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FORENSIC BIOLOGY/DNA
Last Review Date: 10/09/2024

# FORENSIC BIOLOGY/DNA FOREWORD



The body of knowledge which comprises forensic science is a compilation of procedures adapted from other disciplines that encompass many of the physical and natural sciences. During the history of forensic science, a multitude of scientists have greatly contributed to the protocols, methods and procedures that have become a routine part of analysis. Every effort has been made in this manual to give proper recognition to the authors of specific procedures; however, in some instances, the original source of forensic procedures has been lost in antiquity. For others, the general procedures belong to the public domain and are recorded in many basic references concerning forensic science. In addition, many of the procedures described in this manual have been adapted from standard laboratory practices, and the citation of thousands of references which deserve credit for aiding in the development of these procedures is neither practical nor possible. To all those scientists who have contributed to the knowledge of forensic science contained herein, we do extend collective recognition and gratitude.

Procedures manuals which offer reliable information that is then combined with corresponding training manuals serve as the foundation for effective quality management of analyses. Extensive effort has been made to ensure that the routine procedures described herein will produce accurate and valid analytical results. However, not all possible analyses that may be encountered in casework can be appropriately covered in a procedures manual, nor can all possible variations to a described procedure be included. Therefore, this manual is written with the understanding that minor variations that do not significantly alter the described procedure may be used. An analyst may use a non-routine procedure not specifically stated in this manual, provided all the following conditions are met:

## FORENSIC BIOLOGY

1. The procedure used is based upon documented and scientifically accepted practice.
2. A notation is made on the worksheet indicating the procedure followed is not specified in the procedures manual.
3. The analyst also indicates on the work sheet why the particular procedure was selected over a procedure contained in this manual. Rationale must be detailed sufficiently to withstand close scrutiny by independent examiners.
4. The analyst provides documentation showing that the non-routine procedure had been tested prior to application with evidence. Test criteria shall include test samples that approximate the characteristics of the evidence, the results obtained with the routine procedure, and the results obtained with the non-routine procedure. Documentation will also include related data concerning the non-routine procedure's sensitivity, precision and possible sources of error.
5. The non-routine procedure used will be recorded to a standard such that another scientist of similar skills and experience can understand fully the procedure used and the results obtained.

**DNA**

1. The procedure used is based upon documented and scientifically accepted practice.
2. A notation is made on the worksheet indicating the procedure followed is not specified in the procedures manual.
3. The analyst also indicates on the worksheet why the particular procedure was selected over a procedure contained in this manual. Rationale must be detailed sufficiently to withstand close scrutiny by independent examiners.
4. The analyst will cite the internal validation within the case notes to demonstrate that the non-routine procedure had been tested prior to application with evidence.
5. The non-routine procedure with the prior approval of the appropriate Technical Leader will be documented in the case file. Results from the application of the non-routine procedure will be provided to the appropriate Technical Leader prior to issuing a report on findings obtained using a non-routine procedure.

Additionally, there may be procedures which pertain to all sections. Such is the case with laboratory reagents. In order to standardize the testing and monitor the shelf life of reagents used by analytical sections, the Forensic Sciences Command has developed protocols which are universal for all sections. These protocols regarding reagent expiration and testing are found in the Command Quality Manual.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

## PROCEDURE: CLEAN TECHNIQUE

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Reviewed by:

Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

Jeanne M. Richeal
DNA Technical Leader

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

All Forensic Biologists and DNA analysts must utilize clean technique. The purpose of clean technique is to prevent extraneous DNA from entering a sample. Sources of contamination include but are not limited to aerosols, liquid droplets, and dry flakes/dust. These contaminants may be transferred by tools, gloves, equipment, commodities, and lab coats. Evidence examination areas and extraction areas are more at risk for the introduction of extraneous DNA. Proper precautions must be taken to reduce the risk of contamination.

## SAFETY CONSIDERATION

Observe Standard Laboratory Practices.

Warning: Treat all reagents/samples as potential biohazards.

Refer to safety considerations under the DNA Isolation/Methods section.

### Personal Protective Equipment (PPE)

Gloves must be worn when handling samples, reagents, equipment, or instruments used for casework within all areas of the Forensic Biology/DNA laboratory.

While examining evidence, Forensic Biologist/DNA analysts must wear a mask, gloves, and disposable lab coat or a reusable lab coat in conjunction with disposable sleeve covers. A head covering should also be worn when appropriate. The gloves must be cleaned with 10% bleach solution and dried. Gloves must be changed or bleached between exhibits and after handling non-evidence items prior to returning to casework. Non-evidence items may include, but are not limited to, refrigerator/freezers, biohazard waste bins, equipment, computers, and telephones.

Lab coats utilized in post-PCR rooms must be disposable or designated for use only in those rooms. Non-disposable lab coats must be removed from the laboratory in a closed container for cleaning.

## PREPARATION

10% Bleach Solution

## INSTRUMENTATION

Standard Laboratory Instrumentation

## MINIMUM STANDARDS AND CONTROLS

Not applicable.

## PROCEDURE OR ANALYSIS

## General Information

1. Analysts should not take phone calls when working in the laboratory. Conversations between laboratory personnel should be kept at a minimum when analysts are working with evidence.

2. Tube openers should be used to uncap sample tubes. The tubes must be centrifuged prior to opening to minimize aerosols. Open only the tube(s) for one exhibit at a time. Tube openers must be cleaned in a 10% bleach solution after each use.

3. Do not pipette directly from a reagent bottle unless it is stated in a specific procedure. Reagents must be poured from their container into a disposable container. When finished, discard the disposable container and the remaining contents. All reagent bottles must be closed during the extraction process.

4. Exhibits will be processed one at a time. Only one exhibit will be open at a time.

5. Processing of questioned samples and reference standards will be separated by time, space, and/or analyst throughout all DNA processes unless otherwise noted in a specific procedure.

   In situations where it is necessary for an agency to submit a secondary standard (i.e. clothing, toothbrush, etc.), it will be extracted with reference samples.

## Decontamination

1. Decontaminate work surfaces with a 10% bleach solution before and after each use. Ensure the surface is dry before examining evidence. Make a new bleach solution daily.

2. All instruments used to process forensic samples must be cleaned as specified by the manufacturer. Pipettes must be decontaminated with a 10% bleach solution before and after each use.

3. Tools must be decontaminated between samples by rinsing with a 10% bleach solution and drying with a clean disposable cloth or tissue. At the analyst's discretion, tools may also be autoclaved after decontamination.

4. All tubes or tube inserts used in DNA analytical processes must be purchased forensic grade/DNA free or exposed to UV light for 30 minutes prior to use.

   **Note**: Do **NOT** expose the following to UV light:
   Maxwell tubes and plungers
   Optical reaction plates
   1.2 mL round-bottom deep well plates
   12-column pyramid bottom reservoirs

2.2 mL square-well deep well plates
1.1 mL square-well v-bottom deep well plates
100 mL disposable troughs

## Sample Processing – DNA Extraction

1. If available, a biohood should be used. If a biohood is not available, samples may be processed on a decontaminated bench top.

2. Separation of unknown samples and reference standards can be accomplished as follows:

   A. Extraction of reference standards and unknown samples can be conducted by different analysts, on different days, or at different times.

   B. Extraction of reference standards and unknown samples can be conducted in different biohoods.

   C. If unknown samples and reference standards are to be processed on the same day using the same hood, the unknown samples and reagent blanks must be maintained in a separate rack and processed first. The reference standards and reagent blank(s) will be maintained in a different rack and processed after the unknown samples. The hood must be bleached in between processing of unknown samples and reference standards.

   D. When utilizing the Maxwell® 16 for extraction, unknown samples and reference standards will not be combined on the same run.

3. The order the samples are extracted and processed must be clearly documented in the case notes. This documentation must show the separate handling of the unknowns and reference standards

## Amplification Set-up

1. All amplifications must be set up in designated biohoods. The biohood fan must be operating during amplification set-up.

2. One master mix of amplification reagents may be used for both unknown samples and reference standards. The master mix will be added to tubes in the rack of unknown samples first and then to the tubes in the rack of reference standards. All tubes will be closed. The positive and negative amplification controls will be set up last.

3. All samples may be amplified at the same time using the same thermal cycler. Do not set the tube rack down in the post-PCR room while transferring samples to the thermal cycler. If the rack contacts anything in the post-PCR room, decontaminate it with a 10% bleach solution before returning it to the main laboratory.

**Post-Amplification**

1. After working with amplified DNA, an analyst will not work with any biological or pre-amplified evidence.

2. The door to the post-PCR room must remain closed.

3. Unknowns and reference standards may be run on the same capillary electrophoresis plate.

## REPORT WORDING

Not Applicable.

## REFERENCES

Not applicable.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** STAIN IDENTIFICATION

**METHOD:** SEMEN STAIN DETECTION

**PROCEDURE:** ACID PHOSPHATASE PRESUMPTIVE CHEMICAL TEST (INDICATION)

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Reviewed by:

_____

Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

This test is designed as a preliminary screening test to aid in the indication of semen stains. This is not a confirmatory test for semen.

## SAFETY CONSIDERATIONS

AP Chemical Test

Sodium a-naphthyl phosphate: Caution! Irritant!
Naphthanil diazo blue B: Caution! Irritant!
Glacial Acetic Acid: Danger! Corrosive!
Caution! Moderately Toxic!
Caution! Combustible!

## PREPARATIONS

Buffer

| | |
|---|---|
| Glacial acetic acid | 5mL |
| Sodium acetate, anhydrous (.24M) | 10g |
| Distilled water | 500mL |

Step 1 Reagent

| | |
|---|---|
| Buffer | 250mL |
| Sodium a-naphthyl phosphate, 0.25% (w/v) | 0.63g |

Step 2 Reagent

| | |
|---|---|
| Buffer | 250mL |
| Naphthanil diazo blue B, 0.5% (w/v) | 1.25g |

Step 1 reagent and Step 2 reagent can be made up in bulk and aliquoted into test tubes and frozen. When needed, one tube of each reagent can be thawed under warm running water for use.

## INSTRUMENTATION

No Instrumentation Required.

## MINIMUM STANDARDS & CONTROLS

Each new batch of reagent must be tested with a positive and negative control before use in casework. Record the results in the Laboratory Asset Manager (LAM).

A positive control (known semen sample) and negative control (reagents only) must be tested before each set of reagent aliquots are used. Document the controls were verified in the case notes.

## PROCEDURE OR ANALYSIS

1. Place a small piece (2mm x 2mm) of substrate on filter paper or another suitable test paper.

2. Add 1-2 drops of Step 1 reagent and allow to react for 30 seconds.

   No color change should be observed at this stage. If color change is observed prior to application of Step 2 reagent, repeat test using new reagent aliquots. If insufficient substrate is available for retesting, report as inconclusive. Consult with Biology QRC as needed.

3. Add 1 drop of Step 2 reagent. Record results after 10 seconds. The test result is graded on a scale using the following guide:

   - Negative: no color change observed after 10 seconds.

   - Inconclusive: An explanation of the circumstances or observations that resulted in an inconclusive conclusion must be documented.
     +/- Inconclusive color change (for example, color change cannot be determined due to substrate coloration).
     +1 Slow pink color develops on sample substrate.
     +2 Slight purple color develops on sample substrate. May develop slowly.

   - Positive: strong reaction/rapid development indicative of semen.
     +3 Intense purple color develops quickly on sample substrate.
     +4 Intense purple color develops rapidly on sample substrate and bleeds into test paper.

## REPORT WORDING

See Appendix I.

## REFERENCES

1. Gaenesslen, R.E. Sourcebook in Forensic Serology, Immunology, and Biochemistry; U.S. Government Printing Office: Washington, DC, August 1983; pp 155-168.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** STAIN IDENTIFICATION

**METHOD:** SEMEN STAIN DETECTION

**PROCEDURE:** KERNECHTROT PICROINDIGOCARMINE STAIN (KPIC) (IDENTIFICATION)

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Reviewed by:

_____

Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

This staining procedure is used to identify the presence of spermatozoa cells in a sample.

## SAFETY CONSIDERATION

| | |
|---|---|
| Aluminum Sulfate: | Warning! Severe Irritant! |
| Picric Acid: | Warning! Severe Irritant!<br>Danger! Explosive!<br>(purchase saturated solution - do not buy dry product) |
| Nuclear Fast Red: | Caution! Slightly Toxic!<br>Caution! Irritant! |
| Indigocarmine: | Caution! Slightly Toxic!<br>Caution! Irritant! |

## PREPARATIONS

Solution #1: Dissolve 5g. aluminum sulfate in 100mL hot water.
Add 0.1g nuclear fast red dye.
Cool and filter.

Solution #2: Dissolve 1g indigocarmine dye (5,5-indigo-sulfonic acid, disodium salt) in 300mL saturated picric acid solution.

Equivalent preparations may be purchased commercially, if available.

## INSTRUMENTATION

Compound microscope with at least 200, 400, 1000 magnification capabilities.

## MINIMUM STANDARDS & CONTROLS

Each new batch of reagent must be tested before use in casework. Prepare a semen slide using Solution #1 and Solution #2. Confirm spermatozoa appear as red-stained bodies with background materials appearing blue or green. Record the results in the Laboratory Asset Manager (LAM).

## PROCEDURE OR ANALYSIS

1. Gloves must be worn during the handling of evidence, including slides.

2. Clean technique must be followed.

3. Fix the smear or stain extract to the slide by gentle heating. A suggested optimal size stain is approximately a 0.5cm diameter circle.

4. Add Solution #1 to the smear or stain extract on the slide. Allow to stand 5 minutes.

5. Rinse with water.

6. Add Solution #2 to slide and allow to stand 3 seconds.

7. Rinse with ethanol or methanol.

8. Observe at 200x magnification. All nuclear material will stain a red or red-purple color. The background materials will be stained blue or green.

    - Positive: At least one verified sperm cell is observed. Spermatozoa must be verified at 1000x magnification. Grade the abundance of spermatozoa observed at 200x magnification. Spermatozoa will appear as differently-stained red bodies, somewhat oval in shape with a slight pink cast. The acrosomal cap will be stained less intensely red than the nuclear portion of the sperm head. If present, the midpiece and tail sections will be stained green or blue-green.

        Trace rare; extremely difficult to locate
        1+ few; difficult to locate
        2+ some in some fields
        3+ some in many fields; easy to locate
        4+ many in most fields

    - Inconclusive: The microscopic examination for the presence of a sperm cell will be called inconclusive when any of the following factors are ambiguous or indefinite when observed at 1000x magnification: the acrosomal cap, point of attachment, size, shape or staining. An explanation of the circumstances or observations that resulted in an inconclusive conclusion must be documented.

    - Negative: A negative result will be reported when no verified or inconclusive spermatozoa are observed.

9. Prepared slides will be repackaged with the parent item unless otherwise described in the case notes.

## REPORT WORDING

See Appendix I.

## REFERENCES

1. Gaensslen, R.E., *Sourcebook Forensic Serology, Immunology, and Biochemistry*; U.S. Government Printing Office: Washington, DC, 1983; pp150-152.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** STAIN IDENTIFICATION

**METHOD:** SEMEN STAIN DETECTION

**PROCEDURE:** P30 ANALYSIS BY SERATEC (INDICATION)

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Reviewed by:

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Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

This procedure is used as a screening test to aid in the indication of semen. This is not a confirmatory test for semen.

## SAFETY CONSIDERATIONS

Observe standard laboratory practices.

## PREPARATION

PSA Buffer Solution (Seratec)

## INSTRUMENTATION

Standard laboratory instrumentation.

## MINIMUM STANDARDS & CONTROLS

Each lot number of Seratec test cassettes must be tested with 4 ng/mL and 10 ng/mL dilutions of a P30 standard in 200 µL of PSA Buffer Solution (Seratec), and a negative control (blank) that is 200 µL of PSA Buffer Solution (Seratec). Both P30 standards must be positive and the negative control must be negative before the lot can be used in casework. Record the results of the quality control check in the Laboratory Asset Manager (LAM). Each card has an internal standard line and control line that must be observed for the test to be considered valid (the control "C" area of the card consisting of two lines).

## PROCEDURE OR ANALYSIS

1. Place a small piece of sample material (5mm x5mm) in a 1.5 mL microcentrifuge tube.

2. Add 250 µL of PSA Buffer Solution (Seratec) to the tube.

3. Incubate at room temperature for 30 minutes with gentle agitation on a shaker.

4. Pipette 200 µL of extract to the sample well of the test cassette.

5. Read results at 10 minutes:

    - Valid results will occur only if two lines (internal standard line and control line) are present in the control area "C." If the standard line and/or control line are not present, the test is invalid and should be repeated. If repeated testing does not produce a valid test result or if repeated testing is not possible, for example due to substrate size, the result may be reported as inconclusive. Observation of a broken or spotted test line(s) may also warrant repeated testing or an inconclusive conclusion. Consult with Biology QRC as needed. An explanation of the circumstances or observations that resulted in an inconclusive conclusion must be documented.

- A positive result is indicated by the presence of a line in the test area "T," at or before the 10-minute reading.

- A negative result is indicated by the absence of a line in the test area at the 10-minute reading. This may result from either the absence of P30 in the sample or it may be due to "high dose hook effect," which may give false negatives in the presence of high concentrations of P30. If a high concentration of P30 is suspected, a 1:100 dilution of the sample may be made to ensure that a false negative does not occur.

## REPORT WORDING

See Appendix I.

## REFERENCES


1. Hochmeister, M., Budowle, B., Rudin, O., Gehrig, C., Borer, U., Thali, M., Dirnhofer, R., Evaluation of Prostate-Specific Antigen (PSA) membrane Test Assay for the Forensic Identification of Seminal Fluid, *J. For. Sciences*, 1999. This procedure allows identification of human semen.

2. Illinois State Police Joliet Forensic Science Laboratory, Evaluation of the Seratec PSA Semiquant Test Research Project 2014-09.

3. Laux, D.L. and Custis, S. (2004) Forensic Detection of Semen III. Detection of PSA Using Membreane Based Tests: Sensitivity Issues with Regards to the Presence of PSA in Other Body Fluids.

4. Laux, D.L., Tambasco, A.J., Benzinger, E.A. (2003) Forensic Detection of Semen II. Comparison of the Abacus Diagnostics OneStep ABAcard P30 Test and the SERATEC PSA SEMIQUANT Kit for the Determination of the Presence of Semen in Forensic Cases.

5. Seratec. User Instructions: PSA in Body Fluids, Seratec. [online]

6. Seratec. User Instructions: Seratec PSA Semiquant, Seratec. [online]

7. Seratec. Product Insert, Seratec. [online]

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** STAIN IDENTIFICATION

**METHOD:** BLOODSTAIN INDICATION

**PROCEDURE:** KASTLE-MEYER TEST

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Reviewed by:

Forensic Scientist Heather May, Chairperson
Forensic Biology Command Advisory Board

Approved by:

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

This test is utilized as a preliminary screening test for blood. This is not a confirmatory test for blood. If field testing for blood was conducted and described as positive, additional laboratory screening is at the analyst's discretion. DNA testing of an item which field tested positive should be considered regardless of Kastle-Meyer result.

## SAFETY CONSIDERATION

| | |
|---|---|
| Hydrogen Peroxide: | Danger! Corrosive! |
| Phenolphthalein: | Caution! Irritant! |
| Potassium Hydroxide: | Danger! Corrosive! |
| Hydrochloric Acid: | Danger! Corrosive!<br>Severe burns and permanent tissue damage may result upon contact with eyes and skin, or if inhaled or ingested. |

Zinc powder or dust in contact with water or damp air evolves hydrogen. The heat of reaction is sufficient that the hydrogen may ignite. Therefore, zinc should not be discarded in the wastebasket. The following procedure should be followed for less than 20 grams of zinc dust:

1. Follow standard laboratory chemical handling practices and work in the hood with the hood on, wearing safety glasses and rubber gloves. With the zinc in a large beaker, add small amounts of concentrated hydrochloric acid with a pipette. The solution will bubble and give off heat. Proceed slowly. Allow time for the bubbling and heat to dissipate before adding more acid. Continue slowly adding acid until no more bubbles are formed and no gray powder is visible (about 3 mLs HCl for 1 gram of zinc).

2. When all the zinc has dissolved (forming soluble zinc chloride), cautiously neutralize the acid solution by adding small amounts of sodium carbonate. Again, foaming will occur. Continue slowly adding sodium carbonate until no more bubbling occurs (about 2g sodium carbonate for 1 gram of zinc). At this point, all the zinc should now be in the form of zinc carbonate, a white precipitate.

3. The zinc carbonate may be filtered out of solution and disposed of in a trash can since zinc carbonate is nontoxic.

## PREPARATION

| | | |
|---|---|---|
| Stock Solution: | Phenolphthalein | 2 g |
| | Potassium hydroxide | 20 g |
| | Distilled water | 100 mL |
| | Zinc dust | 20 g |

Mix, add a few boiling chips and boil under reflux 2-3 hours or until the solution has lost all its pink color. Cool and decant into a bottle containing some zinc to keep it in the reduced form.

Working Solutions:

| | | |
|---|---|---|
| Solution #1: | Ethanol | 10 mL |
| Solution #2: | Phenolphthalin stock solution | 2 mL |
| | Distilled Water | 10 mL |
| | Ethanol | 2 mL |
| Solution #3: | 3% Hydrogen peroxide | 10 mL |

Equivalent preparations may be purchased commercially, if available.

## INSTRUMENTATION

No instrumentation required.

## MINIMUM STANDARDS & CONTROLS

The production laboratory must test each new batch of stock reagent with a positive and negative control before use in casework and distribution to other laboratories. The results will be recorded in the Laboratory Asset Manager (LAM).

A positive control (known bloodstain) and negative control (reagents only) must be tested each day before the reagents are used. Document the controls were verified in the case notes.

## PROCEDURE OR ANALYSIS

1. A small cutting, swabbing, or extract of the suspected bloodstain is placed on filter paper or spot test paper.

2. Add two to three drops of Working Solution #1 (ethanol) to the substrate sample.

3. Add two drops of Working Solution #2 to the substrate sample.

   No color change should be observed at this stage. If color change is observed prior to application of Working Solution #3, repeat test on a new portion of substrate. Consider retesting with new working solutions. If insufficient substrate is available for retesting, report as inconclusive. Consult with Biology QRC as needed.

4. Add two to three drops of Working Solution #3 (3% hydrogen peroxide) to the substrate sample. Record results within 10 seconds.

- Positive: observation of a pink color indicates peroxidase activity, indicative of hemoglobin.

- Negative: no color change is observed.

- Inconclusive: color change cannot be determined, for example due to substrate coloration. An explanation of the circumstances or observations that resulted in an inconclusive conclusion must be documented.

## REPORT WORDING

See Appendix I.

## REFERENCES

1. Gaensslen, R. E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, U.S. Government Printing Office: Washington, DC, August 1983, pp. 101-116.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** STAIN IDENTIFICATION

**METHOD:** BLOODSTAIN INDICATION

**PROCEDURE:** OUCHTERLONY TEST

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Reviewed by:

_____
Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____
Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

Double immunodiffusion is a method that allows the immunological identification of a protein. The identification is made by simultaneously comparing the reaction of the unknown protein with the reaction of a known protein against a known antiserum.

## SAFETY CONSIDERATIONS

Sodium Azide - Highly Toxic!
Danger! Reproductive Hazard!

## PREPARATIONS

1/2% Agar Gel

| | |
|---|---|
| Agar | 0.5 g |
| Sodium chloride | 0.85 g |
| Sodium azide | 0.01 g (optional preservative) |
| Distilled water | 100 ml |

1. Boil with constant stirring until agar is completely dissolved.

2. Pipette about 3 ml. of agar solution in 30 2-inch disposable plastic petri dishes and allow to cool on a level surface. Place the cover on each petri dish and store upside down in the refrigerator to prevent the gels from dehydrating.

## INSTRUMENTATION

No Instrumentation Required.

## MINIMUM STANDARDS & CONTROLS

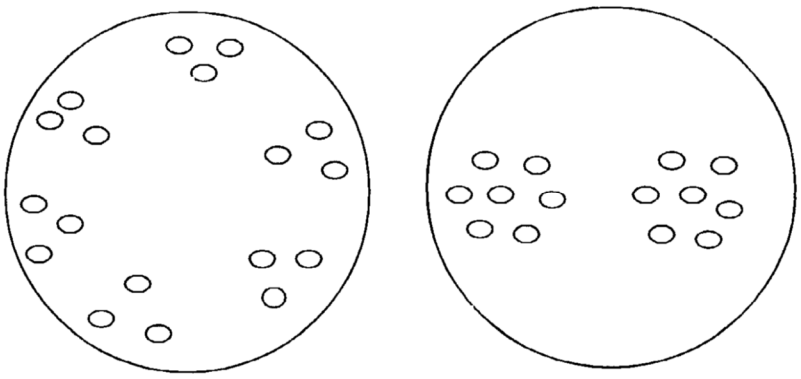
Each new batch of Ouchterlony (agar) plates must be tested with a positive and negative control before use in casework. Record the results in the Laboratory Asset Manager (LAM).

Each new lot of antisera must be quality control tested before use on case samples. See the Quality Assurance Appendix for additional information.

A positive control (portion or extract of known sample to which the antiserum is directed) and a negative control (empty well or extraction liquid, if used) must be tested with each set of samples. Document the controls were verified in the case notes.

## PROCEDURE OR ANALYSIS

1. Using a template drawn on a plastic petri dish cover, punch the desired pattern of wells into the gel. The pattern may be a series of 3 wells forming an equilateral triangle or 7 wells forming a hexagon with a central well. See below:



Note: When screening an unknown bloodstain to determine animal species origin, it is economical to use the hexagonal shape. Place an extract of the stain in the central well and antisera for 6 different species around it. Any antisera that forms a precipitin band must be checked by the conventional method described below. This method is only a screening technique to eliminate a majority of the possible species.

2. When using the customary 3-well pattern, position the wells in a triangle with apex above. (See diagram below.)

   a. In the apex well, place antiserum.

   b. In the right base well, place the known standard antigen extract or diluted serum.

   c. In the left base well, place the questioned stain extract.

Anti-Human Serum

Extract of Questioned Bloodstain — Extract of Known Human Bloodstain

3. Set up a positive control test triangle with the known sample in both left and right wells.

4. Set up a negative control test triangle with a blank on one side and the known sample on the other.

5. Cover the petri dish and leave undisturbed overnight. Refrigeration is not normally required.

6. Read the plate with the aid of a lamp. Precipitin bands that form a continuous arc of convergence (identity band) between the antiserum well and the two extract wells are considered a positive result. A negative result is demonstrated by the presence of a precipitin band between the antiserum well and the known sample well but no precipitin

band between the antiserum well and the questioned well. Precipitin bands that do not converge between the antiserum well and the two extract wells are considered an inconclusive result. An explanation of the circumstances or observations that resulted in an inconclusive conclusion must be documented.

## REPORT WORDING

See Appendix I.

## REFERENCES

1. Gaensslen, R.E. *Sourcebook in Forensic Serology, Immunology and Biochemistry;* U.S. Government Printing Office: Washington, DC, August 1983; pp 221-241.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** STAIN IDENTIFICATION

**METHOD:** SALIVA STAIN INDICATION

**PROCEDURE:** PHADEBAS TEST

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Reviewed by:

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Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

This procedure is a preliminary screening test to aid in the indication of saliva. This is not a confirmatory test for saliva.

## SAFETY CONSIDERATIONS

Observe standard laboratory practices.

## PREPARATIONS

Phadebas® tablets (Magle)

## INSTRUMENTATION

Standard laboratory instrumentation.

## MINIMUM STANDARDS & CONTROLS

A positive control (known saliva stain) and a negative control (reagents only) must be tested with each set of samples. The positive control should have a transparent dark blue supernatant. The negative control should have no color observed in the supernatant. Document the controls were verified in the case notes.

## PROCEDURE OR ANALYSIS

1. Place a small piece of the sample material in a 10 x 75 test tube. In a second tube, place an equal-sized piece of known saliva stain as a positive control. In a third tube add no sample (negative control).

2. Add 1.0 ml. $H_2O$ and 1/4 Phadebas® tablet to each tube using forceps, not fingers, to handle the tablets.

3. Vortex to mix thoroughly.

4. Incubate at 37°C for 30 minutes.

5. Centrifuge for 5 minutes.

6. Record results immediately:

    - Positive: A transparent dark blue supernatant of equal or greater intensity than the positive control is regarded as a positive test for amylase activity, indicative of the presence of saliva.

- Inconclusive: A blue supernatant that is less intense than the positive control but darker than the negative control. An explanation of the circumstances or observations that resulted in an inconclusive conclusion must be documented.

- Negative: no color is observed in the supernatant.

## REPORT WORDING

See Appendix I.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** STAIN IDENTIFICATION/PRESERVATION

**METHOD:** HAIR ROOTS

**PROCEDURE:** SELECTION AND PREPARATION OF HAIRS FOR DNA ANALYSIS

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Reviewed by:

_____

Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

This procedure contains guidelines for selecting and preparing apparent hairs for DNA testing. Apparent hairs may be observed visually or examined microscopically in preparation for DNA analysis. A microscopic examination must be conducted to support a conclusion of no apparent root or tissue. The method used (visual or microscopic) must be documented. Use of a stereomicroscope is considered a visual examination method for purposes of this procedure.

## SAFETY CONSIDERATIONS

Use standard laboratory safety practices.

Chemical/Reagent warning: Permount, xylenes, xylene substitute, and ethanol potentially pose both flammability and health hazards. Consult the Safety Data Sheets (SDS) for further information on safe use of these materials.

## PREPARATIONS

No preparations necessary.

## INSTRUMENTATION

Stereomicroscope
Compound microscope

## MINIMUM STANDARDS AND CONTROLS

Adhere to the procedures in FB-I-A: Clean Technique.

## PROCEDURE

1. If necessary, apparent hairs may be removed from tapings by using a few drops of xylene substitute or another suitable solvent to soften the adhesive.

2. For visual-only observation and preservation of apparent hair(s): remove approximately 2cm from each end of the apparent hair(s), or the entire hair if less than or close to 2cm long. A stereomicroscope may be used to assist visual observation of apparent hair(s).

3. For microscopic examination using a compound microscope, wet mount apparent hairs using mineral oil and examine at 200x with a compound microscope to select apparent hairs that may possess a root or adhering tissue.

4. Remove the apparent hairs from the slide(s).

   If a semi-permanent mounting medium was used, clean the outside of the slide with a 10% bleach solution; then use one of the following methods to remove the cover slip:

   a. Freezing method

Place the slide in the freezer for a few minutes. Pry off the cover slip. Add a drop of xylenes or xylene substitute to the hair to dissolve the mounting medium and loosen the hair.

b. Xylenes method
Soak the slide in xylenes or xylene substitute for several hours until the cover slip can be pried from the slide.

5. To prepare an apparent hair(s) for DNA analysis, cut approximately 2 cm of the proximal (root) end of the apparent hair. If the apparent hair is affixed to a slide, the distal end may be left in the original mounted position. If the apparent hair is less than or close to 2 cm long, the entire hair may be recovered.

6. If necessary, soak the apparent hair in approximately 10 to 20 mL of xylenes or xylene substitute for a few minutes to remove any residual mounting medium. Rinse briefly in ethanol to remove any remaining xylenes or xylene substitute.

7. Place the apparent hair in a microcentrifuge tube and either preserve the apparent hair for future DNA analysis or proceed to extraction using the procedure described in FB-IIIA-3: Isolation of DNA from Non-Semen Stains.

## REPORT WORDING

See Appendix I for specific wording suggestions.

## REFERENCES

Refer to the Reagent Blanks and Controls for Extraction section for appropriate references.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**PROCEDURE:** REAGENT BLANKS AND CONTROLS FOR EXTRACTION

---

Reviewed by:

Forensic Scientist Katherine A. Sullivan, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

Jeanne M. Richeal
DNA Technical Leader

William E. Frank
DNA Technical Leader

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

DNA profiles obtained from biological evidence provide information as to the source of the sample. The goal of DNA extraction is to separate DNA from the substrate, while removing impurities that may be inherent to the sample. The recovery of high molecular weight DNA is essential to downstream processing of the sample. Several extraction procedures are validated for use in casework. The extraction method chosen for each sample will be based on the sample type, sample condition, expected recovery, and whether the sample is suitable for automation.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices.

Warning: Treat all reagents/samples as potential biohazards.

## PREPARATIONS

See individual extraction procedures.

## INSTRUMENTATION

Standard laboratory instrumentation.

## MINIMUM STANDARDS AND CONTROLS

The reagent blank is a laboratory process control that aids in the identification of potential sources of contamination. It serves as a quality check on the reagents used during sample processing and a quality assurance measure for monitoring laboratory clean technique.

I. Reagent blanks are created whenever a sample or set of samples are extracted. A reagent blank will consist of a volume of extraction reagents equivalent to the greatest volume utilized for samples in the extraction set. Reagent blanks will be extracted at the same time using the same methodology and reagents as the samples they represent. In order to optimize detection of potential contaminants, reagent blanks are extracted last and in a fashion equivalent to the most stringent processing applied to any sample within the extraction set.

II. Minimum number of reagent blanks required

A. Extraction set

An extraction set is defined as a group of samples extracted at the same time using the same extraction methodology and reagents.

1. Each extraction set of standards requires at least one reagent blank.

2. Each extraction set of unknowns requires at least two reagent blanks.

3. If a sample is extracted by itself, only one reagent blank is required.

The numbers of reagent blanks above are the minimum requirement for each extraction set. It is the analyst's responsibility to determine the appropriate number of reagent blanks to process with each set of samples.

It may be helpful to extract similar sample types together to prevent exhaustion of the reagent blanks (e.g. low level semen samples, touch samples, etc.).

If all reagent blanks from an extraction set are consumed during processing, no additional analysis of samples from that extraction set will be permitted.

B. Additional reagent blanks may be necessary when an extraction set:

1. Contains a large number of samples.

2. Contains potentially low-level or inhibited samples, or samples that may require additional amplifications (e.g. Y-STR).

3. Contains samples extracted in multiple tubes.

**Note:** Additional reagent blanks are required for Maxwell® 16 extraction sets containing more samples than can be processed with a single run or instrument. See the DNA IQ™ Extraction: Maxwell® 16 procedure for additional information.

III. Processing

A. Extraction

1. The largest volume of reagent added to any sample within the extraction set will also be added to the reagent blanks.

2. If a sample is extracted in multiple tubes for the purpose of combining before resolubilization, the total volume of extraction reagent used for the sample will be determined. This volume will be added to the reagent blanks. This volume may require the reagent blanks to be extracted in multiple tubes.

3. For differential extraction sets, reagent blanks will be created to track the processing of the F1 and F2 fractions. The F2 reagent blank will also represent any F3 fraction that is processed at the same time as the F2 fractions. If an F3 fraction is extracted separately from the F2 fractions, an F3 reagent blank will be created to track that extraction.

B. Resolubilization and Elution

Reagent blanks will be resolubilized/eluted at the lowest volume used for any sample within the extraction set.

C. Diluting

Reagent blanks are never diluted since this would minimize the possibility of detecting and identifying extraneous DNA.

D. Concentration

If a sample from the extraction set is concentrated, an equivalent volume of reagent blank from the same extraction set will be concentrated in the same manner. If the reagent blank is already at the concentrated volume, it does not need to be concentrated provided the percentage of reagent blank remaining is equal to or greater than the percentage of sample to be analyzed. For additional information see the section below on "Assessing whether sufficient reagent blank remains for additional analysis."

E. Additional Purification

If a sample from an extraction set is purified, the equivalent volume of reagent blank from the same extraction set will be purified in the same manner.

F. Combining extracts

1. If a sample is extracted in multiple tubes within an extraction set, those extracts may be combined after resolubilization/elution. The equivalent volume of reagent blank from the same extraction set will be combined in the same manner.

2. If a sample is extracted between two different extraction sets, those extracts may be combined. The equivalent volume of reagent blank from each extraction set will be combined in the same manner. The samples and reagent blanks must be quantified before combining.

IV. Quantification

A. All reagent blanks will be quantified using qPCR.

B. Once a reagent blank has an acceptable quantification, no additional re-quantification is required.

V. Amplification and Profiling

A. A reagent blank is required for each amplification chemistry.

1. If the qPCR results for all reagent blanks within an extraction set are "not detected", any reagent blank may be chosen for amplification. If quantifiable DNA is observed in one or more reagent blanks, there may be restrictions to amplification. See "Detection of Extraneous DNA" for amplification requirements.

2. The volume of reagent blank amplified will be equal to or greater than the largest volume or equivalent volume* of sample amplified within the extraction set.

   *For example, if 10µL of a 1/10 dilution is amplified, the equivalent volume of reagent blank required is 1µL (10µL x 1/10 = 1µL).

