicable.

## REFERENCES

NDIS Procedures Manual

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## D. OFFENDER SAMPLE VERIFICATION

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## INTRODUCTION

Verification must be performed on samples before information is released for hits, discovery, and expungement of analyzed samples. This section describes the interaction between the DNA Indexing Laboratory and casework laboratories when a hit occurs. This procedure requires the use of the STACS-DB Hit Confirmation, Hit Tracking, and Storage Subsystem modules.

The DNA Indexing Laboratory does not issue formal reports of analysis; the Laboratory only issues letters of verification.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all reagents/samples as potential biohazards.
Refer to safety considerations under the individual sections of the DNA Indexing Procedures Manual.

## PREPARATIONS

Refer to the Clean Technique section.

## INSTRUMENT SPECIFICATIONS

Refer to the appropriate extraction and amplification sections.

## MINIMUM STANDARDS AND CONTROLS

Refer to the Interpretation of STRs section.

## PROCEDURE

### Hit Verifications

1. The local CODIS administrator or the case analyst will inform the Indexing Laboratory when a case-to-offender match has been determined. The casework laboratory will request offender verification. The Indexing Laboratory will make every effort to resolve the match to the Indexing Specimen within 30 business days.
2. DNA analysis:
   A. Two independent extractions and amplifications must be conducted.
      1) The hit file will be compiled under the offender sample designated as the original in STACS-DB.

2) If the match is to an unverified offender profile, place the sample on the hit confirmation list in the STACS-DB Hit Confirmation module. Reanalyze a portion of the original offender sample through STACS-DB.
3) If samples from the same offender have been analyzed previously and demonstrate matching profiles, the sample has been verified.
4) Verify that all controls typed correctly from the verification analysis.

B. Matching profiles exist when the two profiles match at a minimum of 6 CODIS autosomal loci, and are consistent at all labeled alleles. Below are the minimum documentation requirements:
1) Hard Copy Manual Data Review
a. Loci that pass interpretation thresholds do not need to be noted with distinguishing marks on the review table.
b. Loci without any labeled alleles (locus drop-out) do not need to be noted with distinguishing marks on the review table. These loci cannot be used for interpretation.
c. If there are loci with any labeled alleles that do not meet interpretation thresholds:
i. The analyst will compare these alleles to the corresponding profile's alleles in CODIS.
ii. The analyst will click off the alleles.
iii. Notations do not need to be made on the Hit Confirmation Report.
iv. The DNA Indexing Technical Leader will be notified of allele discrepancies.
v. When necessary, a printed electropherogram along with its corresponding controls will be included in the hard copy verification file.
d. All artifacts will be identified.
2) Electronic Manual Data Review
a. Review each electropherogram on the computer screen. Enter initials in the GeneMapper project to indicate review.
b. Loci without any labeled alleles (locus drop-out) cannot be used for interpretation.
c. If there are loci with any labeled alleles that do not meet interpretation thresholds:
i. The analyst will compare these alleles to the corresponding profile's alleles in CODIS.
ii. The analyst will click off the alleles.
iii. Notations do not need to be made on the Hit Confirmation Report.
iv. The DNA Indexing Technical Leader will be notified of allele discrepancies.
d. All artifacts will be identified.

3. Circumstances that do not correspond to any of the above will be documented with an explanation, i.e., effects of primer differences.

4. Inked Print – Fingerprint verifications are not a routine part of sample verifications, but may be performed if deemed necessary.
   A. Copy the sample receipt and retain the copy in the laboratory.
   B. Send the original to a latent print examiner for comparison to a Bureau of Identification ten print card.
      1) If the prints match, the original receipt and a copy of the ten print card is returned to the DNA Indexing Laboratory. Ensure that the latent print examiner documented the match with the date and the examiner's initials.
      2) If the prints do not match, the latent print examiner will notify the DNA Indexing Laboratory and perform a search of AFIS. Notify the DNA Indexing Technical Leader if no suitable match is found.
      3) If no Bureau of Identification ten print card is available, latents comparison will not be conducted. Send the appropriate letter to the requesting laboratory.
5. Personal Information
   A. Perform a LEADS and NCIC search.
   B. Compare the names, dates of birth, and identification numbers for consistency.
   C. Ensure that the offender has a qualifying reason listed on the sample receipt. If one is not provided, use available resources to determine the qualifying reason and document it on the sample receipt.
6. Verbal notification of the hit confirmation may be provided after steps 1 through 6 are completed and the offender information and data have been peer reviewed.
   A. The reviewer must document his/her initials, date of the review, and that providing verbal information to the agency is acceptable.
   B. Document the release of information on a conversation sheet and file it with the hit verification file.
7. Hard copy hit verification file reviews
   A. The analyst will ensure the file is complete by placing a check next to each applicable item.
      1) Non-applicable items require an "N/A".
   B. The technical reviewer will document an administrative and technical review by circling the checkmarks made by the analyst on the Verification File Checklist and initialing and dating the Sample Verification Form as the Technical Reviewer.
   C. Refer discrepancies between analysts and reviewers to the Technical Leader.
8. Electronic hit verification file reviews
   A. Reviews by both the analyst and technical reviewer are documented and tracked through the STACS-DB Hit Tracking Module.
   B. Refer discrepancies between analysts and reviewers to the Technical Leader.

**Notification of a Rush Hit Verification**

1. The LDIS Laboratory will notify the DNA Indexing Laboratory of a rush hit verification through email and/or verbal communication.

## LETTER WORDING

1. If the offense does not qualify or if the sample was collected before the effective date of the legislation, the Indexing Laboratory will verify the sample and issue a hit letter as normal. The Indexing Laboratory will then remove the DNA profile from CODIS and print the CODIS Deletion Report. The sample should then be marked as "Rejected" in STACS-DB. Place the report into the Hit Verification File and add a comment in STACS-DB that the sample was deleted from CODIS due to ineligibility.
    A. Notify the Assistant Laboratory Director after the DNA profile has been deleted from CODIS and the sample has been rejected in STACS-DB.
2. Hit Verification Letter guidelines for routine hits:
    A. An example letter can be found in the Appendix.
    B. Letters will only be sent for offender matches.
    C. Letters will only be sent to the requesting laboratories unless documentation exists in the hit file authorizing additional releases.
    D. Send a separate letter for each case when an offender hits to multiple cases.
    E. Send a separate letter for each non-duplicate offender when a forensic specimen hits to multiple offenders.
        1) If the offender samples are from different individuals, the letter should contain separate personal information sections for each offender.
        2) If the offender samples are from the same individual, only one personal information section is necessary.
    F. The search date should be the earliest date in CODIS.
    G. The date of the letter must be on or before the date of notification to the casework laboratory.

## REFERENCES

STACS Software

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## E. RUSH OFFENDER SAMPLE ANALYSIS

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## INTRODUCTION

This section addresses requests to perform DNA analysis of a particular offender sample out of chronological order. This is known as a 'Rush.' This procedure requires the use of the STACS-DB Outsource Batch Receiving, Extraction, Storage Subsystem and CODIS Upload modules.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all reagents/samples as potential biohazards.
Refer to safety considerations under the individual sections of the DNA Indexing Procedures Manual.

## PREPARATIONS

Refer to the Clean Technique section.
Refer to the appropriate extraction and amplification sections.

## INSTRUMENT SPECIFICATIONS

Refer to the appropriate extraction and amplification sections.

## MINIMUM STANDARDS AND CONTROLS

Refer to the appropriate extraction and amplification sections.
Refer to the Interpretation of STRs section.

## PROCEDURE

1. Local Forensic Science Laboratories will submit all requests from law enforcement agencies for the expedited analysis of an offender sample in writing (electronic mail is acceptable) to the Assistant Laboratory Director of the DNA Indexing Laboratory or designee. The request will include:
   A. The offender's name, date of birth, and any other identifying information available from the law enforcement agency.
   B. The case number and exhibit number.
2. The Forensic Science Laboratory will ensure that there is a forensic profile in CODIS with which to search the offender profile. Exceptions will be addressed by the Assistant Laboratory Director.
3. If the sample has been outsourced, select 'Process In-house'. The sample will be placed on the 'Extraction Worklist'.

4. DNA analysis can be performed in conjunction with hit verifications or routine analysis.
5. Rush offender sample analysis is completed when the DNA profile is uploaded to CODIS.
6. The Assistant Laboratory Director or designee will inform the local Forensic Science Laboratory upon completion.
7. The casework laboratory is responsible for searching the forensic sample remotely or may wait for the routine search of the State DNA Index System.
8. All requests and conversation records will be retained.

## REPORT WORDING

Not Applicable

## REFERENCES

Not Applicable.

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## F. EXPUNGEMENT

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## INTRODUCTION

This section describes the policies and procedures for expunging a sample. This procedure requires the use of the STACS-DB Sample Expungement, Submission, and Storage Subsystem modules. Three types of expungements exist in STACS-DB; administrative, legal, and quantity not sufficient (QNS). Administrative removals are expungements of samples that should not have been submitted or were submitted incorrectly. These include collections from persons convicted of a misdemeanor, the subject's name on the receipt and on the envelope were different, etc. Legal expungements are those ordered by the court to remove the sample profile and all of its information from the database. Because STACS-DB does not remove offender information from administrative expungements, both administrative and legal expungements are performed as legal expungements.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices
Warning: Treat all reagents/samples as potential biohazards.
Refer to safety considerations under the individual sections of the DNA Indexing Procedures Manual.

## PREPARATIONS

Refer to the Clean Technique section.
Refer to the appropriate extraction and amplification sections.

## INSTRUMENT SPECIFICATIONS

Refer to the appropriate extraction and amplification sections.

## MINIMUM STANDARDS AND CONTROLS

Refer to the appropriate extraction and amplification sections.
Refer to the Interpretation of STRs section.

## PROCEDURE

1. Requests for expungement will be handled in accordance with the 730 ILCS 5/5-4-3 and the Administrative Rules.
2. Court Ordered Expungements. Take the following actions whenever a valid notification for expungement is received or follow the instructions per court order:
   A. The Assistant Laboratory Director will approve all court ordered expungements.

1. Verify the offender information on the expungement request matches the information on the sample receipt
2. Verify the DNA profile using STACS-DB QA/QC module.

B. Verify there is no other qualifying reason for this individual.
C. Check CODIS for pending matches.
D. Use the STACS-DB Sample Expungement module to delete all computer records correlating the PID and other laboratory files.
E. Purge the PID file of all data correlating the PID files and DNA typing results.
F. Print the STACS-DB Submission Report documenting the expungement and place in the PID file.
G. Retain the court order in an expungement file maintained by the Assistant Laboratory Director.
H. Remove sample and sample DNA results from all levels of CODIS. Include the NDIS reconciliation report in the Expungement file or as directed by a court order.
I. Destroy all forms of the sample following the guidelines for disposing of potentially biohazardous waste.
J. Write a letter to the court/attorneys documenting compliance, if requested.

3. Administrative Removal Expungements. Take the following action when a valid administrative removal notification is received:

A. Ensure that the sample is the one in the request. Verification of the DNA profile must be performed on samples that have been analyzed.
B. Use the STACS-DB Sample Expungement module to delete all computer records correlating the PID and other laboratory files. Print the submission information from the STACS-DB Submission module and place in the PID file.
C. Remove the contents of the PID file and place in the expungement file.
D. Remove all forms of the sample from storage using the STACS-DB Storage Subsystem. Dispose as potentially biohazardous waste.
E. Remove DNA results from all levels of CODIS if DNA analysis has been completed.
1. Print the CODIS Deletion Report and place the report into the Administrative Removal File.
F. Write a letter documenting compliance, if requested.