3. Re-amplification of reagent blanks is required only when a sample from an extraction set is re-amplified at a volume or equivalent volume greater than the previous amplification of the reagent blank. If the required volume of reagent blank is not available, the sample will not be re-amplified.

B. A reagent blank must be run on the same instrument model as the samples it represents.

C. Re-preparation and/or re-injection of samples from an extraction set does not require re-preparation and/or re-injection of the associated reagent blank, unless the sample is run on a different instrument model.

VI. Assessing whether sufficient reagent blank remains for additional analysis of previously processed samples

A. Determine the volumes of sample and reagent blank remaining.

B. Calculate the percentage of original sample required:

   (volume required for new processing)/(original sample volume) = % of sample required

C. Calculate the volume of reagent blank required:

   (% of sample required) x (original reagent blank volume) = volume of reagent blank required

D. If insufficient reagent blank remains, no additional processing of a sample will be conducted.

VII. Detection of Extraneous DNA in Reagent Blanks

1. The reagent blank with the highest quantification results within an extraction set must be selected for amplification. This reagent blank will be amplified at a level expected to produce the most complete profile.

2. The technical leader will be notified when extraneous DNA is observed in a reagent blank and an Incident Form will be used to document the issue.

3. Amplification of additional samples from the associated extraction set may be possible upon consultation with the technical leader.

## PROCEDURE OR ANALYSIS

Refer to the appropriate procedure for the type of sample from which DNA is being extracted. The extracted DNA should be stored in a dedicated Forensic Biology/DNA evidence refrigerator or freezer until analysis is complete.

## REPORT WORDING

Not applicable.

## REFERENCES


1. Millipore Microcon 100 DNA Fast Flow User's Guide.

2. Microcon 100 Filters Study (referenced in Section Advisory Committee memorandum 08-BIO-16).

3. ISP R&D Project: Microcon 100 DNA Fast Flow Filter Performance Check (2012-11).

4. ISP R&D Project: Evaluation of Methods to Remove Inhibitors from DNA Extracts (2013-18).

5. Also see the following early ISP validations studies:

   V9. Extraction Study and the Use of DTT for Bloodstain Extractions.

   V20, V21, V22, V23. Effect of Various Environmental Insults on Recovery and RFLP Analysis of DNA.

   V24. Effect of Two Hour Resolubilization vs. Overnight in TE Buffer.

   V30. Efficiency of the Differential Extraction for Spermatozoa Recovery.

V41. Use of Microconcentrate Tubes vs. Ethanol Precipitation.

V42. Use of Stain Extraction Buffer in the Differential Extraction of Sperm Cell-Containing Mixed Samples.

6. Giusti, A., et al. "Application of Deoxyribonucleic Acid (DNA) Polymorphism to the Analysis of DNA Recovered from Sperm," *Journal of Forensic Sciences*, 1986, Vol. 31, No. 2, pp. 409-417.

7. Hochmeister, M., et al. "Typing of Deoxyribonucleic Acid (DNA) Extracted from Compact bone from Human Remains," *Journal of Forensic Sciences*, Nov. 1991, Vol. 36, No. 6, pp. 1649-1661.

8. Labor, T., et al. "Evaluation of Four Deoxyribonucleic Acid (DNA) Extraction Protocols for DNA Yield and Variation in Restriction Fragment Length Polymorphism (RFLP) Sizes Under Varying Gel Conditions," *Journal of forensic Sciences*, March 1992, Vol. 37, No. 2, pp. 404-424.

9. Lee, H. C., et al. "The Effect of Presumptive Test, Latent Fingerprint and Some Other Reagents and Materials on Subsequent Serological Identification, Genetic Marker and DNA Testing in Bloodstains," *Journal of Forensic Identification*, 1989, Vol. 36, pp. 339-358.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** DNA ISOLATION

**PROCEDURE:** SEMEN:ORGANIC DIFFERENTIAL EXTRACTION

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Reviewed by:

_____

Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____

Jeanne M. Richeal
DNA Technical Leader

_____

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

The goal of DNA extraction is to separate DNA from the substrate, while removing impurities that may be inherent to the sample. The recovery of high molecular weight DNA is essential to downstream processing of the sample.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents. Wear appropriate personal protective equipment and use the fume hood when using these chemicals.

**Chloroform** is toxic and a suspected human carcinogen. It is harmful if inhaled, ingested or exposed to the eyes or skin.

**Phenol** is a toxic and corrosive chemical and a suspected carcinogen. It is harmful or fatal if ingested, inhaled, or exposed to the eyes. Special protection: use of a local exhaust hood is required; use chemical resistant protective gloves (LATEX gloves are NOT a sufficient barrier of protection) such as neoprene or rubber.

**Sodium Dodecyl Sulfate (SDS)** is harmful if inhaled or ingested. It potentially causes skin and eye burns. Special protection: wear a dust mask or respirator; use of a fume hood is required.

**8-Quinolinol** is irritating to the eyes, skin, mucous membranes and upper respiratory tract. Laboratory experiments have shown mutagenic effects.

## PREPARATIONS

Equivalent preparations may be purchased commercially, if available.

**Chloroform/Isoamyl Alcohol (24:1)**

| | |
|---|---|
| Chloroform | 96 mL |
| Isoamyl alcohol | 4 mL |

Purchasing this mixture is highly recommended. If prepared in-house, prepare in fume hood (use the indicated proportions). Store in glass bottles.

**390 mM DTT**

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

Aliquot into convenient size volumes and freeze. Remix after thawing.

**Buffer-Saturated Phenol/Chloroform/Isoamyl Alcohol 25:24:1, pH8**

Optional: 8-Quinolinol may be mixed in at 1 mg per mL of solution.
Purchasing this mixture is highly recommended. If prepared in-house, prepare in fume hood (use the indicated proportions). Store in glass bottles.

**20 µg/µL Proteinase K**

| | |
|---|---|
| Proteinase K | 500 mg |
| ddi $H_2O$ (or equivalent) | 25 mL |

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

**Stain Extraction Buffer**

| | |
|---|---|
| 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. Autoclave.

**$TE^{-4}$**

| | |
|---|---|
| 1.0 M Tris, pH 8.0 | 10 mL |
| 500 mM EDTA, pH 8.0 | 0.2 mL |

Add ddi $H_2O$ (or equivalent) to 1.0 liter. Autoclave.

**TNE**

| | |
|---|---|
| 1.0 M Tris, pH 8.0 | 10 mL |
| NaCl | 5.84 g |
| 500 mM EDTA, pH 8.0 | 4.0 mL |

Add ddi $H_2O$ (or equivalent) to 1.0 liter. Autoclave.

## INSTRUMENTATION

Standard laboratory instrumentation.

## MINIMUM STANDARDS AND CONTROLS

Refer to the Reagent Blanks and Controls for Extraction section.

## PROCEDURE OR ANALYSIS

CW Microfuge tubes should not be used for organic extractions as PCI may leak out during centrifugation.

Since the volume of extraction reagents can be increased to accommodate the substrate material, swabs will be limited to two swabs per microfuge tube. The swab material will be removed from the stick before placing it into the microfuge tube.

All other substrates should be limited so the extraction reagents can sufficiently interact with the substrate. Substrate should never be packed tightly, as that will prevent the extraction reagents from interacting with the entire substrate.

1. Place the sample into an extraction tube. Add:

   500 µL Stain Extraction Buffer
   5 µL Proteinase K (20 µg/ µL)

   Add sufficient reaction mixture to have excess liquid visible if substrate is too absorbent.

   Mix gently and incubate two hours at 37°C.

2. Place the sample in the basket of the extraction tube. Centrifuge for five minutes at approximately 10,000 x g.

3. Divide the sample into three portions for extractions of the F1, F2, and F3 fractions.

   F3 fraction: Place the material retained in the basket into a new extraction tube. The F3 may be processed further at the analyst's discretion.

   Note: Unextracted F3 substrates must be retained when samples are consumed.

   F1 fraction: Transfer the liquid above the sperm cell pellet into a microcentrifuge tube.

   F2 fraction: The sperm cell pellet should be left in the original extraction tube.

4. F2 fraction:

   A. Add the following to the sperm cell pellet:

      500 µL Stain Extraction Buffer
      5 µL Proteinase K (20 µg/µL)

      Mix gently and incubate 30 minutes at 37°C.

   B. Centrifuge for five minutes at approximately 10,000 x g.

   C. Remove the liquid from the sperm cell pellet and discard.

D. Rinse the sperm cell pellet with 1 mL TNE, vortex and centrifuge at maximum speed for ten minutes. Remove and discard the TNE buffer without disturbing the sperm cell pellet. After the wash, 1 µL of the sperm cell pellet may be removed for KPIC.

5. F2 and F3 fractions:

   Add: 350 µL Stain Extraction Buffer
   40 µL 390 mM DTT
   10 µL Proteinase K (20 µg/µL)

   Add sufficient reaction mixture to have excess liquid visible if the F3 substrate is too absorbent.

   Mix and incubate at 37°C for two hours.

6. Place the F3 sample in the basket of the extraction tube. Centrifuge for five minutes at approximately 10,000 x g. Transfer the liquid into a new tube, if preferred.

PURIFICATION

7. Organic Extraction: Add 500 µL phenol/chloroform/isoamyl alcohol (PCI) to the liquid. This step must be done in the fume hood. Vortex for approximately one minute to achieve a milky emulsion. Centrifuge for two minutes at maximum speed.

   At the analyst's discretion, the aqueous and interface may be re-extracted using 500 µL PCI or 500 µL chloroform/isoamyl alcohol.

ISOLATION

8. Place the aqueous phase from the organic extraction into a Microcon 100 tube. Spin to dryness at 500 x g. Use the following equation to calculate the rpm value for any selected centrifuge:

   $N = \sqrt{(RCF/0.000018 \times radius)}$

   N = rpm setting
   RCF = Relative centrifugal force (g)
   Radius = cm from filter surface to center of centrifuge head

9. Add 50 – 100 µL of $TE^{-4}$ to the filter in order to wash residual extraction components from the DNA. Centrifuge to dryness.

10. Add the appropriate volume of $TE^{-4}$ (depending on anticipated DNA recovery), invert filter, vortex, and spin out liquid.

11. If desired, incubate the sample to resolubilize the DNA for at least one hour at 56°C.

12. At the analyst's discretion, extracted DNA samples may be re-extracted using the manual DNA IQ™ or organic methods, or purified using QIAquick silica filters.

    A. For re-extraction with manual DNA IQ™, the entire volume of sample extract should be added to 250 µL of prepared lysis buffer with DTT, as described in the initial lysis step of the procedure. Continue the re-extraction following the remaining steps as described in the DNA IQ™ Extraction: Magnetic Separation Stand procedure.

    B. For organic re-extraction, repeat steps 1 and 7 through 11 above omitting the DTT.

    C. To purify the sample extract using a QIAquick silica filter, follow the steps as described in the Non-Semen: Bone Extraction procedure.

## REPORT WORDING

Not applicable.

## REFERENCES

Refer to the Reagent Blanks and Controls for Extraction section.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** DNA ISOLATION

**PROCEDURE:** NON-SEMEN: ORGANIC EXTRACTION

---

Reviewed by:

Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

Jeanne M. Richeal
DNA Technical Leader

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

The goal of DNA extraction is to separate DNA from the substrate, while removing impurities that may be inherent to the sample. The recovery of high molecular weight DNA is essential to downstream processing of the sample.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents. Wear appropriate personal protective equipment and use the fume hood when using these chemicals.

**Chloroform** is toxic and a suspected human carcinogen. It is harmful if inhaled, ingested or exposed to the eyes or skin.

**Phenol** is a toxic and corrosive chemical and a suspected carcinogen. It is harmful or fatal if ingested, inhaled, or exposed to the eyes. Special protection: use of a local exhaust hood is required; use chemical resistant protective gloves (LATEX gloves are NOT a sufficient barrier of protection) such as neoprene or rubber.

**Sodium Dodecyl Sulfate (SDS)** is harmful if inhaled or ingested. It potentially causes skin and eye burns. Special protection: wear a dust mask or respirator; use of a fume hood is required.

**8-Quinolinol** is irritating to the eyes, skin, mucous membranes and upper respiratory tract. Laboratory experiments have shown mutagenic effects.

## PREPARATIONS

Equivalent preparations may be purchased commercially, if available.

**Chloroform/Isoamyl Alcohol (24:1)**

| | |
|---|---|
| Chloroform | 96 mL |
| Isoamyl alcohol | 4 mL |

Purchasing this mixture is highly recommended. If prepared in-house, prepare in fume hood (use the indicated proportions). Store in glass bottles.

**390 mM DTT**

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

Aliquot into convenient size volumes and freeze. Remix after thawing.

**Buffer-Saturated Phenol/Chloroform/Isoamyl Alcohol 25:24:1, pH8**

Optional: 8-Quinolinol may be mixed in at 1 mg per mL of solution.
Purchasing this mixture is highly recommended. If prepared in-house, prepare in fume hood (use the indicated proportions). Store in glass bottles.

**20 µg/µL Proteinase K**

| | |
|---|---|
| Proteinase K | 500 mg |
| ddi $H_2O$ (or equivalent) | 25 mL |

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

**Stain Extraction Buffer**

| | |
|---|---|
| 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. Autoclave.

**$TE^{-4}$**

| | |
|---|---|
| M Tris, pH 8.0 | 10 mL |
| 500 mM EDTA, pH 8.0 | 0.2 mL |

Add ddi $H_2O$ (or equivalent) to 1.0 liter. Autoclave.

## INSTRUMENTATION

Standard laboratory instrumentation.

## MINIMUM STANDARDS AND CONTROLS

Refer to the Reagent Blanks and Controls for Extraction section.

## PROCEDURE OR ANALYSIS

CW Microfuge tubes should not be used for organic extractions as PCI may leak out during centrifugation.

Since the volume of extraction reagents can be increased to accommodate the substrate material, swabs will be limited to two swabs per microfuge tube. The swab material will be removed from the stick before placing it into the microfuge tube.

All other substrates should be limited so the extraction reagents can sufficiently interact with the substrate. Substrate should never be packed tightly, as that will prevent the extraction reagents from interacting with the entire substrate.

A. Non-Semen Body Fluid Stains and Cellular Material

1. Place the sample into an extraction tube. Add:
   400 µL Stain Extraction Buffer
   10 µL Proteinase K (20 mg/ mL)
   5 µL 390 mM DTT

   Mix and spin briefly to force the sample into the liquid.

2. Incubate unknown samples at 56°C overnight. Standards may be incubated for two hours at 56°C.

3. When appropriate, place the substrate in the basket of the extraction tube. Centrifuge for five minutes at approximately 10,000 x g. Remove and discard the spin basket. Transfer the liquid into a new tube, if preferred.

PURIFICATION

4. Organic Extraction: Add 500 µL phenol/chloroform/isoamyl alcohol (PCI) to the liquid. This step must be done in the fume hood. Vortex for approximately one minute to achieve a milky emulsion. Centrifuge for two minutes at maximum speed.

   At the analyst's discretion, the aqueous and interface may be re-extracted using 500 µL PCI or 500 µL chloroform/isoamyl alcohol.

ISOLATION

5. Place the aqueous phase from the organic extraction into a Microcon 100 tube. Spin to dryness at 500 x g. Use the following equation to calculate the rpm value for any selected centrifuge:

   $N = \sqrt{(RCF/0.000018 \times radius)}$

   N = rpm setting
   RCF = relative centrifugal force (g)
   Radius = cm from filter surface to center of centrifuge head

6. Add 50 – 100 µL of $TE^{-4}$ to the filter in order to wash residual extraction components from the DNA. Centrifuge to dryness.

7. Add the appropriate volume of $TE^{-4}$ (depending on anticipated DNA recovery), invert filter, vortex, and spin out liquid.

8. If desired, incubate the sample to resolubilize the DNA for at least one hour at 56°C.

9. At the analyst's discretion, extracted DNA samples may be re-extracted using the manual DNA IQ™ or organic methods, or purified using QIAquick silica filters.

A. For re-extraction with manual DNA IQ™, the entire volume of sample extract should be added to 250 µL of prepared lysis buffer with DTT, as described in the initial lysis step of the procedure. Continue the re-extraction following the remaining steps as described in the DNA IQ™ Extraction: Magnetic Separation Stand procedure.

B. Organic re-extraction may begin at step 1 (omitting the DTT and incubating for two hours instead of overnight), step 4, or step 5 and processed through step 8.

C. To purify the sample extract using a QIAquick silica filter, follow the steps as described in the Non-Semen: Bone Extraction procedure.

B. Hair with Roots

1. Rinse the hair by adding sterile distilled $H_2O$ (or equivalent) to the microcentrifuge tube and shaking for one hour at room temperature. This will remove any debris adhering to the hair.

2. Rinse the hair briefly in fresh sterile distilled $H_2O$ (or equivalent).

3. Add:
   500 µL Stain Extraction Buffer
   50 µL 390 mM DTT
   15 µL Proteinase K (20 mg/ mL)

   Incubate at 56°C overnight.

4. Vortex the hair/stain extraction buffer mixture for 30 seconds. Add:
   50 µL 390 mM DTT
   15 µL Proteinase K (20 mg/ mL)

   Incubate at 56°C overnight.

5. Spin the microcentrifuge tube at maximum speed to sediment the pigments. Transfer the supernatant to a fresh tube.

6. Follow Section A, Steps 4 through 9.

## REPORT WORDING

Not applicable.

## REFERENCES

Refer to the Reagent Blanks and Controls for Extraction section.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** DNA ISOLATION

**PROCEDURE:** NON-SEMEN: BONE EXTRACTION

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**Reviewed by:**

_____
Forensic Scientist Katherine A. Sullivan, Chairperson
Forensic Biology/DNA Command Advisory Board

**Approved by:**

_____
Jeanne M. Richeal
DNA Technical Leader

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William E. Frank
DNA Technical Leader

_____
Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

Refer to the Reagent Blanks and Controls for Extraction section.

## SAFETY CONSIDERATION

Observe Standard Laboratory Practices.

Warning: Treat all reagents/samples as potential biohazards.

Warning: The following are considered hazardous reagents. Wear appropriate personal protective equipment and use the fume hood when using these chemicals.

**Chloroform** is toxic and a suspected human carcinogen. It is harmful if inhaled, ingested or exposed to the eyes or skin.

**Phenol** is a toxic and corrosive chemical and a suspected carcinogen. It is harmful or fatal if ingested, inhaled, or exposed to the eyes. Special protection: use of a local exhaust hood is required; use chemical resistant protective gloves (LATEX gloves are NOT a sufficient barrier of protection) such as neoprene or rubber.

**Sodium Dodecyl Sulfate (SDS)** is harmful if inhaled or ingested. It potentially causes skin and eye burns. Special protection: wear a dust mask or respirator; use of a fume hood is required.

**8-Quinolinol** is irritating to the eyes, skin, mucous membranes and upper respiratory tract. Laboratory experiments have shown mutagenic effects.

## PREPARATIONS

Equivalent preparations may be purchased commercially, if available.

**Chloroform/Isoamyl Alcohol (24:1)**

| | |
|---|---|
| Chloroform | 96 mL |
| Isoamyl alcohol | 4 mL |

Purchasing this mixture is highly recommended. If prepared in-house, prepare in fume hood (use the indicated proportions). Store in glass bottles.

**390 mM DTT (PCI)**

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

Aliquot into convenient size volumes and freeze. Remix after thawing.

**Buffer-Saturated Phenol/Chloroform/Isoamyl Alcohol 25:24:1, pH8**

Optional: 8-Quinolinol may be mixed in at 1 mg per mL of solution.

Purchasing this mixture is highly recommended. If prepared in-house, prepare in fume hood (use the indicated proportions). Store in glass bottles.

**20 µg/µL Proteinase K (PCI)**

| | |
|---|---|
| Proteinase K | 500 mg |
| ddi $H_2O$ (or equivalent) | 25 mL |

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

**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. Autoclave.

**$TE^{-4}$**

| | |
|---|---|
| 1.0 M Tris, pH 8.0 | 10 mL |
| 500 mM EDTA, pH 8.0 | 0.2 mL |

Add ddi $H_2O$ (or equivalent) to 1.0 liter. Autoclave.

## INSTRUMENTATION

Refer to the Reagent Blanks and Controls for Extraction section.

## MINIMUM STANDARDS AND CONTROLS

Refer to the Reagent Blanks and Controls for Extraction section.

## PROCEDURE OR ANALYSIS

Bone which has been submerged or in the environment for a long period of time may not yield DNA which is amenable to nuclear DNA testing. Therefore, ask for entire bone samples (i.e., ribs or femurs). Request that the medical examiner does NOT saw on the bone.

Note: If soft tissue is present that is not putrid, extract a portion of it. If a DNA profile is not obtained, extract the bone.

1. Clean the bone and remove all of the flesh. Soak the bone in 10% bleach for 1-2 minutes. Rinse in sterile water and dry the bone thoroughly. Using sandpaper or a rotary tool, remove the outer layer of tissue. Avoid the sawed ends of the bone since these may be contaminated. If the sample is putrid, avoid the marrow as it will produce less high molecular weight DNA.

2. Obtain up to 4-15 grams of bone dust. The SPEX Freezer Mill is recommended for this collection as follows:

A. Collect approximately 4 grams of bone. Cut slices of compact bone with an appropriate tool (e.g. a hack saw or rotary tool) small enough to fit in the grinding vial set.

B. Wash all components of a grinding vial set with 10% bleach for 1-2 minutes, rinse the grinding vial components in DNA grade water and allow to dry.

C. Moisten a sterile swab with $TE^{-4}$ buffer or DNA grade water and swab the inside of the grinding cylinder, the impactor and inside facing ends of the end plugs of the grinding vial set. This sample will be used to process a reagent blank.

D. Place one end plug in the grinding cylinder, add the bone sample to be processed, add the impactor to the cylinder and close the grinding vial set with the second end plug. The impactor should be able to move back and forth in the vial.

E. **WEARING CRYOGENIC GLOVES:** Slowly fill the Freezer Mill with liquid nitrogen to the compartment's fill line. Close the unit; a stream of liquid nitrogen fog will be released from a vent in the instrument. When this slows, open the Freezer Mill and refill with liquid nitrogen.

F. **WEARING CRYOGENIC GLOVES** and using the extractor tool, load the grinding cylinder in the Freezer Mill. Standard factory installed settings for a 15 minute initial cool followed by three two minute grind cycles and two minute cool cycles should be selected and the run started.

G. When the run is complete open the Freezer Mill **WEARING CRYOGENIC GLOVES** and remove the grinding vial set with the extractor tool.

H. Allow the sample to warm up before opening to avoid condensation.

I. Transfer portions of approximately 1 g of bone dust to either 15 or 50 mL tubes for optional de-calcification or DNA extraction.

J. Repeat procedure if condition of sample indicates greater than 4 g of bone may be required.

3. Place approximately 1 g of bone dust in a 15 mL or 50 mL conical bottom tube.

4. Optional bone decalcification:

A. Add 500 mM EDTA pH 8.0: approximately 10 mL to 15 mL tubes or 25 mL to 50 mL tubes. Vortex.

Incubate the tube with rocking for 24 hours at 4°C.

Spin the tube for 15 minutes at approximately 2000 x g.

Discard the supernatant.

Repeat this process for a total of 3-5 times.

Process a reagent blank in the same manner.

Repeat the additions of EDTA to the reagent blank and bone sample tubes until the bone dust sample takes on a gelatinous appearance; the de-calcification procedure is complete.

The reagent blank created by extracting the swab from the surfaces of the grinding vial will not require EDTA chelation and may be processed directly with the SDS-based extraction solution. If EDTA chelation is not performed, this will be the only reagent blank processed.

B. Fill the tube to the top with sterile ddi $H_2O$. Vortex.

Spin 15 minutes at approximately 2000 x g.

Repeat this process for a total of three times decanting the water after each spin. Treat the reagent blank tube in the same manner.

5. Add a volume of Stain Extraction Buffer, proteinase K (20µg/µL) and 390 mM DTT (in a ratio of 400/10/5) that is twice the volume of the remaining bone or tissue sample. Incubate overnight at 56°C. Rock periodically until the pellet dissolves. The reagent blank swab and EDTA chelation blank (if created) will be processed with the same volume as the bone or tissue sample.

6. Extract the suspension three times with phenol/chloroform/isoamyl alcohol (PCI). Repeat until the aqueous phase becomes relatively clear (more than three extractions may be required). Follow with a chloroform/isoamyl extraction. Note: If the solution is not clear the filter may become plugged.

7. Remove the DNA from the aqueous phase using Centricon 100 filters or another equivalent filter. Rinse the filters twice with $TE^{-4}$ before collecting DNA.

**Note**: PCR inhibitors are known to co-extract with some human remains samples. If qPCR analysis indicates inhibition, bone samples can be purified further using QIAquick silica filters as follows:

1. Add 5 volumes of the QIAquick Gel Extraction Kit binding buffer to the bone sample DNA extract or reagent blank. Briefly vortex and pulse spin.

2. Assemble a filter apparatus from the QIAquick Gel Extraction Kit for each sample and add the mixture of binding buffer and extract to the filter. Centrifuge on high (approximately 13,000-14,000 rpm) for one minute. Discard fluid from collection tube.

3. Add 750 µL of wash buffer (with ethanol) from the QIAquick Gel Extraction Kit. Centrifuge on high (approximately 13,000-14,000 rpm) for one minute. Discard fluid from collection tube. Centrifuge on high (approximately 13,000-14,000 rpm) for an additional one minute. Transfer filter to a clean standard microfuge tube.

4. Add 30-100 µL of elution buffer from the QIAquick Gel Extraction Kit directly to the membrane. Let the buffer stand on the membrane for one minute then centrifuge on high (approximately 13,000-14,000 rpm) for one minute. Purified DNA will be collected in the microfuge tube. Cap the tube, discard the filter and quantify the purified extract by qPCR.

## REPORT WORDING

Not applicable.

## REFERENCES

Refer to the Reagent Blanks and Controls for Extraction section.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** DNA ISOLATION

**PROCEDURE:** DNA IQ™ EXTRACTION: MAGNETIC SEPARATION STAND

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**Reviewed by:**

Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

**Approved by:**

Jeanne M. Richeal
DNA Technical Leader

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

The Promega DNA IQ™ System paramagnetic resin purifies DNA while eliminating inhibitors. Below is the procedure for the purification of preprocessed samples using a magnetic separation stand.

## SAFETY CONSIDERATIONS

Observe standard laboratory practices.

Warning: Treat all reagents and samples as potential biohazards.

## PREPARATIONS

**Prepared Lysis Buffer (per sample)**

| | |
|---|---|
| 1M DTT | 1µL |
| DNA IQ™ lysis buffer | 100µL |

**DNA IQ 1X Wash Buffer**

| | |
|---|---|
| DNA IQ 2X Wash Buffer (in kit) | 70mL |
| Propanol | 35mL |
| Ethanol | 35mL |

Mix together in 2X wash buffer bottle provided in DNA IQ kit. Relabel as 1X wash buffer solution.

## INSTRUMENTATION

Standard Laboratory Instrumentation
Promega Corporation MagneSphere® Technology Magnetic Separation Stand

## MINIMUM STANDARDS & CONTROLS

Refer to the Reagent Blanks and Controls for Extraction section.

## CRITICAL REAGENTS

Promega Corporation DNA IQ™ Resin

## PROCEDURE OR ANALYSIS:

A. Non-Semen Stains (excluding tissue, hair, and bone samples):

1. Vortex the stock resin bottle for several seconds at high speed to ensure resin is thoroughly mixed. Do not pour out from stock resin bottle. Carefully pipette from stock bottle, using a new pipette tip each time.

2. Add 7µL of DNA IQ™ resin to each preprocessed sample tube.

   **Note:** If processing more than four tubes, vortex the resin every four tubes. This will help ensure maximum and uniform DNA recovery from every sample.

3. Vortex the samples for 3 seconds at high speed. Incubate at room temperature for 5 minutes. Vortex samples for 3 seconds once every minute during the 5-minute incubation.

4. Vortex samples for 2 seconds at high speed. Place each sample in the magnetic stand.

   **Note**: If resin does not form a distinct pellet on the side of a tube, vortex the tube and quickly place it back in the stand.

5. Carefully remove and discard all of the solution without disturbing the resin pellet.

   **Note:** If some resin is drawn up in the pipette tip, gently expel it back into the tube to allow re-separation.

6. Add 100µL of prepared lysis buffer to each sample. Remove each sample from the magnetic stand, vortex for 2 seconds at high speed, and return to the magnetic stand.

7. Discard all lysis buffer from each sample tube.

8. Add 100µL of prepared 1X wash buffer to each sample. Remove each sample from the magnetic stand, vortex for 2 seconds at high speed, and return to the magnetic stand.

9. Discard all wash buffer from each sample tube.

10. Repeat steps 8 and 9 two more times to equal a total of three washes. Be sure that all solution has been removed after the last wash.

11. With the samples still in the magnetic stand, open the lids and air-dry the resin for 5 minutes.

    **Note:** Do not dry for more than 20 minutes, as this may inhibit DNA removal.

12. Add 25µL to 100µL of elution buffer to each sample, depending on expected DNA recovery. Close all tube lids.

13. Vortex samples for 2 seconds at high speed. Incubate at 65ºC for 5 minutes.

14. Remove the samples from the heat source, vortex for 2 seconds at high speed, and immediately place the samples on the magnetic stand.

    **Note:** Tubes must remain hot until placed in the magnetic stand or DNA yield will decrease.

15. Carefully transfer the DNA elution to a microcentrifuge tube.

16. If complete inhibition is observed in the resulting extract, notify the technical leader to discuss a course of action.

## REPORT WORDING

Not applicable.

## REFERENCES


1. Crouse C, et al. Improving efficiency of a small forensic DNA laboratory: validation of robotic assays and evaluation of microcapillary array device. *Croat Med J* 2005; 46(4):563-577.

2. Promega Corporation. DNA Purification from Various Sample Types Using the DNA IQ™ System. *Profiles in DNA* [Online]. (September 2002; 5).

3. Promega Corporation Technical Bulletin, No. 296, DNA IQ™ System - Small Sample Casework Protocol.

4. ISP R&D Project: Evaluation of Methods to Remove Inhibitors from DNA Extracts (2013-18).

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** DNA ISOLATION

**PROCEDURE:** DNA IQ™ EXTRACTION: MAXWELL® 16

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Reviewed by:

Forensic Scientist Katherine Sullivan, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

Jeanne M. Richeal
DNA Technical Leader

William E. Frank
DNA Technical Leader

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

The Promega DNA IQ™ System paramagnetic resin purifies DNA while eliminating inhibitors. Below is the procedure for the purification of preprocessed samples using a Maxwell® 16 instrument.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices.

Reference MSDS for all commodities prior to use.

Warning: Treat all reagents/samples as potential biohazards.

## PREPARATIONS

None

## INSTRUMENTATION

Standard laboratory instrumentation
Promega Corporation Maxwell® 16

## MINIMUM STANDARDS AND CONTROLS

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).

Refer to the Reagent Blanks and Controls for Extraction section for information regarding the minimum number of reagent blanks required for each sample batch type.

## CRITICAL REAGENTS

Promega Corporation DNA IQ™ Casework Pro Kit for Maxwell® 16

## PROCEDURE OR ANALYSIS

### Maxwell®16 LEV Setup

1. Place the number of Maxwell® 16 DNA IQ™ Casework Pro Kit cartridges to be used into the cartridge preparation rack. Place each cartridge into the holder with the tube holder facing towards the numbered side of the rack. Hold the cartridge firmly and remove the seal.

2. Place the plunger into well #8 of each cartridge (well #8 is the closest to the tube holder).

3. Transfer the preprocessed sample into well #1 (well #1 is the well closest to the cartridge label and furthest from the user).

4. Place 0.5 mL elution tubes into the front of the Maxwell® 16 cartridge preparation rack. Add 25 – 50 µL of elution buffer to each tube.

5. Turn the Maxwell® 16 instrument on. The instrument will power up, display the firmware version number (make sure it is set as "Fnsc" on the lower right of the LCD display and LEV on the lower left), proceed through a self-check and home all axes.

6. Scroll to "Run" and press the "Run/Stop" button. Open the door when prompted to do so on the LCD display. Press the "Run/Stop" button to extend the platform.

7. Once the platform has extended, transfer the Maxwell® 16 LEV cartridge rack onto the platform. Ensure the rack is placed onto the Maxwell® 16 with the tube holders closest to the door. The rack will only fit into the instrument in this orientation.

   Note: If you are processing fewer than 16 samples, the reagent cartridges should be centered on the platform and spaced evenly.

8. Prior to starting the automated method, ensure that the desired volume of elution buffer has been added to the elution tubes.

9. Press the "Run/Stop" button. The platform will retract. Close the door.

10. The Maxwell® 16 will immediately begin the purification run. The LCD screen will display the steps performed and the approximate time remaining in the run. When the purification is complete, the LCD screen will display a message that the method has ended. Upon completion, open the door. Check to make sure that all of the plungers have been removed from the magnetic rod assembly. If the plungers have not been removed, push them down gently by hand to remove them from the magnetic rod assembly.

11. Press the "Run/Stop" button to extend the platform.

12. Remove the elution tubes from the heated elution tube slots and close them.

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

    To prevent evaporation of eluted DNA, cap elution tubes immediately after completion of the purification run.

If complete inhibition is observed in the resulting extract, notify the technical leader to discuss a course of action.

13. Remove cartridges and plungers from the instrument platform and discard. Do not reuse reagent cartridges, plungers, or elution tubes. Wipe down the interior of the instrument with 70% ethanol and/or run the UV light program, as needed.

14. Use one of the scroll (up/down) buttons to move the cursor to select "Yes" or "No" to run the purification method again.

    If "Yes" is selected, the Menu screen will appear.

    If "No" is selected, the platform is retracted back into the instrument. You are then prompted to close the door.

## REPORT WORDING

Not applicable.

## REFERENCES


1. Maxwell® 16 Instrument Operating Manual [Online], 2007.

2. DNA IQ™ Casework Pro Kit for Maxwell® 16 Technical Bulletin [Online].

3. The Maxwell® 16 Applications Database.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** DNA ISOLATION

**PROCEDURE:** DNA IQ™ EXTRACTION: MAXWELL® FSC

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Reviewed by:

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Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

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Jeanne M. Richeal
DNA Technical Leader

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Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

The Promega DNA IQ™ System paramagnetic resin purifies DNA while eliminating inhibitors. Below is the procedure for the purification of preprocessed samples using a Maxwell® FSC instrument.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices.

Reference MSDS for all commodities prior to use.

Warning: Treat all reagents/samples as potential biohazards.

## PREPARATIONS

None

## INSTRUMENTATION

Standard laboratory instrumentation
Promega Corporation Maxwell® FSC

## MINIMUM STANDARDS AND CONTROLS

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).

Refer to the Reagent Blanks and Controls for Extraction section for information regarding the minimum number of reagent blanks required for each sample batch type.

## CRITICAL REAGENTS

Promega Corporation Maxwell® FSC DNA IQ™ Casework Kit

## PROCEDURE OR ANALYSIS

**Note:** This procedure is for the Maxwell® FSC and not the Maxwell® RSC 48. Please refer to the Maxwell® RSC 48 Instrument/Maxprep™ Liquid Handler/Portal Software using methods for sample preparation through Capillary Electrophoresis Procedures for specifics in using the Maxwell® RSC 48.

## Maxwell® FSC Setup

1. 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.

2. Place the plunger into well #8 of each cartridge (well #8 is the well closest to the tube holder).

3. Transfer the preprocessed sample into well #1 (well #1 is the well closest to the cartridge label and furthest from the user).

4. Place 0.5 mL elution tubes into the front of the Maxwell® FSC deck tray. Add 25 – 50 µL of elution buffer to each tube.

5. Press **Start** to begin. Select Maxwell® FSC DNA IQ™ Casework analysis method using the tablet touch screen or by scanning the barcode on the kit box. Select sample positions and then press **Proceed.** Press **OK** to open the instrument door.

6. Once the platform has extended, place the Maxwell® FSC deck tray in the instrument. Ensure the rack is placed onto the Maxwell® FSC with the tube holders closest to the door. The rack will only fit into the instrument in this orientation.

   Note: If you are processing fewer than 16 samples, the reagent cartridges should be centered on the platform and spaced evenly.