## REPORT WORDING

Not Applicable.

## REFERENCES

Title 20: Part 1285: Joint Committee on Administrative Rules

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

## APPENDIX A: QUALITY ASSURANCE

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Reviewed by:

Forensic Scientist Kerry M. Reavis, CAB Chairperson
DNA Indexing Command Advisory Board

Approved by:

Forensic Scientist Ashley Y. Flack
Springfield Forensic Science Laboratory
DNA Indexing Technical Leader

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FB/DNA Program Manager
Forensic Sciences Command

## I. GOALS AND OBJECTIVES

1. Overall goals:
   - A. To provide high quality, state of the art offender sample databasing services to the citizens of the State of Illinois;
   - B. To serve as the Illinois State DNA Index System; and,
   - C. To ensure the quality of the offender sample testing and the integrity of the state database.
2. Objectives:
   - A. To have documented DNA Indexing procedures which ensure the output of a quality product;
   - B. To routinely monitor the laboratory's performance and the integrity of the offender database; and,
   - C. To document the identification and correction of problems with analysis.

## II. ORGANIZATION AND MANAGEMENT

1. The DNA Indexing Quality Assurance Program is part of the Command's Quality Assurance Program. The following topics are addressed in the Command QA Manual:
   - A. Command Quality Assurance Program
   - B. Competency Testing
   - C. Proficiency Testing
   - D. Administrative Reviews
   - E. Command Quality Assurance Reviews
   - F. Mock Trial/Court Appearance Rating
   - G. Forensic Biology/DNA Quality Assurance
   - H. External Proficiency Testing
   - I. Blind Proficiency Testing
   - J. Corrective Action

## III. DNA INDEXING PERSONNEL QUALIFICATIONS AND TRAINING

1. DNA Indexing Analyst.

A. Educational Prerequisites

1. Forensic Scientists that have completed the ISP DNA Training Program and were hired into the position to perform DNA analysis including interpretation prior to July 1, 2009, will have met or exceeded the following requirements:

a. Bachelor of Science, Bachelor of Arts or advanced degree in a natural science or its equivalent.

b. Three separate university/college courses totaling at least six cumulative semester hours in the following subject areas:

i. Molecular Biology
ii. Genetics
iii. Biochemistry

c. Statistics and/or population genetics completed through the ISP Training Program or external training.

2. Forensic Scientists that have completed the ISP DNA Training Program and were hired into the position to perform DNA analysis including interpretation between July 1, 2009 and June 30, 2020, will have met or exceeded the following requirements:

a. Bachelor of Science, Bachelor of Arts, or advanced degree in a biology, chemistry, or forensic science-related degrees that must have science and laboratory-based course as an integral component.

b. Three separate university/college courses totaling at least nine cumulative semester hours where each of the following areas of study comprise an integral component for the specified course:

i. Molecular Biology
ii. Genetics
iii. Biochemistry

c. Statistics and/or population genetics completed through the ISP Training Program or external training.

3. Forensic Scientists that have completed the ISP DNA Training Program and were hired into the position to perform DNA analysis including interpretation on or after July 1, 2020, will have met or exceeded the following requirements:

a. Bachelor of Science, Bachelor of Arts, or advanced degree in a biology, chemistry, or forensic science-related degrees that must have science and laboratory-based coursework as an integral component.

b. Three separate university/college courses totaling at least nine cumulative semester hours where each of the following areas of study comprise an integral component for the specified course:

i. Molecular Biology
ii. Genetics
iii. Biochemistry

c. University/college course in statistics and/or population genetics that has been successfully completed.

To qualify, courses do not have to have these titles, but must cover equivalent material. The DNA Indexing Technical Leader will review the course syllabus or letter from the instructor describing course content to determine if the course work/equivalents meet the prerequisite requirements.

B. Training/Qualifying

1) Each individual will complete a formal period of training and evaluation prior to assuming independent Indexing responsibilities. The date the analyst was hired will be used to determine the applicable QAS version for education, experience, and training requirements.

a) New analysts will complete the documented Forensic Biology and DNA Training programs with the exception of the mock and supervised casework modules. The new analysts will then complete the documented DNA Indexing training program.

b) Experienced analysts will have their training program documentation and technical knowledge reviewed

and evaluated; and will complete Indexing training modules.

2) The training/qualifying program will be documented in a training file.
   a) The training coordinator will document the successful completion of the training/qualifying program in a training file. A check list will be maintained summarizing the training.
   b) Upon the completion of the training program, the training file will be sent to the Director of Training or designee.
   c) Upon completion of the training, the Director of Training will provide a copy of the training checklist and a letter confirming the completion of training to the trainee's laboratory director and DNA Indexing Technical Leader.

C. Experience
   1) All individuals will have worked in a human DNA laboratory for a minimum of six months with at least three months in a forensic or database laboratory, prior to assuming independent DNA Indexing responsibilities.

D. Certification
   1) Final approval for conducting independent Indexing analyses rests with the Command Administration.
   2) Initial certification is based on a recommendation by a training coordinator prior to beginning independent DNA Indexing analyses within the Command.
      a) To be certified in this manner, an individual will do the following:
         (i). Demonstrate the ability to analyze blood and body fluids using the appropriate DNA technology;
         (ii). Demonstrate the ability to reproduce accurate and precise results;
         (iii). Demonstrate the ability to conduct analysis on previously analyzed offender samples;
         (iv). Demonstrate theoretical knowledge of DNA analysis;
         (v). Successfully complete competency tests;
         (vi). Successfully complete a mock trial;
         (vii). Successfully complete supervised offender analysis; and
         (viii). Successfully complete a comprehensive examination.

b) Upon completion of Indexing training, each analyst must successfully pass a qualifying test. Five samples must be analyzed.

E. Duties include:
1) Conduct offender sample analysis.
2) Review and interpret offender sample data.
3) Perform hit verifications and report results.
4) Upload data to CODIS.
5) Perform quality assurance and quality control tests.

2. DNA Indexing Technical Leader

A. Technical leadership of the DNA Indexing Section will be provided and conducted in accordance with Command programs and the Quality Assurance Standards for DNA Databasing Laboratories:

B. Education Prerequisites

1) The technical leader must be a full-time employee of the Forensic Sciences Command and have, at a minimum, a Master's degree in a biology-, chemistry-, or forensic science-related area and successfully completed a minimum of 12 semester or equivalent credit hours of a combination of undergraduate and graduate (at least one course of three hours) course work covering the following subject areas:
a) Biochemistry
b) Genetics
c) Molecular Biology
d) Statistics and/or Population Genetics

To qualify, courses do not have to have these titles, but must cover equivalent material. The training coordinator will review the course syllabus or letter from the instructor describing course content to determine if the course work/equivalents meet the prerequisite requirements.

2) If the educational requirements are not satisfied, the Indexing Technical Leader may possess a waiver from the American Society of Crime Laboratory Directors (ASCLD) or other organization designated by the Director of the FBI.

C. Training/ Qualifying

1) The Indexing Technical Leader must have completed a documented training program in DNA analysis in a program that includes the methods, procedures, equipment, and materials used in forensic DNA analysis and their applications and limitations.

D. Experience

1) The Indexing Technical Leader must have a minimum of three years human DNA laboratory experience as a qualified analyst on database or forensic samples.

2) The Indexing Technical Leader shall have previously completed or will complete the FBI sponsored auditor training within one year of appointment.

E. Duties include:

1) Managing the technical issues and technical problem solving of analytical methods for the DNA Indexing Laboratory to include:
   a) Assistance on difficult or non-routine sample analysis and resolution of disagreements between analysts.
   b) Approval of the technical specifications for outsourcing and serving as the technical point of contact for the outsourcing laboratory to assist with technical questions and issues.
   c) The authority to initiate, suspend, and resume DNA operations for the laboratory or an individual.
2) Documenting an annual review of all methods used by the DNA Indexing Laboratory and includes approving all validations and procedures used in DNA Indexing analysis. Newly-appointed technical leaders must document a review of all validations and methodologies currently used by the laboratory.
3) Has oversight responsibility for and will document an annual review of the DNA Indexing training and safety programs to include:
   a) The oversight of training of laboratory staff.
   b) The review of educational qualifications for DNA analysts.
4) Proposing new or modified analytical Indexing procedures.
5) Documenting an annual review of the DNA Indexing Procedure Manual and is responsible for the DNA Indexing quality assurance program to include:
   a) The review of incident reports and approval of corrective action as needed, including the resolution and reporting of any contamination or extraneous DNA issues identified in a sample.
   b) The accountability for the laboratory's quality assurance program to the extent that he or she has the authority to terminate the laboratory's DNA testing in the event of a technical problem until the problem is solved.
   c) The resolution of outsourcing quality issues.
6) Documenting a review of every audit of the DNA Indexing Laboratory. The DNA Indexing Technical Leader will approve any corrective action(s), provide input on technical

matters which arise from audits, and assist with responses to any audit findings.

7) Documenting the review and approval of the proficiency testing program and overseeing the proficiency testing performance of analysts via information provided by the Director of Quality Assurance and Quality Review Coordinator assessments; and is responsible for any corrective action.

8) Accessible to the laboratory to provide on-site, telephonic, or electronic consultation as needed. This includes accurate and timely communication to the DNA analysts regarding Command decisions affecting DNA.

9) Review the academic transcripts and training records for newly qualified analysts and approve their qualification prior to independent database analysis and document such review. Newly-appointed technical leaders must document a review of the educational qualifications and training records of all currently qualified analysts.

10) Review potential conflicts of interest involving contractual employees, hired by the Illinois State Police, who are also employed by other NDIS and/or vendor laboratories.

F. Technical leader succession plan:

1) When the technical leader vacates the position, the Assistant DNA Indexing Technical Leader will serve as the Technical Leader until a permanent replacement is selected. If the Assistant position is vacant at that time, a casework DNA Technical Leader will serve as the DNA Indexing Technical Leader. If there is no one in the Command qualified and willing to be the DNA Indexing Technical Leader, the laboratory will cease new sample analysis and the Command will immediately institute a nationwide search for a qualified individual.

G. An Assistant DNA Indexing Technical Leader:

1) Will be selected from qualified members of the DNA Indexing staff. The duties include functioning as the technical leader when the technical leader is out of the laboratory and serving as the technical leader when that position is vacant.

3. State CODIS Manager

A. Education Prerequisites

1) The State CODIS Manager must meet the education requirements of an analyst.

B. Training/Qualifying

1) The State CODIS Manager must have a working knowledge of the following:
   a) Computers
   b) Computer networks
   c) Computer database management

Formal CODIS training by the FBI provides sufficient knowledge to meet this criterion.

1) The State CODIS Manager must be or have been a qualified analyst and have documented training in mixture interpretation.
2) The State CODIS Manager must have successfully completed FBI DNA auditor training and CODIS software training within six months of appointment if either had not already been completed.

C. Duties include
1) Administration of the laboratory's CODIS network.
2) Scheduling and documentation of the CODIS computer training of database analysts.
3) Assurance that the security of data stored in CODIS is in accordance with state and/or federal law and NDIS operational procedures.
4) Assurance that the quality of data stored in CODIS is in accordance with state and/or federal law and NDIS operational procedures.
5) Assurance that matches are dispositioned in accordance with NDIS operational procedures.
6) Has the authority to terminate an analyst's or laboratory's participation in CODIS in the event of a problem until the reliability and security of the computer data can be assured. This authority extends to all CODIS sites in Illinois.

4. Laboratory Technician

A. Education and Training
1) Will have documented training, education, and experience commensurate with their responsibilities as outlined in the job description.
2) Training/Qualifying
   a) Must receive on the job training specific to their job function.
   b) Must complete a qualifying test before performing analytical tests with Indexing samples.

B. Duties include:
1) Reagent preparation, monitoring, laboratory maintenance, and performing instrument maintenance and checks.
2) Receive, prepare, and store offender samples.

3) Receive and store reagents, commodities, and equipment.
4) Maintain DNA offender samples in accordance with the Sample Control Policy and using STACS-DB modules.
5) Enter personal data using STACS-DB modules.

5. Continuing Education

A. Each analyst, the Indexing Technical Leader, and the CODIS Manager must be responsible for keeping abreast of current developments within the field. Each DNA analyst, the Indexing Technical Leader, and CODIS Manager must complete eight hours of annual continuing education. Annual is defined as per calendar year. Examples of how this may be accomplished include:

1) Professional organizations and their meetings;
2) In-service training;
3) Attendance at formal training courses;
4) Participation at in-house technical meetings/ courses/ seminars; and
5) College course work.

NOTE: Training provided to qualify an analyst to perform an analytical procedure in DNA Indexing offender analysis will not be considered continuing education.

In addition, each analyst will at a minimum read nine publications related to DNA per year. This will be documented in LIMS.

B. The laboratory director will provide the opportunity to participate in these activities as outlined in the following directives:

1) Tuition Reimbursement
2) Society Memberships
3) Command Advisory Board (CAB)
4) Attendance at Professional Meetings
5) Out-of-State Travel Requests

## IV. SAMPLE HANDLING AND FACILITY REQUIREMENTS

1. All DNA analysts/technicians will follow clean technique as documented in the DNA Indexing Procedures Manual.
2. The Indexing Laboratory will conduct the following activities either in a separate space or at a separate time:
   A. Sample receipt and preparation
   B. DNA extraction
   C. PCR set up
3. The Indexing Laboratory will conduct the following activity in a separate, dedicated space
   A. Amplification
   B. Post-PCR analysis
4. Aqueous amplified products will be contained in a room separate from non-amplified product. Amplified product may be removed from the amplification area for disposal only. If amplified product is removed from the amplification area it will be sealed in a closed container.
5. The laboratory will follow the decontamination procedures outlined in Clean Technique found in the DNA Indexing Procedures Manual.
6. The laboratory administration will monitor clean technique practices. Monitoring will include, but not be limited to, a review of the bleach logs. This is done to ensure that all surfaces and equipment are being bleached properly. Clean technique practices will be evaluated during laboratory inspections and QA visits.
7. Cleaning and Sterilization Procedures
   A. Non-disposable glassware and plastic containers will be cleaned with detergent and completely rinsed with tap water.
   B. All waste from the PCR room will be sealed in a closed container before being removed from the PCR room.
   C. The laboratory will follow the decontamination procedures outlined in Clean Technique found in the DNA Indexing Procedures Manual.
      1) The analytical laboratory floor will be mopped using a freshly prepared 10% bleach solution once a week. The PCR room must be mopped last. This must be documented in logs located in the Indexing laboratory.
      2) The entire analytical laboratory (countertops, equipment, etc.), including the PCR room, must be bleached once a week with a freshly prepared 10% bleach solution. This must be documented in logs located in the Indexing laboratory.
      3) The bleach log is used to monitor decontamination of facilities and equipment according to the *Quality Assurance Standards for DNA Databasing Laboratories*.

## V. SAMPLE CONTROL

1. The laboratory will have a documented sample control system to ensure the integrity of offender samples. This is outlined in the following DNA Indexing Policies/Procedures:
   - A. Sample Receipt
   - B. Sample Files
   - C. Sample Recollection
   - D. Sample Duplicates
   - E. Sample Preservation
   - F. Contractual Analysis
   - G. Expungement
   - H. Clean Technique
   - I. Quality Control Analysis Measures
   - J. Quality Control Search Measures
2. Retention of Offender Samples and Records:
   - A. All sample files, sample verification files, and laboratory analysis files will be retained indefinitely at the Indexing Laboratory or at a secure facility for archival storage.
     - 1) Maintain original sample receipt forms and all laboratory-generated documents.
     - 2) Maintain electronic and/or paper documents generated during DNA analysis.
     - 3) Maintain a record of the sample number, current location, a notation if sample is consumed, and date.
   - B. Offender samples will be retained indefinitely.
     - 1) Materials generated by PCR analysis:
       - a) Extracted DNA remaining after analysis will only be maintained on samples if no original sample remains.
       - b) Amplified DNA will be destroyed after analysis.
3. Sample Storage
   - A. Storage requirements are defined in the Sample Preservation section.

## VI. QUALITY CONTROL SAFEGUARDS

Quality control safeguards have been developed for the Indexing Laboratory to ensure:

- correct data is uploaded to CODIS
- correct case-to-offender database hits are identified
- expungement of the correct sample and results from CODIS
- release of correct information for an offender sample in response to a request for discovery or under the Freedom of Information Act

1. Analysis Measures
   - A. Swab receipts and envelopes are bar coded by the manufacturer.
   - B. Sample bar coding: Each sample and the corresponding sample receipt are uniquely bar-coded when received.
   - C. Sample Tracking: Analytical and storage plates are uniquely bar coded and tracked through the analytical process by STACS-DB.
   - D. Known Orientation Control: A known control is placed on each analytical plate in a position selected by the computer. The location of the control is suggested by STACS-DB so that consecutive plates do not have the control in the same location. When analysis is completed, the known orientation control must be located in the correct position and must type correctly with a minimum of six (6) autosomal CODIS loci. Exceptions must be approved by the DNA Indexing Technical Leader. If the control results are incorrect, the plate is discarded and sample analysis is repeated. An orientation control is not required on plates containing previously typed or manually extracted samples.

2. Search Measures
   - A. Duplicate Samples: The offender Index is searched for duplicates. These serve as blind proficiency samples.
   - B. Conviction Matches: These result from matches between an offender and a forensic profile previously matched to a suspect, or an individual who has been convicted in that forensic case. These serve as blind proficiency tests.
   - C. Sample Verification: Offender sample results are verified with each case-to-offender hit, before expunging offender samples and results, and before releasing results for discovery and Freedom of Information requests.
   - D. Hit Confirmation: A case-to-offender hit is confirmed after an evidentiary sample is drawn from the suspect identified through a database hit. This confirmatory sample is analyzed and directly compared to the forensic evidence profile by the casework laboratory. This is the ultimate test of the quality of the entire convicted offender DNA program, testing proper collection by

agencies over which ISP has no control and our own sample analysis and databasing.

## VII. VALIDATIONS

1. The laboratory will use validated methods and procedures as outlined in the Command Directives and meet *Quality Assurance Standards for DNA Databasing Laboratories*. (Standard 8.1).
2. References for STR Validation
   - A. Baylor College of Medicine, Inventors. Perkin-Elmer Applied Biosystems Corporation, assignee. Amp*Fl*STR Blue PCR Amplification Kit. U.S. Patent 5,364,759.
   - B. Baylor College of Medicine, Inventors. Perkin-Elmer Applied Biosystems Corporation, assignee. Amp*Fl*STR Profiler Plus PCR Amplification Kit. U.S. Patent 5,364,759.
   - C. Baylor College of Medicine, Inventors. Promega Corporation, assignee. *GenePrint* PowerPlex Fluorescent STR System.
   - D. Budowle B., Sprecher C.J. Concordance Study on Population Database Samples Using the PowerPlex 16 Kit and Amp*Fl*STR Profiler Plus Kit and Amp*Fl*STR COfiler Kit. J. Forensic Science 46:637-641.
   - E. Comey C.T., Koons B.W., Presley K.W., Smerick J.B., Sobieralski C.A., Stanley D.M., Baechtel F.S., DNA Extraction Strategies for Amplified Fragment Polymorphism Analysis. J. Forensic Sci. 39:1254-1269.
   - F. Crouse C.A., Rogers S., Amiott E., Gibson S. and Masibay A. Analysis and Interpretation of Short Tandem Repeat Microvariants and Three Banded Allele Patterns Using Multiple Allele Detection Systems. J. Forensic Sci. 1999;44:87-94.
   - G. Edwards A., Civitello A., Hammond H.A., Caskey C.T. DNA Typing and Genetic Mapping with Trimeric and Tetrameric Tandem Repeats. Am J. Hum Genet 1991; 49:746-756.
   - H. Edwards A., Hammond H.A., Jin L., Caskey C.T., Chakraborty R. Genetic Variation at Five Trimeric and Tetrameric Tandem Repeat Loci in Four Human Population Groups. Genomics 1992; 12:241-253.
   - I. Finis C.J. Megaplex STR Analysis from a Single Amplification: Validation of the PowerPlex 16 System. Profiles in DNA; January 2001:3-6.
   - J. Fregeau C.J., Bowen K.L. and Fourney R.M. Validation of Highly Polymorphic Fluorescent Multiplex Short Tandem Repeat Systems Using Two Generations of DNA Sequences. J. Forensic Sci. 1999;44:133-166.
   - K. GeneScan and Genotyper [Computer Programs] Macintosh Version, 1997.
   - L. Gentra Systems, Inc. Puregene DNA Isolation Kits.

M. Hammond H.A., Jin L., Zhong Y., Caskey C.T., Chakraborty R. Evaluation of 13 Short Tandem Repeat Loci for Use in Personal Identification Applications. Am J. Hum Genet 1994;55:175-189.
N. ISP DNA Indexing Validations.
O. Perkin-Elmer Applied Biosystems Amp*Fl*STR Cofiler PCR Amplification Kit User's Manual, 2001.
P. Perkin-Elmer Applied Biosystems Amp*Fl*STR Cofiler PCR Amplification Kit User's Manual, 2001. Krenke B.E., Tereba A., Anderson S.J., Buel E., Culhane S., Finis C., Tomsey C., Zachetti J., Masibay A., Rabbach D., Amiott E., Sprecher C.J. Validation of a 16-locus Fluorescent Multiplex System. J. Forensic Science 47:773-785.
Q. Perkin-Elmer Applied Biosystems Amp*Fl*STR Profiler Plus PCR Amplification Kit User's Manual, 1997.
R. Perkin-Elmer Applied Biosystems QuantiBlotTM Human DNA Quantitation Kit User's Manual, 1996.
S. Perkin-Elmer Applied Biosystems. Performance Optimized Polymer 6 (POP-6) U.S. Patent 5,552,028.
T. Perkin-Elmer Applied Biosystems. Performance Optimized Polymer 4 (POP-4) US Patent 5,552,028.
U. Promega Matrix FL-JOE-TMR-CXR Technical Bulletin, 2001.
V. Promega PowerPlex®16 System Technical Manual, 2000.
W. Promega PowerPlex Matrix Standards, 3100 Technical Bulletin, 2001.
X. Qiagen. QIAmp 96 DNA Swab BioRobot Kit Handbook. 2001.
Y. Sprecher C., Krenke B., Amiott B., Rabbach, Grooms K. The PowerPlex®16 System. Profiles in DNA; August 2000:3-6.
Z. Walsh P.S., Fildes NJ, Reynolds R. Sequence Analysis and Characterization of Stutter Products at the Tetranucleotide Repeat Locus vWA. Nucleic Acids Res. 1996;24:2807-2812.
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CC. Promega PowerPlex Y23 Technical Manual, 2012.
DD. Promega PowerPlex Fusion Technical Manual, 2020
EE. GeneMapper ID-X Software Manual Version 1.5
Publication Number 100031707 Revision A