7. Verify proper deck tray set up:

   a. Cartridges have been placed in the designated positions and seals have been completely removed

   b. Pre-extracted samples have been placed in well #1 of each cartridge

   c. Elution tubes have been placed in their correct positions with caps opened and desired volume of elution buffer added

   d. Plungers have been placed in well #8 of each cartridge

8. Select **Start.** The platform will retract, and the door will close automatically.

9. The Maxwell® FSC will immediately begin purification. The tablet touch screen will display the steps performed and the approximate time remaining in the run. When purification is complete the tablet touch screen will display a message that the method has ended. Upon completion, select **Open Door.**

10. Remove the deck tray, retrieve the elution tubes, and close them.

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

    To prevent evaporation of eluted DNA, cap the elution tubes immediately after completion of the purification run.

    If complete inhibition is observed in the resulting extract, notify the technical leader to discuss a course of action.

11. Remove cartridges and plungers from the deck tray and discard. Do not reuse reagent cartridges, plungers, or elution tubes. Wipe down the interior of the instrument with 70% ethanol and/or run the **Sanitize** method, as needed.

12. Select the **Home** image to go back to the Start menu.

    Select the **Door** image to open and close the door.

## REPORT WORDING

Not applicable.

## REFERENCES


1. Maxwell® FSC Instrument Operating Manual. [Online, 9/19]

2. Maxwell® FSC DNA IQTM Casework Kit Technical Manual. [Online, 05/18]

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** DNA ISOLATION

**PROCEDURE:** NON-SEMEN PREPROCESSING FOR DNA IQ™ EXTRACTION

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Reviewed by:

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Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____

Jeanne M. Richeal
DNA Technical Leader

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Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

DNA extraction techniques isolate DNA from nucleated cells contained in biological samples. Below is the procedure for the preprocessing of non-semen samples (excluding tissue, hair, and bone) using the Promega DNA IQ™ System.

## 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.

## PREPARATIONS

### 18 mg/mL Proteinase K

1. Add 556µL of Nuclease-Free Water (from Casework Extraction Kit) to the tube of lyophilized Proteinase K and gently invert to dissolve.

2. Store Proteinase K solution at -20°C. Prior to use, thaw and remix.

   Note: Proteinase K from sources other than the Casework Extraction Kit may be used as long as it is prepared at the appropriate concentration.

### 1M DTT

| | |
|---|---|
| Dithiothreitol | 20g |
| DI $H_2O$ | 130mL |

1. Combine and mix to dissolve. Aliquot into convenient volumes and freeze.

2. Prior to use, thaw and remix.

## INSTRUMENTATION

Standard laboratory instrumentation

## MINIMUM STANDARDS AND CONTROLS

Refer to the Reagent Blanks and Controls for Extraction section.

## CRITICAL REAGENTS

None

## PROCEDURE

Samples to be extracted may be placed in either 1.5mL microcentrifuge tubes, CW Microfuge tubes, or ClickFit Microtubes. If the Maxprep™ Liquid Handler is being used for non-semen sample preprocessing for a Maxwell® instrument, the type of microcentrifuge tube **must** be consistent for all samples within the extraction batch.

Substrate from swabs will be limited to one swab per microcentrifuge tube. The swab material will be removed from the stick before placing it into the microcentrifuge tube.

All other substrates should be limited so the extraction reagents can sufficiently interact with the substrate. Substrate should never be packed tightly, as that will prevent the extraction reagents from interacting with the entire substrate.

When CW Microfuge tubes or ClickFit Microtubes are used instead of 1.5mL microcentrifuge tubes, the substrate may need to be limited even further to account for the additional space taken up by the CW Spin Basket. When substrate is placed into a basket prior to incubation, a CW Spin Basket will be used. DNA IQ™ Spin Baskets cannot be used prior to incubation.

A. EXTRACTION USING A MAGNETIC SEPARATION STAND

1. Prepare lysis buffer by adding 1µL of 1M DTT per 100µL of DNA IQ™ lysis buffer for 350µL of prepared lysis buffer per sample.

   Ex. For extraction of 10 samples: 3500µL lysis buffer + 35µL 1M DTT.

2. Add between 150µL and 250µL of the prepared lysis buffer with DTT to each sample, ensuring the substrate is covered with buffer.

3. Incubate the samples at 70ºC for 30 minutes.

4. Briefly centrifuge samples. When appropriate, transfer the substrate and prepared lysis buffer to a spin basket seated in an extraction tube and centrifuge for 2 minutes at maximum speed. Remove and discard the spin basket.

5. Complete the extraction following the steps in the DNA IQ™ Extraction: Magnetic Separation Stand procedure.

B. EXTRACTION USING A MAXWELL® INSTRUMENT

1. Prepare Extraction Mix by adding 386µL of Casework Extraction Buffer, 10 µL of the Proteinase K solution and 4 µL of 1-Thioglycerol per sample.

   **Note:** 1-Thioglycerol is viscous. Pipet slowly.

2. Dispense 400µL of Extraction Mix to each sample. Briefly vortex the samples.

3. Incubate samples at 56°C for 30 minutes.

4. Briefly centrifuge samples. When appropriate, place the substrate in a spin basket and place back in the extraction tube. Centrifuge for 2 minutes at maximum speed. Remove and discard the spin basket.

5. Add 200µL of the DNA IQ™ Lysis Buffer. Vortex the sample for 5 – 10 seconds and centrifuge.

   **Note:** If the Maxprep™ Liquid Handler is being used then DNA IQ™ Lysis Buffer will be added by this instrument. For further information refer to Forensic Biology/DNA Maxwell® RSC 48 Instrument/Maxprep™ Liquid Handler/Portal Software using methods for sample preparation through Capillary Electrophoresis procedure.

6. At this step, samples may be left at room temperature overnight and processing completed the next day. Do not refrigerate or freeze samples.

7. Complete the extraction following the steps in the appropriate DNA IQ™ Extraction: Maxwell® procedure.

C. EXTRACTION USING THE TECAN FREEDOM EVO® 150 AUTOMATED WORKSTATION

1. Prepare extraction master mix as follows:

   (N = number of samples)

   | | | |
   |---|---|---|
   | (N+1) x | 100µL | 1x Casework Extraction Buffer |
   | (N+1) x | 300µL | Autoclaved water |
   | (N+1) x | 10µL | Proteinase K |
   | (N+1) x | 4µL | 1-Thioglycerol |

   **Note**: 1-Thioglycerol is viscous. Pipet slowly.

2. Dispense 400µL of extraction master mix to each sample.

3. Incubate the samples at 56°C for 30 minutes.

4. Centrifuge the CW Microfuge Tubes for 2 minutes at maximum speed. Remove and discard the CW Spin Basket.

5. At this step, samples may be left at room temperature overnight and processing completed the next day. Do not refrigerate or freeze samples.

6. Complete the extraction following the steps in the Tecan with Promega Methods procedure.

## REPORT WORDING

Not applicable.

## REFERENCES


1. Tecan Freedom EVO® 150 Operating Manual. Version 3.1 or above [CD], 2006.

2. Promega Corporation. DNA Purification from Various Sample Types Using the DNA IQ™ System. *Profiles in DNA* [Online]. (September 2002; 5).

3. Promega Corporation Technical Bulletin, No. 296, DNA IQ™ System - Small Sample Casework Protocol.

4. DNA IQ™ Casework Pro Kit for Maxwell® 16 Technical Bulletin [Online].

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7. Stangegaard, M., et al. Automated extraction of DNA and PCR setup using a Tecan Freedom EVO® liquid handler. Forensic Science International: Genetics Supplement Series 2 (2009) 74-76.

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9. Morf, N. V., et al. Internal validation of Tecan robots (Freedom EVO® 150 and 75) for PCR and capillary electrophoresis setup. Forensic Science International: Genetics Supplement Series 3 (2011) e89-e90.

10. Crouse C, et al. Improving efficiency of a small forensic DNA laboratory: validation of robotic assays and evaluation of microcapillary array device. *Croat Med J* 2005; 46(4):563-577.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** DNA ISOLATION

**PROCEDURE:** SEMEN PREPROCESSING FOR DNA IQ™ EXTRACTION

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Reviewed by:

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Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____

Jeanne M. Richeal
DNA Technical Leader

_____

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

DNA extraction techniques isolate DNA from nucleated cells contained in biological samples. Below is the procedure for the preprocessing of samples potentially containing semen using the Promega DNA IQ™ System.

## 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.

## PREPARATIONS

### 18 mg/mL Proteinase K

1. Add 556µL of Nuclease-Free Water (from Casework Extraction Kit) to the tube of lyophilized Proteinase K and gently invert to dissolve.

2. Store Proteinase K solution at -20°C. Prior to use, thaw and remix.

   Note: If prepared at the appropriate concentration, Proteinase K from sources other than the Casework Extraction Kit may be used.

## INSTRUMENTATION

None.

## MINIMUM STANDARDS AND CONTROLS

Refer to the Reagent Blanks and Controls for Extraction section.

## CRITICAL REAGENTS

None.

## PROCEDURE

Substrate from swabs will be limited to one swab per microcentrifuge tube. The swab material will be removed from the stick before placing it into the microcentrifuge tube.

All other substrates should be limited in the microcentrifuge tubes so the extraction reagents can sufficiently interact with the substrate. Substrate should never be packed tightly, as that will prevent the extraction reagents from interacting with the entire substrate.

**Note**: CW Spin Baskets **must not** be used for extraction of semen samples, as the CW Spin Basket is made with a proprietary chemical that binds sperm cells.

## EXTRACTION ON THE MAXWELL® INSTRUMENT OR TECAN FREEDOM EVO® 150 AUTOMATED WORKSTATION

1. Prepare non-sperm digestion buffer as follows:

   (N = number of samples)

   | | | |
   |---|---|---|
   | (N+1) x | 100µL | 1x Casework Extraction Buffer (CEB) |
   | (N+1) x | 300µL | Autoclaved water or equivalent |
   | (N+1) x | 10µL | Proteinase K (ProK) |

2. Dispense 400µL of non-sperm digestion buffer to each sample. Briefly vortex the samples.

3. Incubate samples at 56°C for 30 minutes.

4. Briefly centrifuge samples. Place the substrate in a spin basket and place back in the extraction tube. Centrifuge for 5 minutes at 10,000 x g. Move the substrate into a new microcentrifuge tube if the mixed fraction (F3) will be extracted or if the substrate is being retained for possible future analysis.

   Note: Unextracted F3 substrates must be retained when samples are consumed.

5. Remove the non-sperm (F1) fraction by transferring the liquid above the sperm cell pellet to a new microcentrifuge tube. Processing of the non-sperm (F1) fraction resumes at step 12.

   - **Using a Maxwell® instrument only:** Add 200 µL of DNA IQ™ Lysis Buffer to each non-sperm fraction (F1) sample. Vortex and centrifuge.
   - **Using a Promega MaxPrep™ Liquid Handler or the Tecan Freedom EVO® Automated Workstation:** DNA IQ™ Lysis Buffer will be added once the sample is placed on the platform and the extraction method is launched.

6. Prepare second digestion buffer as follow:

   (N= number of samples)

| | | |
|---|---|---|
| (N+1) x | 100µL | 1x Casework Extraction Buffer (CEB) |
| (N+1) x | 300µL | Autoclaved water or equivalent |
| (N+1) x | 10µL | Proteinase K (ProK) |

7. Dispense 400 µL of digestion buffer to each F2 sample. Briefly vortex.

8. Incubate samples at 56°C for 30 minutes.

9. Vortex and centrifuge the tubes for 5 minutes at 10,000 x g. Remove the liquid from the sperm cell pellet and discard. 1µL of the sperm cell pellet may be removed for KPIC

10. Prepare sperm lysis buffer as follows:

    (N = number of samples)

| | | |
|---|---|---|
| (N+1) x | 400µL | DNA IQ™ Lysis Buffer |
| (N+1) x | 4µL | 1-Thioglycerol |

    **Note**: 1-Thioglycerol is viscous. Pipet slowly.

11. Add 400µL of sperm lysis buffer to each sample including the mixed fraction (F3), if necessary. Vortex vigorously for 10 seconds and centrifuge briefly.

12. At this step, samples may be left at room temperature overnight and processing completed the next day. Do not refrigerate or freeze samples.

13. Complete the extraction of the samples following the procedure for the Maxwell® instrument or the Tecan Freedom EVO®150.

## REPORT WORDING

Not applicable.

## REFERENCES


1. Tecan Freedom EVO® 150 Operating Manual. Version 3.1 or above [CD], 2006.

2. DNA IQ™ Casework Pro Kit for Maxwell® 16 Technical Bulletin [Online].

3. Wells, F., et al. DNA IQ High Throughput Method Testing on the Tecan Freedom EVO. Scientific Applications Report, 08/18.

4. Stangegaard, M., et al. Automated extraction of DNA and PCR setup using a Tecan Freedom EVO® liquid handler. Forensic Science International: Genetics Supplement Series 2 (2009) 74-76.

5. Frégeau, C. J., et al. Validation of a DNA IQ-based extraction method for TECAN robotic liquid handling workstations for processing casework. Forensic Science International: Genetics 4 (2010) 292-304.

6. Morf, N. V., et al. Internal validation of Tecan robots (Freedom EVO® 150 and 75) for PCR and capillary electrophoresis setup. Forensic Science International: Genetics Supplement Series 3 (2011) e89-e90.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** DNA ISOLATION

**PROCEDURE:** FIRED CARTRIDGE CASE PREPROCESSING FOR DNA IQ™ EXTRACTION

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Reviewed by:

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Forensic Scientist Heather May
Forensic Biology/DNA Command Advisory Board

Approved by:

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Jeanne M. Richeal
DNA Technical Leader

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Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

Below is the procedure for the preprocessing and recovery of DNA from fired cartridge cases or similar substrates using the Promega DNA IQ™ System.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices.

Reference MSDS 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.

## PREPARATIONS

### 18 mg/mL Proteinase K

1. Add 556µL of Nuclease-Free Water from Casework Extraction Kit to the tube of lyophilized Proteinase K and gently invert to dissolve.

2. Store Proteinase K solution at -20°C. Prior to use, thaw and remix.

Note: Proteinase K from sources other than the Casework Extraction Kit may be used if it is prepared at the appropriate concentration.

### 1 M DTT

Dithiothreitol 20g
Deionized $H_20$ 130mL

1. Combine and mix to dissolve. Aliquot into convenient volumes and freeze.

2. Prior to use, thaw and remix.

### BTmix

2 mg/mL Bovine Serum Albumin (BSA)
62.5 mg/mL Gly-Gly-His (GGH) tripeptide
Deionized (DI) water

1. Prepare 125 mg/mL GGH solution: Add 3715µL of DI water to 500 mg of GGH. Mix to dissolve.

2. Prepare 4mg/mL BSA solution: Add 3680 uL of DI water to 320µL 50 mg/mL BSA. Mix to dissolve.

3. Combine approximately equal volumes of GGH solution and BSA solution (prepared above) and mix. Aliquot into convenient volumes and store at -20°C.

## INSTRUMENTATION

Standard laboratory instrumentation

## MINIMUM STANDARDS AND CONTROLS

Refer to the Reagent Blanks and Controls for Extraction section.

## CRITICAL REAGENTS

BTmix Solution

## PROCEDURE OR ANALYSIS

1. Prepare a swabbing solution by combining 500 µL of Casework Extraction Buffer and 30uL of BTmix per sample. Gently mix the solution.

2. Hold the cartridge case from the inside using rubber-tipped forceps. Hold the cartridge case with the head stamp facing down over an empty, disposable 15 mL beaker.

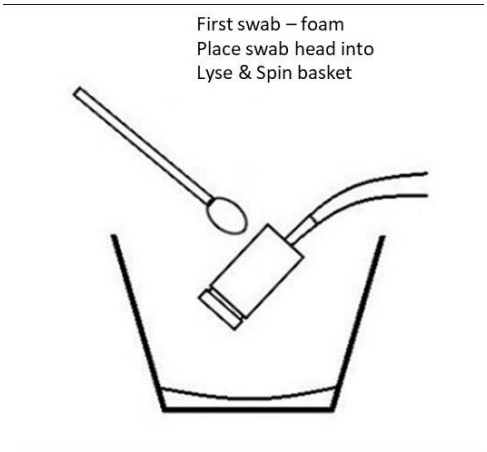
   **Note:** Collection can be performed on each cartridge case individually or on up to 3 cartridge cases together, depending on the size, condition, and case information.

3. Draw 500 µL of swabbing solution into a P1000 pipette tip.

4. Perform four rinses with the swabbing solution by dispensing over the cartridge case body and rotating 1/4 turn after each rinse. A fifth rinse will be used to dispense the swabbing solution over the head stamp area.

   **Note:** The swabbing solution is collected in the 15 mL beaker and re-used for each rinse.

5. Swab all outer surfaces of the cartridge case with one sterile foam swab. Place swab head into the CW Spin basket by breaking the shaft near the foam head.




6. Repeat the five rinses as described above using approximately 400 µL volume of swabbing solution already present in the 15 mL beaker

7. Swab all outer surfaces of the cartridge case with a second sterile foam swab. Place swab head into the CW Spin basket with first foam swab by breaking the shaft near the foam head.

8. Pipette the entire volume of swabbing solution into the CW Spin basket.

9. Immediately following this procedure, thoroughly rinse lysis buffer from cartridge cases using 70% ethanol to prevent corrosion. This can be accomplished by submerging the cartridge case in 70% ethanol and agitating for at least 5 seconds. Cartridge cases can be air-dried or dried with a Kimwipe or something similar prior to packaging.

10. Prepare a master mix by combining 10 µL of Proteinase K solution and 4 µL of 1-Thioglycerol per sample. Gently mix the solution and add 14 µL to each sample.

11. Incubate samples at 56°C for 30 min. Continue per ISP procedure for DNA IQ™ chemistry on a Maxwell®.

## REPORT WORDING

Not applicable.

## REFERENCES

1. Bille et al. An improved process for the collection and DNA analysis of fired cartridge cases. Forensic Sci Int Genet. 2020 May; 46:102238.

2. ISP Research Project: Method to collect cellular material from fired cartridge cases for DNA analysis (2022-01)

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** DNA ISOLATION

**PROCEDURE:** DNA IQ™ EXTRACTION: MAXWELL® RSC 48

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Reviewed by:

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Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____

Jeanne M. Richeal
DNA Technical Leader

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Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

The Promega DNA IQ™ System paramagnetic resin purifies DNA while eliminating inhibitors. Below is the procedure for the purification of the preprocessed samples using a Maxwell® RSC 48 instrument.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices.

Reference MSDS 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.

## PREPARATIONS

None.

## INSTRUMENTATION

Standard laboratory instrumentation

Promega Corporation Maxwell® RSC 48

## MINIMUM STANDARDS AND CONTROLS

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 reagents blank(s).

Refer to the Reagent Blanks and Controls for Extraction section for the information regarding the minimum number of reagent blanks required for each sample batch type.

## CRITICAL REAGENTS

Promega Corporation Maxwell® FSC DNA IQ™ Casework Kit

## PROCEDURE or ANALYSIS

### Maxwell® RSC 48 Setup

1. Place the number of Maxwell® FSC cartridges to be used into the deck tray(s) and press down firmly to snap the cartridges into place. Hold the cartridge firmly and remove the seal.

2. Place the plunger into well #8 of each cartridge. This is the well closest to the tube holder.

3. Transfer the preprocessed sample into well #1. This is the well closest to the cartridge label and furthest from the user.

4. Place the 0.5 mL elution tubes into the front of the Maxwell® RSC 48 deck tray. Add 25 – 50 µL of elution buffer to each tube.

5. Select **Start** to begin the extraction run. Select Maxwell® FSC DNA IQ™ Casework analysis method using the tablet touch screen or by scanning the barcode from the Maxwell® FSC DNA IQ™ Casework Kit, then select **Proceed**.

   If the method was selected using the touch screen, scan the barcode of the Maxwell® FSC DNA IQ™ Casework Kit into the "SCAN BARCODE" screen and select **OK**.

   If the method was selected by scanning the barcode, the "SCAN BARCODE" screen will not be displayed. Select **OK** to open the door.

   **Note**: The Maxwell® RSC 48 does not require that the samples be balanced as it is required with Maxwell® instruments that have placement for 16 samples.

6. Extend the platform and place the Maxwell® RSC deck trays on the instrument. Ensure the back deck tray is placed onto the Maxwell® RSC 48 furthest from the door and the front deck tray closest to the door. Place the tube holder(s) containing the elution tubes in front of the deck tray(s).

   **Note:** The deck trays will fit the instrument in only one configuration.

7. Verify proper deck tray setup:

   a. Cartridges have been placed in the designated positions and the seals have been completely removed.

   b. The pre-processed sample have been placed in well #1 for each cartridge.

   c. Elution tubes with the desired volume of elution buffer have been placed in their correct positions in the tube holder with caps opened towards the front of the instrument.

   d. Plungers have been placed in well #8 of each cartridge.

8. Select **Start**. The platform will retract, and the door will close automatically.

   The Maxwell® RSC 48 has the Vision System and will detect the following issues before the extraction run can proceed:
   - Cartridges are present for all active cartridge positions
   - Plungers are present in well #8 for active cartridge positions
   - Elution tubes are present and open for active cartridge positions

   If a Machine Vision Error is displayed, then the issue will need to be resolved before the extraction run can proceed.

9. The Maxwell® RSC 48 will immediately begin the extraction run if no issues are detected by the Vision System. The touch screen of the tablet will display the steps performed and the approximate time remaining in the run. When the extraction run is complete, the touch screen of the tablet will display a message that the method has ended. Upon completion, select **Open Door.**

10. Remove the deck trays. If the Maxprep™ Liquid Handler is being used immediately for quantification setup, the deck trays may be placed in the appropriate location on the deck of the Maxprep™ Liquid Handler.

    If the samples are not being used immediately for quantification set-up on the Maxprep™ Liquid Handler, then the elution tubes should be immediately closed to prevent evaporation.

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

11. Remove cartridges and plungers from the deck tray and discard. **Do not** reuse reagent cartridges, plungers, or elution tubes. Wipe down the interior of the instrument with 70% ethanol and/or run the **Sanitize** method, as needed.

12. Select the **Home** image to go back to the Start menu.

    Select the **Door** image to open and close the door.

## REPORT WORDING

Not applicable.

## REFERENCES


1. Maxwell® RSC 48 Instrument Operating Manual. [Online, 12/22]

2. Maxwell® FSC DNA IQ™ Casework Kit Technical Manual. [Online, 08/21]

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** DNA QUANTITATION

**PROCEDURE:** Quantitative PCR (qPCR): PowerQuant™ System

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**Reviewed by:**

Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

**Approved by:**

Jeanne M. Richeal
DNA Technical Leader

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

STR analysis is dependent on the quantity of DNA present in an evidentiary sample. The PowerQuant™ System is a quantitative PCR (qPCR) multiplex that provides human specificity, the potential to identify the ratio of male to female DNA in a mixed sample, an assessment of the degree of DNA degradation, and an evaluation of sample inhibition.

The sample information generated using the PowerQuant™ System may be used to guide decisions regarding sample re-purification, sample dilution, and the optimal template volume and system for amplification.

All samples will have acceptable quantification results prior to amplification. Samples may be quantified in duplicate at the analyst's discretion.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices.
Reference MSDS for all commodities prior to use.
Warning: Treat all reagents/samples as potential biohazards.

## INSTRUMENTATION

Standard laboratory instrumentation.
Applied Biosystems® 7500 Real-Time PCR System

## SOFTWARE

Applied Biosystems® HID Real-Time PCR Analysis Software, version 1.1 or higher
Applied Biosystems® 7500 Software, version 2.0.6 or higher

## MINIMUM STANDARDS & CONTROLS

Included in the PowerQuant™ System kit is a pooled human male genomic DNA (gDNA) standard. This standard is used to prepare a serial dilution series representing the following approximate quantities: 50 ng/µL, 12.5 ng/µL, 3.125 ng/µL, 0.781 ng/µL, 0.195 ng/µL, 0.049 ng/µL, 0.012 ng/µL, and 0.003 ng/µL.

Serial dilutions of PowerQuant™ Male gDNA Standard will be prepared with the PowerQuant™ Dilution Buffer and can be stored for up to one week at 4°C.

A standard curve is generated using the amplification results from the dilution series. This curve is required to determine the DNA concentration of unknown DNA samples.

## CRITICAL REAGENTS

The quantitative PCR (qPCR) analysis kits are critical reagents. The kits and their components will be stored using manufacturer recommendations. Refer to Appendix IV-B for critical reagent quality control procedures.

## PROCEDURE

1. Prepare the PowerQuant™ Male gDNA Standard dilution series as follows:

| Standard | DNA (µL) | Buffer (µL) |
|---|---|---|
| A (50 ng/µL) | 20 | 0 |
| B (12.5 ng/µL) | 10 of A | 30 |
| C (3.125 ng/µL) | 10 of B | 30 |
| D (0.781 ng/µL) | 10 of C | 30 |
| E (0.195 ng/µL) | 10 of D | 30 |
| F (0.049 ng/µL) | 10 of E | 30 |
| G (0.012 ng/µL) | 10 of F | 30 |
| H (0.003 ng/µL) | 10 of G | 30 |

2. Each reaction requires 1 µL of primer/probe/internal positive control (IPC) mix, 10 µL of master mix and 7 µL of amplification grade water. Add 18 µL of the reaction mixture to each required well of the reaction plate.

3. Add 2 µL of standard, DNA sample, reagent blank, or control to the appropriate wells of the reaction plate. Seal with an optical adhesive cover and centrifuge briefly. Analyze the plate on the Applied Biosystems® 7500 Real-Time PCR System.

4. Evaluate the $R^2$ and the slope of the quantitation curves. Sample results may be accepted when the standard curve $R^2$ values are equal to or greater than 0.98 and slopes are -3.1 to -3.6.

   The standard curves will be established with at least one replicate of each standard in the dilution series. Exceptions may be approved by the technical leader and will be documented in an Incident Form, which will be included in the notes packet for all effected cases.

5. Evaluate each sample for inhibition by reviewing the IPC $C_T$ values.

   Inhibited samples may be diluted, purified or re-extracted before amplification. Samples exhibiting complete inhibition should not be amplified unless re-quantification indicates the inhibitor was reduced.

6. Evaluate the degradation index (DI) for each sample. Samples with elevated DI values may require amplification of additional target DNA to obtain the most complete profile possible.

7. The internal validation data (e.g. sensitivity study) will be utilized to select the extract(s) for amplification that are expected to provide the most informative STR results.

   Extracts selected for amplification will be concentrated if the amount of DNA in the maximum volume of neat extract is insufficient to produce autosomal profile results suitable for CODIS search or a complete Y-STR haplotype. If the concentrated amount of DNA is still insufficient and there is additional substrate remaining, an extraction of additional substrate should be considered so that extracts may be combined for amplification. Every attempt should be made to obtain an optimal amplification target. Extracts obtained using the Tecan Freedom EVO® 150 will be concentrated using Microcon® filters.

8. Analysts may defer amplification of any remaining extracted items once all case questions have been answered, when the case has been adjudicated, or when the case has been canceled by the agency.

   Additionally, an analyst may choose to defer autosomal amplification of an item based on the following quantification results:

   - The male to female quantification ratio indicates the sample is better suited for Y-STR analysis.
   - The quantification result for a sample is undetermined. Analysts may choose to amplify these items when reference standards are available for comparison.

   The reason for deferment of any evidentiary items will be documented. If necessary, the submitting agency can request supplemental examinations be conducted on items that were deferred or not examined (see Command Directive ADM15).

9. Refer to the Reagent Blanks and Controls and the DNA Quality Assurance sections of the Forensic Biology/DNA Procedures Manual for the procedures regarding quantifiable amounts of DNA detected in reagent blanks.

10. The notes packet will contain the following information and data:

    A. Sample well position, sample name, target name, task, IPC $C_T$, autosomal quantity, degradation quantity, Y quantity, male to female ratio (MTFR), and the degradation index.

    B. Slope, $R^2$ values, and Y-intercept values.

11. Electronic data (.eds file) will be attached to the quantification plate in LIMS.

## REPORT WORDING

See Appendix I.

## REFERENCES


1. PowerQuant™ System Technical Manual; TMD047, version 3/15.

2. ISP R&D Internal Validation: A Comparison of Next Generation Quantification Kits (Quantifiler Trio®/ PowerQuant™/InnoQuant™). Project 2015-02.

3. ISP R&D Project: Amplification of Low Level and Not Detected Samples (2018-01).

4. Ewing, M. M., et. al. "Human DNA quantification and sample quality assessment: Developmental validation of the PowerQuant™ System." *Forensic Science International: Genetics.* 23 (2016) 166-177.

5. Ewing, M. M., et. al. "The PowerQuant™ System: A New Quantification Assay for Determining DNA Concentration and Quality." Promega Corporation, 2014.

6. Green R. L., et. al. "Developmental Validation of Quantifiler Real Time PCR Kits for the Quantification of Human Nuclear DNA Samples." *Journal of Forensic Sciences*. July 2005, Vol 50, No 4, pp. 809-825.

7. Richard, M. L. et. al. "Developmental Validation of a Real Time Quantitative PCR Assay for Automated Quantification of Human DNA." *Journal of Forensic Sciences*. September 2003, Vol 48, No 5, pp. 1041-1046.

8. Andreasson H. and Allen, M. "Rapid Quantification and Sex Determination of Forensic Evidence Materials." *Journal of Forensic Sciences*. November 2003, Vol 48, No 6, pp. 1280-1286.

9. Nicklas, J. A. and Buel, E. "Development of an Alu-based Real Time PCR Method for Quantitation of Human DNA in Forensic Samples." *Journal of Forensic Sciences*. September 2003, Vol 48, No 5, pp. 936-944.

10. Van der horst, E. H. et. al. "Taq-Man®-based Quantification of Invasive Cells in the Chick Embryo Metastasis Assay." *Biotechniques*. 2004, Vol 37, No 6, pp. 940-945.

11. Barbisin, M., et. al. A multiplexed system for quantification of human DNA and male DNA and detection of PCR inhibitors in biological samples. *Forensic Science International: Genetics Supplement Series 1.* August 2008, pp. 13-15.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** PCR

**PROCEDURE:** AMPLIFICATION AND ELECTROPHORESIS OF STRs: POWERPLEX® Y23

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Reviewed by:

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Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

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Jeanne M. Richeal
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Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

Short tandem repeat (STR) genetic markers are polymorphic DNA loci that contain a repeated nucleotide sequence. The STR repeat unit can be from two to seven nucleotides in length. The number of times a unit is repeated at an STR locus differs from individual to individual, resulting in alleles of different lengths. This polymorphism makes them useful for human identification purposes.

The PowerPlex® Y23 System amplifies the following loci: DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385a/b, DYS456 and YGATAH4.

The alleles within each locus as well as the loci themselves are separated by size using capillary electrophoresis. The use of multicolor dye-labeled primers allows loci that have alleles with overlapping size ranges to be distinguished from one another during the course of the capillary electrophoresis run.

## SAFETY CONSIDERATIONS

Use standard laboratory practices.

Warning: extracted and amplified samples may contain potential biohazards.

| | |
|---|---|
| Warning: Hazardous Reagents: | Formamide: An irritant and suspected teratogen. Causes irritation to the eyes, skin and mucous membrane. Do not inhale or ingest. |
| Electrical Shock Hazard: | The capillary electrophoresis unit contains a high voltage power supply. Handle with caution. Under no circumstances should any safety system be bypassed. Arcing may result from incomplete drying of instrument components. |
| Laser Hazard: | The capillary electrophoresis unit contains a laser. Operate only with doors closed. Service by Applied Biosystems® personnel only. |

## PREPARATIONS

Promega PowerPlex® Y23 PCR Amplification Kit (Critical Reagent)

Upon receipt the allelic ladder and internal lane standard will be placed in the Post-PCR room. See Appendix IV for quality control information.

2800M Control DNA

Prepare a dilution of the concentrated control DNA before use.

Deionized Formamide

Aliquot into convenient volumes and freeze with protection against defrosting. If the aliquot is not frozen, discard immediately. Formamide may be stored for a maximum of one year from the date the stock bottle was received.

WEN Internal Lane Standard (ILS) 500 Y23

**Note:** The ILS contains amplified DNA and must be stored in the Post-PCR room.

Add 0.5µL ILS to 9.5µL formamide and vortex for 10-15 seconds. Make fresh before each use.

PowerPlex® Y23 Amplification Master Mix

| | |
|---|---|
| PCR Reaction Mix | 5 µL |
| Primer Set | 2.5 µL |

Prepare a volume sufficient for the number of samples. Briefly vortex and spin. Solution must be made fresh before each use.

## INSTRUMENTATION

Standard laboratory instrumentation

Applied Biosystems® GeneAmp® 9700™ and ProFlex™ Thermal Cyclers

See Appendix IV-B for maintenance instructions.

Applied Biosystems® 3500 Genetic Analyzer

See Appendix IV-B for instrument specific maintenance and spectral instructions.

## SOFTWARE

Applied Biosystems® 3500 Series Genetic Analyzer Data Collection Software, version 4.0.1 or higher
GeneMapper® ID-X Analysis Software, version 1.6 or higher
Promega PowerPlex® panels and bin sets for PowerPlex® Y23

## MINIMUM STANDARDS AND CONTROLS

Amplification controls associated with samples being typed shall be amplified concurrently in the same instrument and with the same primers as the forensic samples.

Positive Amplification Control:

One or both of the following samples may be used as a positive amplification control:

2800M Control DNA (~0.5ng)
NIST Traceable control (~0.5ng)

The following are the DNA profiles of each sample:

| | 2800M | NIST Traceable |
|---|---|---|
| DYS576 | 18 | 18 |
| DYS389I | 14 | 13 |
| DYS448 | 19 | 20 |
| DYS389II | 31 | 30 |
| DYS19 | 14 | 16 |
| DYS391 | 10 | 11 |
| DYS481 | 22 | 21 |
| DYS549 | 13 | 12 |
| DYS533 | 12 | 12 |
| DYS438 | 9 | 11 |
| DYS437 | 14 | 14 |
| DYS570 | 17 | 22 |
| DYS635 | 21 | 23 |
| DYS390 | 24 | 24 |
| DYS439 | 12 | 11 |
| DYS392 | 13 | 11 |
| DYS643 | 10 | 10 |
| DYS393 | 13 | 13 |
| DYS458 | 17 | 16 |
| DYS385a/b | 13,16 | 11,14 |
| DYS456 | 17 | 15 |
| YGATAH4 | 11 | 12 |

Complete haplotypes for at least one positive amplification control must be obtained or the Technical Leader will be notified.

Negative Amplification Control:

17.5 µl sterile ddi water, or equivalent

To verify an amplification, a negative control should not contain extraneous DNA. The Technical Leader will be notified if extraneous DNA is observed in a negative amplification control.

## PROCEDURE FOR AMPLIFICATION

1. In cases where DNA from questioned samples will be amplified in either autosomal or Y-STRs, the suspect reference standard(s) must be typed with an autosomal chemistry for entry into CODIS. In cases where no questioned samples will be amplified, profiling of reference standards may be performed at the analyst's discretion.

2. Determine an appropriate quantity of sample DNA to dilute or concentrate to 17.5 µL with sterile ddi water, or equivalent. This quantity is dependent upon the quality of the DNA and the sensitivity of the capillary electrophoresis instrument.

3. Add 17.5 µL of sample DNA/water to 7.5 µL of the amplification master mix in an appropriately labeled microamp tube.

4. Prepare the positive and negative DNA amplification controls and the reagent blanks in the same manner as case samples.

5. Amplify in a thermal cycler using the following parameters:
    - 1 cycle at 96°C for 2 minutes
    - 30 cycles (94°C for 10 seconds, 61°C for 60 seconds, 72°C for 30 seconds) at max ramp rate
    - 1 cycle at 60°C for 20 minutes
    - Soak at 4°C

6. After amplification, the samples may be stored in a dedicated amplified DNA refrigerator for up to two weeks. Samples to be stored longer should be frozen in a dedicated amplified DNA freezer. Amplified samples should be stored in a closed container labeled with identification of the specific amplification set enclosed.

## PREPARATION OF AMPLIFIED DNA SAMPLES FOR CAPILLARY ELECTROPHORESIS

1. Pipette 10 µL of formamide/ILS mixture into the necessary wells of a 96-well plate. A capillary should never inject from an empty plate well. Add additional wells of formamide or formamide/ILS mixture as needed. Add 1 µL of amplified sample, amplification control, allelic ladder or reagent blank to the appropriate wells.

2. Close plate with septum and spin briefly.

## CAPILLARY ELECTROPHORESES DATA COLLECTION

1. Create a Plate Record for the run. Input the appropriate sample name, assay, file name convention, and results group.

   The run and injection times are set by the instrument protocol included in the assay selected for each plate. Run times will be determined by the initial evaluation of each instrument and must be sufficiently long enough to capture all the peaks required for the sizing method. The default injection time is 15 seconds.