FF. GeneMapper ID-X User Bulletin Version 1.6
Publication Number 100073905 Revision B

GG. GeneMapper ID-X Software Getting Started Guide Version 1.5
Publication Number 10031701 Revision B

## VIII. ANALYTICAL PROCEDURES

1. Procedures. The laboratory will have approved written analytical procedures.
   - A. Procedures used in DNA Indexing will be approved by the DNA Indexing Technical Leader and according to the Command Directives.
   - B. Procedures being developed as part of the R&D Program may be used in offender databasing with approval by the DNA Indexing Technical Leader and with Command approval.
2. Reagents. The laboratory will use reagents that are suitable for the methods employed.
   - A. Reagents, chemicals, and analytical commodities will be tracked in STACS-DB.
   - B. Reagents may be prepared with non-sterile, deionized water.
   - C. The laboratory will maintain a log for documenting commercial biological reagents utilized in the laboratory. This log will be maintained in the STACS-DB program.
     1) The log will be maintained on all chemicals received in the laboratory.
     2) Information contained in the log will include the:
        a) manufacturer,
        b) date received,
        c) lot numbers received,
        d) quantity of each lot received,
        e) storage conditions
        f) expiration date, if appropriate.
     3) An annual inventory of these reagents will be conducted by a designated person in the laboratory.
     4) The laboratory will maintain a log for documenting the formulation of all reagents prepared in the laboratory.
     5) The formulations for all reagents are found in the DNA Indexing Procedure Manual and STACS-DB.
     6) Information kept in the log must include the:
        a) Preparation date,
        b) The lot numbers of chemicals used to prepare the reagent,
        c) The concentration and quantity of the reagent prepared,
        d) The identity of the individual preparing the reagent,
        e) The storage conditions of the reagent.
   - D. Laboratory prepared reagents will be labeled with the:
     1) Identity of the reagent
     2) Concentration of the reagent
     3) Date of preparation

4) Date of expiration
5) Identity of the individual preparing the reagent
6) Storage conditions.
7) A hazard warning, if necessary

E. Purchased reagent containers will be labeled with the following:
1) STACS-DB barcode to include date received, expiration date, and initials
2) Storage conditions

F. Expiration Dates
1) Use the expiration date listed.
2) If there is no manufacturer's suggested expiration date, then the expiration date will be set at one year from the date opened.
3) Reagents that are prepared and frozen immediately have an indefinite expiration date. However, once the reagent is thawed, it has a one year expiration date from the date it was thawed.
4) All other prepared reagents expire one year from the date prepared.

G. Critical Reagents.
1) The following reagents have been defined as critical reagents for DNA Indexing:
a) DNA amplification kit components
b) DNA Internal Lane standard
2) Critical reagents must be quality controlled in-house.
a) Procedures for quality control of these reagents are found in this manual.
(i) In use testing is acceptable. Quality control procedures may be performed during routine laboratory processing.
(ii) A critical reagent log will be maintained documenting all quality control procedures performed on a particular lot of a reagent. STACS-DB will maintain the log.
(iii) If a particular supply, chemical, reagent or material does not meet the required quality control standard(s), the DNA Indexing Technical Leader and the manufacturer will be notified and the entire lot rejected. Reject the lot in STACS-DB.
(iv) The procedures for critical reagents do not have to be run individually but may be combined with other procedures as appropriate.
(v) Quality control records will be maintained indefinitely.

H. In quality control procedures specifying use of a previously controlled lot for comparison to a new lot, appropriate controls and known standards will be compared if an old lot is available.

I. Non-Critical Reagents. These are not critical reagents but are tested prior to use.

1) Yield Gel Standards

3. Standards & Controls

A. The following standards and controls will be used in PCR STR analysis of offender samples and are included in the DNA Indexing Procedures. These standards and controls must work properly.

1) Quantitation standards for estimating the amount of DNA (for manual processing only).

2) Positive and negative amplification controls.

3) Allelic ladder and internal lane standard for variable number tandem repeat sequence PCR based systems.

4) Manipulation blanks, reagent blanks and/or negative amplification controls will be used to monitor clean technique. If DNA is present above the detection threshold in a blank or negative, it must be reported to the Indexing Technical Leader. The sample batch will be re-extracted or reamplified as appropriate.

a) The Indexing Technical Leader will inform the Quality Assurance Manager when DNA profiles appear in a blank. An incident report must be made by the Indexing Technical Leader and placed in the Incident Notebook located in the laboratory. A copy of the report must be given to the Quality Assurance Manager.

b) The Orientation Control will be used to monitor the position of samples and the successful orientation of the plate on the robot decks. An orientation control is not required on plates containing hit verification samples. The orientation control must be located in the proper position and must type correctly at a minimum of six (6) autosomal CODIS loci. Exceptions must be approved by the DNA Indexing Technical Leader. If the orientation control is not positioned correctly or does not type correctly, all samples in the batch/plate will be reanalyzed. Inform the Indexing Technical Leader.

4. Reporting frequency estimates.

A. For a given population(s) and/or hypothesis of relatedness, the probability of observing a particular forensic unknown profile in the offender database will be estimated as the Random Match Frequency of the forensic unknown multiplied by the number of unique profiles in the offender database. The Random Match Frequency calculations

will be derived from a documented population database appropriate for the calculation.

## A. Verification of Yield Gel Standards

1. Purpose: To compare newly prepared standards against previously tested standards or commercially prepared standards to ensure consistency.
2. Procedure:
   - A. Prepare new yield gel standards.
   - B. Test new standards against previously tested standards.
   - C. Record the results on the "Verification of Yield Gel Standards Form."
3. Assessment of Results:
   - A. Compare the band intensity and width of the previously tested standards against the new standards.
   - B. Accept standards that are comparable to the previous set of standards.
   - C. Discard all of the standards and repeat the procedure if unacceptable.
4. Perform this procedure for each new preparation of yield gel quantitation standards.
5. Store Frozen.
6. Expiration dates
   - A. Indefinitely until thawed.
     - 1) Expires one year from date thawed.

**Verification of Yield Gel Standards Form**

| Cathodal Region | | | |
|---|---|---|---|
| Lane | Sample | Volume | DNA (ng) |
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| 6 | | | |
| 7 | | | |
| 8 | | | |
| 9 | | | |
| 10 | | | |
| 11 | | | |
| 12 | | | |
| 13 | | | |
| 14 | | | |
| Middle Origin | | | |
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| 6 | | | |
| 7 | | | |
| 8 | | | |
| 9 | | | |
| 10 | | | |
| 11 | | | |
| 12 | | | |
| 13 | | | |
| 14 | | | |

Analyst _____ Date _____

Lot # _____

Date of Dilution _____

Previously Tested Standards:

Lot # _____

Date of Dilution _____

Notes:

Are the band intensities and widths of the new standards comparable to the previous lot? _____

**Attach**

**Photograph**

**Here**

B. **Verification of Primer Mixture**

1. Purpose: To demonstrate that a new lot of primer mixture can produce accurate typing results.
2. Procedures:
   A. Prepare an amplification reaction mixture using the new lot of primer mixture.
   B. Amplify a positive, reagent blank, and negative amplification control. The positive is located in the kit.
   C. Analyze the amplified products using a genetic analyzer and associated software.
   D. Record the results on the "Verification of Primer Mixture Form" and attach the electropherograms from the positive and negative controls.
3. Assessment of Results.
   A. Verify that all of the control samples typed correctly.
   B. Reject the lot number of the primer if incorrect or incomplete typing results are obtained. Notify the DNA Indexing Technical Leader and the manufacturer.
4. Storage Conditions.
   A. PowerPlex Y23: Store frozen.
   B. PowerPlex Fusion: Store frozen.
5. Expiration Dates:
   A. Use the expiration date on the box. If the outer packaging has a different expiration date than the reagent, use the date from the packaging and retain the packaging until the reagent is consumed or discarded.

## Verification of Primer Mixture Form

QC Date_____ Analyst_____

Primer Lot #_____ Kit type_____

Expiration Date_____

**Reference Positive Lot#** _____

**Reference Master Mix Lot#** _____

**Reference ddi$H_2O$ (or equivalent) Lot#** _____

**Results and Conclusions:**

Do all controls exhibit expected alleles at sufficient peak height?_____

Are the negative controls blank? _____

Did this lot pass the QC test? _____

**Notes:**

**Include the positive control and negative control electropherograms along with this sheet.

## C. Verification of PowerPlex 5X Master Mix

1. Purpose: To demonstrate that a new lot of 5x Master Mix can produce accurate typing results.

2. Procedures:
   - A. Prepare an amplification reaction mixture using the new lot of 5x Master Mix.
   - B. Amplify a positive, reagent blank, and negative amplification control. The positive control is 2800M and is located in the kit.
   - C. Analyze the amplified products using a genetic analyzer and associated software.
   - D. Record the results on the Verification of PowerPlex 5x Master Mix Form and attach the electropherograms from the positive and negative controls.
3. Assessment of Results.
   - A. Verify that all of the control samples typed correctly.
   - B. Reject the lot number of the 5x Master Mix if incorrect or incomplete typing results are obtained. Notify the DNA Indexing Technical leader and the manufacturer.
4. Storage Conditions:
   - A. PowerPlex Y23 and PowerPlex Fusion 5x Master Mix are stored frozen.

## Verification of PowerPlex Master Mix Form

QC Date_____ Analyst_____

5X Master Mix Lot #_____ Plate #_____

Expiration Date_____ Kit Type_____

Reference 2800M Lot# _____

Reference Primer Lot# _____

Reference ddi$H_2O$ (or equivalent) Lot# _____

Results and Conclusions:

Do all controls exhibit expected alleles at sufficient peak height?

_____

Are the negative controls blank? _____

Did this lot pass the QC test? _____

Notes:

**Include the positive control and negative control electropherograms along with this sheet. **

## D. Verification of Positive Control

1. Purpose: To demonstrate that a new lot of positive control can produce accurate typing results.
2. Procedures:
   A. Amplify the positive control, reagent blank, and a negative amplification control.
   B. Analyze the amplified products using a genetic analyzer and associated software.
   C. Record the results on the "Verification of Positive Control Form" and attach the electropherograms from the positive and negative controls.
3. Assessment of Results.
   A. Verify that the positive control typed correctly. Ensure that the peak heights are acceptable.

| PowerPlex Fusion Locus | 2800M Positive Control | Y STR Locus | 2800M Positive Control |
|---|---|---|---|
| Amelogenin | X,Y | DYS576 | 18 |
| D3S1358 | 17,18 | DYS389I | 14 |
| D1S1656 | 12,13 | DYS448 | 19 |
| D2S441 | 10,14 | DYS389II | 31 |
| D10S1248 | 13,15 | DYS19 | 14 |
| D13S317 | 9,11 | DYS391 | 10 |
| Penta E | 7,14 | DYS481 | 22 |
| D16S539 | 9,13 | DYS549 | 13 |
| D18S51 | 16,18 | DYS533 | 12 |
| D2S1338 | 22,25 | DYS438 | 9 |
| CSF1PO | 12,12 | DYS437 | 14 |
| Penta D | 12,13 | DYS570 | 17 |
| TH01 | 6,9.3 | DYS635 | 21 |
| vWA | 16,19 | DYS390 | 24 |
| D21S11 | 29,31.2 | DYS439 | 12 |
| D7S820 | 8,11 | DYS392 | 13 |
| D5S818 | 12,12 | DYS643 | 10 |
| TPOX | 11,11 | DYS393 | 13 |
| DYS391 | 10 | DYS458 | 17 |
| D8S1179 | 14,15 | DYS385a/b | 13,16 |
| D12S391 | 18,23 | DYS456 | 17 |
| D19S433 | 13,14 | YGATAH4 | 11 |
| FGA | 20,23 | | |
| D22S1045 | 16,16 | | |

B. Verify that the negative controls are blank.

C. Reject the lot number of the positive control if incorrect or incomplete typing results are obtained. Notify the DNA Indexing Technical Leader and the manufacturer.