2. Place the prepared plate into the instrument and link to the Plate Record created above.

3. Start the run.

## GENEMAPPER® ID-X ANALYSIS

1. Create an analysis project. Add samples to the project.

2. Select the ADVANCED Analysis Method and use the following analysis parameters:

   - 125 RFU analytical threshold
   - minimum peak half width of 2
   - Local Southern Sizing Method
   - smoothing option set to light

3. Assign the appropriate Panel for each sample.

4. Assign an appropriate Size Standard for each sample.

5. Review each sample's raw data and select an appropriate analysis range to include the peaks required for the sizing method.

6. Input the start and stop data points of the analysis range under the Peak Detector tab within Analysis Method (double-click to open).

7. Click the Analyze button to analyze the data.

8. Review the size quality (SQ) flags for all samples. A green flag indicates a successful sizing. For non-passing samples (yellow or red flags), evaluate the sizing analysis in Size Match Editor to determine if any adjustments can be made that would allow the sizing to pass.

9. Peak labels may be renamed but not removed. Labels for sample peaks must include base pairs, peak height and allele call, with the exceptions of the internal lane standard and the allelic ladder. The internal lane standard requires only the base pair labels. Allelic ladders require only the allele call labels, except when sizing an off-ladder allele.

## ELECTRONIC DATA

Attach all the .hid sample files from each run directly to the 3500 plate or to the Fusion Allele Table panel in LIMS.

## REPORT WORDING

See Appendix I.

## REFERENCES


1. Promega Corp. PowerPlex® Y23 System Technical Manual, Part #TMD035, Revised 4/17.

2. Applied Biosystems® GeneAmp® PCR Systems 9700 User's Manual, PN 4316011, Rev. D., 5/06.

3. Applied Biosystems® ProFlex PCR System User Guide, PN MAN0007697, Rev. B.0, 6/16.

4. Applied Biosystems® 3500/3500xl Genetic Analyzer User Guide, PN 4401661 Rev. C., 06/2010.

5. Applied Biosystems® 3500/3500xl Genetic Analyzer User Bulletin, June 2011.

6. GeneMapper® ID-X Software, v 1.0 Getting Started Guide, PN 4375574 Rev. A, 10/2007.

7. GeneMapper® ID-X Software, v 1.5, User Bulletin, PN 100031708 Rev. A, 18 May 2015.

8. ISP R&D Internal Validation: Thermal Cycler Model 480 and 9700 Comparisons.

9. ISP R&D Internal Validation: CE3500 Validation (2012-17).

10. ISP R&D Internal Validation: PowerPlex® Y23 System and Expansion of YSTR Mixture Frequency Calculators (2013-07).

11. ISP R&D Internal Validation: PowerPlex® Y23 Validation on the AB 3500 Platform (2016-08).

12. ISP R&D project: Performance Check of the GeneMapper® ID-X Software for Use in Analyzing PowerPlex® Y23 Data (2014-03).

13. ISP R&D project: Capillary Life Study (IP05-03).

14. ISP R&D Internal Validation: Applied Biosystems® 9700™ vs ProFlex™ (2016-10).

15. Thompson, J. M., et. al. Developmental Validation of the PowerPlex Y23: A Single Multiplex Y-STR Analysis System for Casework and Database Samples Forensic Science International: Genetics, Volume 7, Issue 2, February 2013, Pages 240–250.

16. Frank, W. E., et al. Y-chromosome STR Haplotypes and Allele Frequencies in Illinois Caucasian, African American and Hispanic Males, J. Forensic Sci., Vol. 51, Issue 5.

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# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**PROCEDURE:** INTERPRETATION: POWERPLEX® Y23

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Reviewed by:

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## INTRODUCTION

These guidelines provide a framework to address the majority of casework interpretations for DNA analysis using short tandem repeats (STR) via PowerPlex® Y23. These guidelines are based on validation studies for PowerPlex® Y23 and information obtained in the published literature. In addition, the interpretation of DNA results is a matter of professional judgement and expertise by the DNA analyst that is scientifically supported by the analytical data.

It is not possible that every situation will be covered by these guidelines and there may be situations that require an analyst to deviate from the stated guidelines. The use of alternative interpretation approaches must be approved by a technical leader and documented in the case file.

## SAFETY CONSIDERATIONS

Use standard laboratory practices.

## PREPARATIONS

None required.

## INSTRUMENT SPECIFICATIONS

No instrumentation required.

## SOFTWARE

GeneMapper® ID-X Analysis Software, version 1.6 or higher

YHRD.ORG
ISP Y-STR Haplotype Frequency Calculator for Two-Person Mixtures, version 3.0.0

## MINIMUM STANDARDS AND CONTROLS

1. A successful electrophoresis run must contain a usable allelic ladder, usable internal sizing standard, and a correctly typed positive amplification control which exhibits no locus or allele drop-out. If an electrophoresis run is deemed unusable and no data is analyzed, the reason the run was not used will be noted on the 3500 worksheet. All .hid files from the run must be attached to the assignment.

2. Negative amplification controls and reagent blanks should not exhibit interpretable levels of Y-STR results that are reproducible in any of the dyes within the size ranges covered by any of the loci. A technical leader will be contacted if interpretable Y-STR results are detected. An Incident Form will be used to document the event. If the analyst and technical leader agree the compromised control does not affect interpretation of the associated case haplotypes, the case haplotypes may be used.

3. For each amplification set and electrophoresis run, a positive amplification control must type correctly. Either the kit positive amplification control or a NIST traceable standard may be used to validate the amplification set or electrophoresis run. If multiple injections or amplifications are made of a positive or negative control sample within an electrophoresis run, only one representative electropherogram from each control used to validate the run or amplification needs to be included in the notes packet.

## PROCEDURE

Y-STR haplotypes will be defined by the number of alleles greater than or equal to 125 RFU. Inclusions and exclusions will be determined using interpretable Y-STR data greater than or equal to 125 RFU.

Inclusions will be reported as "**cannot be excluded (is included)**." Exclusions will be reported as "**excluded**."

The loci DYS570 and DYS576 have been defined as rapidly mutating Y-STRs, as their mutation rate is greater than 1%. The ISP R&D Laboratory has also identified a single locus mutation at DYS385a/b in a paternal line study. Paternal line standards may be utilized due to the absence of a direct standard. Exclusions of paternal line standards at a single locus or only rapidly mutating loci will not be reported without first being reviewed by a technical leader.

The following are comparison conclusions:

1. Cannot be excluded (is included)

    a. A single haplotype is present in the unknown evidentiary haplotype at the loci being used for interpretation. The unknown evidentiary haplotype is concordant with another haplotype at each of these loci.

    b. A single haplotype (major and/or minor) is differentiated from a mixture at the loci being used for interpretation. The unknown evidentiary haplotype is concordant with another haplotype at each of these loci.

    c. More than one possible haplotype is present in the unknown at the loci being used for interpretation. The haplotype of the standard is contained as a possible haplotype at each locus.

2. Excluded
   A failure to meet the criteria of cannot be excluded (is included).

3. Inconclusive
   No comparison can be made since the evidentiary profile has been determined to be unsuitable for comparison.

## ASSESSMENT OF DATA

1. Interpret with caution any Y-STR haplotype that exhibits the following:

    - Degradation
      A marked decrease in peak heights from smaller to larger base pair loci (left to right) within a given dye.

    - Inhibition
      Locus specific peak height diminishment and/or allele or locus dropout.

    - Preferential amplification
      Preferential amplification is the unequal sampling of alleles, which can result in peak height imbalance and may be locus specific. Variability occurs particularly when amplifying lower amounts of DNA template.

    - Locus dropout that is inconsistent with degradation.

    - Stochastic effects
      Amplification of low template DNA samples can be subject to stochastic effects, where an allele fails to amplify to a detectable level. Stochastic effects within an amplification may affect one or more loci, irrespective of allele size.

2. Each sample will be examined for fluorescent pull-up. Pull-up is defined as a peak caused by under-compensation of the spectral calibration, present in one or more dyes within 0.5 base pairs of an allele in another dye. Peaks determined to be fluorescent pull-up must be noted and do not require re-injection. If they appear often, a new spectral calibration should be performed.

3. Each sample will be examined for artifacts that fall within the analytical range and may be present at a given base pair size in two or more dyes. Re-amplification or re-injection of a sample may be necessary to determine if a peak is an artifact. Artifacts will include those observed and reported in the PowerPlex® Y23 System Technical Manual. All artifacts will be documented on the electropherogram. Use caution when artifacts are present in a mixture, as the artifact may be masking an allele.

4. It is permissible to interpret data across multiple electropherograms.

## ASSESSMENT OF A PEAK

1. Peaks are defined as alleles if they are greater than or equal to 125 RFU and are assigned an allelic designation by GeneMapper® ID-X. Off-ladder microvariants are included in this definition.

2. Interpretation of off-scale data will be conducted with caution. Re-amplification with less template DNA is suggested prior to interpreting this type of sample.

3. Peaks located at a position one base pair smaller than a major peak may be interpreted as incomplete A nucleotide addition (minus A) and noted as such on the electropherogram.

4. Peaks representing alleles that have been designated as "OL- allele" by GeneMapper® ID or ID-X will be evaluated against the allelic ladder (or the average of allelic ladders run) and an allelic assignment made. The calculations for the allelic assignment will be documented in the notes packet for each sample and run. This allelic assignment will be listed on the allele table.

5. Peaks may be considered stutter if the ratio of their peak heights to their primary alleles are equal to or less than the percentages listed below. An analyst may accept stutter artifacts outside the values identified. This needs to be supported by the data and documented in the notes packet.

| Fluorescent Dye | Locus | N-2 Stutter | N-1 Stutter | -2bp Artifact | N+1 Stutter |
|---|---|---|---|---|---|
| Fluorescein (Blue) | DYS576 | 1% | 13% | - | 2% |
| | DYS389I | 4% | 8% | - | - |
| | DYS448 | - | 5% | - | - |
| | DYS389II | - | 16% | - | - |
| | DYS19 | - | 9% | 14% | - |
| JOE (Green) | DYS391 | - | 10% | - | - |
| | DYS481 | 6% | 27% | - | 6% |
| | DYS549 | 1% | 11% | - | 4% |
| | DYS533 | 3% | 10% | - | 6% |
| | DYS438 | - | 5% | - | - |
| | DYS437 | - | 9% | - | 1.1% |
| TMR-ET (Yellow) | DYS570 | 2% | 18% | - | 4% |
| | DYS635 | - | 10% | - | 2% |
| | DYS390 | - | 13% | - | - |
| | DYS439 | - | 9% | - | - |
| | DYS392 | 2% | 13% | - | 9% |
| | DYS643 | - | 4% | - | - |
| CXR-ET (Red) | DYS393 | 1% | 16% | - | 4% |
| | DYS458 | - | 15% | - | 3% |
| | DYS385a/b | - | 15% | - | - |
| | DYS456 | - | 19% | - | 3% |
| | YGATAH4 | - | 12% | - | 2% |

Additional artifacts and stutter peaks may be observed for off-scale data associated with amplification of excess DNA.

Promega Corporation reports the observance of the following low-level artifact/stutter peaks:

- At DYS19, in the plus 2 base pair position.
- At DYS448, in the minus 9 and minus 15 base pair positions.

6. The GeneMapper® ID-X software uses a proprietary algorithm to identify signals with poor morphology and designates them as "Spike". If the appearance of a spike interferes with the evaluation of other observed peaks, the sample should be re-injected.

## SINGLE HAPLOTYPES:

1. A single haplotype is characterized by single interpretable alleles at all loci, with the exception of DYS385a/b where there may be two peaks. A single allele at DYS385a/b will not be considered complete unless the RFU value is greater than 781.

   While rare, gene duplications have been observed and will be considered prior to reporting a sample as single-source. Duplications at DYS19, DYS385a/b and DYS448 were reported in the ISP R&D Illinois and Southern India population database studies. Duplications will be confirmed by a technical leader.

## MIXTURES INVOLVING TWO CONTRIBUTORS

1. Two interpretable alleles at any locus may be indicative of a mixture.

2. If the amount of male DNA amplified is greater than 100 pg and the ratio of contributors is 3:1 or greater and within +/- 50% of this ratio across all loci, the contributing haplotypes (major/minor) may be interpreted. For example, 3:1 would encompass a range of ratios from 1.5:1 to 4.5:1.

   Stochastic effects are expected in mixtures where these ratio expectations are exceeded. Loci with single alleles will be interpreted based on the observed ratio of major and minor contributors. The ratio of major and minor contributors will be included in the evaluation of complete haplotypes observed at DYS385a/b. Expected peak height imbalance within a dye set will be considered prior to accepting single peaks as from two contributors.

   In the absence of inhibition or degradation, peak balance across loci within the JOE, TMR-ET and CXR-ET dye sets is relatively uniform. Across loci in the FL dye set, higher peak heights may be observed at the DYS576 and DYS448 loci. Allele and/or locus dropout may be observed when amplifying less than 100 pg of XY source DNA or when samples are affected by degradation or inhibition.

3. If the contributors to a mixed Y-STR haplotype are not resolved, inclusions will be based upon all possible contributors for the alleles defined at each locus.

## MIXTURES INVOLVING GREATER THAN TWO CONTRIBUTORS

1. If the ratio of contributors to a mixture of Y-STR haplotypes is 3:1 or greater, resolvable profiles from one or two contributors may be determined.

2. Unresolved mixtures of more than two contributors are unsuitable for comparison to other unknown haplotypes or reference standards.

## APPLYING POPULATION FREQUENCY DATA TO STR RESULTS

1. All probative associations will be accompanied by a statistical evaluation. If a reference standard has locus drop-out, or allele drop-out at DYS385a/b, the statistic will not be calculated at that locus for the evidentiary profile.

2. Since all loci are located on the Y-chromosome, haplotype frequencies are estimated using the counting method. Haplotype frequencies (p) are calculated using the formula p = x / n, where x is the number of observations of a given haplotype in the database and n is the total number of haplotypes in the database.

3. Single-source Y-STR haplotypes will be searched in the YHRD Haplotype Reference Database (yhrd.org) population database. DYS385a/b will be considered one locus for purposes of the database search. Haplotypes will be searched twice; first using all PowerPlex® Y23 loci and then a second time using only the Yfiler™ loci (which excludes DYS576, DYS481, DYS549, DYS533, DYS570, and DYS643 from the search). The most discriminating results of the two searches will be reported.

   Haplotype frequencies will be reported with a 95% confidence upper bound using the Clopper-Pearson method to account for sampling variation and the size of the database. This is defined as the profile probability. If a haplotype has been observed in the database, the profile probability will be calculated using this formula:

$$\sum_{k=0}^{x} \binom{n}{k} p_0^k \, (1 - p_0)^{n-k} \; = 0.05$$

   If a haplotype has not been observed in the database, the profile probability will be calculated using this reduced formula:

$$1 - (0.05)^{1/n}$$

   Statistical values will be reported for the African American, Caucasian, and Hispanic populations groups.

4. Per SWGDAM Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Laboratories (2014), it is recognized that population substructure exists for Y-STR haplotypes; noting the substructure parameter values (Θ) are small for most populations and inversely proportional to the number of loci reported in a haplotype. Therefore, a population substructure correction will be applied to frequencies for single-source haplotypes with fewer than 10 loci by reporting match probability.

   Match probability values are provided by yhrd.org and are calculated using this formula:

$$\Pr(A|A) = \Theta + (1 - \Theta)\, pA$$

A is the haplotype of interest, Pr (A|A) is the probability of observing A given that it has already been observed once in another individual in the same subpopulation, and pA is the profile probability.

The match probability value calculated from the pooled US African American, Asian, Caucasian, and Hispanic population groups will be reported.

5. As noted above, single-source haplotypes will be searched twice. This may result in a situation where one search will be conducted with 10 or more loci and the other will be conducted with fewer than 10 loci. In this case, the fractional results of the profile probability and match probability will be compared and the more discriminating statistic will be reported.

6. If single-source autosomal STR and Y-STR results are obtained from an exhibit, the product of the respective match probabilities for the African American, Caucasian, and the more common of the Southeastern Hispanic and Southwestern Hispanic population groups may be calculated. For the purposes of this combined statistic, the pooled match probability value may not be used. Individual match probabilities will need to be determined for each of the three reported population groups. The resulting population statistics for the African American, Caucasian, and Hispanic population groups will be reported.

7. If an unresolved mixture of two males is identified, the Illinois State Police Y-STR Haplotype Frequency Calculator For Two-Person Mixtures will be used. This calculator determines all possible combinations of haplotypes and searches the Illinois Combined Y-STR database. The reported frequency estimate will be the sum of all the observations divided by the size of the database (i.e. counting method) at the 95% confidence limit. If there are no observations of the haplotype in the database, the haplotype frequency will be reported as 2/(N+2).

## REPORT WORDING

See Appendix I.

## REFERENCES


1. Promega Corp. PowerPlex® Y23 System Technical Manual, Part #TMB035, Revised 4/17.

2. GeneMapper® ID-X Software, v 1.0, Getting Started Guide, 2007.

3. GeneMapper® ID-X Software, v 1.5, User Bulletin, 18 May 2015.

4. ISP R&D project: YHRD Haplotype Reference Database Performance Check (2018-14).

5. ISP R&D Internal Validation: PowerPlex® Y23 Validation on the AB 3500 Platform (2016-08).

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7. ISP R&D project: YSTR 2-Person Mixture Profile Frequency Calculator (2006B-03).

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20. Scientific Working Group on DNA Analysis Methods (SWGDAM). (2014). SWGDAM Interpretation Guidelines for Y-chromosome STR Typing by Forensic DNA Laboratories.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** PCR

**PROCEDURE:** INTERPRETATION AND REPORTING GUIDELINES FOR PARENTAGE TESTING

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Reviewed by:

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Approved by:

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## INTRODUCTION

Parentage interpretation, as specified in Command Directive EVH 4, may be performed on cases involving criminal paternity or maternity, identification of human remains, or cases in which an evidentiary stain is used in lieu of a reference standard from a common parentage trio (mother, father and child).

## SAFETY CONSIDERATIONS

None required.

## PREPARATIONS

None required.

## INSTRUMENT SPECIFICATIONS

No instrumentation required.

## SOFTWARE

Popstats Parentage Calculator within CODIS software, version 7.0 or higher

## MINIMUM STANDARDS AND CONTROLS

None required.

## PROCEDURE

Throughout this document the unknown parent has been referred to as the alleged father (AF) for the sake of convenience. This should be changed to alleged mother (AM) when appropriate.

I. General Information
For specific information regarding case acceptance, please refer to Command Directive ESH Appendix 1.

A. Profiles for at least two of the three individuals of the common parentage trio must have been developed from reference standards.

B. Criminal parentage interpretations may be possible when only the profiles of the child and one alleged parent are available. In these cases, only exclusions may be reported. Interpretations that do not result in an exclusion will be forwarded to an accredited parentage laboratory for further evaluation.

C. The obligate parental allele must be identifiable for at least three loci in order for a comparison to be possible.

D. For mixtures, the profile in question must be fully differentiated.

Cases in which the questioned profile cannot be fully differentiated from a mixture should be sent to an American Association of Blood Banks (AABB) accredited laboratory for interpretation.

II. Interpretation of Submitted Profiles

A. Interpretations Performed

1. Criminal Paternity
Interpretation will be conducted to determine whether the child or product of conception could have inherited the obligatory paternal alleles from the alleged father.

2. Identification of Human Remains
Interpretation will be conducted to determine whether the profile from the human remains is consistent with one of the parents of a parentage trio or from an offspring of the parentage trio.

3. Evidentiary Stain Used in Lieu of a Reference Standard
Analysis will be conducted to determine whether the profile from the evidentiary stain is consistent with one of the parents of a parentage trio or with an offspring of the parentage trio. Comparisons will be made only for evidentiary stains exhibiting a minimum of three loci and either single source profiles or mixtures from which a single source profile can be fully differentiated.

B. Possible Conclusions

1. Consistency at all observed loci will be considered as consistent with relatedness.

2. For those cases in which up to two inconsistencies are observed, a determination of relatedness will not be rendered. Because the mutation rates at the STR loci profiled are significant, interpretation and/or testing at additional loci by an AABB accredited laboratory will be suggested.

3. Three or more inconsistencies will be considered an exclusion.

III. Application of Population Frequency Data to Parentage Interpretations
Statistical analysis for those cases reported as consistent will be conducted using the Popstats parentage calculator in the CODIS software. Due to the potential for linkage between the vWA and D12S391 loci of the PowerPlex® Fusion System, these loci will not be used in the statistical analyses. In addition, DYS391 will not be used.

Statistics will be calculated for each of the Black, White, and Hispanic racial groups and only the statistical values from the most conservative racial group will be listed in the report, without naming the racial group.

Types of Statistics Utilized

A. Combined Parentage Index (CPI)
The combined parentage index is a likelihood ratio (LR) comparing the likelihood that the mother (M) and alleged father (AF) are the parents of the child (C) in question with the likelihood that an untested, randomly chosen man and the mother are the parents. CPI is a measure of the strength of the genetic evidence alone. CPI is calculated as the product of the parentage indices (PI) for each locus.

CPI will be used for statistical evaluation of questioned paternal or questioned maternal profiles. Generated frequencies will be truncated to four significant figures for reporting.

An offspring and two parental profiles versus an offspring and one known and one unknown parental profile (as described by I.W. Evett and B.S. Weir in "Interpreting DNA Evidence"; pg. 168 by Sinauer Associates, Inc. 1998).

| Mother (or Father) | Child | Alleged Father (AF) or Alleged Mother (AM) | LR Formula |
|---|---|---|---|
| BD | AB | AC | 1/2a |
| BC | AB | AC | 1/2a |
| BC | AB | AB | 1/2a |
| BC | AB | A | 1/a |
| B | AB | AC | 1/2a |
| B | AB | AB | 1/2a |
| B | AB | A | 1/a |
| AB | AB | AC | 1/[2(a+b)] |
| AB | AB | AB | 1/(a+b) |
| AB | AB | A | 1/(a+b) |
| AB | A | AC | 1/2a |
| AB | A | AB | 1/2a |
| AB | A | A | 1/a |
| A | A | AB | 1/2a |
| A | A | A | 1/a |

B. Reverse Combined Parentage Index (RCPI)
The reverse combined parentage index is a likelihood ratio comparing the likelihood that the child (offspring) is the product of the parental pair in question with the

likelihood that the offspring is the product of an untested, randomly chosen parental pair. RCPI is calculated as the product of the reverse parentage indices (RPI) for each locus.

RCPI will be used for statistical evaluation of questioned offspring profiles. Generated frequencies will be truncated to four significant figures for reporting.

An offspring and two parental profiles versus an offspring and two unknown parental profiles (as described by M.N. Hochmeister, et al., Journal of Forensic Science 41(1): pg. 158. 1996).

| Offspring (present or missing) | Parent 1 (purported) | Parent 2 (purported) | LR Formula |
|---|---|---|---|
| A | A | A | $1/a^2$ |
| A | A | AC | $1/2a^2$ |
| A | AC | AC or AD | $1/4a^2$ |
| AB | A | B | 1/2ab |
| AB | A | AB or BC | 1/4ab |
| AB | AB | AB | 1/4ab |
| AB | AC | AB, BC or BD | 1/8ab |

C. Probability of Exclusion (PE)

The probability of exclusion is defined as the probability of excluding a random individual as a parent, given the alleles of the child and the biological parent's profile. It is equal to the frequency of all the people in the population who do not possess an allele that matches the obligate parental allele of the child.

PE gives the probability that a person randomly selected from the population can be excluded from being a biological child of two known parents.

The probability of exclusion will be calculated and used to derive the probability of inclusion (PI) according to the following formula:

1 – PE (probability of exclusion) = PI (probability of inclusion)

D. Probability of Inclusion (PI)

The probability of inclusion is defined as the probability of including a random individual as a parent, given the alleles of the child and the biological parent's profile. It is equal to the frequency of all the people in the population who possess an allele that matches the obligate parental allele of the child.

The PI will be derived from the calculated probability of exclusion (PE) according to the following formula:

1 – PE (probability of exclusion) = PI (probability of inclusion)

Probability of inclusion will be evaluated for all human remains cases and those where an evidentiary stain is being used in lieu of a reference standard. Generated percentages will be truncated to three decimal places for reporting purposes.

E. Probability of Parentage (W)
The probability of parentage is based on Bayes' Theorem and is a means of expressing the likelihood that the alleged father (AF) is in actuality the biological father based on all the evidence from the case, both genetic (derived from parentage testing) and non-genetic (derived from testimonials of involved parties).

Probability of parentage will be evaluated for all cases. Generated percentages will be truncated to three decimal places for reporting.

It is expressed by the formula:

W = CPI x P/[CPI x P + (1-P)], where P represents a prior probability of paternity

In the United States, the court system has made the assumption that the prior probability of paternity is equal to 0.5; the argument being the tested man is either the true father or he is not. Based on the assumption the prior probability is equal to 0.5, the equation simplifies to:

W = CPI/(CPI + 1)

The Popstats parentage calculator generates probability of paternity (W) statistics with the prior probability equal to 0.5.

## REPORT WORDING

When comparisons are based on fewer than the total number of loci amplified, a statement listing the interpreted loci must be added.

I. Paternity Cases

A. No inconsistencies
(AF Name) cannot be excluded as the biological father of (Child Name). It is XXX times more likely to see these genetic results if (AF Name) is the biological father of (Child Name) than if an untested, unrelated man is the father. The probability of parentage is XXX% as compared to an untested, unrelated male (Prior probability = 0.50).

The statistical analysis is based on the DNA results obtained at [list the appropriate loci or the PowerPlex® Fusion loci with the exception of vWA, DYS391, and D12S391.]

B. Inconsistencies at up to two loci
No determinations will be made regarding paternity due to the inconsistencies at the following loci: XXX and XXX.

The DNA profiles identified in Exhibits XXX, XXX, and XXX will be forwarded to XXX for further interpretation and their results will be the subject of a separate report.

Statistical evaluation of mutation rates at these loci as well as testing of additional loci by an American Association of Blood Banks (AABB) accredited laboratory is suggested to provide conclusive parentage results.

C. Inconsistencies at three or more loci
(AF Name) can be excluded as the biological father of (Child Name).

II. Human Remains Cases / Evidentiary Stains Used In Lieu of a Reference Standard

A. The remains/evidentiary stain are the suspected offspring
The DNA profile obtained from Exhibit WWW (remains or stain description) was interpreted for reverse parentage. The reverse combined parentage index is XXX. The probability that the profile from Exhibit WWW represents a biological offspring of Exhibit YYY (AF Name) and Exhibit ZZZ (AM Name) is approximately 99.999%. The probability that the profile from Exhibit WWW does not represent a biological offspring of Exhibit YYY (AF Name) and Exhibit ZZZ (AM Name) is approximately 0.001%.

The statistical analysis is based on the DNA results obtained at [list the appropriate loci or the PowerPlex® Fusion loci with the exception of vWA, DYS391, and D12S391.]

B. The remains/evidentiary stain are a suspected parent
The DNA profile obtained from Exhibit WWW (remains or stain description) was interpreted for parentage. The combined parentage index is XXX. The probability that the profile from Exhibit WWW represents a biological mother/father to Exhibit CCC (Alleged Offspring's Name) is approximately 99.999%. The probability that the profile from Exhibit WWW does not represent a biological parent to Exhibit CCC (Alleged Offspring's Name) is approximately 0.001%.

The statistical analysis is based on the DNA results obtained at [list the appropriate loci or the PowerPlex® Fusion loci with the exception of vWA, DYS391, and D12S391.]

## REFERENCES


1. Evett, I.W. and Weir, B.S. *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists*, Sinauer Associates, Inc., Sunderland, Massachusetts, 1998.

2. Hochmeister, M.N., Budowle, B., Eisenberg, A., Borer, U.V. and Dirnhofer, R. "Using Multiplex PCR Amplification and Typing Kits for the Analysis of DNA Evidence in a Serial Killer Case". Journal of Forensic Science 41(1):155-162. 1996.

3. Butler, J.M. *Advanced Topics in Forensic DNA Typing: Interpretation*, Elsevier Academic Press, San Diego, California, 2015.

4. Gill, P., Phillips, C., McGovern, C., Bright, J., Buckleton, J. "An Evaluation of potential allelic association between the STRs vWA and D12S391: Implications in criminal casework and applications to short pedigrees". Forensic Science International: Genetics 6 (2012) 477-486.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** PCR

**PROCEDURE:** AMPLIFICATION AND ELECTROPHORESIS OF STRs: POWERPLEX® FUSION

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Reviewed by:

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## INTRODUCTION

Short tandem repeat (STR) genetic markers are polymorphic DNA loci that contain a repeated nucleotide sequence. The STR repeat unit can be from two to seven nucleotides in length. The number of times a unit is repeated at an STR locus differs from individual to individual, resulting in alleles of different lengths. This polymorphism makes them useful for human identification purposes.

STR loci can be amplified using the polymerase chain reaction (PCR) process. The PowerPlex® Fusion System allows simultaneous amplification of the following loci: Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, DYS391, D8S1179, D12S391, D19S433, FGA, and D22S1045.

The alleles within each locus as well as the loci themselves are separated by size using capillary electrophoresis. The use of multicolor dye-labeled primers allows loci with alleles of overlapping size ranges to be distinguished from one another during the course of the capillary electrophoresis run.

## SAFETY CONSIDERATIONS

Use standard laboratory safety practices.

| | |
|---|---|
| Biohazard warning: | Extracted and amplified samples may contain potential biohazards. |
| Chemical/Reagent warning: | Anode and cathode buffers, formamide, and POP-4 polymer may cause eye, skin, and respiratory tract irritation. Formamide is also a suspected teratogen. Avoid breathing vapor. Use with adequate ventilation. |
| Electrical Shock Warning: | The genetic analyzer contains a high voltage power supply. Under no circumstances should any safety system be bypassed. |
| Laser warning: | The genetic analyzer contains a solid-state laser. Operate only with doors closed. Service is to be performed by authorized personnel only. |

## PREPARATIONS

PowerPlex® Fusion System (Critical Reagent)

Upon receipt, the allelic ladder and internal lane standard from the kit must be placed in the Post-PCR room. See Appendix IV for quality control information.

2800M Control DNA

Prepare a dilution of the concentrated control DNA before use.

Deionized Formamide

Aliquot into convenient volumes and freeze with protection against defrosting. If the aliquot is not frozen, discard immediately. Formamide may be stored for a maximum of one year from the date the stock bottle was received.

WEN Internal Lane Standard (ILS) 500

**Note:** The internal lane standard contains amplified DNA and must be stored in the Post-PCR room.

Add 0.5µL ILS to 9.5µL formamide and vortex for 10-15 seconds. Make fresh before each use.

PowerPlex® Fusion PCR Amplification Mix

| | |
|---|---|
| 5X Master Mix | 5µL |
| 5X Primer Pair Mix | 5µL |

Centrifuge Master Mix and Primer Pair Mix briefly to bring contents to the bottom, then vortex reagents for 15 seconds before each use. Do not centrifuge the Master Mix or Primer Pair Mix after vortexing, as this may cause concentration of these reagents at the bottom of the tube. Prepare a volume of amplification mix sufficient for the number of samples. Vortex and then pipet into each reaction tube. Solution must be made fresh before each use.

## INSTRUMENTATION

Standard laboratory instrumentation
Applied Biosystems® GeneAmp® 9700™ and ProFlex™ Thermal Cyclers
Genetic Analyzers

See Appendix IV-B for instrument specific maintenance and spectral instructions.

## SOFTWARE

Applied Biosystems® 3500 Series Data Collection Software, version 4.0.1 or higher
GeneMapper® *ID-X* Analysis Software, version 1.6 or higher

## MINIMUM STANDARDS AND CONTROLS

Amplification controls associated with samples being typed shall be amplified concurrently in the same instrument and with the same primers as the forensic samples.

Positive Amplification Control:

One or both of the following samples may be used as a positive amplification control:

2800M Control DNA (~0.5ng)
NIST Traceable control (~0.5ng)

The following are the DNA profiles of each sample:

| | 2800M | NIST Traceable |
|---|---|---|
| Amelogenin | X, Y | X, Y |
| D3S1358 | 17, 18 | 16, 18 |
| D1S1656 | 12, 13 | 15, 16 |
| D2S441 | 10, 14 | 10, 14 |
| D10S1248 | 13, 15 | 13, 15 |
| D13S317 | 9, 11 | 9, 12 |
| Penta E | 7, 14 | 11, 16 |
| D16S539 | 9, 13 | 10, 13 |
| D18S51 | 16, 18 | 12, 13 |
| D2S1338 | 22, 25 | 17, 24 |
| CSF1PO | 12, 12 | 10, 11 |
| Penta D | 12, 13 | 10, 13 |
| TH01 | 6, 9.3 | 7, 9.3 |
| vWA | 16, 19 | 14, 18 |
| D21S11 | 29, 31.2 | 32, 32.2 |
| D7S820 | 8, 11 | 8, 10 |
| D5S818 | 12, 12 | 11, 12 |
| TPOX | 11, 11 | 9, 10 |
| DYS391 | 10 | 11 |
| D8S1179 | 14, 15 | 12, 12 |
| D12S391 | 18, 23 | 17, 23 |
| D19S433 | 13, 14 | 13, 14 |
| FGA | 20, 23 | 21, 22 |
| D22S1045 | 16, 16 | 14, 15 |

Complete genotypes for at least one positive amplification control must be obtained or the Technical Leader will be notified

Negative Amplification Control:

15 µL sterile ddi water or equivalent

To verify an amplification, a negative control should not contain extraneous DNA. The Technical Leader will be notified if extraneous DNA is observed in a negative amplification control.

## PROCEDURE FOR AMPLIFICATION

1. In cases where DNA from questioned samples will be amplified in either autosomal or Y-STRs, the suspect reference standard(s) must be typed with an autosomal chemistry for entry into CODIS. In cases where no questioned samples will be amplified, profiling of reference standards may be performed at the analyst's discretion.

2. Determine an appropriate quantity of sample DNA to dilute or concentrate to 15µL with sterile ddi water, or equivalent. This quantity is dependent upon the quality of the DNA and the sensitivity of the genetic analyzer.

3. Add 15µL of sample DNA/water to 10µL of amplification mix in a labeled microamp tube.

4. Prepare the positive and negative DNA amplification controls and the reagent blanks in the same manner as case samples.

5. Amplify in a thermal cycler selecting Max Mode as the ramp speed and using the following parameters:

   96°C for 1 minute

   94°C for 10 seconds
   59°C for 1 minute
   72°C for 30 seconds
   for 29 cycles

   60°C for 10 minutes

   4°C soak

6. After amplification, the samples may be stored for up to two weeks in a dedicated amplified DNA refrigerator. Samples to be stored longer should be frozen in a dedicated amplified DNA freezer. Amplified samples should be stored in a closed container labeled for clear identification of the specific amplification set enclosed. Amplified DNA will be discarded following case completion.

## PREPARATION OF AMPLIFIED DNA SAMPLES FOR CAPILLARY ELECTROPHORESIS

<u>Applied Biosystems® 3500/3500xL Genetic Analyzer</u>

1. Pipette 10µL of formamide/ILS mixture into the necessary wells of a 96-well plate. If needed, add formamide or formamide/ILS mixture to fill the remaining wells in a column since a capillary should never inject from an empty well. Add 1 µL of amplified sample, amplification control, allelic ladder or reagent blank to the appropriate wells.

2. Close plate with septum and centrifuge briefly.

3. Denature samples at 95°C for 3 minutes, then immediately chill on ice or in a freezer for 3 minutes. Denature samples just prior to loading the plate on the instrument.

## CAPILLARY ELECTROPHORESIS DATA COLLECTION

1. Create a Plate Record for the run. Input the appropriate sample name, assay, file name convention, and results group.

   The run and injection times are set by the instrument protocol included in the assay selected for each plate. Run times will be determined by the initial evaluation of each instrument and must be sufficiently long enough to capture all the peaks required for the sizing method. The default injection time for each model instrument will be used:

   15 seconds for the 3500 Genetic Analyzer
   24 seconds for the 3500xL Genetic Analyzer

2. Place the plate into the instrument and link to the Plate Record created above.

3. Start the run.

## GENEMAPPER® *ID-X* ANALYSIS

1. Create an analysis project and add samples to the project.

2. Assign the sample type, set the panel to PowerPlex_Fusion_Panels_IDX_v2.0 or STRmix PowerPlex_Fusion_Panels_IDX_v2.0, and set the size standard to WEN_ILS_500_IDX.

3. Select the appropriate analysis method and verify the following settings on the Peak Detector tab:

| | |
|---|---|
| Analysis Range | Determined from the raw sample data |
| Sizing Range | 60 to 500 bases |
| Smoothing | Light |
| Baseline Window | 51 pts |
| Size Calling Method | Local Southern Method |

Analytical Threshold

| Fluorescent Dye | Binary | STRmix |
|---|---|---|
| Fluorescein | 190 RFU | 190 RFU |
| JOE | 190 RFU | 150 RFU |
| TMR-ET | 190 RFU | 190 RFU |
| CXR-ET | 190 RFU | 190 RFU |
| WEN | 190 RFU | 190 RFU |

Min. Peak Half Width 2 pts
Polynomial Degree 3
Peak Window Size 15 pts
Slope Threshold 0.0 to 0.0

Note: If necessary, the polynomial degree may be increased to 5 and the peak window size may be decreased to 13 to improve peak resolution.

4. Click Analyze and correct Analysis Requirement flags, as needed.

5. Review the Analysis Summary to verify the quality of allelic ladders, controls, and samples.

6. Peak labels may be changed but not removed. Labels must include base pairs, peak height and allele call, with the exceptions of the size standard and the allelic ladder. The size standard requires only base pair labels. Allelic ladders require only allele call labels, except when sizing an off-ladder allele.

## ELECTRONIC DATA

Attach all the .hid sample files from each run directly to the 3500 plate or to the Fusion Allele Table panel in LIMS.

## REPORT WORDING

See Appendix I.

## REFERENCES


1. Promega Corporation. PowerPlex® Fusion System Technical Manual, Part #TMD039, Revised 3/15.

2. Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide, PN 4401661 Rev. C, 06/2010.

3. Applied Biosystems® 3500/3500xL Genetic Analyzer User Bulletin, June 2011.

4. GeneMapper® *ID-X* Software, v 1.0 Getting Started Guide, PN 4375574 Rev. A, 10/2007.

5. GeneMapper® *ID-X* Software, v 1.5 User Bulletin, PN 100031708 Rev. A, 18 May 2015.

6. Applied Biosystems® GeneAmp® PCR System 9700 User's Manual, PN 4316011, Rev. D., 5/06.

7. Applied Biosystems® ProFlex PCR System User Guide, PN MAN0007697, Rev. B.0, 6/16.

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9. ISP R&D Internal Validation: Thermal Cycler Model 480 and 9700 Comparisons.

10. ISP R&D Internal Validation: PowerPlex Fusion and Applied Biosystems 3500 Genetic Analyzer (2014-08).

11. ISP R&D project: Evaluation of Peak Window Size and Polynomial Degree (07B-09).

12. ISP R&D project: Capillary Life Study (IP05-03).

13. ISP R&D Internal Validation: Applied Biosystems® 9700™ vs ProFlex™ (2016-10).

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15. Moretti, T., et al. Validation of Short Tandem Repeats (STRs) for Forensic Usage: Performance Testing of Fluorescent Multiplex STR Systems and Analysis of Authentic and Simulated Forensic Samples, J. Forensic Sci., Vol. 46, Issue 3, May 2001, pp. 647-660.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**PROCEDURE:** INTERPRETATION: PowerPlex® Fusion

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Reviewed by:

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## INTRODUCTION

The guidelines provide a framework to address the majority of casework interpretations for DNA analysis using short tandem repeats (STR) via PowerPlex® Fusion. These guidelines are based on validation studies for PowerPlex® Fusion and information obtained in the published literature. In addition, the interpretation of DNA results is a matter of professional judgment and expertise by the DNA analyst that is scientifically supported by the analytical data.

It is not possible that every situation will be covered by these guidelines and there may be situations that require an analyst to deviate from the stated guidelines. The use of alternative interpretation approaches must be approved by a technical leader and documented in the assignment.

## SAFETY CONSIDERATIONS

Use standard laboratory practices.

## PREPARATIONS

None required.

## INSTRUMENT SPECIFICATIONS

No instrumentation required.

## SOFTWARE

GeneMapper® ID-X Analysis Software version 1.6, or higher
ISP STR Calculator, version 5
CODIS version 11, or higher

## MINIMUM STANDARDS AND CONTROLS

I. An electrophoresis run must contain the following:

   A. An allelic ladder that correctly sizes and types the positive control(s) and samples.

   B. The positive amplification control may be either the one provided in the amplification kit, a NIST traceable standard, or both. All genotypes must be complete for a positive control to verify the electrophoresis run.

II. An electrophoresis run may also contain the following, when appropriate:

   A. Negative amplification control(s)

   B. Reagent blank(s)

Negative amplification controls and reagent blanks should not exhibit interpretable STR results that are reproducible in any of the dyes within the size ranges covered by the respective loci.

The technical leader will be contacted if interpretable STR results are detected. The Incident Documentation Form will be used to document the event, which will be included in the notes packet for all associated cases. If the analyst and technical leader determine that the compromised control does not affect interpretation of the associated evidentiary profiles, the evidentiary profiles may be used.

III. Case documentation

A. Electropherograms

For each amplification and/or capillary electrophoresis run, at a minimum, the following will be included in the notes packet:

- An allelic ladder; if multiple ladders are used for sizing only one representative electropherogram is required in the notes packet
- A positive and negative control; if controls are amplified or injected multiple times, only one representative electropherogram is required in the notes packet
- Reagent blank(s)

B. .hid files

All .hid files produced for all allelic ladders, controls, reagent blanks, and samples must be attached to the case assignment.

IV. If an electrophoresis run is deemed unusable and no data is analyzed, the reason will be noted on the 3500 worksheet. All .hid files from the capillary electrophoresis run must be attached to the assignment.

**PROCEDURE**

I. Each profile will be evaluated for the following:

A. Degradation

A decrease in peak heights from low molecular weight loci to high molecular weight loci within a fluorescent dye. Degradation may also be identified when using a quantification system that contains a degradation index (DI). The term has been used to describe the decrease in peak heights due to environmental factors. This term has also been applied to the diminishment of peak heights due to increasing molecular weight of alleles as a consequence of the amplification chemistry. While the cause may not be known, it is important to evaluate the profile for decreasing peak heights.

B. Inhibition

Locus specific peak height diminishment and/or allele or locus dropout may first be evidenced by an increase in the CT value of the qPCR Internal PCR Control or an increased DI value. In some samples, inhibition may be overcome by reducing the volume of extracted DNA amplified. In other samples it may be beneficial to perform an additional purification of the extracted DNA prior to amplification. The ISP Internal Validation on PowerPlex® Fusion has shown that Amelogenin is often the first locus affected by some PCR inhibitors.

C. Preferential amplification

Uneven amplification of alleles within a locus attributable to large differences in base pair size. This may also be observed due to primer binding site mutations.

D. Low template DNA

Amplification of low template DNA may be subject to increased stochastic variation in the peak height ratios and/or stutter percentages. This may result in allele and/or locus dropout.

E. Off-scale data

Data obtained outside the limits of linearity (LOL) may not accurately reflect the relative fluorescent units (rfu) of the respective peaks. Therefore, off-scale peak heights should not be used for any mathematical calculations. This data may include excessive fluorescent pull-up, elevated stutter and other artifacts. When the quantitative values from on-scale peak heights are important, off-scale data should be re-amplified.

II. Assessment of a peak

A. Data will be assessed to determine whether a peak is allelic or an artifact. Some peaks may be indistinguishable as an allele or artifact. It may be beneficial to re-amplify or re-inject the sample to aid in the determination of the peak being allelic or an artifact. If additional analysis has not been conducted or the additional analysis doesn't provide resolution as an allele or an artifact, the peak will be deemed inconclusive.

B. Alleles are peaks that are equal or greater than the analytical threshold and assigned an allelic designation. Alleles will also include:

1. Microvariants

An allele that contains an incomplete repeat unit (e.g. 9.3 at TH01).

2. Off-ladder (OL) alleles

   Peaks representing alleles that have been designated as "OL-allele" by the genotyping software will be evaluated against the closest useable allelic ladder and an allelic assignment will be made, as long as that assignment does not exceed ±2 repeats units outside of the lowest and highest end of the ladder range. The calculations for the allelic assignment will be documented for the sample and each run.

   When the allelic assignment cannot be determined within a precision of +/- 0.5 base pairs, the allele will be assigned < the smallest allele or > the largest allele for the locus. This locus will not be used for any statistical calculation.

3. Genetic anomalies

   a. Aberrant Di-Allelic Pattern: An imbalanced peak height ratio at a locus which may be the result of a somatic mutation of a homozygote, a chromosomal rearrangement or a primer binding mutation in one of the alleles of the heterozygote resulting in reduced amplification efficiency. As discussed in the Clayton, T.M., et al. (2004) publication, the minor allele may be 50% to 10% of the major allele. If the di-allelic pattern cannot be confirmed by an additional exhibit or second amplification then the locus will not be used for statistical calculations.

   b. Tri-Allelic Pattern: Three alleles at a locus may be the result of an extra fragment of a chromosome or the entire chromosome. A tri-allelic pattern is characterized by two types. The Type 1 pattern is where the sum of the two smaller peak heights are approximately equal to the peak height of the larger allele and the Type 2 pattern is where all three peaks are relatively balanced. If the tri-allelic pattern cannot be confirmed by an additional exhibit or a second amplification then the locus will not be used for statistical calculations.

C. Artifacts

Re-amplification or re-injection of a sample may be necessary to determine if the peak is an artifact. It is important to note that some artifacts are reproduceable and will not resolve with additional analysis. Artifacts will include those observed and reported in the PowerPlex® Fusion System Technical Manual and the Illinois State Police Internal Validation. This is not an exhaustive list of artifacts so it still possible to observe artifacts that have not been documented by Promega or the Illinois State Police Internal Validation. All artifacts will be documented on the electropherogram.

The following are the most common artifacts found in STR analysis:

1. Stutter

   Stutter is an amplification artifact that may occur as result of strand slippage during DNA synthesis. The most common type is one repeat unit shorter than the associated allelic peak (N-1). The majority of loci in PowerPlex® Fusion System are tetranucleotide repeats. In addition, the multiplex chemistry contains one trinucleotide repeat, D22S1045, and two pentanucleotide repeats, Penta D and Penta E. In general, a trinucleotide repeat will exhibit more stutter than a tetranucleotide repeat and a pentanucleotide repeat will exhibit less stutter than either a trinucleotide or tetranucleotide repeat.

   The high stutter value reported in the Illinois State Police Internal Validation and the values reported in the Developmental Validation by Promega Corporation for PowerPlex® Fusion were compared. The higher of the two values was accepted for each locus and then rounded to the next whole percentage. The values are found in the chart below and are expected for on-scale data within the LOL of the instrument.

   While less common than N-1 stutter, it is also possible to have stutter that is two repeat units shorter (N-2) and one repeat unit larger (N+1) than the associated allelic peak. These values were obtained in the Illinois State Police Internal Validation of PowerPlex® Fusion have been truncated to two significant figures and are included in the table below.

   Alleles that are separated by one repeat unit may also have elevated stutter which exceeds the accepted N-1value due to the additive effect of N-1 and N+1 stutter (i.e. the alleles are 18, 20 with a 19 peak in the stutter position).

   A stutter artifact accepted outside the values identified in the chart may be the result of data outside the LOL or low template DNA. Peaks in stutter position to an off-scale allele may not be properly filtered. Therefore, caution should be applied when accepting the peak as stutter. Stutter artifacts need to be supported by the data and documented.

| Fluorescent Dye | Locus | N-1 Stutter | N+1 Stutter | N-2 Stutter |
|---|---|---|---|---|
| Fluorescein (Blue) | D3S1358 | 12% | 1.0% | 1.0% |
| | D1S1656 | 15% | 1.6% | 0.90% |
| | D2S441 | 10% | 1.3% | 0.50% |
| | D10S1248 | 13% | 0.40% | 1.2% |
| | D13S317 | 10% | 1.4% | 0.90% |
| | Penta E | 8% | 0.90% | 0.90% |
| JOE (Green) | D16S539 | 11% | 1.1% | 1.0% |
| | D18S51 | 15% | 1.4% | 1.1% |
| | D2S1338 | 14% | 1.0% | 1.2% |
| | CSF1PO | 10% | 1.8% | 0.60% |
| | Penta D | 7% | not observed | not observed |
| TMR-ET (Yellow) | TH01 | 5% | 1.1% | 1.1% |
| | vWA | 12% | 2.0% | 1.1% |
| | D21S11 | 12% | 2.5% | 0.80% |
| | D7S820 | 11% | 1.0% | 1.0% |
| | D5S818 | 10% | 1.8% | 0.50% |
| | TPOX | 6% | 0.70% | 0.40% |
| | DYS391 | 9% | 2% | 0.80% |
| CXR-ET (Red) | D8S1179 | 14% | 1.5% | 1.1% |
| | D12S391 | 16% | 0.70% | 0.50% |
| | D19S433 | 11% | 1.2% | 1.7% |
| | FGA | 13% | 0.72% | 0.80% |
| | D22S1045 | 17% | 8.6% | 1.3% |

2. Incomplete 3' A nucleotide addition

   Peaks that are one base pair shorter than the associated major peak may be interpreted as incomplete A nucleotide addition (-A). If it is deemed necessary, samples exhibiting excessive incomplete A nucleotide addition may be re-amplified.

   It is important to note when incomplete A nucleotide addition peaks are labeled, peaks in N-1 stutter position may not be properly filtered by the genotyping software.

3. Fluorescent pull-up

   Under-compensation by the spectral may result in peaks that are fluorescent "pull-up" in the allelic call ranges. Even applying an appropriate spectral to DNA results with approximate 6,000 rfu or less will exhibit pull-up peaks at approximately 1-3% from the under-compensated allele. Therefore, DNA results with greater than 6,000 rfu through the linear range of the instrumentation may demonstrate a greater percentage of fluorescent pull-up from an under-compensated allele. Pull-up may also occur when there are two alleles separated by generally one repeat unit forming a bridge-like peak in another fluorescent dye. This information will need to be used to properly evaluate whether a peak is allelic or potentially fluorescent pull-up.

   A peak determined to be fluorescent pull-up does not require re-injection. However, if these artifacts appear often and apparently are not due to increased rfu, a new spectral calibration should be performed.

4. Spikes

   Peaks that are generally tall and thin and are observed in one or more of the fluorescent dyes. If a spike interferes with the evaluation of other peaks, the sample should be re-injected.

   **Note:** Polymer crystals may cause spikes. If spikes continue to appear within a capillary electrophoresis run, replacing the polymer may resolve the issue.

5. Dye artifacts

   These are detached fluorescent primer tags that have also been commonly referred to as "dye blobs". Dye artifacts that occur in samples from the same amplification set or those that have been identified by the manufacturer of the amplification chemistry will not require additional analysis for clarification. A dye artifact that has not been documented by Promega Corporation or in the ISP Internal Validation may require the sample to be re-amplified or re-injected to clarify the interpretation. Also, if the dye artifact interferes with the evaluation of other observed peaks, the sample may be re-amplified or re-injected to clarify the interpretation.

D. Sub-analytical threshold peaks

   Consideration of peaks observed below the analytical threshold will be strictly limited to those samples exhibiting low DNA template levels as evidenced by locus or allele

dropout and in which only a limited number of peaks are present above the analytical threshold. In addition, the sub-analytical threshold peaks under consideration must:

- Be distinguishable from noise at the locus
- Not occupy an N-1 stutter position
- Display normal Gaussian morphology

In those low template samples where sub-analytical peaks warrant consideration, these peaks will only be used to assist in evaluating potential number of contributors and whether the quality of the profile is suitable or not suitable for making comparisons.

Sub-analytical threshold peaks will not be used in any statistical calculations or for any quantitative evaluations.

III. Interpretation guidelines for single source and mixed profiles

A. The following parameters and/or guidelines will be used:

1. The analytical threshold is set at a level to reliably distinguish an allelic peak from the instrument's noise. The analytical threshold is 190 rfu.

2. The stochastic threshold is the value above which it is reasonable to assume allelic dropout has not occurred in a single-source sample. The stochastic threshold is 1160 rfu.

3. The peak height ratio (heterozygote balance) is the ratio of peak heights between the two alleles of a heterozygote. The expected peak height ratio is at least 50% for all loci.

   The relationship between peak heights and the starting template is always stochastic since variability is introduced at sampling, extraction, PCR and capillary electrophoresis stages of DNA analysis. It is expected that there will be increased variance of the peak height ratio as allelic peak heights decrease. Therefore, as peak heights decrease it is appropriate to consider a heterozygote that falls below the established peak height ratio. The peak height ratio alone may not be an accurate indicator for contributor number and will be applied with caution below the stochastic threshold.

4. Establishing a mixture ratio between contributors in a mixture may assist in differentiating the individual profiles. These ratios are best determined at loci where there is no sharing of alleles, no allele is in a stutter position and all alleles are above the stochastic threshold. If a profile does not have a locus meeting these criteria, then the mixture ratios can be derived by identifying a locus where

the major contributing alleles are above the stochastic threshold and the minors are not affected by n-1 stutter positions. For those loci exhibiting allele sharing, alleles in stutter positions or alleles which fall below the stochastic threshold, caution must be exercised in defining a mixture ratio.

For a two-person mixture, a major and minor contributor may be differentiated or resolved where the ratio of the contributors is at least 3:1 and appears consistent throughout the profile. It is acceptable to assign a range around the mixture ratio to aid in restricting genotypes. **Note:** Exercise caution when accepting a mixture ratio range that exceeds +/- 50% of the calculated/accepted ratio or when peak height ratios are below expectations. These conditions are expected when limited template contributors are a part of the mixture.

If the mixture ratio is less than a 3:1 in a two-person mixture, the genotype combinations cannot be assigned as belonging to only the major or minor contributor and the mixture will be considered unresolved. In these instances, the peak height ratio may be applied to restrict genotypes for both contributors.

For a mixture of greater than two contributors, the mixture ratio of 3:1 or greater between the tallest minor and the major contributor(s) above the stochastic threshold may be used to resolve the major profiles.

5. DNA results for the same sample may be interpreted across multiple electropherograms. The electropherograms used for interpretation will be clearly identified.

6. As appropriate, the genotypes for the unknown evidentiary profiles must be determined before a comparison to a reference standard.

7. If the DNA analysis for a particular exhibit/sample does not provide usable information, evaluate whether additional DNA analysis such as increasing or decreasing the DNA template for amplification, re-purification or another extraction may be warranted.

8. All assumptions used for interpretation will be documented.

B. Identification of the Number of Potential Contributors

The determination of the number of contributors is an assumption based on observed alleles above the analytical threshold. The analytical threshold is set at a level to reliably distinguish an allelic peak from the instrument's noise.

Sub-analytical threshold peaks may be used to assist in the evaluation of the potential number of contributors. This will be strictly limited to those samples exhibiting low

template DNA levels as outlined in Section II.D. If sub-analytical threshold peaks are used to assist in the determination of the contributor number, lowered Y-scale data should be included.

The number of contributors will be documented in the notes and included in the report.

1. Single-source

   A profile is generally considered to have originated from a single contributor if there are no more than two alleles observed at a given locus unless the locus is determined to be tri-allelic.

2. Mixtures

   A sample is generally considered to be a mixture if three or more alleles are present at one or more loci and/or the peak height ratio is below expectations. Allele counting may aid in determining the minimum number of contributors but it is also possible that allele sharing may result in an underestimate of the actual number of contributors. Relative peak heights between apparent major and minor alleles (mixture ratios) may assist in determining the extent of allele sharing. Therefore, it is important that the entire profile be evaluated in determining the profile is a mixture.

   If a mixture cannot be determined to be consistent with two or three contributors then the profile may be reported as being from "at least" the minimum number of contributors based on allele counting. For example, a profile with five alleles at any locus barring any genetic anomalies would be consistent with a mixture of at least three individuals.

C. Determining Possible Genotype Combinations

1. Single source

   It is expected that peak height ratios may fall below the expected value with samples that are degraded, low template, have extreme differences in allele sizes, or those with a primer binding site mutation.

   In general, a heterozygous locus will be considered complete when two alleles are above or below the stochastic threshold and a homozygous locus will only be considered complete when a single allele is equal to or greater than the stochastic threshold.

2. Mixture of two contributors

Profiles consisting of two contributors may have loci that are resolvable, unresolvable or a combination of the two.

A mixture can be differentiated using the peak height ratio (50%) and mixture ratio (3:1) to restrict the genotype possibilities for major and minor contributors. It is acceptable to assign a range around the calculated mixture ratio. The mixture ratio will be documented.

The use of peak height ratios and mixture ratios to restrict genotypes will be applied with caution to those alleles below the stochastic threshold.

An obligate minor allele may be considered a complete genotype when it is equal to or greater than the stochastic threshold. The following are genotype considerations:

- Homozygote
- Heterozygote with its sister allele masked in a stutter peak if the minor allele is of similar peak heights
- Heterozygote with its sister allele shared with an allele of the major contributor

Caution should be used when determining that the only possible sister allele to an obligate minor allele is a peak being masked in a slightly elevated stutter position.

If the obligate minor allele is below the stochastic threshold, the sister allele will generally be considered inconclusive.

At the approximate base pair size or the locus where it has been determined that the minor contributor has dropped below the analytical threshold or is possibly being shared with a major allele with no apparent impact on the interpretation of the major profile, the remaining loci may be interpreted according to the guidelines for a single source profile. This will be documented on the electropherogram. Reduced Y-scale data will be included in support of these interpretations.

a. Four Allele Loci

i. Resolved

The two alleles with the tallest peak heights and the two alleles with the smallest peak heights may be paired together as heterozygotes.

ii. Unresolved

The genotypes will be defined as all possible heterozygote combination of alleles.

b. Three Allele Loci

i. All alleles above stochastic threshold (complete genotypes)

(a) Resolved

If there is one tall allele with two smaller alleles, the genotypes will be determined to be a major homozygote with a minor heterozygote.

If there are two tall alleles with one small allele, the genotypes will be determined to be a major heterozygote and the minor genotype may be restricted based on the mixture ratio.

(b) Unresolved

The genotypes of each contributor will be defined as all possible heterozygote and homozygote genotype combinations that support the relative relationship between the contributors.

ii. One or two alleles below stochastic threshold

(a) Resolved

If one allele is above the stochastic threshold and two alleles are below, the genotypes may be determined to be a major homozygote with a minor heterozygote (complete genotypes).

If two alleles are above the stochastic threshold and one allele is below, the two taller alleles will be considered a heterozygote and the smallest allele should be evaluated based on the overall mixture ratio and relative peak height to establish whether the minor genotype is complete.

If the minor genotype is incomplete, then the sister allele to the minor allele will be considered inconclusive.

(b) Unresolved

(1) Two alleles above stochastic threshold

If the locus is considered complete based on relative peak heights, the genotypes will be defined as all possible heterozygotes and homozygotes that support the relative relationship between the contributors.

If there is uncertainty as to whether the locus is complete, the genotypes will be restricted to all combinations of the two alleles above the stochastic threshold; the pairing of each of these alleles with the allele below the stochastic threshold; the sister allele to allele below stochastic threshold will be considered inconclusive.

(2) One allele above stochastic threshold

If the locus is considered complete based on relative peak heights, the genotypes will be defined as all possible heterozygotes and homozygotes that support the relative relationship between the contributors.

If there is uncertainty as to whether the locus is complete, the genotypes will be restricted such that the allele above the stochastic threshold will be considered a homozygote with the remaining two alleles a heterozygote pair; the allele above stochastic threshold pairing with each of the two alleles below the stochastic threshold; the sister allele to each of two alleles below stochastic threshold will be considered inconclusive.

iii. All three alleles below stochastic threshold

(a) Resolved

If there are two tall alleles with one small allele, the genotypes will be determined to be a major heterozygote with the sister to the obligate minor allele considered inconclusive.

(b) Unresolved

At loci exhibiting all three alleles below the stochastic threshold the sister allele for each of the three alleles will be considered inconclusive.

c. Two allele loci

i. Resolved

If it has been determined that the minor contributor is still present and has not completely dropped out, the appropriate genotypes for the minor contributor will be restricted based on relative peak heights and the mixture ratio.

ii. Unresolved

(a) Two alleles above stochastic threshold

If the locus is considered complete based on relative peak heights, the genotypes will be defined as the heterozygote and the homozygotes for each contributor.

(b) One or both alleles below stochastic threshold

These loci are considered inconclusive since it is not possible to determine the extent of allele drop out.

d. One allele loci

i. Above stochastic threshold

(a) Resolved

If it has been determined that the minor contributor is still present and has not completely dropped out, the genotype for the minor contributor will be determined as the observed allele with a sister allele that is inconclusive. The major contributor will be determined to be the homozygote.

(b) Unresolved

The genotype for both contributors will be determined as the observed allele with a sister allele that is inconclusive.

ii. Below stochastic threshold

(a) Resolved

The genotype for the major contributor will be determined to be the observed allele with a sister allele that is inconclusive.

(b) Unresolved

The genotypes for both contributors will be considered inconclusive.

e. Assuming a known contributor

The assumption of a profile from a reference standard within a mixture may be considered when an individual has had an intimate and timely association with the evidentiary item.

It is also acceptable to assume a profile identified from a specific individual. This would include assuming a profile resolved from one of the fractions of a differential extraction to resolve a mixture in another fraction. As appropriate, an assumption of a known contributor's profile may also be used to restrict genotypes or assist in the determination of mixture ratios. This will be documented on the electropherogram.

All assumptions will be documented and reported.

It is not acceptable per NDIS to assume an unknown single source profile from one exhibit to resolve a mixture in a different exhibit.

f. Differential extractions exhibiting carry-over

Incomplete separation of non-sperm or sperm fractions may result in carry-over into the other fraction. This is commonly observed as minor alleles detected in one fraction that are consistent with the major profile in the other fraction.

When carry-over is observed in the non-sperm and/or sperm fractions, the exhibit may be reported as a whole. This would require the major profile in each fraction to be resolved to a single genotype at each locus, without assuming. The observance of a mixture in any fraction that is not attributable to carry-over will require that each fraction be reported separately.

If the fractions of the differential extraction are reported separately, any mixtures identified will be interpreted as outlined in the procedures for resolved/unresolved mixtures of two contributors. The report wording will include a mixture statement defining the number of contributors and

all comparisons to both the major and minor contributors. If an assumption of a profile is used to resolve a fraction, it must be stated in the report. See Appendix I for additional information.

3. Mixture of greater than two contributors

The determination that a mixture consists of greater than two contributors may be based on the number of alleles at a locus, peak height imbalances, mixtures ratio inconsistencies between loci and/or the presence of subthreshold peaks.

It will need to be ascertained if there is a "definitive" contributor number or if the contributor number is "indeterminate". Regardless of whether the mixture has a definitive or indeterminate number of contributors, the mixture will be evaluated to determine if a major contributor or two major contributors can be differentiated from the mixture.

If the mixture can definitively be identified as consisting of three contributors, it may be possible to interpret a minor profile in addition to the major profiles. As in two person mixtures, it is acceptable to assume a known contributor to aid interpretation of three person mixtures. The assumption of one of the contributors may aid the assessment of the contributor number as well as the mixture ratio between the contributors.

In mixtures where the minor contributor(s) have been determined to have dropped below the analytical threshold or are possibly being shared with a major allele but having no apparent impact on the major profile, the remaining two contributors may be interpreted at the appropriate loci according to the guidelines for two person mixtures. This will be documented on the electropherogram.

a. Resolving one major contributor

A major contributor may be differentiated from the mixture provided:

- The mixture ratio is at least 3:1 between the major contributor and the tallest minor contributor(s) at the interpretable loci
- In general, peak height ratios are equal to greater than 50%
- Evidence for the sharing of a minor allele with a major allele may best be determined by examining the peak height relationship between the major and minor contributors across the entire profile.

b. Resolving two major contributors

Two major contributors may be differentiated from the mixture but not necessarily from each other provided:

- The mixture ratio is at least 3:1 between the two major contributors and the minor contributor(s) at the interpretable loci
- In general, peak height ratios are equal to or greater than 50% but it is most likely that all possible genotype combinations will be assigned to each major contributor.

Two major contributors may be further resolved from one another by:

- Assuming a known contributor
- Application of the mixture ratio as described for two person mixtures

Caution should be taken to ensure that the minor profile(s) are sufficiently below the stochastic threshold or have dropped out entirely so as not impact interpretation above the stochastic threshold.

c. Unresolvable mixture of greater than two contributors

If a mixture from at least two contributors is considered unresolvable, it is generally due to imbalances in peak heights, alleles below the stochastic threshold and possible allele dropout. In these instances, it is not possible to determine genotypes. Such mixtures are not suitable for making comparisons to other unknown evidentiary profiles or reference standards.

Amplification of additional DNA may be considered at the discretion of the analyst.

IV. Comparisons between two or more unknown evidentiary profiles or the unknown evidentiary profiles to reference standards

A. Inclusions will be reported as **cannot be excluded (is included)**. Exclusions will be reported as **excluded**.

B. If a reference standard is reported as **cannot be excluded (is included)** from a probative unknown evidentiary profile it will be accompanied by a statistical calculation. For exhibits on which a differential extraction has been performed, only the most informative statistic will need to be reported when the probative contributor(s) is identified in more than one fraction.

C. An open profile is an unknown evidentiary sample that is excluded from the reference standard(s). Open profiles should be compared and reported to the agency to provide information as to the number of unique contributors.

If an open profile is not eligible for CODIS upload and has at least six searchable STR loci, it must be keyboard searched against the laboratory personnel DNA database.

- If no associations are returned, the Local Match Detail Report from the keyboard search will be included in the notes packet to document the correct entry of the profile.

- If an association is excluded, a technical review for correct entry and the exclusion will be conducted and documented. The Local Match Detail Report will not be included in the notes packet.

- If an association cannot be excluded, the Technical Leader will be notified. Refer to Command Directive TCH 21. If further evaluation does not support exclusion, the Local Match Detail Report from the keyboard search will be included in the notes packet.

DNA profiles or mixtures of at least six loci not suitable for CODIS upload or a search of the laboratory personnel DNA database should be compared against the analyst's own profile. If the analyst cannot be excluded from the sample, the technical leader will be notified.

D. In the review of an exclusion, the unknown evidentiary profile and a reference standard profile may share a lot of genetic similarity. If the two profiles share at a minimum 70% of the alleles at the CODIS core loci, then Y-STR analysis may be conducted to determine if this is a potential relative. If the two profiles do not share a minimum of 70% of the alleles but the analyst determines the partial match warrants additional consideration, an exception may be made upon consultation with a technical leader.

1. Additional analysis using Y-STRs will only be conducted if the following conditions have been met:

    - There is sample remaining from the unknown evidentiary sample and the reference standard.

    - The unknown evidentiary profile must be from a male and is from a single source. This also includes a deduced profile with a single genotype at all interpreted loci.

- If DYS391 results have been identified for the two profiles, the haplotypes must match.

- The evidentiary profile will need to have DNA results at ten of the thirteen original CODIS core loci or at fifteen of the twenty expanded CODIS core loci.

- The reference standard is a male.

2. If either one of the profiles is female, then no further DNA analysis will be conducted by ISP and the partial match will not be reported. In addition, if there is insufficient sample to perform Y-STR analysis then the partial match will not be reported. For additional information, please consult the Command Directives.

E. The following are the definitions of the conclusions for a comparison:

1. Cannot be excluded (is included)

a. A single genotype has been identified in the unknown evidentiary profile at the loci being used for interpretation. The genotypes of the unknown evidentiary profile are concordant with another profile at each of these loci.

b. A single genotype (major and/or minor) is differentiated from a mixture at the loci being used for interpretation. The genotypes of the unknown evidentiary profile are concordant with another profile at each of these loci.

c. More than one possible genotype is present in the unknown evidentiary profile at the loci being used for interpretation. The genotypes of the standard are contained in the possible genotypes at each of these loci, including loci with allelic dropout.

2. Excluded
A failure to meet the criteria of cannot be excluded (is included).

3. Inconclusive
No comparison can be made since the evidentiary profile has been determined to be unsuitable for comparison.

V. Applying Population Frequency Data to STR Results

A. All probative associations will be accompanied by a statistical evaluation. For exhibits on which a differential extraction has been performed, only the most

informative statistic will need to be reported when the probative contributor(s) is identified in more than one fraction.

B. The Illinois State Police will be using the allele frequencies from the 2015 Expanded FBI STR Population Data that has been compiled by the FBI Laboratory for the African American, Caucasian, Southeastern Hispanic and Southwestern Hispanic populations.

C. The minimum allele frequency at a given locus for each population is calculated as 5/2N, where N = the number of individuals in the database for the autosomal STR loci. The reported frequency estimates for Y-STR locus, DYS391, will include a 95% confidence limit. The frequency will be calculated using the ISP STR Calculator incorporated into LIMS.

D. The formulas used for calculating the individual autosomal STR loci are based on National Research Council II recommendation 4.1b.:

1. Heterozygote: $2pq$

2. Homozygote: $p^2 + p(1-p)\theta$, where $\theta = 0.01$

3. Incomplete genotype: $2p$

4. Tri-allelic: $2pq$, where p and q are the two most common allele frequencies

E. The profile probability for the Y haplotype is estimated applying a 95% confidence upper bound to the haplotype frequency using the Clopper-Pearson method.

F. The product rule is used to calculate the combined frequency for the profile. This calculation is made by multiplying together the individual frequencies for each locus. For loci with more than one possible genotype, the sum of the frequencies of each possible genotype is used.

G. Statistics will be reported as a Random Match Probability (RMP).

1. Statistics will be calculated for the African American, Caucasian, Southeastern Hispanic, and Southwestern Hispanic populations. Only the most common statistical value from the population groups will be reported with no reference to the population group.

2. The statistic will be reported in the format of "1 in 1/combined frequency of occurrence." The reported statistic will be truncated to 2 significant figures. No profile frequencies will be rounded. For example, 1 in 1,490,000,000 = 1 in 1.4 billion.

3. If the statistic is calculated to be 1 in 1, the frequency of the profile will be reported as a percentage. The percentage will be rounded up to 2 significant figures.

4. When calculating a 2p statistic:

    a. If a population's frequency at a locus is calculated to be greater than 1, a frequency of 1 will be used.

    b. When the statistic includes only one locus and a population's frequency is greater than 1, it will be reported that no individuals can be excluded without a standard for comparison.

5. If a reference standard has locus drop-out, the statistic will not be calculated at that locus for the evidentiary profile. If a reference standard has allele drop-out at a locus, only the allele available for comparison will be used in the calculation (2p).

## REPORT WORDING

See Appendix I for reporting guidelines.

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# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** LIKELIHOOD RATIO WITH PROBABILISTIC GENOTYPING

**PROCEDURE:** INTERPRETATION: STRmix™ for PowerPlex® Fusion

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## INTRODUCTION

STRmix™ v2.9 has been validated for the interpretation of PowerPlex® Fusion profiles. STRmix™ is a fully continuous software program using likelihood ratios with probabilistic genotyping for the interpretation of autosomal STR DNA results.

## GENERAL

The following is the general interpretation approach using STRmix™:

- The number of contributors will be assigned prior to analyzing the profile in STRmix™. To aid in determining the number of contributors, the stutter filters in GeneMapper™ ID-X for binary interpretation may be used.
- DNA profiles containing up to four contributors can be analyzed in STRmix™.
- STRmix™ may be used for evidentiary profiles of mixtures up to four contributors that have no reference standards for comparison but may yield a CODIS eligible profile upon deconvolution.
- STRmix™ outputs will be reviewed and all appropriate results will be reported.
- A reference standard that has been interpreted using binary methods may be manually compared with the STRmix™ results of the evidentiary profile in the following situations:
    - Exclusions
    - Non-probative reference standard that cannot be excluded (i.e. included) from the STRmix™ results of the evidentiary profile and it is not necessary to calculate a likelihood ratio.

## ASSESSING THE SUITABILITY OF A PROFILE

A. Profile analysis

Artifacts will be removed from the evidentiary profile. These include the following:

1. Peaks that may be stutter that were not modelled in the validation.

   The following stutters were modelled during validation:
   a) n+1
   b) n-1
   c) n-2bp at D1S1656

2. Incomplete 3’ A nucleotide addition

3. Spikes

4. Fluorescent pull-up

5. Dye artifacts

Please note: that for reference standards, all stutter peaks and artifacts will be removed.

B. Determination of contributor number

The assignment of the number of contributors to an evidentiary profile will be made prior to the comparison of the profile to any reference standards unless conditioning/assuming is used to aid this determination. An assumed/conditioned contributor is an individual whose DNA profile is reasonably expected to be observed in an evidentiary item based on the case scenario. This should be supported by the electropherogram. It is also acceptable to use DNA results that are from a single contributor in another fraction of the same item for assuming/conditioning. The assumed/conditioned contributor must be resolved to one genotype at each locus or that locus must not be used.

The number of contributors should be the number most likely to explain the profile.