4. Storage Conditions:

A. The PowerPlex 2800M Positive Control concentrate is stored in the refrigerator.

## Verification of Positive Control Form

QC Date_____ Analyst_____

Positive Lot #_____ Plate #_____

Expiration Date_____ Kit Type_____

Reference Primer Lot# _____

Reference Master Mix Lot# _____

Reference ddi$H_2O$ (or equivalent) Lot# _____

**Results and Conclusions:**

Does the positive control exhibit expected alleles at sufficient peak height? _____

Are the negative controls blank? _____

Did this lot pass the QC test? _____

Notes:

**Include the positive control and negative control electropherograms along with this sheet.**

## E. Verification of Ladder

1. Purpose: To demonstrate that the new lot of ladder can produce accurate typing results.
2. Procedures:
   A. Mix appropriate amounts of the correct internal lane standard with deionized formamide.
   B. Prepare a positive control and ladder.
   C. Analyze the samples using a genetic analyzer and associated software.
   D. Record the results on the "Verification of Ladder Form" and attach the electropherograms from the positive control and ladder.
3. Assessment of Results:
   A. Verify that the ladder typed correctly, and peak heights are sufficient.
   B. Verify that the positive control typed correctly.
   C. If the reagent fails, reject the lot number. Notify the DNA Indexing Technical Leader and the manufacturer.
4. Storage Conditions:
   A. PowerPlex Y23 and PowerPlex Fusion ladder are stored frozen.

## Verification of Ladder Form

QC Date_____ Analyst_____

Ladder Lot #_____ Kit Type_____

Expiration Date_____

**Reference internal lane standard lot#** _____

**Reference plate # for positive control** _____

**Results and Conclusions:**

Does the ladder exhibit expected alleles at sufficient peak height?_____

Did the positive control type correctly?_____

Did this lot pass the QC test? _____

Notes:

**Include the positive control and ladder electropherograms along with this sheet. **

## F. Verification of Internal Lane Standard

1. Purpose: To test a new lot of Internal Lane Standard for sizing of fragments separated during capillary electrophoresis.
2. Procedure:
   A. Mix appropriate amounts of the new lot of Internal Lane Standard with deionized formamide.
   B. Prepare a positive control, negative controls, and ladder.
   C. Analyze the samples using a genetic analyzer and associated software.
   D. Record the results on the "Verification of Internal Lane Standard Form" and attach the GeneMapper data.
3. Assessment of Results:
   A. PowerPlex Y23 WEN Internal Lane Standard 500: Verify that the peaks corresponding to the 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 base pair fragments are sharp and well defined with a fluorescence intensity of 50 or higher.
   B. PowerPlex Fusion WEN Internal Lane Standard 500: Verify that the peaks corresponding to the 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 base pair fragments are sharp and well defined with a fluorescence intensity of 50 or higher.
   C. Verify that the positive and negative controls typed correctly.
   D. Reject the lot number of Internal Lane Standard if incorrect or incomplete typing results are obtained. Notify the DNA Indexing Technical Leader and the manufacturer.
4. Storage:
   A. Y23 WEN Internal Lane Standard 500 and PowerPlex Fusion WEN Internal Lane Standard 500 are stored frozen upon receipt and stored in the refrigerator upon first thaw.
5. Expiration Dates:
   A. Use the expiration date located on the reagent tubes.

## Verification of Internal Lane Standard Form

QC Date_____ Analyst_____

ILS Lot #_____ Kit Type_____

Expiration Date_____

Reference plate # for controls: _____

**Results and Conclusions:**

Are the base pair fragments sharp and well defined?_____

Are the RFU's greater than 50?_____

Do all controls exhibit expected alleles?_____

Did this lot pass the QC test?_____

Notes:

*** Include the positive control and ladder electropherograms along with this sheet

## H. Annual System Verification with NIST-Traceable Standard

1. Purpose: To annually verify that the entire PCR system is functioning within accepted criteria by the use of a standard which is traceable to the NIST SRM 2391D. The documentation on the traceability of the standard to the NIST SRM is located at the ISP DNA Indexing Laboratory.
2. Procedure:
   A. Extract a NIST Traceable Orientation Control as per ISP protocol.
   B. Amplify using ISP protocol for the appropriate kit.
   C. Analyze the samples using a genetic analyzer and appropriate software.
   D. Attach the positive control, negative controls, and Orientation Control electropherograms to the "Annual System Verification Form."
3. Assessment of Results:
   A. Compare the results to the known results for the standard. Any discrepancies will be re-analyzed and resolved.
   B. The profile for the Orientation Control is recorded in STACS-DB.

## Annual System Verification Form

Date:_____ Analyst:_____

Lot # orientation control:_____ Kit Type:_____

Did the NIST-traceable orientation control standard type correctly?_____

Did the positive control type correctly? _____

Were the negative controls blank? _____

Notes:

***Attach the positive control, negative controls, and the orientation control sample electropherograms to this sheet.***

## I. NIST-Traceable Standard Evaluation

1. Purpose: To make an in-house DNA sample traceable to the NIST SRM 2391D.
2. Procedure:
   A. Obtain a sufficient quantity of biological material (usually blood). This sample is the orientation control.
   B. Ensure a homogenous mixture if multiple collections were obtained.
   C. Amplify the orientation control, positive control, negative controls, and the NIST SRM 2391D using ISP protocol for appropriate loci.
   D. Analyze the samples using a genetic analyzer and appropriate software.
   E. Attach the positive control, negative controls, and all sample electropherograms, to the "NIST Traceable Standard Evaluation Form."
3. Assessment of Results:
   A. Verify that the NIST SRM 2391D typed correctly.
   B. Verify that the positive and negative controls typed correctly.
   C. Any discrepancies will be re-analyzed and resolved.

## NIST-Traceable Standard Evaluation Form

Analyst_____ Date_____

Indexing Technical Leader _____ Date_____

Reference orientation control lot #: _____

Reference NIST SRM 2391D lot#: _____

Did the in-house standard type correctly? _____

Did the positive control type correctly? _____

Were the negative controls blank? _____

| | NIST SRM | In-house Standard |
|---|---|---|
| D3S1358 | | |
| TH01 | | |
| D21S11 | | |
| D18S51 | | |
| PentaE | | |
| D5S818 | | |
| D13S317 | | |
| D7S820 | | |
| D16S539 | | |
| CSF1PO | | |
| PentaD | | |
| Amel. | | |
| vWA | | |
| D8S1179 | | |
| TPOX | | |
| FGA | | |
| Y STR Locus | | |
| DYS576 | | |
| DYS389I | | |
| DYS448 | | |
| DYS389II | | |
| DYS19 | | |
| DYS391 | | |
| DYS481 | | |
| DYS549 | | |
| DYS533 | | |
| DYS438 | | |
| DYS437 | | |

| | | |
|---|---|---|
| DYS570 | | |
| DYS635 | | |
| DYS390 | | |
| DYS439 | | |
| DYS392 | | |
| DYS643 | | |
| DYS393 | | |
| DYS458 | | |
| DYS385 | | |
| DYS456 | | |
| YGATAH4 | | |
| Y indel | | |
| D2S441 | | |
| D19S433 | | |
| D22S1045 | | |
| SE33 | | |
| D10S1248 | | |
| D1S1656 | | |
| D12S391 | | |
| D2S1338 | | |

Notes:

***Attach the positive control, negative control, and all of the sample electropherograms to this sheet.***

## J. Evaluation of a New Genetic Analyzer

1. Purpose: When a new Applied Biosystems Genetic Analyzer is received into the laboratory it must be evaluated before routine use. The following studies must be conducted.

2. Procedure:

Note: The following Instrument Run Times are validated. Ensure all internal lane standard base pairs are detected when these run times are used:

2000 seconds

A. Spectral Calibration

1) Prepare a run of the appropriate Matrix Standards for the Genetic Analyzer.
2) Follow the protocol described in the corresponding literature.

B. Precision and Reproducibility

1) Choose five samples that have been previously characterized and prepare these samples with the appropriate ladder to run on the Genetic Analyzer. It is only necessary to run samples in one amplification kit.
2) Inject each sample 20 times. Inject the ladder at least once and more frequently as necessary.
3) Analyze the collected data using the validated data analysis software according to ISP protocol.
4) Determine the allele base pair size average, standard deviation, minimum, and maximum values for the alleles at each locus for each sample.
5) Compare the obtained profiles to the known profiles.

C. Sensitivity and Injection Parameter Check - Establishing a Baseline Level of Sensitivity

1) Amplify a positive control in twenty-two separate wells.
2) Amplify a negative control.
3) Run the samples on the ABI Genetic analyzer with a ladder. Inject the samples with the validated injection parameters.
4) Analyze the collected data using the validated data analysis software according to ISP protocol.
5) Determine the average, standard deviation, minimum, and maximum values for peak height ratios, homozygote alleles, and heterozygote alleles.

D. Optional Concordance Check

1) A selection of previously analyzed samples may be chosen to run on the new ABI Genetic Analyzer after the above checks are completed and before entering the instrument into routine use.

3. Assessment of Results:

A. Spectral Calibration

1) PowerPlex Fusion and PowerPlex Y23

a) The Q-value must be greater than 0.95.

b) The condition number, or c-value, must fall below 13.5.

2) PowerPlex Y23/PowerPlex Fusion

a) Examine the data for an increased or negative baseline.

b) Up to 3 capillaries may fail resulting in three borrowing events.

B. Precision and Reproducibility

1) The precision of the instrument must be within ±0.5 bp using 3X the standard deviation. Any deviations must be approved by the DNA Indexing Technical Leader.

2) All samples must type correctly.

C. Sensitivity and Injection Parameter Check - Establishing a Baseline Level of Sensitivity

1) Peak heights must be appropriate for the amount of input DNA and suitable for the amplification system used.

2) Peak height ratios should be above 50%.

3) Homozygote peak heights must be greater than the minimum acceptable value.

4) Compare peak height averages determined above to those obtained on other genetic analyzers used in the laboratory. If the new genetic analyzer exhibits a significantly different level of sensitivity, modify injection parameters appropriately.

D. Optional Concordance Check

1) All samples examined must type correctly.