If the number of contributors cannot be determined or there are greater than four contributors, the profile will not be analyzed in STRmix™. The reason for the determination will be documented in the notes packet.

C. Method for assigning the number of contributors

The following guidelines can be used in assigning the number of contributors to an evidentiary profile:

1. Review the entire profile. Assess the level of degradation, presence of low-level peaks, appearance of the baseline, and the general quality of the profile.

2. Identify likely forward and reverse stutter peaks using allele stutter ratio expectations.

3. Find the locus with the highest number of unambiguously allelic peaks (A). If A is an odd number, add 1. A/2 is the initial postulate of the number of contributors to the profile.

4. Review peak height imbalances at the most informative locus, which is the one containing the greatest number of alleles. If there is too much imbalance between alleles, this may indicate the presence of an additional contributor above that suggested by allele count alone.

5. If one or more contributors at the most informative locus is either a low-level contributor or a major contributor, evaluate as to whether this pattern is represented at other loci.

6. Apply the general pattern of contributors (number and mixture proportion) to other loci in the evidentiary profile. If it is consistent, assign this number of contributors to the profile. If it is inconsistent, consider the addition or subtraction of a contributor.

7. If available, the DNA results from DYS391 and PowerPlex® Y23 may be used to aid in assigning the contributor number.

8. Additional information to consider when determining the number of contributors:

    a. Discriminating loci such as D1S1656, Penta E, D2S1338, D21S11, and FGA are likely to be the most informative when determining the number of likely contributors to a profile.

    b. The presence of one or two low-level peaks may indicate drop-in and not an additional contributor.

    c. If unintuitive weights/mixture proportions are obtained for the contributors, it may be the contributor number is incorrect and needs to be re-evaluated. As STRmix™ is attempting to determine the genotypes of the trace contributor, it can reduce the weights of these other contributors.

    d Peaks below the analytical threshold but not in a stutter position may suggest the presence of an additional trace contributor. Examine the mixture for support of a potential trace contributor above the analytical threshold through imbalance in peak heights and/or mixture proportions before determining the number of contributors. If the sub-analytical peaks are not within range of alleles above analytical threshold, then the contributor is unlikely to affect the interpretation of the contributor(s) above analytical threshold. While an additional contributor may not greatly affect the LR, it will result in STRmix™ modelling an additional contributor for comparison. It is important to note that STRmix™ will only model allelic/stutter peaks above analytical threshold. Therefore, the most likely number of contributors above analytical threshold should be used to explain the data.

    e Consideration should be made as to whether additional DNA analysis (i.e. re-extraction or re-amplification) could potentially aid in determining the number of contributors to the item

D. Manual (binary) profile comparison

1 A manual comparison may be made between the STRmix™ deconvolution results for evidentiary profiles and reference standards that have been interpreted using binary methods. The binary interpretation of the reference standard will be documented in the Allele Table. Based on these comparisons the following conclusions can be drawn: cannot be excluded (is included), excluded, or inconclusive. The reason for the conclusion will be documented in the notes packet. If the conclusion is cannot be excluded (is included) for the probative reference standard or inconclusive then the likelihood ratio should be calculated using STRmix™.

2 If a profile is not interpreted in STRmix™, but is used for comparison to reference standards, the binary interpretation will be documented in the Allele Table.

E. Profiles suitable for STRmix™ interpretation

The following profiles types are suitable for STRmix™ but this list may not be exhaustive:

- All probative evidentiary profiles from which a reference standard cannot be excluded (is included) or for which the comparison is inconclusive.
- Evidentiary profiles up to four contributors with results at two or more loci. It is important to note that evidentiary profiles with limited data are likely to result in low likelihood ratios for true contributors and the chance of an adventitious match of a non-contributor is increased. Refer to the sensitivity and specificity plots within the Illinois State Police STRmix™ validation document for details.
- Probative evidentiary profiles from which a reference standard(s) has been excluded upon manual comparison. STRmix™ may be considered if it may be beneficial in order to generate one or more CODIS profiles via the excel-based software tools, POPSTR and FORESTR.

The following profile types are **not** suitable for STRmix™:

- The number of contributors cannot be assigned.
- There are more than four contributors.
- There are results at only one STR locus.

## ANALYZING IN STRMIX™

A. A text file created in GeneMapper™ ID-X will be used for analysis in STRmix™ for each unknown sample with the following analytical threshold for each fluorescent dye:

Fluorescein = 190 RFU
JOE = 150 RFU
TMR-ET = 190 RFU
CXR-ET = 190 RFU
WEN = 190 RFU

In addition, the text files require the following information depending on the sample type:

- Unknown samples need to include peaks in stutter positions that were modelled during validation. The export file will also need to contain Samples name, Marker, Allele designations, size and height.

- Known samples will not include stutter. The text file will also need to contain Sample name, Marker and Allele designations. It is not necessary for the file to contain the size and height for known samples. The reference standard will be interpreted using binary interpretation guidelines to create a text file. Loci with allele drop-out will be omitted from the text file. Reference standards from legacy chemistries and outsourced cases also may be used to create a text file for these profiles.

B. The text file(s) will be exported for STRmix™ entry.

C. Setting the propositions for calculating the likelihood ratio (LR) and/or deconvolution

The likelihood ratio assesses the probability of the evidence given two competing propositions (e.g. $H_1$ and $H_2$). The $H_1$ generally aligns with the person of interest that cannot be excluded (is included) and the $H_2$ generally aligns with the exclusion of the person of interest.

As appropriate, reference standards should be assumed/conditioned in the proposition set since this reduces the number of unknowns in the $H_2$. Reducing the number of unknown contributors in the $H_2$ will reduce the LR. Therefore, propositions should be constructed in order to minimize the LR.

Analysis should be set up with the $H_1$ ($H_p$ in STRmix™) comprising the person of interest and known or unknown individuals up to the number of contributors (e.g. for a three-person mixture $H_p$ could be the person of interest (POI) and two unknowns). The $H_2$ ($H_d$ in STRmix™) should consist of any assumed (or conditioned) individuals and then all unknown individuals up to the number of contributors (e.g. for a three-person mixture with no assumed contributors, $H_d$ would be three unknowns).

The $H_1$ and the $H_2$ will contain the same number of contributors unless discussed with the Technical Leader.

The following are examples:

1. Two-person mixture from a swab collected from the victim. DNA profile corresponds to POI and victim.

   $H_1$: DNA originates from victim and POI
   $H_2$: DNA originates from victim and unknown

2. Three-person mixture from firearm located at scene. One component corresponds to POI.

   $H_1$: DNA originates from POI and two unknowns
   $H_2$: DNA originates from three unknowns

3. Three-person mixture from firearm located at scene. One component corresponds to POI #1 and another to POI #2. When multiple POIs are considered in the $H_1$ together this is considered a compound LR. Below are examples of LRs that may be calculated.

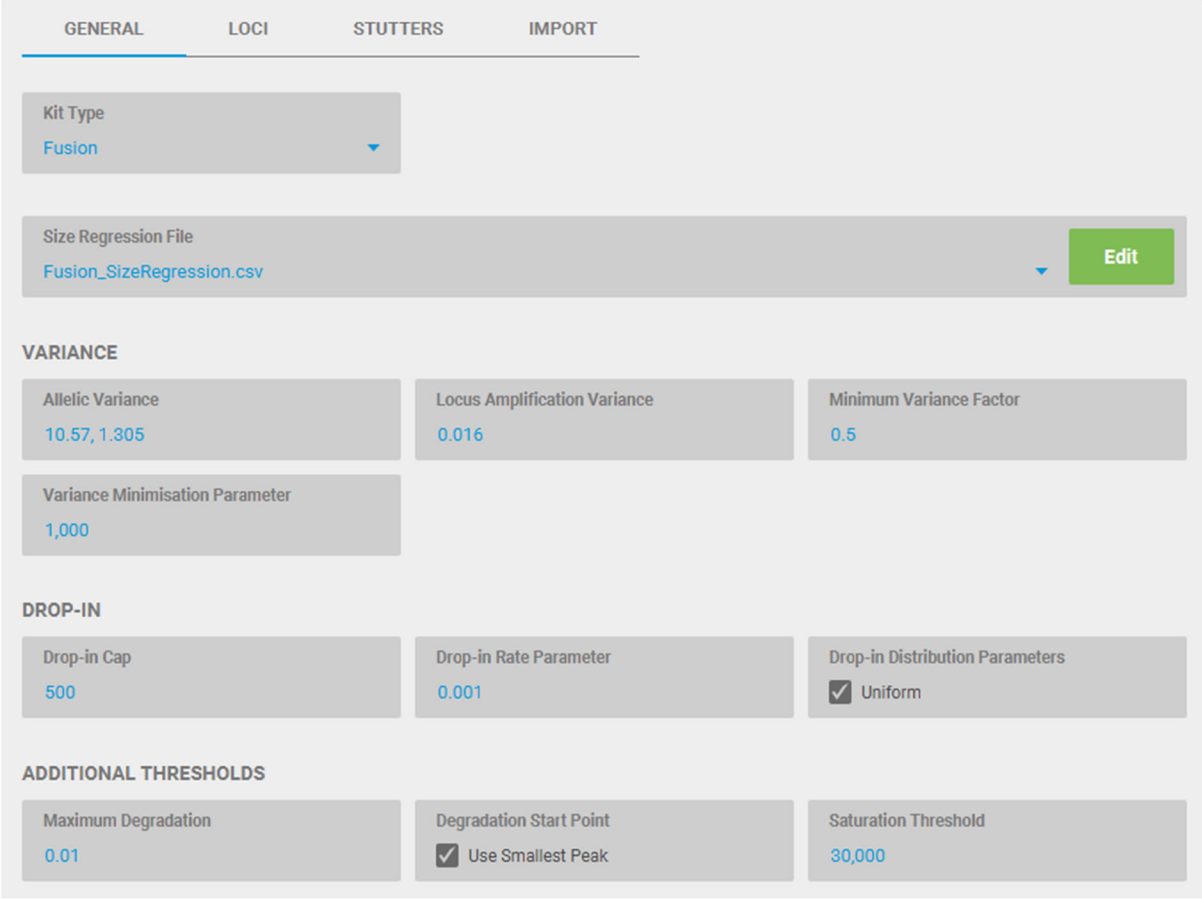
   $H_{1a}$: DNA originates from POI #1 and two unknowns
   $H_{1b}$: DNA originates from POI #2 and two unknowns
   $H_{1c}$ (compound LR): DNA originates from POI #1 and POI #2 and one unknown
   $H_2$: DNA originates from three unknowns

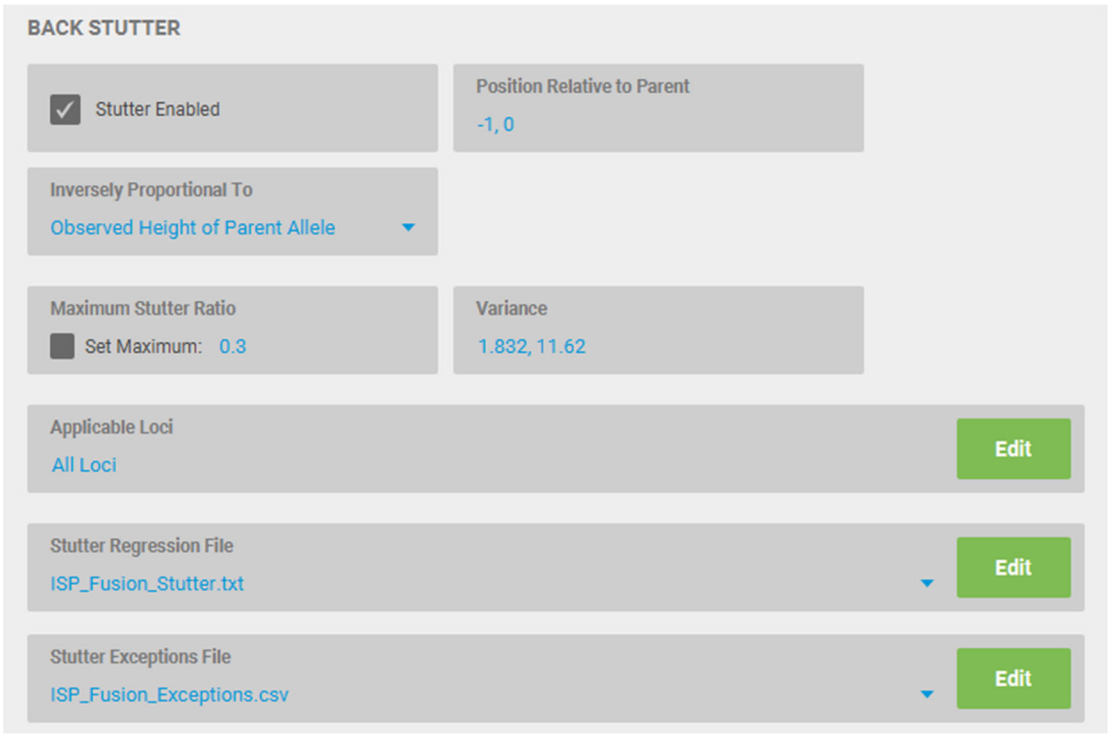
   As appropriate, a compound LR should only be considered if each POI has an LR that supports the $H_1$. If the $H_1$ of the compound LR is supported, the resultant LR will generally be additive. Therefore, if one of the POIs have a very high LR and the other POI has a very low LR then the resultant LR will be more representative of the contributor with the greater LR. This LR will be available in the electronic file. The report will contain only the individual LRs since the compound LR may be misinterpreted or misrepresented by the courts if the analysts is not able to provide proper context. If a compound LR results in zero, then the individuals cannot occur in the mixture together and this information along with the individual LRs will be provided in the report.

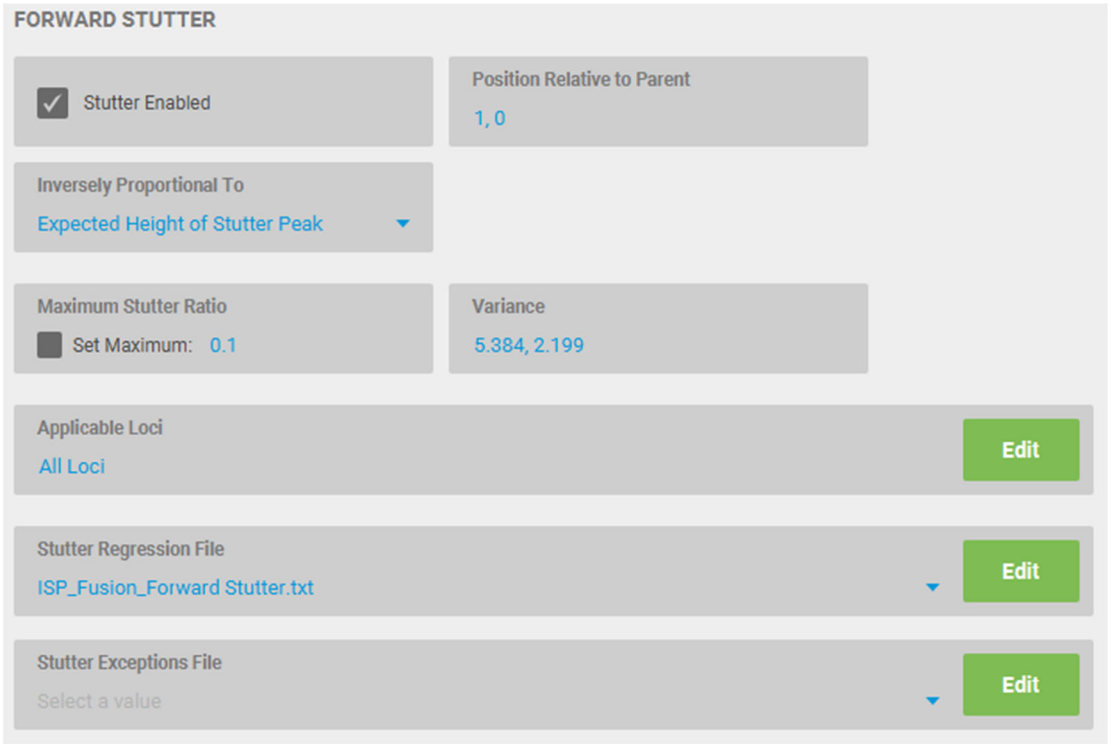
## INTERPRETING PROFILES IN STRMIX™

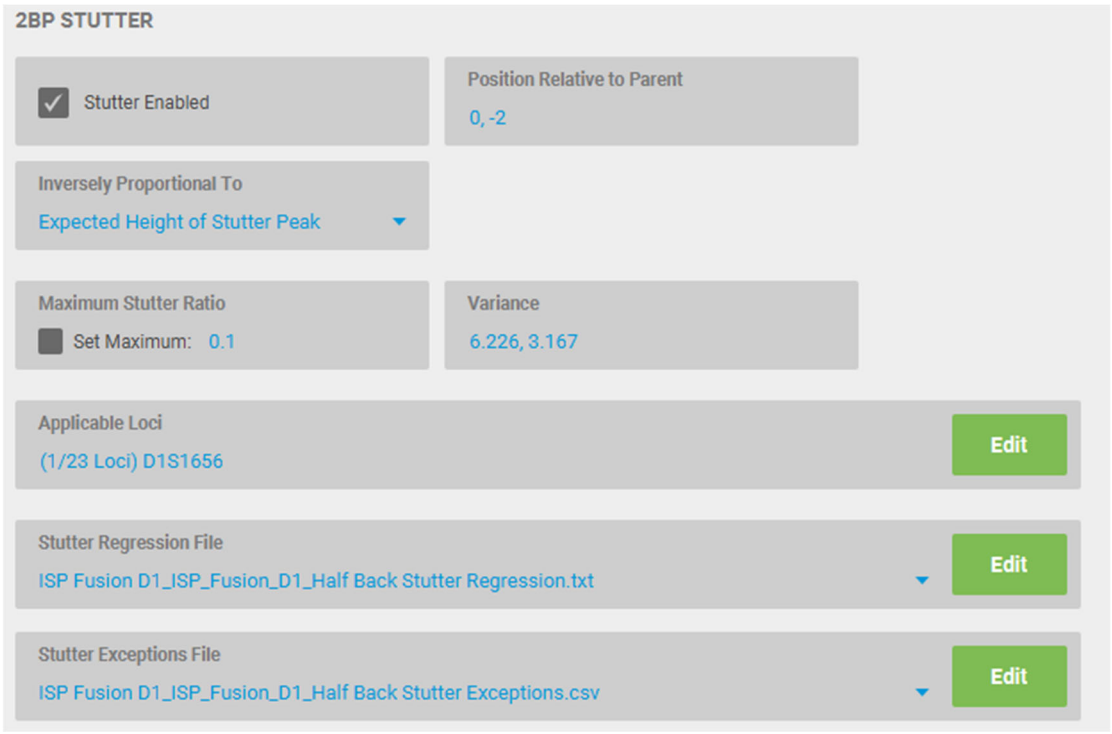
A. Run parameters

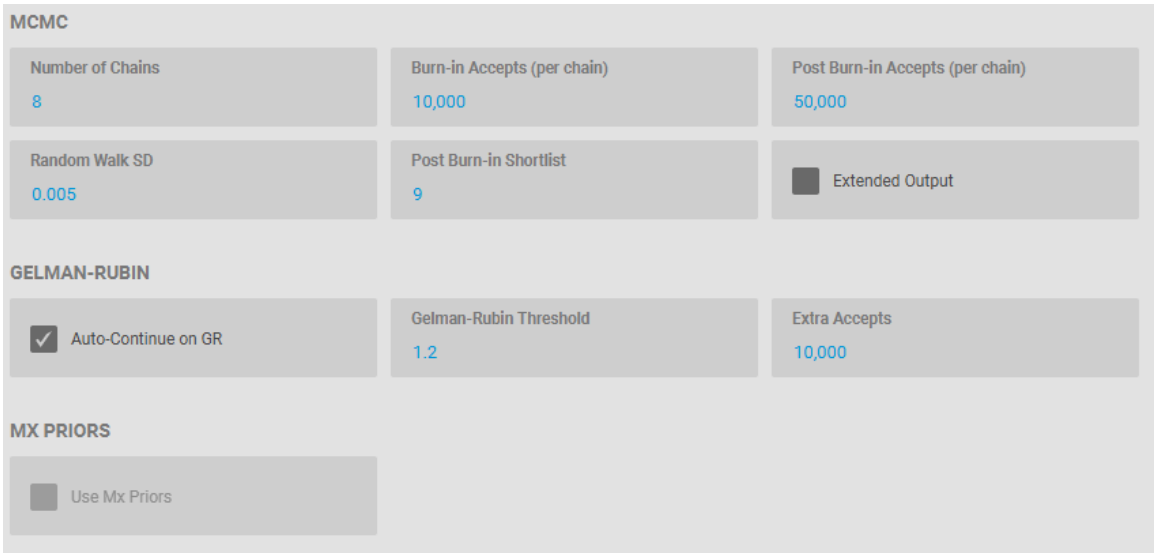
The PowerPlex Fusion® settings are found in the screen shots below. The Auto-Continue under Gelman-Rubin should be selected using the default setting of 10,000 extra accepts. This Auto-continue only affects post burn-in. Therefore, it may be necessary to increase the burn-in and post burn-in to resolve issues. The stutter values can be found within the Illinois State Police STRmix™ validation document.





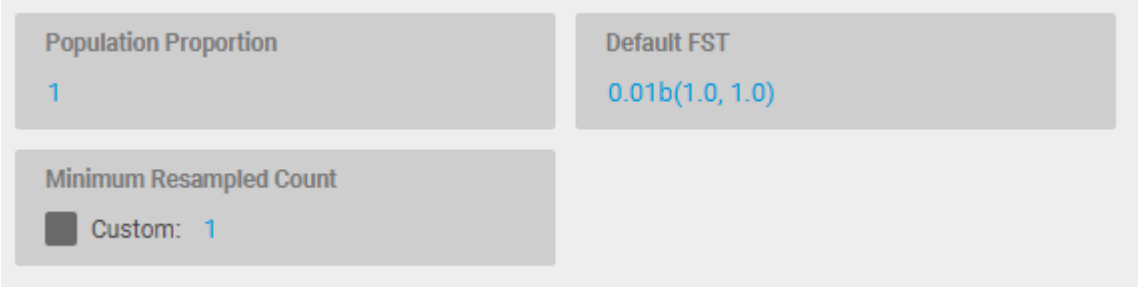



B. Population parameters

A sub-source likelihood ratio will be calculated with the allele frequencies from the 2015 Expanded FBI STR Population Data for African American, Caucasian, Southeastern Hispanic, and Southwestern Hispanic population groups where $\theta = 0.01$. Sampling variation will be accounted for using 99% high posterior density, MCMC uncertainty, and allele frequency uncertainty. The lowest likelihood ratio will be reported with no reference to the population group.

The Minimum Resampled Count (MRC) will be set to one. The MRC is designed to prevent large differences between the sub-source and HPD LRs due to alleles not previously observed within the population.




C. In the following situations "Ignore Locus" function within the Kit Settings of STRmix™ will be used:

1. Primer binding site mutations
2. Tri-allelic patterns
3. Somatic mutations
4. Null alleles
5. Analytical issues (e.g. loci compromised by potential pull-up, poor resolution such as a 9.3/10 at TH01 that is not resolved)
6. Rare situations that the modelling within STRmix™ is unable to accommodate.
7. The reference standard has allele drop-out. However, this is not necessary if the text file was edited prior to conducting the STRmix™ deconvolution. If the text file is not edited, then "Ignore Locus" function must be used for the particular locus when using LR for Previous.

The reason for using the "Ignore Locus" function will be documented in the comments box of the STRmix™ run. If the "Ignore Locus" function was used for the evidentiary profile, all reference standards will be manually compared to the omitted locus.

D. Off-ladder (OL) alleles

Alleles labelled as OL alleles are not permitted within the STRmix™ input file. These alleles will require an allelic size designation to be used in the STRmix™ input file. If an allele exceeds ±2 repeat units outside of the lowest or highest end of the ladder range,

then the locus should be removed from the deconvolution using the "Ignore Locus" option. The reason for the omission will be documented in the comments box of the STRmix™ run. If a LR is calculated, any reference standard should be manually compared at this locus.

E. Reference standards in a legacy amplification kit

A reference standard that has been previously profiled in an amplification kit other than PowerPlex® Fusion can be used with STRmix™. The results will be for those loci in common between the two kits.

F. Replicates

A replicate within STRmix™ is an additional amplification of the same extracted DNA and can be combined within one STRmix™ interpretation for a sample. In general, this can provide more informative DNA results by improving the power of discrimination.

It is not required that a replicate be included in the STRmix™ interpretation. For example, it is not necessary if a full profile was obtained from one amplification and no further information was obtained from additional amplification(s). In addition, if replicate amplifications of the sample indicate different numbers of contributors are present, the replicate with greatest assigned number of contributors should be used and reported. In some situations, it may be appropriate to report both replicates as separate STRmix™ interpretations.

G. LR from Previous

If STRmix™ has been previously used to conduct a deconvolution on an item, "LR from Previous" will be used when additional reference standards are submitted. However, if the additional reference standard is used for assuming (conditioning), a new proposition set will be analyzed in STRmix™ since this generally provides a more common LR value.

## REVIEW OF STRMIX™ OUTPUT

A. Primary diagnostics

If the values for the following primary diagnostics are counter-intuitive, then the results will need to be further evaluated:

1. Weights

Where possible, the weights generated by STRmix™ should be assessed at each locus as to whether they are intuitive. The weights are a primary diagnostic but should be reviewed in conjunction with the profile and the other diagnostics.

2. Mixture proportions

The template values are the average of the modes of the accepted iterations of each chain of the post burn-in phase of the interpretation. These should also be assessed as to whether they are intuitive with the profile.

a. Mx Priors:

The Mx priors feature may be helpful when the mixture proportions are not intuitive given the observed profile. This feature will only be used through approval from the Technical Leader.

3. Per locus likelihood ratios

If there are one or more loci that do not fit the pattern for the majority of loci, further evaluation will be required. (i.e. One locus has LR=0 and all the other loci have LR>1).

B. Secondary diagnostics

If the values for the secondary diagnostics are excessive, then the results will be further evaluated.

1. Total iterations/acceptance rate

The value indicates the total number of post burn-in iterations that the Markov chain Monte Carlo (MCMC) ran during analysis and this value should be less than **2.15 billion**. This value and the number of accepts chosen for the analysis is how often a new proposed set of parameters was accepted. This is the acceptance rate.

In the example found below the analysis consisted of 400,000 post burn-in accepts spread across 3,562,462 total iterations giving an acceptance rate of 1 in 8.91.

A very low acceptance rate (e.g. 1 in thousands to millions) in combination with the other diagnostics may indicate that the analysis needs to be run for additional iterations. On its own and with no other indication of sub-optimal results a low acceptance rate is not an indication that rework is necessary.

**POST BURN-IN SUMMARY**

| | | | |
|---|---|---|---|
| Total iterations | 3,562,462 | Acceptance rate | 1 in 8.91 |
| Effective sample size | 29,032.76 | log(likelihood) | 44.37 |
| Gelman-Rubin convergence diagnostic | 1.1 | | |
| Allele variance (mode = 11.106) | 8.065 | Back Stutter variance (mode = 10.303) | 10.465 |
| Forward Stutter variance (mode = 9.611) | 11.951 | 2bp stutter variance (mode = 8.776) | 17.999 |

2. Locus Specific Amplification Efficiency (LSAE)

   A high or excessive value may indicate that the peak heights are quite variable across the profile. This should be evaluated as to whether that is accurate.

3. Average log(likelihood)

   This value shows the average $\log_{10}$(likelihood) for the entire post burn-in MCMC. This is the log of the average likelihood (or probability) value created at each of the post burn-in MCMC iterations.

   The larger this value the better STRmix™ has been able to describe the observed data.

   A negative value may suggest STRmix™ was not able to describe the data well given the information it has been provided. The following may explain why this value may be low or negative.

   a. The profile is very low level and there is very little data being used for the likelihood.

   b. The number of contributors is incorrect and there are forced stochastic events in the STRmix™ run as a result (e.g. large heterozygote peak imbalances or variation in mixture proportions across the profile).

   c. Data has been erroneously removed and STRmix™ will not account for this as dropout.

   d. An artifact has been left labeled and STRmix™ will not account for this as drop-in.

   It is important to note good quality mixtures are likely to give higher average $\log_{10}$(likelihood) values than good quality single-source profiles. Therefore, a low average $\log_{10}$(likelihood) value alone is not necessarily an indicator of an issue.

4. Gelman-Rubin value

This diagnostic provides information on whether the MCMC analysis has likely converged. STRmix™ uses multiple chains to carry out the MCMC analysis and ideally each chain will be sampling in the same space after burn-in. If the chains spend their time in different spaces, then it is likely the analysis has not run long enough.

Whether or not the chains have spent time in the same space can be gauged by the within-chain and between-chain variances. These two variances are used to calculate the variance of what is called the 'stationary distribution'. If all chains have sampled the same space, then the within-chain variance and the variance of the stationary distribution will be approximately equal. If chains have spent time in different spaces, then the variation between the chains is likely to be larger than the variation within the chains. This is calculated by the Gelman-Rubin convergence diagnostic (GR), which is a ratio of the stationary distribution and within-chain variances. For a converged analysis the Gelman-Rubin will be 1.

If the Gelman-Rubin value is greater than 1.2, then it is possible the analysis has not converged and the results should be evaluated. The burn-in and post burn-in accepts per chain can be increased to 10x to 20x the default values, which may reduce the Gelman-Rubin to less than 1.2.

5. Allele variance and stutter variance constants

   These values are the average value for variance and stutter variance constants across the entire post burn-in MCMC analysis. These values can be used as a guide as to the level of stochastic variation in peak heights that is present in the profile.

   If the variance constant has increased markedly from the mode of the prior distribution, then this may indicate the DNA profile is sub-optimal or the number of contributors is incorrect.

   Used in conjunction with the average $\log_{10}$(likelihood), a large allele variance or stutter variance constant can indicate poor amplification.

   If the sample is simply low-level, this should result in a low average $\log_{10}$(likelihood) and an average variance constant.

   If data has been omitted, the stutter filter was on, or data has been misinterpreted, this may result in a low average $\log_{10}$(likelihood) and high variances.

6. Effective sample size

   Effective sample size (ESS) is the number of independent samples the MCMC has taken from the posterior distribution of all parameters.

A low ESS in relation to the total number of iterations suggests the MCMC has not moved very far with each step or has had a low acceptance rate. A low absolute value of ESS (e.g. 10s or 100s) means there is potential for a large difference in weights if the analysis was run again. This will be taken into account during high posterior density (HPD) interval generation in any *LR* calculations unless the genotype sets are completely resolved on a single combination. In this case there will be no effect of ESS on the HPD interval. Please note a low ESS on its own is not an indication that rework is required.

7. Additional troubleshooting

The following are examples where the STRmix™ results obtained do not seem intuitively correct:

- Large LRs (greater than 1) are obtained for each locus except one where the LR is 0.
- The mixture proportions do not reflect what is observed.
- The degradation does not reflect what is observed.
- The interpreted contributor genotypes do not appear intuitively correct.
- Large variance between sub-source LR and 99% one-sided lower bound highest probability density interval (HPD). A 1-2 order of magnitude difference is expected between sub-source LR and 99% one-sided lower bound HPD interval. This issue may be addressed by increasing the burn-in and post burn-in by up to 20X the default values.

The following may be reasons for these issues to occur:

- The MCMC has not run for enough iterations. The burn-in and post burn-in accepts per chain can be increased to up to 20x the default values.
- The number of contributors has not been correctly chosen.
- There is an artifact that should have been removed from the text file prior to STRmix deconvolution
- The amplification has been affected by inhibition, etc.
- Incomplete separation of alleles that are separated by one base pair during capillary electrophoresis.

- Unexpected large imbalance between peaks.

Additional analysis should be conducted if warranted by the weights and/or the other diagnostics.

## REPORTING

As appropriate, the information below will be provided in the report.

A. Assumptions

The assigned number of contributors and assumed/conditioned contributors will be stated in the report.

B. Likelihood ratio

1. The hypotheses for the proposition sets used to calculate any likelihood ratios.

2. The likelihood ratio that will be reported is the 99% lower bound HPD that is truncated to two significant figures for PowerPlex® Fusion profiles. The LR will be calculated for the African American, Caucasian, Southeastern Hispanic, and Southwestern Hispanic population groups. The lowest LR will be reported with no reference to the population group.

   If the LR is greater than 1, it supports the $H_1$. If the LR is less than 1, it supports the $H_2$. The inverse LR (1/LR) of this value will be used so a whole number can be reported. If the LR equals 1, then this provides no scientific support for either the $H_1$ or $H_2$.

   If the LR equals zero, then the DNA profile from the reference standard will be excluded from the profile. However, if the LR equals zero when evaluating whether two or more individuals can comprise a mixture then the conclusion is that these individuals cannot comprise the mixture together. This information should be reported along with the individual LRs.

3. If a compound LR results in support for the H1, then the individual LRs will be reported. This is due to the fact the combination of these individuals may be misinterpreted or misrepresented by the stakeholders if the analyst is not able to provide proper context.

4. If the compound LR results in zero, then the compound LR will be reported in addition to the individual LRs because these individuals cannot exist in the DNA mixture together.

If requested or if case circumstances indicate an alternate LR is more suitable (e.g. a LR for a relative), then that LR may be reported. If the LR supports the $H_2$ then the inverse LR (1/LR) will be used so a whole number will be reported.

5. At minimum, the most informative LR will be reported for each item.

C. Verbal scale

A statement of opinion as to the scientific significance of the DNA results is based on the following verbal scale from the recommendations of the SWGDAM Ad Hoc Working Group on Genotyping Results Reported as Likelihood Ratios.

| Likelihood Ratio | Verbal Qualifier |
|---|---|
| 1 | Uninformative |
| 2 – 99 | Limited support |
| 100 – 9,999 | Moderate support |
| 10,000 – 999,000 | Strong support |
| ≥ 1,000,000 | Very strong support |

## CASE DOCUMENTATION

- Run folder for all STRmix™ interpretations will be attached in the LIMS
- STRmix™ report(s)
- Staff Database Search:
    - If an open profile is not eligible for CODIS upload due to scenario or not meeting the Moderate Match Estimate (MME) of 1 in 70,000 and has at least six STR loci, it must be keyboard searched against the Laboratory Personnel DNA Database.

    - The keyboard search profile will be developed from one of the following methods: FORESTR 90% or STRmix Component Interpretation Summary ≥ 99%.

    - If the method used does not provide six loci, the other method must also be evaluated. This will be documented in the notes packet. If a potential association is made, the analyst will notify the technical leader.

If a summary report was generated, this report along with the full STRmix™ report will be included in the electronic data for the case.

## REFERENCES

Foundational Knowledge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2. Illinois State Police Internal Validation of STRmix™ v2.6 for PowerPlex® Fusion

3. Illinois State Police Internal Validation of STRmix™ v2.7 for PowerPlex® Fusion

4. Illinois State Police STRmix™ v2.9 Upgrade: Parameter Estimation and Performance Check

5. STRmix™ v2.9 Operations Manual

6. STRmix™ v2.9 Implementation and Validation Guide

7. STRmix™ v2.9 User's Manual

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9. Bright et al. (2018). Internal Validation of STRmix$^{TM}$; A multi laboratory response to PCAST. Forensic Science International: Genetics, 34, 11-24.

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28. Taylor, D et al. (2016). Factors affecting peak height variability for short tandem repeat data. Forensic Science International: Genetics, 21, 126-133.

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29. Taylor, D. (2014). Using continuous DNA interpretation methods to revisit likelihood ratio behavior. Forensic Science International: Genetics, 11, 144-153.

30. Curran, J. and Buckleton, J. (2011). An investigation into the performance of methods for adjusting for sampling uncertainty in DNA likelihood ratio calculations. Forensic Science International: Genetics, 5, 512-516.

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# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** DILUTION AND CONCENTRATION

**PROCEDURE:** DILUTION AND CONCENTRATION

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Reviewed by:

_____
Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____
Jeanne M. Richeal
DNA Technical Leader

_____
Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

Refer to the Reagent Blanks and Controls for Extraction section.

## SAFETY CONSIDERATIONS

Refer to the Reagent Blanks and Controls for Extraction section.

## PREPARATIONS

Refer to the Reagent Blanks and Controls for Extraction section.

## INSTRUMENTATION

Refer to the Reagent Blanks and Controls for Extraction section.
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## MINIMUM STANDARDS AND CONTROLS

Refer to the Reagent Blanks and Controls for Extraction section.

## PROCEDURE

### Dilution

Extracted DNA samples can be diluted using TE-4 buffer, sterile distilled/deionized water, or other equivalent diluent. The volumes listed below will be used to prepare dilutions unless otherwise documented.

1/10 = 2µL DNA / 18µL diluent

1/100 = 2µL DNA / 198µL diluent

1/1000 = 1µL DNA / 999µL diluent

### Concentration

Extracted DNA samples can be concentrated using one of two methods.

**Note**: A reagent blank must be processed to the same degree as the associated samples.

A. Microcon® DNA Fast Flow Filters

1. Add 1 µl of cRNA (1 µg/µl) to the extracted DNA that will be concentrated. If combining samples, cRNA should only be added to one of the tubes of extracted DNA that will be combined.

2. Vortex and briefly centrifuge the samples. The extracted DNA with the cRNA can be placed directly onto a Microcon ® DNA Fast Flow Filter and processed as described in

the isolation procedures with the analyst selecting the final volume for sample recovery. As appropriate, extracted DNA from multiple microfuge tubes can be combined at this step. Extracted DNA obtained using the Tecan Freedom EVO® 150 must be concentrated using this method.

B. Vacuum Centrifuge Dehydration

A vacuum centrifuge can be used to dehydrate or reduce the volume of a previously quantified DNA sample. These samples will be resolubilized in an appropriate volume of sterile, distilled/deionized water (or the equivalent). Using the vacuum centrifuge method increases the final concentration of EDTA in the recovered sample. Concentrations of EDTA exceeding 10mM can lead to inhibition upon amplification. Caution should be exercised when concentrating large volumes of DNA extract, to minimize the effects of inhibition.

## REFERENCES


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2. ISP R&D project: AmpFℓSTR Identifiler Plus PCR Inhibition Study II: EDTA, Hematin, and Humic Acid Effects (2012-08).

3. ISP R&D project: Concentration Methods (Command Advisory Board memorandum 20-BIO-08)

4. ISP R&D project: Method to increase DNA yield when using Microcon® filter concentration with addition of cRNA (2021-07).

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** AUTOMATION

**PROCEDURE:** TECAN WITH PROMEGA METHODS

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Reviewed by:

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Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

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Jeanne M. Richeal
DNA Technical Leader

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Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

This procedure is to be used for the processing of samples from extraction through amplification using the Tecan Freedom EVO® 150 Automated Workstation and Promega methods. As necessary, any automated steps may be replaced with manual methods.

## 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.

## PREPARATIONS

**DNA IQ 1X Wash Buffer**

| | |
|---|---|
| DNA IQ 2X Wash Buffer (in kit) | 70mL |
| Propanol | 35mL |
| Ethanol | 35mL |

Mix together in 2X wash buffer bottle provided in DNA IQ kit. Relabel as 1X wash buffer solution

## INSTRUMENTATION

Standard laboratory instrumentation
Tecan Freedom EVO® 150 Automated Workstation

## MINIMUM STANDARDS AND CONTROLS

Refer to the Reagent Blanks and Controls for Extraction section.

## CRITICAL REAGENTS

Promega Corporation DNA IQ™ Resin

## PROCEDURE

### Extraction

1. Place the tubes containing the lysate onto the deck of the Tecan Freedom EVO® 150 Automated Workstation according to the plate layout on the Tecan Worksheet – Other in the Laboratory Information Management System (LIMS).

2. Launch the EVOware® software and select the Extraction_v2_0_ISP_122018 method.

3. In the Graphical User Interface (GUI), verify the selection of "aqueous" and "tubes" as the extraction type. Follow the instructions in the GUI for reagent preparation and deck set-up.

   Note: The volume calculated by the GUI for each reagent placed on the deck includes a dead volume required to ensure pipetting accuracy. The instrument adds the following reagent volumes to each sample:

| | |
|---|---|
| DNA IQ™ Resin | 14 µL |
| Lysis Buffer | 100 µL |
| 1X Wash Buffer | 100 µL per wash |
| Elution Buffer | 50 µL – 100 µL |

## Quantification

1. Launch the EVOware® software and run the Quant_v2_0_0_ISP method.

2. Follow the prompts in the GUI and when prompted, import the Tecan Quant Import file from LIMS.

3. Upon completion of the method, transfer the 96-well plate to the 7500 Real-Time PCR System. For further information refer to Forensic Biology/DNA Quantitative PCR (qPCR): PowerQuant™ System.

4. Upon completion of the run on the Applied Biosystems® 7500 Real-Time PCR System, export the data and import into LIMS.

## Normalization and Amplification

1. In LIMS, select items for amplification on the Tecan PowerQuant Worksheet – Amp Setup page. Navigate to Tecan Fusion Setup and generate the Tecan Amp Import file.

2. Launch the EVOware® software and run the Amp_v3_0_1_ISP method.

3. Follow the prompts in the GUI, and when prompted, import the Tecan Amp Import file from LIMS.

4. Under Review Extraction Plate adjust the amplification targets as needed.

5. Upon completion of the method, export the Normalization Data file from the Tecan Freedom EVO® 150 Automated Workstation and import it into LIMS to create the Tecan Fusion Amp Worksheet.

6. Transfer the 96-well plate to the Applied Biosystems® GeneAmp® 9700™ or ProFlex™ Thermal Cycler. For further information refer to Forensic Biology/DNA Amplification and Electrophoresis of STRs: PowerPlex® Fusion.

## REPORT WORDING

Not applicable.

## REFERENCES


1. Tecan Freedom EVO® 150 Operating Manual. Version 3.1 or above [CD], 2006.

2. Wells, F., et al. DNA IQ High Throughput Method Testing on the Tecan Freedom EVO. Scientific Applications Report, 08/18.

3. Stangegaard, M., et al. Automated extraction of DNA and PCR setup using a Tecan Freedom EVO® liquid handler. Forensic Science International: Genetics Supplement Series 2 (2009) 74-76.

4. Frégeau, C. J., et al. Validation of a DNA IQ-based extraction method for TECAN robotic liquid handling workstations for processing casework. Forensic Science International: Genetics 4 (2010) 292-304.

5. Morf, N. V., et al. Internal validation of Tecan robots (Freedom EVO® 150 and 75) for PCR and capillary electrophoresis setup. Forensic Science International: Genetics Supplement Series 3 (2011) e89-e90.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** AUTOMATION

**PROCEDURE:** Maxwell® RSC 48 Instrument/Maxprep™ Liquid Handler/Portal Software using methods for sample preparation through Capillary Electrophoresis

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Reviewed by:

Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

Jeanne M. Richeal
DNA Technical Leader

Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

This procedure is to be used for the processing of samples from preparation through capillary electrophoresis. As necessary, any automated steps may be replaced with validated manual methods.

## 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.

## PREPARATIONS

18 mg/mL Proteinase K

1. Add 556µL of Nuclease-Free Water (from Casework Extraction Kit) to the tube of lyophilized Proteinase K and gently invert to dissolve.

2. Store Proteinase K solution at -20°C. Prior to use, thaw and remix.

**Note:** Proteinase K from sources other than the Casework Extraction Kit may be used as long as it is prepared at the appropriate concentration.

## INSTRUMENTATION

Promega Maxprep™ Liquid Handler with Portal software
Promega Maxwell® RSC 48 Instrument
Standard laboratory instrumentation

## MINIMUM STANDARDS AND CONTROLS

Refer to the Reagent Blanks and Controls for Extraction section.
Refer to Amplification & Electrophoresis of STRs: PowerPlex® Fusion.

## CRITICAL REAGENTS

Maxwell® FSC DNA IQ™ Casework Kits
PowerQuant™ System
PowerPlex® Fusion System

## PROCEDURE

### Pre-processing <u>non-semen</u> samples:

1. Non-semen samples should be placed in a CW basket inside a CW tube. Substrate should be limited to one swab per microcentrifuge tube.

    - Substrate from swabs will be limited to one swab per microfuge tube. The swab material will be removed from the stick before placing it into the microfuge tube.
    - All other substrates should be limited so the extraction reagents can sufficiently interact with the substrate. Substrate should never be packed tightly, as that will prevent the extraction reagents from interacting with the entire substrate.
    - If CW Microfuge tubes are used instead of another type of microcentrifuge tube, the substrate may need to be limited even further to account for the additional space taken up by the CW Spin Basket.

2. Barcode labels should be placed on the side of the tube opposite the hinge and just below the lip of the tube. Samples will then be scanned into the Extraction Setup worksheet in LIMS.

3. Prepare Extraction Mix by adding 386µL of Casework Extraction Buffer, 10µL of the Proteinase K solution and 4µL of 1-Thioglycerol per sample.

    **Note**: 1-Thioglycerol is viscous. Pipet slowly.

4. Dispense 400µL of Extraction Mix to each sample. Briefly vortex the samples.

5. Incubate the samples at 56ºC for 30 minutes.

6. Centrifuge samples at maximum speed for 5 minutes. Remove and discard the spin basket.

7. Continue to DNA IQ™ Setup on the Maxprep™ Liquid Handler.

### Pre-processing <u>Semen</u> samples:

1. Semen samples will be placed in a microcentrifuge tube.

    - A CW tube **<u>must not</u>** be used for extraction, as the CW Spin Basket is made with a proprietary chemical that binds sperm cells.

- The type of microcentrifuge tube should be consistent for all samples within the extraction batch. The F1s and the F2s are considered separate extraction batches.
- The substrate should be limited to one swab per microcentrifuge tube. The swab material will be removed from the stick before placing it into the microfuge tube.
- All other substrates should be limited in the 1.5mL microcentrifuge tubes so the extraction reagents can sufficiently interact with the substrate. Substrate should never be packed tightly, as that will prevent the extraction reagents from interacting with the entire substrate.

2. Barcode labels should be placed on the side of the tube opposite the hinge and just below the lip of the tube. Samples will then be scanned into the Extraction Setup worksheet in LIMS.

3. Prepare non-sperm digestion buffer as follows:

   (N = number of samples)

   (N+1) x 100µL 1x Casework Extraction Buffer (CEB)
   (N+1) x 300µL Autoclaved water or equivalent
   (N+1) x 10µL Proteinase K (ProK)

4. Dispense 400µL of non-sperm digestion buffer to each sample. Briefly vortex the samples.

5. Incubate the samples at 56ºC for 30 minutes.

6. Briefly centrifuge the samples. Place the substrate in a DNA IQ™ spin basket and place back in the extraction tube. Centrifuge for 5 minutes at 10,000 x g. Move the substrate into a new microcentrifuge tube if the mixed fraction (F3) will be extracted or if the substrate is being retained for possible future reanalysis.

   **Note**: Unextracted F3 substrates must be retained when samples are consumed.

7. Remove the non-sperm (F1) fraction by transferring the liquid above the sperm cell pellet to a new DNA IQ™ microcentrifuge tube.

8. Prepare a second digestion buffer as follows:

   (N = number of samples)

   (N+1) x 100µL 1x Casework Extraction Buffer (CEB)
   (N+1) x 300µL Autoclaved water or equivalent
   (N+1) x 10µL Proteinase K (ProK)

9. Dispense 400µL of digestion buffer to each F2 sample. Briefly vortex.

10. Incubate samples at 56ºC for 30 minutes.

11. Vortex and centrifuge the tubes for 5 minutes at 10,000 x g. Remove the liquid from the sperm cell pellet and discard. 1µL of the sperm cell pellet may be removed for KPIC.

12. Prepare sperm lysis buffer as follows:
(N = number of samples)

    (N+1) x 400µL DNA IQ™ Lysis Buffer
    (N+1) x 4µL 1-Thioglycerol

    **Note**: 1-Thioglycerol is viscous. Pipet slowly.

13. Add 400µL of sperm lysis buffer to each sample including the mixed fraction (F3), if necessary. Vortex vigorously for 10 seconds and centrifuge briefly.

14. Continue to DNA IQ™ Setup on the Maxprep™ Liquid Handler.

**DNA IQ™ Setup on the Maxprep™ Liquid Handler**

1. Launch the software for the Maxprep™ Liquid Handler from the desktop and select Maxwell® FSC DNA IQ™ - Tubes method.

2. Follow the Graphical User Interface (GUI) prompts for running the method.

3. Prepare the deck trays. Microfuge tubes for elution should be empty and open with the Maxwell® cartridges unsealed and placed in the appropriate locations on the deck tray.

    - Do not leave gaps between cartridges – positions on the deck tray should not be skipped.
    - Do not center cartridges on the deck tray – the Maxwell® RSC 48 does not need to be balanced.

4. Pre-processed samples will be placed on the deck of the Maxprep™ Liquid Handler as prompted by the GUI.

5. Select “Run.”

**Note:** The Maxprep™ Liquid Handler will add plungers to the cartridges, add elution buffer to the elution tubes and add 200µL lysis buffer to each sample then transfer the lysis buffer-sample to the cartridge.

6. Upon completion of the method, export the output file and upload to the LIMS Pre-processing worksheet.

**DNA IQ™ Extraction on the Maxwell® RSC 48**

1. Launch the software for the Maxwell® RSC 48 from the desktop and on the home screen select "Start".

2. Scan the barcode on the front deck tray and if applicable scan the barcode on the back deck tray.

3. Select the "DNA IQ Casework" method and then select "Proceed".

4. Follow the GUI prompts to run the method.

    - The Maxwell® RSC 48 does not need to be balanced for the extraction to proceed.
    - The Maxwell® RSC 48 also has the Vision System, which will detect certain issues before the extraction starts. For instance, if a plunger has been transferred to cartridge well #8 and whether the elution tube is present and open. However, the Vision System cannot detect whether the elution tubes have elution buffer.

**Quantification Setup on the Maxprep™ Liquid Handler**

1. Launch the software for the Maxprep™ Liquid Handler and select "Start".

2. Select the PowerQuant® System Setup method.

    - If there are multiple variants, select the appropriate variant (i.e. one variant may set up a standard curve, whereas another variant may prepare the calibrators for a virtual curve).

3. Follow the GUI prompts to run the method.

    **Note**: Ensure that all sample tubes are open before running the method.

4. Upon completion of the method, export the output file and import into the quantification worksheet in LIMS. The import file for the Applied Biosystems® 7500 will be created in LIMS.

5. Seal the plate with optical film and briefly centrifuge. Transfer the 96-well plate to the 7500 Real-Time PCR System. For further information refer to Forensic Biology/DNA Quantitative PCR (qPCR): PowerQuant™ System.

6. Create a plate in the Applied Biosystems® 7500 software using the LIMS import file.

7. Upon completion of the run on the Applied Biosystems® 7500 Real-Time PCR System, export the data and import into LIMS.

**Normalization and Amplification Setup on the Maxprep™ Liquid Handler**

1. In LIMS, select items for amplification and generate an amplification worksheet.

2. Identify and indicate which samples need to be concentrated and/or combined. Combine and/or concentrate the samples using the SpeedVac. Resolubilize samples in the appropriate amount of sterile water.

3. Prepare a Maxwell® RSC 48 deck tray for amplification by placing the microcentrifuge tubes on the deck tray in the order dictated by the LIMS worksheet.

4. From LIMS, create an amplification setup export file.

    a. In the Portal on the Maxprep™ Liquid Handler computer, navigate to Labware on the sidebar menu and select "Import" tab.

    b. Change the Format to "Maxwell®48-Position" and the Template to "Maxwell® Deck Tray".

    c. Select "+ Select Import File…" and import the amplification setup file from LIMS.

    d. Navigate to Concentration Data on the sidebar menu and select the PowerQuant template from the table.

    e. Select "Import Concentration Data," then select "+ Select File to Import…"and import the same amplification setup file from LIMS.

5. Launch the software on the Maxprep™ Liquid Handler and on the home screen select "Start".

6. Select the Promega DNA Normalization and STR Setup method.

    - If there are multiple variants, select the appropriate variant.

**Note:** Ensure that all sample tubes are open before running the method.

7. Upon completion of the method, export the STR Setup and Normalization output files and upload to the LIMS amp worksheet.

8. Briefly centrifuge the 96-well plate and transfer to a thermal cycler. For further information refer to Forensic Biology/DNA Amplification and Electrophoresis of STRs: PowerPlex® Fusion.

### Capillary Electrophoresis Setup on the Maxprep™ Liquid Handler

1. Launch the software on the Maxprep™ Liquid Handler and on the home screen select "Start".

2. Select the Capillary Electrophoresis Sample Setup method.

    - If there are multiple variants, select the appropriate variant (i.e. one variant may be for use with a 3500 genetic analyzer and another variant may be for use with a 3500xl genetic analyzer).

3. Follow the GUI prompts to run the method.

    **Note**: The Maxprep™ Liquid Handler can accept up to four amplification plates to setup one capillary electrophoresis plate.

4. Upon completion of the method, export the output file and upload to the Capillary Electrophoresis worksheet in LIMS.

5. From LIMS, create a genetic analyzer import file.

6. Import the file into the genetic analyzer to create a plate map.

7. Cover the 96-well plate with a septum and centrifuge briefly.

8. Denature the samples at 95$^{o}$C for 3 minutes, then immediately place on ice or in a freezer for 3 minutes. This should be conducted just prior to loading the plate on the CE instrument.

## REPORT WORDING

Not applicable.

## REFERENCES


1. Maxprep™ Liquid Handler Operating Manual. [Online, 11/21]

2. Maxprep™ Liquid Handler Method for Preprocessing of Maxwell® FSC DNA IQ™ Casework Kit Samples in Tubes. [Online, 11/19]

3. Preprocessing Methods for the Maxprep™ Liquid Handler Protocol. [Online, 12/21]

4. Maxwell® RSC 48 Instrument Operating Manual. [12/22]

5. PowerQuant® System Setup Method for the Maxprep™ Liquid Handler. [Online, 02/21]

6. Promega DNA Normalization and STR Setup Method for the Maxprep™ Liquid Handler Technical Manual. [Online, 12/21]

7. Capillary Electrophoresis Sample Setup Method for the Maxprep™ Liquid Handler Technical Manual. [Online, 10/21]

8. Portal Access Technical Manual. [Online, 08/21]

9. Illinois State Police Internal Validation: Maxwell® RSC 48 Instrument/Maxprep™ Liquid Handler/Portal Software using methods for purification thru Capillary Electrophoresis Preparation.

10. Loten et al. Developmental Validation of the Maxwell® FSC DNA IQ™ Casework Kit on the Maxwell® RSC 48 Instrument. March 2018 [Promega White Paper]

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** RAPID DNA

**PROCEDURE:** BUCCAL SWAB STANDARD PROFILING USING THE APPLIED BIOSYSTEMS™ RAPIDHIT™ DNA SYSTEM

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Reviewed by:

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Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

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Jeanne M. Richeal
DNA Technical Leader

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Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

This procedure is used for profiling buccal swab standards using the Applied Biosystems™ RapidHIT™ DNA System with the RapidLink Software and GeneMarker® HID STR Human Identity Software v2.9.5. Buccal swab standards are processed using the RapidHIT™ ID ACE GlobalFiler Express Sample Cartridge. The GlobalFiler Express multiplex STR amplification kit contains 21 autosomal loci, including the 20 CODIS core loci, two Y-chromosome loci, and the Amelogenin locus.

## SAFETY CONSIDERATIONS

Observe Standard Laboratory Practices.

Reference SDS for all commodities prior to use.

Warning: Treat all reagents/samples as potential biohazards.

## PREPARATIONS

Store Primary Cartirdge (housing) at room temperature for up to 12 months, or 6 months once installed on the instrument.

Store the RapidHIT™ ACE GFE Sample Cartridge at 4°-10°C for up to 12 months.

Store the RapidHIT™ ID Utility Cartridge at 4°-10°C for up to 12 months.

Store the GFE Control (Ladder) Cartridge at 4°-10°C for up to 12 months.

Store the RapidHIT™ ID AB ACE GFE Positive Control Cartridge at 4°-10°C for up to 12 months.

Store the RapidHIT™ ID AB ACE GFE Negative Control Cartridge at 4°-10°C for up to 12 months.

## INSTRUMENTATION

Applied Biosystems™ RapidHIT™ ID DNA System

## SOFTWARE

RapidHIT™ System Software v.1.3.2.2 GeneMarker HID Software v.2.9.5

## MINIMUM STANDARDS AND CONTROLS

1. A RapidHIT ™ ID DNA System run must contain an appropriate allelic ladder and size standard. An allelic ladder is installed on the instrument during the primary cartridge installation. Each sample contains and Internal Lane Standard (ILS) that is comprised of peaks from 80-500 base pairs. If the ILS fails, Applied Biosystems™ will be contacted.

2. A RapidHIT™ AB ACE GFE Positive Control Cartridge and RapidHIT™ AB ACE GFE Negative Control Cartridge are required to evaluate each new lot number of RapidHIT™ ID ACE GFE Sample Cartridges

3. Reagent blanks are not required for a run because this is a closed system.

## CRITICAL REAGENTS

RapidHIT™ ACE Sample Cartridges

## PROCEDURE

### Performing Analysis

1. Open the RapidHIT™ ID ACE GFE Sample Cartridge and insert the swab into the sample location on the cartridge. The swab should be approximately 3 inches long or less to be inserted into the sample location on the cartridge. Break and/or cut the swab stick to the appropriate length.

2. Log in to the instrument.

3. Scan the swab barcode and place the buccal swab into the appropriate chamber, ensuring that the cap clicks into place. Enter the sample identification into the instrument.

4. Insert the sample cartridge into the instrument.

5. The instrument run will automatically begin. The run time is approximately 90 minutes.

6. Upon completion of the run, select "DONE" on the instrument and remove the sample cartridge from the instrument.

### Data Analysis

1. Results are analyzed using the RapidLink™ and GeneMarker® HID DNA Analysis Software. Each sample is given a green check, yellow X, or red X.

    a. A green check indicates the sample passed at the 20 CODIS core loci and is ready for upload.

    b. A yellow X indicates the sample was flagged and is not ready for upload into CODIS. Possible reasons include a rare off-ladder allele or a tri-allelic pattern at a CODIS core locus. These samples will be re-profiled using PowerPlex® Fusion 5C.

c. A red X indicates the sample failed and is not ready for upload into CODIS. Possible reasons include RFUs that are either too low or too high, or a mixture is present. These samples will be re-profiled using PowerPlex® Fusion 5C.

2. An .xml file is generated for each sample that has a CODIS eligible profile.

   a. Edit sample header information as to the appropriate sample name, specimen category, suffix, source ID, and specimen partial.

   b. Include edited CMF into case assignment.

3. The electropherograms and raw data must be attached to the case assignment in LIMS.

## REFERENCES


1. RapidHIT™ ID System v1.0 Site Preparation Guide.

2. RapidHIT™ ID System v1.3.2 User Guide.

3. Applied Biosystems RapidHIT™ ID System Internal Validation Report.

4. Tan, E., et al. Fully integrated, fully automated generation of short tandem repeat profiles. Investigative Genetics 2013; 4:16.

5. Turingan, R., et al. Rapid DNA analysis for automated processing and interpretation of low DNA content samples. Investigative Genetics 2016; 7:2.

6. Manna, A., et al. Developmental validation of the DNAscan* Rapid DNA Analysis™ instrument and expert system for reference sample processing. Forensic Science International: Genetics 2016; 25: 145-156.

7. Grover, R., et al. FlexPlex27 – highly multiplexed rapid DNA identification for law enforcement, kinship, and military applications. International Journal of Legal Medicine. 03 March 2017.

8. Carney, C., et al. Developmental validation of the ANDE™ rapid DNA system with FlexPlex™ assay for arrestee and reference buccal swab processing and database searching. Forensic Science International: Genetics 2019; 40:120-130.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

**PROTOCOL:** DNA ANALYSIS

**METHOD:** PRESERVATION OF EXTRACTED DNA AND REAGENT BLANKS

**PROCEDURE:** PRESERVATION OF EXTRACTED DNA AND REAGENT BLANKS

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Reviewed by:

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Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____

Jeanne M. Richeal
DNA Technical Leader

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Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

This procedure will be used to dry down extracted DNA from evidence samples and their associated reagent blanks.

## SAFETY CONSIDERATIONS

Observe standard laboratory practices.

Warning: Treat all reagents/samples as potential biohazards.

## PREPARATIONS

None

## INSTRUMENTATION

Vacuum centrifuge(s)

## MINIMUM STANDARDS AND CONTROLS

Not applicable

## PROCEDURE

1. Extracted DNA and the associated reagent blanks that are frozen will need to be thawed. All samples will be spun down to remove condensation from the lids.

2. Decontaminate the vacuum centrifuge with 50% methanol, 50% ethanol, or mild detergent per the manufacturer's recommendation.

3. If using one vacuum centrifuge, the samples will be dried down separately in the following order:

    a. Unknown samples
    b. Reference standards
    c. Reagent blanks

    If multiple vacuum centrifuges are available, each type of sample may be dried down concurrently in separate instruments.

4. Centrifuge the samples at high temperature for thirty minutes, then check the tubes for dryness. Repeat, as necessary, in thirty-minute increments until the sample is fully dry.

5. Package the samples from evidentiary items for return to the agency. The reagent blanks will be retained and tracked by the laboratory.

## REFERENCES

1. ISP R&D project: Effects of Drying Times and Temperatures on the Preservation of Extracted DNA (IP04-01).

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

## APPENDIX IA: FORENSIC BIOLOGY REPORT WORDING

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Reviewed by:

_____
Forensic Scientist Katherine A. Sullivan, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____
Brenda Danosky
FB/DNA Program Manager

## APPENDIX IA
## FORENSIC BIOLOGY REPORT WORDING

This appendix includes suggested wording for a number of different analytical situations. The analyst should consider all available case information when formulating their conclusions. Each case is different and not all situations can be covered by the guidelines below.

Forensic Biology reports are written using this basic format:

### I. Exhibit number and description

This section contains a list of the exhibits submitted. A description of the packaging is not required.

### II. Findings

This section contains a brief description of the results of the examination or analysis. In many cases, the findings will consist of the name of the substance indicated/identified or a description of the materials observed. The results of any confirmatory tests performed will be reported, even if negative. This may require the results of several tests to be reported separately. Inconclusive results should only be reported when there is not enough sample to repeat the test and other test results are not available.

| **Sample/Test Performed** | **Suggested Wording** |
|---|---|
| Blood or Buccal Standards | "Preserved." or "Portion preserved." |
| Bloodstains | |
| a. Small stains that would be consumed in testing | "No testing at this time due to small sample size. Stain preserved." |
| b. Positive results | |
| Kastle-Meyer (KM) test | "Blood indicated." or "Blood indicated. Further analysis will be conducted upon request." |
| Species test | "Human material identified." |
| KM and species tests | "Human material identified. (Blood indicated.)" |

**Note:** Species testing should only be conducted in those instances when identification of species is needed prior to DNA testing.

c. Inconclusive results

| | |
|---|---|
| KM test | "Examination for blood inconclusive." |
| Anti-human test | "Could not be identified as human material." |
| Anti-species (non-human) test | "Species origin could not be determined." |

d. Negative results/insufficient samples

| | |
|---|---|
| KM test | "No blood indicated." |
| Anti-human test | "Could not be identified as human material." |
| Anti-species (non-human) test | "Species origin could not be determined." |
| Insufficient sample | "Insufficient sample for further testing." |

<u>Saliva</u>

| | |
|---|---|
| a. Positive results | "Saliva indicated." or "Amylase detected in levels indicative of saliva. Amylase is found in high levels in saliva but can also be found in other body fluids." |
| b. Inconclusive results | "Examination for saliva inconclusive." |
| c. Negative results | "No saliva indicated." |

<u>Semen</u>

a. Positive results

| | |
|---|---|
| Sperm search (KPIC), regardless of the result of any other test for semen | "Semen identified." and/or "Spermatozoa identified." |
| P30 test, when KPIC is not performed or the result is negative | "Semen indicated." or "P30 detected in levels indicative of semen. P30 is found in high levels in semen but can also be found in other body fluids." |
| Acid phosphatase (AP) test +3 or +4, when AP is the only test performed | "Semen indicated." or "Acid phosphatase detected in levels indicative of semen. Acid Phosphatase is found in high levels in semen but can also be found in other substances." |

b. Inconclusive Results

| | |
|---|---|
| KPIC test, P30 test, or AP +2 or lower | "Examination for semen inconclusive." and/or "Examination for spermatozoa inconclusive." |

c. Negative results/insufficient sample

| | |
|---|---|
| P30 test and KPIC, regardless of AP result (if performed) | "No semen identified." |
| KPIC only | "No spermatozoa identified." |
| P30 test, regardless of AP result | "No semen indicated." |
| AP test, when AP is the only test performed | "No semen indicated." |

| | |
|---|---|
| Fingernail scrapings/clippings | "Apparent debris observed."<br>"No apparent debris observed."<br>"Swabbing collected." |
| Hairs/Fibers/Debris | "Apparent hairs/fibers/debris observed/ collected." or "This item was taped for removal of apparent hairs, fibers, and debris." |
| Hairs (examined for DNA potential) | "Apparent hair(s) observed. Preserved for DNA analysis."<br>"No apparent root or tissue observed."<br>"Does not appear human in origin." |
| Touch samples | "Preserved." or "Swabbing collected." |

## III. Requests

This section may include:

- Requests for additional samples or standards to be submitted.
- Notifications regarding microscopy evidence, exhibits not examined, consumption requirements, or due diligence.
- Statements encouraging contact with the agency or attorneys involved.

Suggested phrases

"At such time as known blood or buccal samples from XXX are submitted, the possible source of the XXX indicated/identified on the evidence may be determined."

"At such time as known blood or buccal samples from XXX are submitted, the possible source of the samples collected/preserved from the evidence may be determined."

"If during the source of your investigation a suspect is developed, please submit a known blood or buccal standard for comparison with the samples collected from the evidence."

"When all appropriate standards are submitted, further analysis may be conducted."

"In the event sexual contact occurred at any time within seven days prior to the alleged sexual assault, it is necessary to obtain known blood or buccal standards from individuals with whom sexual contact had occurred."

"Pending DNA results, no examinations were performed on Exhibit # at this time. If at a later date it is determined that the value of this evidence can significantly aid this case, please advise."

"Other exhibits were received in this case but were not analyzed."

"Microscopy evidence (specify type) was observed/collected in this case. Please notify the laboratory if additional analysis would aid in your investigation."

"In order for the laboratory to exercise due diligence in analyzing this case, it is necessary for you to contact us as soon as a trial date is established. The DNA analytical process does take a significant amount of time and without sufficient notification it may not be possible for the DNA analyst to meet court deadlines."

"The samples collected from Exhibits XXX may need to be consumed in DNA analysis due to limited sample size. Authorization is required before DNA analysis can be conducted. Please contact the Forensic Biology Section at your earliest convenience."

"If you have any questions regarding this report, please feel free to contact me."

## IV. Evidence Disposition

The disposition of all exhibits referenced in the report must be mentioned in this section, whether transferred to another section, retained by the laboratory, returned to the submitting agency, ready for agency pick-up, or destroyed.

Suggested phrases

"Portions of Exhibit(s) XXX will be submitted for DNA analysis. If analyzed, those findings will be the subject of a separate report."

"Exhibit(s) XXX will be retained in our evidence vault and should be picked up at your earliest convenience."

"Evidence from this case will be returned to your agency."

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

## APPENDIX I-B: DNA REPORT WORDING

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**Reviewed by:**

_____
Forensic Scientist Katherine Sullivan, Chairperson
Forensic Biology/DNA Command Advisory Board

**Approved by:**

_____
Forensic Scientist Jeanne M. Richeal
DNA Technical Leader

_____
Forensic Scientist William E. Frank
DNA Technical Leader

_____
Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

Upon completion of analytical testing and the interpretation of those results, a laboratory report will be provided to the submitting agency and/or their designee. Expectations regarding format and content of DNA reports, as well as report wording suggestions can be found in this appendix.

## SECTIONS OF THE REPORT

### Evidence Description

The first section of the report will consist of two columns with the headings, Exhibit and Description. Analysts may choose to list the exhibits in one of the following ways:

1. List only the exhibits analyzed.

2. List all exhibits submitted with a notation specifying exhibits that were not analyzed.

### Results

This section of the report will contain the following information for each exhibit analyzed:

1. A statement describing the technology (i.e. STR or Y-STR) and the loci amplified (i.e. the amplification kit or a list of the individual loci). For outsourced cases, the vendor laboratory conducting the analysis will be identified.

2. The type of profile identified.

   A. No human DNA profile.

   B. A single-source human DNA profile.

   C. A mixture of human DNA profiles.

      For all mixtures, the number of contributors must be stated if it can be determined.

3. Partial profiles.

   Partial profiles must include either a list of the loci identified or a statement indicating the number of loci identified.

   **Note**: Female DNA profiles without results at DYS391 are considered complete.

4. A comparison statement.

   A. Cannot be excluded (is included)
   The reporting of an association between an unknown profile and a reference standard. This wording will be used in the following situations:

1. Single-source profiles or those deduced to a single genotype or haplotype at the interpreted loci when reference standard and/or other unknown profiles are concordant.

2. Mixtures where profiles cannot be deduced to a single genotype or haplotype at the interpreted loci when reference standard and/or other profiles cannot be excluded.

B. Excluded
The reporting that no association exists between an unknown profile and a reference standard. This wording will be used in the following situations:

1. Single-source profiles or those deduced to a single genotype or haplotype at the interpreted loci when reference standard and/or other unknown profiles are not concordant.

2. Mixtures where profiles cannot be deduced to a single genotype or haplotype at the interpreted loci when reference standard and/or other profiles can be excluded.

C. Inconclusive
Applies to profiles that are unsuitable for comparison. Reporting a list of loci identified or the number of detected loci is not required for profiles that are unsuitable for comparison.

5. A statistical statement to report the weight of the match/inclusion for all probative samples. For exhibits on which a differential extraction has been performed, only the most informative statistic will need to be reported when the probative contributor(s) is identified in more than one fraction. If the statistic is being calculated using fewer loci than those identified in the profile, a list of the loci or the number of loci must be included in the statistical statement. This does not apply to the absence of DYS391 in female DNA profiles.

6. A CODIS statement, when appropriate.

7. Any additional information about the evidence.

**Requests**
This section of the report is used to request items from the submitting agency. It will also be used to reference prior reports.

**Evidence Disposition**
A disposition must be provided for all exhibits listed in the report. This may include: transferred to another section, retained by the laboratory, returned to the submitting agency, ready for agency pick-up, or destroyed.

A statement is required when an item has been consumed in analysis and must indicate whether extracted DNA remains for further testing.

## EXAMPLES OF REPORT WORDING STATEMENTS

The report wording suggestions on the following pages may require modification based on specific case situations. The analyst will need to determine the wording that is technically correct for their analytical results. The technical reviewer will confirm the wording is appropriate for the analysis conducted.

### Exhibits Not Examined

Exhibit XXX was not analyzed/examined [at this time].

### Team approach evidence handling

DNA analysis was conducted by Forensic Scientists XXX and the results were interpreted by the author of this report.

### qPCR Only

The following suggestions are for use in those situations when exhibits are quantified but not amplified:

> Quantitative PCR indicates a mixture of female and male DNA.
>
> The proportion of male and female DNA identified in the tested portion of Exhibit(s) XXX indicates this sample should be directed to Y-STR analysis.
>
> Exhibit XXX was not profiled due to the proportion of male and female DNA identified.
>
> Quantitative PCR did not detect any human/male DNA [in the tested portion of] Exhibit XXX. Further analysis of this evidence has been deferred at this time.
>
> Exhibit XXX was not profiled [at this time].

### Method of Amplification and Loci

[Short Tandem Repeat (STR) or Y Chromosome Short Tandem Repeat (Y-STR)] analysis was performed on Exhibits X and Y at the following loci: (to be followed by a list of the appropriate loci).

[Short Tandem Repeat (STR) or Y Chromosome Short Tandem Repeat (Y-STR)] analysis was performed by [vendor name] on Exhibits X and Y at the following loci: (to be followed by a list of the appropriate loci).

DNA from Exhibits X and Y was amplified and profiled at the [Short Tandem Repeat (STR) or Y Chromosome Short Tandem Repeat (Y-STR)] loci characterized by the PowerPlex® [name of amplification kit] System.

[Short Tandem Repeat (STR) or Y Chromosome Short Tandem Repeat (Y-STR)] analysis was performed on Exhibits X and Y using the PowerPlex® [name of amplification kit] System.

## Type of Profile Identified

1. **Single-source**

   Complete profile:
   A human DNA profile/Y-STR haplotype was identified in Exhibit X.

   Partial profile:
   A human DNA profile/Y-STR haplotype was identified in Exhibit X at the XXX loci.

   A human DNA profile was identified in Exhibit X at XXX of XXX loci.

2. **Mixture**

   A mixture of human DNA profiles was identified in Exhibit X that has been interpreted as a mixture of XXX people.

   A mixture of human DNA profiles was identified in Exhibit X that has been interpreted as a mixture of at least XXX people.