4. Documentation:

A. Record the results.

B. Routine maintenance must also be recorded in STACS-DB.

## K. Annual Verification of the Genetic Analyzer

1. Purpose: An Applied Biosystems Genetic Analyzer must be evaluated on a yearly basis or more often as necessary.

   When repair, service, or calibration is performed, a performance check must be conducted before being used for sample analysis. The type of performance check depends on the type of maintenance. For example, spectral matrix and sensitivity are required when changes or adjustments have been made to the optical system Repairs to or servicing of the polymer system, auto sampler, etc. may be checked in use. Use the controls and ladder to verify the correct operation. The Technical Leader may approve additional and/or alternative checks.

2. Procedure
   - A. Spectral
     - 1) PowerPlex Fusion/PowerPlex Y23
       - a) Prepare a run of the appropriate Matrix Standards for the Genetic Analyzer.
       - b) Follow the protocol described in the corresponding literature.

   - B. Sensitivity For Standard Input DNA and Injection Parameter Check.
     - 1) Amplify a positive control in twenty-two separate wells.
     - 2) Amplify a negative control.
     - 3) Run the samples on the AB Genetic Analyzer with a ladder at the previously determined injection parameters for the individual instrument.
     - 4) Analyze the collected data using the validated data analysis software according to ISP protocol.
     - 5) Determine the average, standard deviation, minimum, and maximum values for peak height ratios, homozygote alleles, and heterozygote alleles.

3. Assessment of Results
   - A. Spectral
     - 1) The Q-value must be greater than 0.95.
     - 2) The condition number, must fall below 13.5 for PowerPlex Fusion and PowerPlex Y23.
     - 3) Examine the data for an increased or negative baseline.
     - 4) Up to 3 capillaries may fail resulting in three borrowing events.
   - B. Sensitivity for Standard Input DNA and Injection Parameter Check
     - 1) Peak heights must be appropriate for the amount of input DNA and suitable for the amplification system used.
     - 2) Peak height ratios should be above 50%.

3) Homozygote peak heights must be greater than the minimum acceptable value.
4) Compare peak heights and peak height ratios to those of the previous Sensitivity for Standard Input DNA Study done on the same instrument. Discuss any significant differences with the DNA Indexing Technical Leader. Compare peak height averages determined above to those from the previous Injection Parameter Checks done on the same instrument. Modify injection parameters appropriately if necessary.

4. Documentation
   A. Spectral
      1) Attach the Spectral Calibration File.
      2) The file can be found in: the 3500 Data Collection software under the Maintenance tab/Spectral/Spectral Report.
      3) Insert the documentation into the appropriate logbook.
   B. Sensitivity for Standard Input DNA and Injection Parameter Check
      1) Attach electropherograms from the 22 positive controls and the negative control.
      2) Attach the calculations of the mean total peak heights.
      3) Insert the documentation into the appropriate logbook.

Instrument barcode:

| | Matrix Standards Used | Date Completed | Analyst Initials | Technical Leader Initials |
|---|---|---|---|---|
| Spectral | | | | |
| Sensitivity for Standard Input DNA | | | | |

## L. Evaluation of a ProFlex Thermal Cycler

1. An evaluation must be conducted when one of the following occur:
   A. A new thermal cycler unit is received into the laboratory.
   B. Repair, service, or calibration is performed and whenever an instrument is shipped back into the laboratory.
2. An evaluation will consist of:
   A. Conduct the Maintenance Tests:
      1) Self-verification test
      2) Heated cover test
      3) Temperature verification test
      4) Verify cycle performance
      5) Temperature non-uniformity test
   B. Reproducibility
      1) Amplify the positive control in twenty-two separate wells on the same plate.
      2) Amplify a negative control.
      3) Run the samples on the AB Genetic Analyzer.
      4) Verify that the positive controls type correctly and interpretable results are achieved.
   C. Assessment of Results
      1) Peak heights must be typical for the amount of input DNA and suitable for the amplification system used.
      2) Peak height ratios must be greater than the minimum acceptable value.
      3) Homozygote peak heights must be greater than the minimum acceptable value.
   D. Documentation
      1) Record the results on the "Evaluation of a ProFlex Thermal Cycler Form", export the results to the ProFlex maintenance records folder, and add the paperwork to the log book.
      2) If the thermal cycler fails the evaluation, do not use the instrument and contact the manufacturer. Attach an "out of service" sticker. Fail the maintenance in STACS-DB.

**Evaluation of a ProFlex Thermal Cycler Form**

1. STACS Instrument Number: _____

2. Serial Number of Base: _____

3. Serial Number of Sample Block: _____

4. Maintenance Tests
   A. Conduct the following tests and record the results in the log book.
      1) Self-verification test
      2) Heated cover test
      3) Temperature verification test
      4) Verify cycle performance
      5) Temperature non-uniformity test
   B. The ProFlex generated result file for each test should be exported to the applicable thermal cycler folder within the ProFlex Maintenance Records Folder.

5. Reproducibility
   A. Using the instrument, amplify the positive control in twenty-two separate wells on the same plate and a negative control for all amplification kits.
   B. Characterize the samples using the ABI Genetic Analyzer.
   C. Assessment of Results:

| | | | |
|---|---|---|---|
| 1) | Did all positive controls type correctly? | YES | NO |
| 2) | Was the negative controls blank? | YES | NO |
| 3) | Are the peak heights typical for the amount of input DNA and suitable for the amplification system used? | YES | NO |
| 4) | Are the peak height ratios greater than the minimum acceptable value for the amplification system and amplification volume used? | YES | NO |
| 5) | Are the homozygote peak heights greater than the minimum acceptable value for the amplification system and amplification volume used? | YES | NO |

Thermal Cycler barcode:

| Evaluation Study | Date Completed | Analyst Initials | Technical Leader Initials |
|---|---|---|---|
| Temperature Non-Uniformity Test | | | |
| Self-Verification Test | | | |
| Heated Cover Test | | | |
| Temperature Verification Test | | | |
| Verify Cycle Performance | | | |
| Reproducibility Fusion | | | |
| Reproducibility Y23 | | | |

## M. Evaluation of a New Maxprep or Maxwell Instrument

1. Purpose: A new Maxprep or Maxwell instrument must be evaluated before use. When repair or service is performed, a performance check may be necessary before being used for sample analysis.

2. Procedure

   A. New instrument

      1) When a new Maxprep instrument is received, perform the daily and weekly maintenance in STACS-DB.

      2) When a new Maxwell FSC is received, turn on the instrument. The instrument will automatically run a self-test. Record the maintenance in STACS-DB.

      3) When a new Maxprep or Maxwell instrument is received, an alternating pattern study must be conducted before it may be used for production samples.

   B. Alternating Pattern Study

      1) Extract eight previously characterized blood or buccal standards and eight reagent blanks per the approved procedure for DNA IQ Extraction: Maxprep Liquid Handler and Maxwell FSC. Set up the cartridge rack or deck tray using an alternating pattern of known standards and reagent blanks.

      2) Amplify the standards and blanks and run on the AB Genetic Analyzer.

      3) Examine the electropherograms to determine if contamination is present and that the known standards generated the expected results.

   C. Repair or service

      1) When major equipment repairs or service are necessary for the Maxwell® instruments, the above study may be required.

3. Assessment of Results

   1) Record the results of the evaluation

2) If the instrument fails the evaluation, do not use the instrument and contact Promega. Attach the "Out of Service" sticker.

## N. Annual Performance Check of the Maxprep or Maxwell Instrument

1. Purpose: The Maxprep or Maxwell instrument must be evaluated annually.

2. A. Procedure

1) Perform the daily and weekly maintenance in STACS-DB.

2) Turn on the instrument. The instrument will automatically run a self-test. Record the maintenance in STACS-DB.

3) An alternating pattern study must be conducted. Extract eight previously characterized blood or buccal standards and eight reagent blanks per the approved procedure for DNA IQ Extraction: Maxprep Liquid Handler and Maxwell FSC. Set up the cartridge rack or deck tray using an alternating pattern of known standards and reagent blanks.

4) Amplify the standards and blanks and run on the AB Genetic Analyzer.

5) Examine the electropherograms to determine if contamination is present and that the known standards generated the expected results.

B. Frequency: Annually

C. Results: Record all results in STACS-DB.

D. Course of Action: If the instrument fails the evaluation, do not use the instrument and contact Promega. Attach the "Out of Service" sticker.

Maxprep or Maxwell barcode:

| Evaluation Study | Date Completed | Analyst Initials | Technical Leader Initials |
|---|---|---|---|
| Daily/Weekly Maintenance | | | |
| FSC Self- Test | | | |
| Alternating Pattern Study | | | |

## IX. EQUIPMENT MONITORING AND MAINTENANCE

1. Monitoring
   A. For each piece of equipment requiring monitoring, the following sections will be listed:
      1) Procedure
      2) Frequency
      3) Results
      4) Course of action
   B. Records will be maintained for equipment monitoring.
2. Maintenance
   A. Where appropriate, maintenance procedures and schedules will be listed for equipment.
   B. Records will be maintained for maintenance.
3. Critical Equipment
   A. The following equipment is defined as critical
      1) Pipettes
      2) Thermometers traceable to NIST used for conducting performance checks
      3) Incubators, heat blocks, and ovens
      4) Robotic systems
      5) Thermal cyclers
      6) Thermal cycler temperature verification systems
      7) Genetic Analyzers

## A. Balances

1. Monitoring
   - A) Procedure
     1) Clean and level.
     2) Transfer standard masses using cotton gloves or forceps.
     3) Use a minimum of three masses (high, low, and medium) that represent the normal range measured on the balance.
   - B) Frequency
     1) Monitor monthly, or if used less frequently, prior to use.
   - C) Results
     1) Record the results in STACS-DB.
     2) Computerized records will be backed up.
   - D) Course of action
     1) The measurements must be within the following tolerance windows:
        a. Ohaus Adventurer AR2140: ±0.2 mg
        b. Ohaus Adventurer ARA520: ±0.02 g
     2) If the measurements are not within the specified tolerance, a second mass set will be used to verify all balance readings.
     3) If the measurements remain out of the tolerance window, then contact a qualified service company for repairs.

## B. Certification of Weights

1. Monitoring between certifications is not required.
2. Certification Procedure
   A) Standard mass certification will be contracted to either the manufacturer or a certified agency.
   B) The company must be ISO 17025 certified.
3. Frequency
   A) Refer to QM-11.
4. Results
   A) Document action in STACS-DB. Retain contractor's certificate or documentation.
5. Maintenance:
   A) No routine maintenance is required.

## C. Ovens

1. Monitoring
   - A) Procedure
     1) Set temperature of oven at desired setting for laboratory procedures.
     2) Use a NIST traceable thermometer.
     3) Tolerance range: +/- 5 degrees°C.
   - B) Frequency
     1) Monitor monthly, but observe temperature before each use.
   - C) Results
     1) Record results in STACS-DB.
   - D) Course of Action
     1) Adjust the temperature dial if necessary and retest.
     2) Call the repair company for any needed repairs if the oven is out of tolerance.
2. Maintenance
   - A) No routine maintenance is recommended by the manufacturer.

## D. Freezer/Refrigerator

1. Monitoring
   A) Use a NIST traceable thermometer.
2. Frequency
   A) Monitor monthly.
3. Results
   A) Record the results on the appropriate form.
4. Course of Action
   A) The range for refrigerators is 1 to 8°C and the range for freezers is -2°C or lower.
   B) If the temperature is outside of the specified tolerance window, adjust the temperature control and retest.
   C) If the temperature remains outside of the specified tolerance window, contact the manufacturer or repair company for repairs.
5. Maintenance
   A) No regular maintenance is required. Defrost/clean as necessary. Contact the repair company for repairs.

**E. Pipette**

1. Calibration Procedure
   - A) Pipette calibration will be contracted to either the manufacturer or a certified agency.
   - B) The company must be ISO 17025 certified.
2. Frequency
   - A) Calibrate annually.
3. Results
   - A) Document action in STACS-DB. Retain contractor's certificate or documentation.
4. Course of Action
   - A) If the pipette cannot be certified, remove from service.
   - B) Submit for repair and subsequent recalibration.
5. Maintenance
   - A) Clean with each use.

**Reference**

STACS-DB Chemical Receiving Module

## F. Thermal Cycler

1. Thermal Cycler Monitoring
   - A) Procedure:
     1) Perform the verification and uniformity tests.
   - B) Frequency: Conduct Temperature Verification and Temperature Uniformity every six months.
   - C) Results
     1) Record the results in STACS-DB.
     2) Export ProFlex test results to appropriate network folder
   - D) Course of Action for test failure
     1) Remove from service and attach the "Not in Service" magnet.
     2) Call manufacturer if the thermal cycler notifies the user that the temperatures are out of range.
2. Temperature Verification Kit Calibration
   - A) Procedure: Send to a company qualified to provide certification for calibration.
   - B) Frequency: Calibrate annually.
   - C) Record the results in STACS-DB
   - D) Course of Action: If the kit fails calibration, discontinue its use.
3. Maintenance

   No regular maintenance is required

**Reference**

ProFlex User's Manual

## G. NIST Traceable Thermometers

NIST Traceable thermometers do not require performance checks. They may be used until the expiration date on the certificate. Maintain the certificate.

## X. PROFICIENCY TESTING

1. Proficiency testing is performed in accordance with the Command QA Manual, which includes the following:
   A) Competency Tests
   B) DNA Quality Assurance
   C) Internal Proficiency Tests
   D) Blind Proficiency Testing
   E) External Proficiency Testing
2. The results of proficiency test results will be checked and compared to the standards by the Quality Assurance Manager as outlined in the Command QA Manual.

## XI. CORRECTIVE ACTION

1. An Incident Report is used for reporting and resolving incidents that could adversely affect the outcome of a DNA analysis. This may include instances of procedural nonconformance, significant sample or data loss, possible contamination or sample switching, etc.

2. For minor incidents such as minimal loss of sample or data, or non-systemic contamination, the individual identifying the incident may develop and follow a course of action. The individual will notify the Technical Leader of an incident and the action taken. The Technical Leader may modify the action. For more serious situations, the individual will notify the Technical Leader before proceeding with a course of action. The Technical Leader must approve a course of action. The Technical Leader will initiate an incident report.

3. The Technical Leader will review the results of the course of action and determine if follow-up action is necessary.

4. Documentation of the incident and follow-up will be maintained by the Technical Leader.

5. The Technical Leader will notify the Laboratory Quality Manager (LQM) of the incident.

6. The Technical Leader, in consultation with the individual and the LQM, may recommend a Quality Issue Report (QIR) be initiated. See QM-8 of the Command Quality Manual. The individual(s) involved will be informed if a QIR is initiated.

7. The Command Quality Manual (QM-8) addresses the Correction Action policies for the laboratories.

## XII. REPORTS/ DOCUMENTATION

1. Procedures for generating and maintaining documentation for database samples are found in the DNA Indexing Procedures Manual: Sample Files and Access to Data and Information.

## XIII. REVIEWS

1. Technical reviews:
   - A) Hard Copy Manual Data Review:
     - 1) The Review Table is used to assist with communications between the technical reviewer and the analyst. The technical reviewer's concurrence with the profile results is indicated by his/her initials on the Review Table for each sample. The review table is the authoritative documentation for acceptance of alleles/profiles.
     - 2) The technical reviewer will conduct an administrative review of the file and initial the Review Table by each sample.
   - B) Electronic Data Review:
     - 1) The technical reviewer's concurrence with the profile results is indicated by his/her initials in the GeneMapper project for each sample.
2. Supervisory reviews are tracked on the Indexing File Review Log. The purposes of a supervisory review are:
   - A) To ensure that proper documentation procedures are followed for the receipt and analysis of offender samples.
   - B) To ensure that proper documentation procedures are followed for the upload, searching, and verification of offender samples.
3. Court monitoring is covered under:
   - A) Administrative Reviews in the Command Directives
   - B) Court Appearance Rating in the Quality Manual

## XIV. SAFETY

1. The safety program is found in the Command Safety Manual and the Springfield Forensic Science Laboratory Safety Manual.

## XV. AUDITS

1. The Command's inspection program is covered in the Command QA Program.
2. In addition to the Command's inspection program, external auditors will review the DNA section in the laboratory once every two years according to the accrediting body's criteria and the Quality Assurance Standards for DNA Databasing Laboratories.
   A) A record of the audit report will be maintained in the laboratory.
   B) A copy of the external audit report and addressed findings will be forwarded to the NDIS Custodian for NDIS Panel review.

## XVI. SOFTWARE VERSION TRACKING

1. Upgrade history and versions will be tracked for analytical software.
2. The laboratory will maintain a list of software that requires tracking.
3. Items to be documented:
   - A) Software name
   - B) Version number
   - C) Date installed
   - D) Summary of changes
   - E) Installer's initials if possible
     1) If the installer is off site, someone in-house will have to record the installer's initials.

## XVII. ABBREVIATIONS

Laboratory analysis files may contain the following abbreviations:

| | |
|---|---|
| AMP | Amplification |
| CE | Capillary Electrophoresis |
| DEG | Degradation/Degraded |
| EPG | Electropherogram |
| ER | Excessive Residual |
| EXT | Extract/Extracted/Extraction |
| INJ | Injection |
| ND | Not Detected |
| NEL | Needs Extended Loci |
| NHMWB | No High Molecular Weight Band |
| NGP | No Genomic Peaks |
| OS | Off-Scale |
| PHR | Peak Height Ratio |
| PPP | Primer Peaks Present |
| PU | Pull-up |
| RA | Reamplify |
| RA+ | Reamp More |
| RA- | Reamp Less |
| RE | Reextract |
| RXN | Reaction |
| SLD | Sealed |
| TFS | Too Few Swabs |
| TMS | Too Many Swabs |

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

# APPENDIX B: FORMS

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Reviewed by:

Forensic Scientist Kerry M. Reavis, CAB Chairperson
DNA Indexing Command Advisory Board

Approved by:

Forensic Scientist Ashley Y. Flack
Springfield Forensic Science Laboratory
DNA Indexing Technical Leader

Brenda Danosky
Acting FB/DNA Program Manager
Forensic Sciences Command

# APPENDIX B: FORMS

The following are representations of the approved forms used in offender sample analysis.

**I. SAMPLE RECEIPT** - (Note: This is an example of the information obtained at sample collection. Alternate versions of this form are in use. Information requested on alternate versions may be modified with the approval of ISP. The layout of this form may be modified as necessary.)

**DNA INDEXING DATABASE SAMPLE RECEIPT**

*Laboratory Barcode*

**(1) Donor's Identifying Information**
**PLEASE PRINT CLEARLY**

Last Name

First Name MI

Signature of Donor

**ATTENTION COLLECTOR:** If signed name doesn't match printed name ***exactly*** (e.g. nickname, maiden name, or hyphenated name), correct person is being collected and initial here _____.

Birth Date: Mo Day Year

Race (check one) ◯Asian ◯Black ◯Hispanic ◯Native American ◯White ◯Other_____

Gender (check one) ◯Male ◯Female

**(2) Collection Certification**

I hereby certify that I have the identity of the donor named in section (1) prior to witnessing/performing the DNA collection.

**(3) Collection Date**

Mo Day 2 0 Year

**(4) Identification Numbers**

SID # I L

IDOC #

DC #_____

Cause/Court #_____

**(5) Qualifying Reason(s)** (check all that apply)

◯ Adjudicated guilty of a Felony (Juvenile Court Act)
◯ Convicted of a Felony (Adult Criminal Code)
◯ Court Ordered (attach order) RARE
◯ Court Ordered Misdemeanant (attach order)
◯ Incarcerated in IDOC or IDJJ
◯ Interstate Transfer from _____
◯ Qualifying Indictee
◯ Sex Offender Registrant (includes SDP/SVP)
◯ Voluntary (must complete consent form on back) RARE

**(6) Agency** (please print clearly or use a label)

Responsible Illinois Agency

Collection Agency Name (if not the Responsible Illinois Agency)

Street Address

City State Zip

Telephone Number Contact Name

**For Laboratory Use Only**

**(7) Adjudication/Conviction/Order/Registration**

Date Mo Day 2 0 Year

**For Laboratory Use Only**

**For Latents Use Only**

**(8) Right Thumbprint**

Print is to be taken at the time of swab collection. Samples will not be accepted without a legible Indicate if a different

**Right Thumbprint**

REV01 05-2014 No. ILBSK1001

ADHERE SWAB ENVELOPE HERE
*(Envelope should overhang off edge of Receipt Form)*

**DNA Indexing Database**
**Buccal Swab Collection Kit**

**ILLINOIS STATE POLICE**
*Division of Forensic Services*
DNA Indexing Laboratory
3710 East Lake Shore Drive
62712

(217) 786-6160 (voice) * (217) 786-6956 (fax)
1 (800) 255-3323 (TDD)

REV01 05-2014 No. ILBSK1001

## BUCCAL SWAB COLLECTION INSTRUCTIONS

Prior to collection, check the Offender DNA Database Search site or call or fax the DNA Indexing Laboratory to make sure a

**Phone: (217) 786-6160 Fax (217) 786-6956**
**http://fscwebsa.isp.state.il.us/DnaSplash**
**Email: DNA_Indexing_Lab@isp.state.il.us**

1) Personnel from the Responsible Agency or designee will collect the buccal swabs from the donor.
2) The following collection procedure must be performed on one donor at a time.
3) Read the entire instruction sheet prior to collecting the sample.
4) Do not use the kit if the kit has been opened or damaged.
5) Do not handle the cotton bulb of the swabs.

STEP 1 Fill out all information requested on the DNA Sample Receipt Form and the envelope to the end of the receipt.

STEP 2 Using the inked strip provided, take a right thumbprint of the donor.

STEP 3 Have the donor remove gum, tobacco, etc. from mouth.

STEP 4 Put on biohazard barrier gloves.

STEP 5 Open one of the sterile swab packages containing two swabs.

STEP 6 Using the two swabs, vigorously swab the inside of the donor's cheek at least 6 times. Remove swabs from the donor's mouth, completely air dry and replace in the opened sterile swab package.

STEP 7 Repeat Step 6 twice using the remaining two packages of swabs.

STEP 8 Place both swab packages into the Swab Envelope. Remove the backing from the envelope and press to seal. DO NOT DETACH the swab envelope from the receipt.

STEP 9 Remove gloves and discard in an appropriate waste receptacle. DO NOT place used gloves in the envelope.

STEP 10 Refold the completed kit and place it into the preprinted return envelope. Remove the backing from the and press to seal the envelope. Attach the Kit Shipping Seal and initial.

STEP 11 Mail the completed kit.

**If you have any questions, call the Laboratory at (217) 786-6160.**

## INSTRUCTION FOR COMPLETING INFORMATION PORTION OF KIT

**1) Donor's Identifying Information:** Print donor's name; have donor sign. Print date of birth and check race and gender. PRINT CLEARLY. Verify the printed name matches the signature. If they are different, ensure the correct person is being collected and initial the blank indicating that the name difference is acceptable. This is especially important for donors with and hyphenated names.

**2) Collection Certification:** Read and sign stating that you have .

**3) Collection Date:** Date the sample was collected.

**4) Identification Numbers:** Enter appropriate numbers for the donor.

**5) Qualifying Reason:** Select the reason why the donor is eligible/ required to donate a sample. Enclose a copy of the signed court order for court ordered samples. If the donor is transferring to Illinois from another state, record the other state in the space provided.

**6) Agency:** The Responsible Agency is the agency in Illinois that is responsible to collect the donor's sample. The Collection Agency is the agency conducting the collection on behalf of the Responsible Agency. Please list the complete address, including zip code, of the agency collecting the sample. Include a contact's name and telephone number. The use of printed address labels is encouraged.

**7) Adjudication/Conviction/Order/Registration:** Enter date of the adjudication, conviction, court order or sex offender registration.

**8) THUMBPRINT:** Peel apart the black inked sheet and place on a surface with inked side up. Roll the donor's right thumb on the inked sheet from the inside edge of the thumb toward the donor's body. If the right thumb cannot be printed, the left thumb or another digit may be used. Label print, if not right thumb. Roll the inked thumb on the thumbprint block in the same manner. The print should extend from side to side and from the tip of the to just below the joint. The print should consist of clear, distinct ridges. Let the print dry completely. NOTE: If a clear print is not obtained, a white

***SAMPLE WILL NOT BE ACCEPTED WITHOUT A CLEAR THUMBPRINT.***

## CONSENT TO PROVIDE A VOLUNTARY DNA SPECIMEN

***(NOT required for any other Qualifying Reason listed in Section 5)***

Voluntary Sample:

I, _____,
voluntarily consent to provide a buccal swab specimen to the Illinois State Police. I understand information obtained from the swab(s) may be used for investigations and may be retained in a record keeping system for future reference. I voluntarily consent to allow the Illinois State Police to use the swab(s) and the information obtained from the swab(s) for any authorized purpose. I understand that I am free to refuse to provide a buccal swab specimen, but that I voluntarily do so and consent to the terms of this statement.

_____
Signature of Voluntary Donor

_____
Date/Time

_____
itness

_____
itness

**If the voluntary donor is a Juvenile, a parent or legal guardian must co-sign this consent form.**

_____
Printed Name of Parent or Legal Guardian

_____
Signature of Parent or Legal Guardian

**II. YIELD GEL WORKSHEET**

Extracted By: _____ Date: _____

Quantitated By: _____ Date: _____

| Cathodal Origin | | | | | |
|---|---|---|---|---|---|
| Lane | Sample | Resolub. Volume (µL) | DNA (ng/4 µL) | Dilution for Amp | |
| | | | | µL Sample | µL Water |
| 1 | λDNA Dilution Series | | 125 | | |
| 2 | | | 63 | | |
| 3 | | | 31 | | |
| 4 | | | 15 | | |
| 5 | | | 8 | | |
| 6 | | | 4 | | |
| 7 | | | | | |
| 8 | | | | | |
| 9 | | | | | |
| 10 | | | | | |
| 11 | | | | | |
| 12 | | | | | |
| 13 | | | | | |
| 14 | | | | | |
| Middle Origin | | | | | |
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5 | | | | | |
| 6 | | | | | |
| 7 | | | | | |
| 8 | | | | | |
| 9 | | | | | |
| 10 | | | | | |
| 11 | | | | | |
| 12 | | | | | |
| 13 | | | | | |
| 14 | | | | | |

Samples extracted by ISP organic method.

# Sample Verification Form

III. Sample Verification Form

**«Sample____»**
*Blank*

**Eligible for Database:**

**YES/NO** _____

**(Circle One)** **Initials**

| Hit Date | Analyst | | Technical Review | | Supervisory Review | | Inserted Pages | Total # of Pages |
|---|---|---|---|---|---|---|---|---|
| Date | Date | Initials | Date | Initials | Date | Initials | | |
| | | | | | | | | |
| | | | | | | | | |
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| | | | | | | | | |
| | | | | | | | | |

***STACS-DB documentation not included in the file can be accessed through the Sample History Report module in STACS-DB.***

**Page 1**
**(Version 8)**

IV. Verification File Checklist

VERIFICATION FILE CHECKLIST

Version #7

___ All pages contain sample # and original initials

___ Indexing Verification Letter

- ___ Indexing sample # listed correctly
- ___ Laboratory case number(s) listed correctly
- ___ Offender information listed correctly

___ Sample Verification Form

- ___ Total # of pages listed
- ___ Pages numbered correctly

___ Hit Confirmation Report or QA/QC Report

___ CODIS Match Report

___ Request for Verification

___ Copy of Sample Receipt

___ Latent Print documentation

___ NCIC

___ Letter Sent to Casework Laboratory

- ___ Date ___ Initials

# ILLINOIS STATE POLICE

# DNA INDEXING PROCEDURES MANUAL

# APPENDIX C: HIT VERIFICATION LETTERS

---

Reviewed by:

Forensic Scientist Kerry M. Reavis, CAB Chairperson
DNA Indexing Command Advisory Board

Approved by:

Forensic Scientist Ashley Y. Flack
Springfield Forensic Science Laboratory
DNA Indexing Technical Leader

Brenda Danosky
FB/DNA Program Manager
Forensic Sciences Command

## I. Hit Verification Letter

Note: The following is a representation of a letter used in an offender sample verification. A letter may be modified to address specific situations.

**ILLINOIS STATE POLICE**
*Division of Forensic Services*

*Governor* *Director*

June 14, 2005

Forensic Scientist John Smith
Illinois State Police
Forensic Science Center at Chicago
1941 West Roosevelt Road
Chicago, IL 60608-1229

Dear Mr. Smith:

A search of the DNA Index on January 3, 2005, resulted in a computer match between specimen number C02-XX-F2 and Illinois Indexing specimen number Iyy-000000. The DNA profile has been verified. The name and date of birth listed below are as they appear on the sample receipt. Other names and dates of birth may exist for this individual.

John Q. Offender
DOB mm/dd/yyyy
IDOC# X00000
SID# IL00000000

The Indexing specimen is not an evidentiary sample. This information is for use in obtaining a confirmatory standard from the above individual. Our policy recommends that the law enforcement agency obtain an additional biological sample from this subject and submit the sample to your laboratory for confirmatory analysis. Please do not hesitate to contact me at (217) 786-6160 if you have any questions.

Sincerely,

Jane Doe
Forensic Scientist

## II. Hit Verification Letter With Duplicates

Note: The following is a representation of a letter used in an offender sample verification. A letter may be modified to address specific situations.

**ILLINOIS STATE POLICE**
*Division of Forensic Services*

*Governor* *Director*

June 14, 2005

Forensic Scientist John Smith
Illinois State Police
Forensic Science Center at Chicago
1941 West Roosevelt Road
Chicago, IL 60608-1229

Dear Mr. Smith:

A search of the DNA Index on January 3, 2005, resulted in a computer match between specimen number C02-XX-F2 and Illinois Indexing duplicates, specimen numbers Iyy-###### and Iyy-######. The DNA profile has been verified. The name and date of birth listed below are as they appear on the sample receipts. Other names and dates of birth may exist for this individual.

John Q. Offender
DOB mm/dd/yyyy
DOC# X00000
SID# IL00000000

The Indexing specimens are not evidentiary samples. This information is for use in obtaining a confirmatory standard from the above individual. Our policy recommends that the law enforcement agency obtain an additional biological sample from this subject and submit the sample to your laboratory for confirmatory analysis. Please do not hesitate to contact me at (217) 786-6160 if you have any questions.

Sincerely,

Jane Doe
Forensic Scientist

## III. Hit Verification Letter for Identical Siblings

Note: The following is a representation of a letter used in an offender sample verification. A letter may be modified to address specific situations.

**ILLINOIS STATE POLICE**
*Division of Forensic Services*

*Governor* *Director*

June 14, 2005

Forensic Scientist John Smith
Illinois State Police
Forensic Science Center at Chicago
1941 West Roosevelt Road
Chicago, IL 60608-1229

Dear Mr. Smith:

A search of the DNA Index on January 3, 2005 resulted in a computer match between specimen number C02-XX-F2 and Illinois Indexing specimen numbers Iyy-###### and Iyy-######. The DNA profiles have been verified. The names and dates of birth listed below are as they appear on the sample receipts. Other names and dates of birth may exist for these individuals.

Apparent identical siblings:

| | |
|---|---|
| Iyy-###### | Iyy-###### |
| John Q. Offender | Jim A. Offender |
| DOB mm/dd/yyyy | DOB mm/dd/yyyy |
| DOC# X00000 | IDOC# X00000 |
| SID# IL00000000 | SID# IL00000000 |

The Indexing specimens are not evidentiary samples. This information is for use in obtaining a confirmatory standard from the above individuals. Our policy recommends that the law enforcement agency obtain additional biological samples from the subjects and submit the samples to your laboratory for confirmatory analysis. Please do not hesitate to contact me at (217) 786-6160 if you have any questions.

Sincerely,

Jane Doe
Forensic Scientist

**ILLINOIS STATE POLICE**
*Division of Forensic Services*

*Governor* *Director*

[CURRENT_ DATE]

Statewide Terrorism Intelligence Center
2100 S. Dirksen Parkway
Springfield, IL 62703
(217) 782-7938

Re: SEARCH REQUEST [SEARCH_ID]

To Whom it May Concern:

The DNA Indexing Laboratory conducted a Familial Search of the DNA profile for specimen [CODIS_SPECIMEN]. The search is designed to identify potential first degree relatives to the contributor of the DNA profile in the associated case within the Illinois DNA Database. The qualified candidates were then subjected to Y-STR analysis for further refinement of the search results.

The following candidates could not be excluded as a potential first degree relative:

Name:
DOB mm/dd/yyyy
IDOC# X00000
SID#IL00000000
Gender:
Other Number:

An Indexing specimen is not an evidentiary sample. Please be aware this information is provided only as an investigative lead. The above named individuals are indirect associations based on a potential genetic relationship and not the source of the forensic unknown DNA profile.

Additionally, this search result only indicates the possibility of a biological relationship; it does not confirm that the named individual is biologically related to the source of the forensic unknown profile. Please do not hesitate to contact me at (217) 786-6160 if you have any questions.

Sincerely,

Jane Doe
Forensic Scientist

**ILLINOIS STATE POLICE**
*Division of Forensic Services*

*Governor* *Director*

[CURRENT_ DATE]

Statewide Terrorism Intelligence Center
2100 S. Dirksen Parkway
Springfield, IL 62703
(217) 782-7938

Re: SEARCH REQUEST [SEARCH_ID]

To Whom it May Concern:

The DNA Indexing Laboratory conducted a Familial Search of the DNA profile for specimen [CODIS_SPECIMEN]. The search is designed to identify potential first degree relatives to the contributor of the DNA profile in the associated case within the Illinois DNA Database. The qualified candidates were then subjected to Y-STR analysis for further refinement of the search results.

No potential first degree relatives were identified at this time.

An Indexing specimen is not an evidentiary sample. Please be aware this information is provided only as an investigative lead. Please do not hesitate to contact me at (217) 786-6160 if you have any questions.

Sincerely

Jane Doe
Forensic Scientist