   The Y-STR haplotype results identified in Exhibit X were interpreted as a mixture of DNA from XXX males.

3. **No STR results**

   No human DNA profile/Y-STR haplotype was identified in Exhibit X.

## Comparison Statements

1. **Single-source DNA profiles/Y-STR haplotypes, major/minor profiles differentiated from a mixture with multiple genotypes at one or more loci, or unresolved mixtures.**

   A [major/minor] [male/female] human DNA profile/Y-STR haplotype was identified in Exhibit X from which XXX can be excluded/cannot be excluded (is included).

   Assuming this is a mixture of XXX and one other individual, a [male/female] DNA profile/Y-STR haplotype was identified from which YYY can be excluded/cannot be excluded (is included).

XXX can be excluded/cannot be excluded (is included) as having contributed to the [major/minor] DNA profile/mixture of DNA profiles identified in Exhibit X.

XXX can be excluded/cannot be excluded (is included) as having contributed to the [major/minor] Y-STR haplotype/mixture of Y-STR haplotypes identified in Exhibit X.

2. **Inconclusive comparisons**

A DNA profile/mixture of DNA profiles was identified in Exhibit X that is unsuitable for comparison.

A Y-STR haplotype/mixture of Y-STR haplotypes was identified in Exhibit X that is unsuitable for comparison.

## Statistical Statements

The expected frequency of occurrence for this profile/mixture of DNA profiles was calculated [at the number/names of loci] for the African American, Caucasian, and Hispanic population groups and was found to be no more common than approximately 1 in XXX unrelated individuals.

With a 95% upper confidence limit, this haplotype would be expected to occur in approximately 1 in XXX unrelated African American males, 1 in XXX unrelated Caucasian males, and 1 in XXX unrelated Hispanic males based on a database of XXX African Americans, XXX Caucasians, and XXX Hispanics at the DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS438, DYS437, DYS635, DYS390, DYS439, DYS392, DYS393, DYS458, DYS385a/b, DYS456, and YGATAH4 loci.

Based on these Y-STR haplotype results, it is at least XXX times more likely that XXX or a paternal relative is the contributor than if the source of the evidence is a randomly selected individual at the DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS438, DYS437, DYS635, DYS390, DYS439, DYS392, DYS393, DYS458, DYS385a/b, DYS456, and YGATAH4 loci.

These Y-STR haplotype results were/This mixture of Y-STR haplotypes was searched against a pooled known database consisting of unrelated African American, Caucasian, and Hispanic males. This mixture of [major/minor] Y-STR haplotypes would be expected to occur in approximately X% of unrelated males [at the number/names of loci] based on a 95% upper confidence limit.

The combined results of the autosomal DNA profile and the Y-STR haplotype identified in Exhibit XXX would be expected to occur in approximately 1 in XXX African American, 1 in XXX Caucasian, and 1 in XXX Hispanic unrelated individuals.

## Additional Situations

1. **Differential extractions exhibiting carry over - reporting on the whole exhibit:**

This is generally applied when the major profile in each fraction can be resolved to a single genotype at each locus without assuming and minor types are observed that are attributable to another fraction. When mixtures are observed in any fraction that are not attributable to carry-over, the fractions must be reported separately.

**2. Partial match/potential-relative match**

The wording suggestions below are not intended to replace any other wording necessary to report case findings. These statements may be combined with other necessary wording to ensure a clear and concise report. Please note the statements below may need to appear in separate reports.

A. Potential-relative match with a signed Potential-Relative DNA Match Acknowledgment Form

Benchwork comparison

Autosomal report: An autosomal DNA profile was identified in Exhibit XXX from which XXX was excluded but there were genetic similarities to warrant Y-STR analysis for these exhibits.

Y-STR report: A Y-STR haplotype was identified in Exhibit XXX from which XXX cannot be excluded (is included).

CODIS comparison

Autosomal report: An autosomal DNA profile was identified in Exhibit XXX that has been searched against the Combined DNA Index System (CODIS). This profile has genetic similarities to a profile in CODIS that warrant Y-STR analysis of this exhibit.

Y-STR report: A Y-STR haplotype was identified in Exhibit XXX from which XXX cannot be excluded (is included).

Mandatory for all reports

These results should be evaluated in conjunction with the results reported by Forensic Scientist XXX of the XXX Laboratory.

The Potential-Relative DNA Match Acknowledgment Form has been signed and the information has been released to XXX.

B. Potential-relative match where the Potential-Relative DNA Match Acknowledgment Form has not been signed

Benchwork comparison

Autosomal report: An autosomal DNA profile was identified in Exhibit XXX from which XXX was excluded; however, additional DNA analysis was warranted for these exhibits.

Additional DNA analysis was conducted on Exhibits XXX; however, the laboratory will not be able to release this information until further discussion has occurred with the agency and the appropriate documentation has been obtained.

Y-STR report: Y-STR haplotypes were identified in Exhibits XXX and a comparison was conducted. However, the laboratory will not be able to release this information until further discussion has occurred with the agency and the appropriate documentation has been obtained.

<u>CODIS comparison</u>

Autosomal report: An autosomal DNA profile was identified in Exhibit XXX that had no associations in the Combined DNA Index System (CODIS). However, additional DNA analysis was warranted for this exhibit.

Additional DNA analysis was conducted on Exhibit XXX; however, the laboratory will not be able to release this information until further discussion has occurred with the agency and the appropriate documentation has been obtained.

Y-STR report: A Y-STR haplotype was identified in Exhibit XXX to which an association was identified in the Combined DNA Index System (CODIS). However, the laboratory will not be able to release this information until further discussion has occurred with the agency and the appropriate documentation has been obtained.

<u>Mandatory for all reports</u>

These results should be evaluated in conjunction with the results reported by Forensic Scientist XXX of the XXX Laboratory.

C. Partial match and Y-STR analysis has excluded the individual as a paternal relative of the source of the DNA profile/haplotype

Report will include the results of the analytical findings only, using the standard report wording in preceding sections.

## CODIS Statements

The DNA profile/Y-STR haplotype identified in Exhibit X has been included in the DNA Index.

The DNA profile/Y-STR haplotype from Exhibit X has been included in the DNA Index and will continue to be compared to other profiles. You will be notified if a consistent profile is detected.

This profile has been searched against the DNA Index. The search did not detect a profile consistent with the profile in this case.

The DNA profile identified in Exhibit X was searched against the DNA Index. The search detected a possible association with XXX, from (State), SID # XXX. This individual demonstrates a DNA profile that is consistent with the evidence profile and could be the donor of the biological material identified.

This profile has been searched against the DNA Index. The search detected a possible association to Laboratory Case # XXX (Agency Name, Agency Case Number), Exhibit Y.

## Requests Statements

This information can be used for investigative purposes only. Please submit an additional [standard] [sample] from XXX for confirmatory forensic analysis.

Other exhibits were received in this case, but were not analyzed.

Please refer to the evidence receipt for a list of all exhibits received on this case.

Please contact (Officer's name) of (Agency name) at (phone number) for further information.

For results of previous biological examinations, please refer to the laboratory report by Forensic Scientist Jane Doe from (laboratory name).

For results of previous biological examinations, please refer to the laboratory report by Forensic Scientist Jane Doe from (laboratory name) dated XXX.

For results of previous biological examinations, please refer to my laboratory report dated XXX.

For results of previous DNA analysis, please refer to my laboratory report dated XXX.

Upon submission of additional standards, further analysis can be conducted to resolve the source of the open profile identified in Exhibit X.

A portion of Exhibit(s) XXX was tested. Please notify the laboratory if additional analysis of this evidence would aid in your investigation.

## Evidence Disposition Statements

The evidence will be held in the laboratory vault and should be picked up within thirty days.

The evidence will be held in the laboratory vault and may be picked up at your earliest convenience.

The evidence has been returned to your agency via registered mail.

Please note that Exhibit X was consumed in analysis. Extracted DNA remains for further testing.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

## APPENDIX II: FORMS

Note: These pages contain representations of approved forms not contained in LIMS. The electronic versions of these forms are found on the transfer server, in the Biology-DNA worksheets folder (\\statepolice.il\ISP\DFS\FSC\Statewide\FSC_Worksheets\Biology-DNA).

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Reviewed by:

_____
Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____
Jeanne M. Richeal
DNA Technical Leader

_____
Brenda Danosky
FB/DNA Program Manager

**INCIDENT DOCUMENTATION FORM**

**Analyst:**_____ **Laboratory:**_____

**Case Number:**_____ **Date of Incident:**_____

**Briefly describe the incident (Analyst):**

**Initials Date**

**Analyst:**_____

**Supervisor:**_____

**TL:**_____

**Course of action by TL:**

**Initials Date**

**TL:**_____

**Resolution of course of action by Analyst:**

**Initials Date**

**Analyst:**_____

**Supervisor:**_____

**TL:**_____

**Follow-up by TL:**

**Initials Date**

**TL:**_____

DNA 8 (5/08)

**[Enter Title of Study]**

**[Enter Laboratory Name] SITE-SPECIFIC ASSESSMENT**

**Precision Assessment**

Summary of Data:

[Enter Precision Summary here]

**Sensitivity Assessment**

Summary of Data:

[Enter Sensitivity Summary here]

**Contamination Assessment**

Summary of Data:

[Enter Contamination Summary here]

**Conclusion**

[Enter Conclusion here]

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Supervisor Review: _____ Date: _____

TL Approval: _____ Date: _____

Laboratory Implementation Date: _____

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DNA 18 (7/18)

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

## APPENDIX IV-A: FORENSIC BIOLOGY QUALITY ASSURANCE

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Reviewed by:

_____

Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____

Brenda Danosky
FB/DNA Program Manager

# APPENDIX IV-A
# FORENSIC BIOLOGY QUALITY ASSURANCE
# TABLE OF CONTENTS

## FORENSIC BIOLOGY QUALITY ASSURANCE
## GOALS AND OBJECTIVES

I. Overall goals:

To provide comprehensive, uniformly accessible, high quality, state of the art forensic biology services to the citizens of the State of Illinois; and

To ensure the quality of this Forensic Biology testing.

II. Objective:

To have documented Forensic Biology procedures which ensure the output of a quality product;

To routinely monitor Forensic Biology testing; and,

To document the identification and correction of problems with Forensic Biology testing.

## FORENSIC BIOLOGY QUALITY ASSURANCE PROGRAM
## ORGANIZATION AND MANAGEMENT

I. The Forensic Biology Quality Assurance Program is part of the Command's Quality Assurance Program. The following topics are addressed in the Command QA Manual:

Command Quality Assurance Program
Competency Testing
Proficiency Testing
Administrative Reviews
Command Quality Assurance Reviews
Mock Trial/Court Appearance Rating
Forensic Biology Quality Assurance
Corrective Action

## FORENSIC BIOLOGY QUALITY ASSURANCE
## FORENSIC BIOLOGY PERSONNEL QUALIFICATION AND TRAINING

I. Personnel Conducting Forensic Biology casework

A. Prerequisites for Forensic Biology training/casework
Prior to assuming casework responsibilities, each analyst must have a bachelor's degree in a natural science or its equivalent.

B. Training/Qualifying

1. Each individual will complete a formal period of training or evaluation prior to assuming independent casework responsibilities.

   a. New analysts will complete the documented Forensic Biology training program.
   b. Experienced analysts will have their training program documentation and technical knowledge reviewed and evaluated.

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 designate.

C. Certification
Final approval for conducting independent casework rests with the Command Administration.

Initial certification is based on a recommendation by a training coordinator prior to beginning independent casework within the Command.

To be certified in this manner, an individual will do the following:

1. Demonstrate the ability to analyze blood and body fluid stains;
2. Demonstrate the ability to reproduce accurate and precise results;
3. Demonstrate the ability to conduct analysis on non-probative cases;
4. Demonstrate theoretical knowledge of Forensic Biology analysis;
5. Successfully complete criterion tests;
6. Successfully complete a mock trial; and
7. Successfully complete supervised casework.

D. Continuing Education (CE)
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

III. The Forensic Biology Training Coordinators duties are as follows:

A. Deliver training to new employees assigned to the Forensic Biology section and provide cross-training to existing employees when required.

B. Create, administer, and grade written and practical examinations.

C. Review all supervised casework generated by trainees.

D. Ensure that all quality control requirements/quality assurance guidelines are followed by individuals in training.

E. Monitor court testimony of trainees by actual viewing of testimony.

F. Lend assistance to analysts in court preparation.

G. Provide accurate and timely communications to Forensic Biology trainees regarding Command decisions affecting Forensic Biology analysis.

H. Review internal proficiency tests by taking the proposed annual test to determine its validity.

IV. Laboratory Technicians

A. Will have documented training, education and experience commensurate with their responsibilities as outlined in job description;

B. Will perform duties commensurate with their level of training such as bleaching, performance checks, reagent preparation, and QA/QC testing.

C. Will return DNA evidence in accordance with the Evidence Control Policy.

## FORENSIC BIOLOGY QUALITY ASSURANCE
## SAMPLE HANDLING AND FACILITY REQUIREMENTS

I. All Forensic Biology analysts will follow clean technique as documented in the Forensic Biology/DNA Procedures Manual.

II. Cleaning and Sterilization Procedures

A. Appropriate glassware and plastic containers will be cleaned and completely rinsed with tap water by hand or using a dishwasher. Before laboratory use, these items will be rinsed with distilled water or equivalent. Items that come in contact with Forensic Biology/DNA samples will be cleaned with detergent and 10% bleach solution, followed by a rinse with distilled water or equivalent.

B. The reagent preparation section details those reagents which require sterilization by autoclaving.

C. The laboratory will follow the decontamination procedures outlined in the Clean Technique section. A bleach log is used to monitor decontamination of facilities and equipment and will include the items below:

1. The Forensic Biology/DNA laboratory floor will be mopped using a freshly prepared 10% bleach solution once a week. The PCR room must be mopped last unless there is a dedicated mop in the PCR room. This must be documented in the Laboratory Asset Manager (LAM).

2. The entire Forensic Biology/DNA laboratory (computer tops, equipment, etc.), including the PCR room, must be bleached once a week with a freshly prepared 10% bleach solution. This must be documented in the LAM.

## FORENSIC BIOLOGY QUALITY ASSURANCE
## EVIDENCE CONTROL

I. Illinois State Police laboratory system has a documented evidence control system to ensure the integrity of physical evidence. This is outlined in the following Command directives:

Evidence Receipt Forms
Submission of Physical Evidence by Mail
Submission of Forensic Biology Evidence
Blood Evidence
Submission of Evidence to the FBI
Collection of Biological Standards
Access to Physical Evidence
Evidence Packaging
Transferring Cases Between Laboratories
Case Tracking
Destruction of Physical Evidence
Documentation of Case Related Phone Calls or Conversations
Signature Requirements for Case Reports
Minimum Standards for Evidence Marking
Internal Evidence Chain
Case Acceptance Policy for DNA Analysis
Clean Technique
Uniform Guidelines for Mailing Evidence

II. Biological Evidence Retention and Return Policy
All items of evidence including parent exhibits and sub-exhibits must be returned after analysis is completed.

## FORENSIC BIOLOGY QUALITY ASSURANCE
## VALIDATIONS

I. The laboratory will use validated methods and procedures as outlined in the Command Directives. Original copies of all validations study materials will be maintained at the Research and Development Laboratory. Electronic copies will be distributed to each regional laboratory.

II. Access to the status of current validation studies can be found in the R&D Laboratory section of the ISP intranet site.

III. References
References for Forensic Biology tests are found in the section for each test.

# FORENSIC BIOLOGY QUALITY ASSURANCE
# ANALYTICAL PROCEDURES

I. Procedures

The laboratory will have approved, written analytical procedures.

A. Procedures used in Forensic Biology analysis will be approved according to the Command Directives.

B. Procedures being developed as part of the R&D Program may be used in casework with Command approval.

II. Reagents

The laboratory will use reagents that are suitable for the methods employed.

A. The LAM will be used for documenting manufactured reagents and stock chemicals utilized in the laboratory.

Information recorded will include the manufacturer, the date a chemical or reagent was received, the lot numbers received, and the quantity received. The expiration date will also be recorded when appropriate.

B. The LAM will be used for documenting the preparation of all reagents used in the laboratory.

1. The formulas for all reagents are found in the Procedures Manual.

2. Information recorded must include the date the reagent was prepared, the lot numbers of chemicals used to prepare the reagent, the quantity prepared, the expiration date of the reagent, the identity of the preparer, and the date the reagent was quality checked.

C. Labeling Requirements

1. Stock chemicals and manufactured reagents will be labeled with the identity of the receiver, the date of receipt and the expiration date. If the chemical does not expire, it must be labeled accordingly.

2. Reagents made in the laboratory will be labeled with the name of the reagent, the concentration, the expiration date, storage conditions, a hazard warning and the identity of the preparer.

D. Expiration Dates

1. Stock chemicals will expire according to the manufacturer's listed expiration date, if any. If no date is specified, the chemical is considered stable and does not expire.

2. Reagents

    a. Manufactured reagents will expire according to the manufacturer's listed expiration date. If there is no date specified, the expiration date will be one year from the date of receipt at the laboratory.

    b. Reagents made in the laboratory will expire one year or less from the date of preparation, regardless of their storage conditions.

    c. Aliquots of species anti-sera will expire one month from the date they are thawed for use.

E. Critical Reagents

Species testing antisera have been defined as critical reagents.

F. Critical Reagent Quality Control

1. Procedures for quality control of these reagents are found in Appendix IV-A.

2. The LAM will be used for documenting all quality control procedures performed on a particular lot of a reagent.

3. If a particular supply, chemical, reagent or material does not meet the required quality control standard(s), the manufacturer will be notified and the entire lot rejected.

4. The quality control procedures for critical reagents do not have to be run individually but may be combined with other procedures as appropriate.

5. Quality control records will be maintained indefinitely.

III. Standards and Controls

The laboratory will monitor the analytical procedures using appropriate controls and standards. Minimum standards and controls are defined within each analytical procedure. Reagents should not be used in casework if the corresponding controls fail to perform as expected. The standards and controls used in Biology testing are qualitative internal reference materials and do not require re-authentication.

## FORENSIC BIOLOGY QUALITY ASSURANCE
## NON-CONFORMANCE

I. The non-conformance should be brought to the attention of the analyst's supervisor. Upon initial notification of the issue, the Director of Quality Assurance in conjunction with the appropriate Bureau Chief, will determine if a Quality Issue Report (QIR) form is necessary.

II. If a QIR is issued, a copy of the QIR will be maintained by the laboratory and the Director of Quality Assurance.

## FORENSIC BIOLOGY QUALITY ASSURANCE
## CRITICAL REAGENT QUALITY CONTROL OF SPECIES ANTISERA

I. Purpose

To compare new lot numbers of antisera (human and animal) to the appropriate series of known standards which are human, swine, bovine, deer, goat, cat, dog, sheep, chicken or duck (bird), rat, hamster or rabbit (rodent) as outlined in the Forensic Biology/DNA Procedures Manual.

II. Procedure

A. Punch the gel with a series of 7 wells to form a hexagon with a central well.

B. Place the antiserum being checked in the central well and bloodstains for 6 different species in the surrounding wells.

C. Cover the petri dish and leave undisturbed at room temperature overnight. The petri dish can be placed in a 37°C oven to decrease the incubation period or in the refrigerator for a longer incubation period.

D. Any known stain which forms a precipitin band with the antiserum must be checked using the following triangular 3-well Ouchterlony pattern:

Set up a positive control: known blood sample in both left and right wells of the triad.

Set up a negative control: known blood sample in one well and a negative (blank) in the other.

Place the antisera being checked in the third well.

E. Cover the petri dish and leave undisturbed overnight at room temperature. The petri dish can be placed in a 37°C oven to decrease the incubation period or in the refrigerator for a longer incubation period.

F. Record the results in the LAM.

III. Assessment of Results

Precipitin bands which form a continuous arc of convergence (identity) between the antiserum and the two extract wells are considered positive results.

If no or partial precipitin bands form and a positive result is expected, repeat test. If a positive result is noted for a species other than what antisera it is directed against, repeat test.

If after repeating the test, the results do not coincide with the expectations of the test, do not use that lot antiserum for casework. Notify the manufacturer.

## FORENSIC BIOLOGY QUALITY ASSURANCE
## EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE

I. Calibration and Performance Checks

A. For each piece of equipment requiring calibration or a performance check, the following information will be listed:

1. Procedure
2. Frequency
3. Results
4. Course of Action

B. Records will be maintained for calibration and performance checks.

C. Tolerance windows, where applicable, will be indicated in the LAM.

II. Maintenance

A. Where appropriate, maintenance procedures and schedules will be listed for equipment.

B. Records will be maintained in the LAM.

# FORENSIC BIOLOGY QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# BALANCES

I. Performance Check

A. Procedure: Use NIST certified weights. Handle them with cotton gloves or forceps. Check a minimum of three weights spanning the range of the balance to include 0.1 g, 1 g, and 10 g.

B. Frequency: Check monthly, or if used infrequently, prior to use.

C. Results: Record the results in the LAM.

D. Course of Action: If the balance does not read within ±2 readability units for each checked weight, then contact a repair company.

II. Maintenance

No regular maintenance is recommended by the manufacturer.

# FORENSIC BIOLOGY QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# OVEN TEMPERATURE CHECKS

I. Temperature Checks

A. Procedure: Record the temperature of the oven using a NIST traceable thermometer. Adjust the temperature dial if necessary and recheck.

B. Frequency: Check annually but observe temperature before each use.

C. Results: Record the results in the LAM.

D. Course of Action: If the temperature is not within the specified tolerance window, contact a repair company.

II. Maintenance

No regular maintenance is required.

# FORENSIC BIOLOGY QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# REFRIGERATORS AND FREEZERS

I. Temperature Checks

A. Procedure: Record the temperature of the refrigerator or freezer using a NIST traceable thermometer. Adjust the temperature dial if necessary and recheck.

B. Frequency: Check monthly.

C. Results: Record the results in the LAM.

D. Course of Action: If the temperatures cannot be maintained within the specified tolerance window, contact a repair company.

II. Maintenance

No regular maintenance is required. Defrost and clean as necessary. Contact the repair company for repairs.

# FORENSIC BIOLOGY QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# PIPETTES

I. Calibration

A. Procedure:

1. New pipettes that arrive from the manufacturer with a certificate of performance may be immediately used in casework. These pipettes should be scheduled for calibration at the next annual date.

2. All pipettes will be calibrated by a company qualified to provide certification for their accuracy and precision.

3. Each pipette must be checked in at least two settings (one low and one high) in its range of use.

B. Frequency: Annually.

C. Results: Each setting tested must be within the contract specifications for each pipette. Document the calibrations in the LAM.

D. Course of Action: Any pipette whose measurements are outside of the contract specifications must be removed from casework. Submit the pipette for repair and calibration.

II. Maintenance
No regular maintenance is required.

III. Use
Pipettes should not be used to collect or dispense any volume outside their calibration ranges.

# FORENSIC BIOLOGY QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# MICROSCOPES

I. Checks

A. Procedure: Microscopes will be cleaned and checked by a qualified professional. Optimum illumination will be established, where applicable.

B. Frequency: At least every two years, or as needed.

C. Results: Document the check in the LAM.

D. Course of Action: If problems are identified, contact a repair company.

II. Maintenance - refer to the Microscopy Procedures Manual

A. Clean microscopes as necessary.

B. Cover all microscopes after use.

# FORENSIC BIOLOGY QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# ALTERNATE LIGHT SOURCE

I. Checks

A. Procedure: Visually examine a 1:50 dilution of semen. This will be prepared and distributed by the Biology QRC as needed.

B. Frequency: Check monthly. If used less frequently, check before each use.

C. Results: Document the check in the LAM.

D. Course of Action: If the dilution is not visualized, retest using a new dilution. If it is still not visualized, contact a repair company.

II. Maintenance

No regular maintenance is required.

# FORENSIC BIOLOGY QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# WATER BATHS

I. Temperature Checks

A. Procedure: Record the temperature of the water in the bath using a NIST traceable thermometer. Adjust the temperature dial if necessary and recheck.

B. Frequency: Check monthly.

C. Results: Document the check in the LAM.

D. Course of Action: If the temperature cannot be maintained within +/- 2°C of the required setting, contact a repair company.

II. Maintenance

Empty, clean and refill with fresh water as needed.

## FORENSIC BIOLOGY QUALITY ASSURANCE
## EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
## THERMOMETERS

I. Only thermometers with ISO/IEC 17025 Certificate of Calibrations may be used. These thermometers must be traceable to national or international standards (e.g. NIST).

II. The calibration certificate for each thermometer will be entered in the LAM.

III. The vendor's calibration is the performance check. The thermometer may be placed into use immediately and used until the expiration date on its certificate.

# FORENSIC BIOLOGY QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# HEAT BLOCK TEMPERATURE CHECKS

I. Temperature Checks

A. Procedure: Record the temperature of the heat block using a NIST traceable thermometer. Adjust the temperature dial if necessary and recheck.

B. Frequency: Check monthly but observe temperature before each use. If not used regularly, check before use.

C. Results: Document the check in the LAM.

D. Course of Action: If the temperature is not within the specified tolerance window, repeat the test. If it continues to fall outside the range, replace the unit or contact a repair company.

II. Maintenance

No regular maintenance is required.

# FORENSIC BIOLOGY QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# BIOHOOD CERTIFICATION

I. Certification

A. Procedure:

1. Biohoods will be maintained by a company qualified to provide a certification of the biohoods' specifications.

2. All biohoods used for DNA will be certified before use in casework.

3. Certification should include, but is not limited to, a check of: the HEPA filter for leaks, the velocity of the air movement, and electrical safety.

B. Frequency: Annually.

C. Results: Document the certification in the LAM.

D. Course of Action: If the certification reveals parameters that do not meet specifications, contact a vendor or manufacturer for repairs and/or replacement parts.

II. Maintenance

Change light bulbs when needed and UV light bulbs biennially, or as indicated by the annual certification.

## FORENSIC BIOLOGY QUALITY ASSURANCE
## PROFICIENCY TESTING

I. Proficiency testing is performed in accordance with Command QA Manual, which includes the following:

Competency Tests
Internal Proficiency Tests
Blind Proficiency Testing
External Proficiency Testing

II. 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.

## FORENSIC BIOLOGY QUALITY ASSURANCE
## AUDITS

I. All Forensic Biology laboratories will be internally audited once a year according to guidelines established in the Command Quality Manual.

A record of the audit report will be maintained in the laboratory.

II. In addition to the Command's inspection program, external auditors will review the Forensic Biology section in the laboratory once every two years.

A. A record of the audit report will be maintained in the laboratory.

B. A copy of the external audit report will be sent to the Quality Assurance Program Administrator with an action memo addressing issues identified by the auditor.

## FORENSIC BIOLOGY QUALITY ASSURANCE
## REPORTS/NOTES PACKETS

I. All pages of the notes packet will be automatically appended with page numbers and initials of the analyst who authors the report.

II. The following notations will be included for examined items, when applicable:

A. Evidence Seals. Documentation of the sealed state of evidence upon receipt and completion of analysis (i.e. sealed, resealed, will reseal). Documentation describing the seal(s), such as color or type (e.g. heat, tape), is not necessary.

Shared packaging will be documented through creation of a case container. Shared packaging is defined as a single sealed package, created by the agency, which contains multiple parent items.

B. Description of evidence item(s). The location, size, and appearance of staining preserved for DNA analysis or staining with positive or inconclusive body fluid test results must be documented. The size of stain portion(s) preserved must be documented, including from which part of the stain it was removed. An annotated photograph may satisfy these requirements.

Documenting visual appearance or size of staining is not required for swabs or reference standards.

C. Disposition of evidence items and/or remaining stain specimen, including repackaging and any sub-items produced.

D. All testing performed and the results.

E. Documentation of pipettes is required.

III. Reporting information is found in Appendix I of the FB/DNA Procedures Manual.

# FORENSIC BIOLOGY QUALITY ASSURANCE REVIEWS

I. **Author Review**

Prior to submission of a case for administrative and technical review, all cases must be reviewed by the author of the report for clerical accuracy, technical accuracy and completeness. Documentation of the author review will be the signature of the forensic scientist on the report.

II. **Administrative and Technical Review**

Biology case files will be administratively and technically reviewed according to the guidelines established in the Command Quality Manual, QM-7.

Questions or concerns identified by the technical reviewer should be brought to the attention of the forensic scientist for resolution. If a difference of opinion occurs between the forensic scientist and the technical reviewer regarding this concern or question, the issue should be handled as outlined in the Quality Manual, QM-7 for all biology cases.

III. **Supervisory Review**

These are outlined in the Command Directives and in the Command Quality Manual. Supervisory reviews will be conducted as required.

IV. **Court Monitoring**

This is covered in the Command Quality Manual under Administrative Reviews and Courtroom Testimony Reviews.

## FORENSIC BIOLOGY QUALITY ASSURANCE
## SAFETY

I. The safety program is found in the Command Safety Manual and laboratory's Facility Operations Manual.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

## APPENDIX IV-B: DNA QUALITY ASSURANCE

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Reviewed by:

_____

Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

Approved by:

_____

Jeanne M. Richeal
DNA Technical Leader

_____

Brenda Danosky
FB/DNA Program Manager

# APPENDIX IV-B

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## DNA QUALITY ASSURANCE
## GOALS AND OBJECTIVES

I. Overall goals:
To provide comprehensive, uniformly accessible, high quality, state of the art DNA testing services to the citizens of the State of Illinois; and,

To ensure the quality of this DNA testing; and,

To meet FBI Quality Assurance Standards (QAS) as part of ISO 17025 requirements.

II. Objective:
To have documented DNA procedures which ensure the output of a quality product;

To routinely monitor DNA testing; and,

To document the identification and correction of problems with DNA testing.

## DNA QUALITY ASSURANCE PROGRAM
## ORGANIZATION AND MANAGEMENT

I. The DNA Quality Assurance Program is part of the Command's Quality Assurance Program. The following topics are addressed in the Command QA Manual:

Command Quality Assurance Program
Competency Testing
Proficiency Testing
Administrative Reviews
Command Quality Assurance Reviews
Mock Trial/Court Appearance Rating
Forensic Biology/DNA Quality Assurance
Corrective Action

II. Current Forensic Sciences Command (FSC) organizational charts are posted on the FSC intranet site.

A. The casework and Indexing Laboratory DNA technical leader assignments are identified on FSC organizational charts.

1. A qualified forensic scientist in the FSC will serve as the back-up casework technical leader when the casework technical leader is absent or permanently leaves. A list of qualified individuals in the FSC will be maintained at Command Headquarters. Qualifications are described in the DNA Personnel Qualifications section of this appendix.

2. If a qualified individual is not available to serve as a casework technical leader the FBI will be contacted with a contingency plan within 14 days of the vacancy. New case assignments will be suspended and only analysis of open cases will continue until the contingency plan is approved by the FBI.

3. The Indexing Laboratory assistant technical leader will serve as the back-up to the Indexing Laboratory technical leader when this individual is absent from the laboratory. The casework technical leader will serve as the back-up when the Indexing Laboratory assistant technical leader is absent.

4. Should the Indexing Laboratory technical leader and assistant technical leader permanently leave the FSC, the casework technical leader will temporarily cover Indexing Laboratory technical leader duties until a qualified replacement is identified. A list of qualified individuals in the FSC will be maintained at Command Headquarters. Qualifications are described in the DNA Personnel Qualifications section of this appendix.

B. Individuals assigned as technical leaders on a full-time basis will review validation studies of current technologies and methodologies utilized by the laboratory system.

   Individuals assigned as technical leaders on a full-time basis will review educational requirements and training records for currently qualified analysts.

C. FSC casework CODIS Administrator duties are assigned to individuals identified as Local CODIS Administrator. This position or the assigned alternate must be filled before a laboratory can upload profiles to NDIS.

D. Qualifications and responsibilities for individuals working in the DNA section are described in the DNA Personnel Qualifications section of this appendix. Retention of training documents will follow Command Directives policy TRN 12.

# DNA QUALITY ASSURANCE
# DNA PERSONNEL QUALIFICATION AND TRAINING

I. Personnel Conducting DNA Casework

A. Educational Requirements for DNA Analysts

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

      i. Biochemistry
      ii. Genetics
      iii. Molecular Biology

   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. Biochemistry
      ii. Genetics
      iii. Molecular Biology

   c. Statistics and/or population genetics completed through 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. Biochemistry
        ii. Genetics
        iii. Molecular Biology

    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 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 or evaluation prior to assuming independent casework responsibilities.

    a. Newly-hired analysts will complete the documented DNA training program.
    b. Newly-hired analysts who have previous experience will have their prior training documentation and technical knowledge reviewed and evaluated by their assigned technical leader. Documentation and assessment of any modified training provided by the Illinois State Police will be reviewed and approved by the assigned technical leader. All training requires completion of a competency test(s).

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 designate.

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 technical leader.
d. Upon the technical leader's review and approval of the individual's release from training and/or a new qualification, the director of training or designate will notify the individual's assigned laboratory director.
e. Upon release of the newly-hired analyst from their initial training program, any qualification records will be maintained by their assigned laboratory.

C. Experience

All individuals will have worked in a DNA laboratory for a minimum of six months prior to assuming independent DNA casework responsibilities.

D. Certification

Job Descriptions (CMS 104) for Forensic Scientists performing DNA analysis are maintained by the laboratory director.

Final approval for conducting independent casework rests with the Command Administration.

Initial certification is based on a recommendation by a training coordinator and approval of that recommendation by the technical leader prior to beginning independent casework within the Command.

1. To be certified in this manner, an individual will do the following:

a. Demonstrate the ability to analyze non-semen and semen stains using the appropriate DNA technology
b. Demonstrate the ability to reproduce accurate and precise results
c. Demonstrate the ability to conduct analysis on non-probative cases
d. Demonstrate theoretical knowledge of DNA analysis
e. Demonstrate competency in mixture interpretation
f. Successfully complete criterion tests
g. Successfully complete a mock trial
h. Successfully complete supervised casework
i. Successfully complete an oral board examination

E. Continuing Education (CE)

1. Each analyst must be responsible for keeping abreast of current developments within the field. Each DNA analyst must complete annual continuing education as required by Standard 5 of the FBI DNA Quality Assurance Audit Document. Annual is defined as per calendar year. A minimum of eight documented hours of CE is required for each analyst.

   **NOTE: Training provided to qualify an analyst to perform an analytical procedure and/or interpretation in casework will not be considered continuing education.**

   Internal training documentation will include title of presentation, record of the presentation, date(s) of training, attendance list and CV of presenter.

   Examples of how this may be accomplished include:
   a. Professional organizations and their meetings
   b. In-service training
   c. Attendance at formal training courses
   d. Participation at in-house technical meetings/courses/seminars
   e. College course work
   f. Internet courses that have been approved by the technical leader(s).

   In addition, documentation that each DNA analyst has reviewed current literature must be kept.

2. The Laboratory Director will provide the opportunity to participate in these activities as outlined in the following directives:
   a. Tuition Reimbursement
   b. Society Memberships
   c. Command Advisory Board (CAB)
   d. Attendance at Professional Meetings
   e. Out-of-State Travel Requests

F. Literature Review
   Each DNA analyst will at minimum read nine publications that relate to DNA per year. This will be documented in LIMS.

II. Technical leadership of the DNA section will be provided and conducted in accordance with Command Programs and FBI Quality Assurance Standards for Forensic DNA Testing Laboratories. Technical leaders and individuals selected to fill a vacant technical leader position will meet the following criteria:

Individuals assigned as the DNA technical leader must have a Master's Degree in Biology, Chemistry or Forensic Science Related area. Course work completed toward the degree(s) must include 12 semester credit hours covering Molecular Biology, Genetics, Biochemistry and Statistics with at least three semester credit hours at the graduate level for one of these topic areas.

Individuals assigned as the DNA technical leader must have three years of forensic DNA laboratory experience obtained at a laboratory where forensic DNA testing was conducted for the identification and evaluation of biological evidence in criminal matters.

Within one year of his/her assignment as a DNA technical leader, an individual will successfully complete the most current FBI sponsored DNA auditor's training class and review all Illinois State Police validation studies, the current DNA Procedures Manual, and the educational and training records of currently qualified analysts.

Job descriptions (CMS-104) for each DNA technical leader are maintained by his/her supervisor.

DNA technical leader's duties are as follows:

A. Responsible for managing the technical issues and problem solving of analytical methods for the Forensic Sciences Command (FSC) DNA laboratories to include:

    1. Assistance on difficult or non-routine cases and resolution of disagreements between analysts.
    2. May provide assistance in court preparation, preparation of affidavits in response to special case issues, and testify as needed, to include Frye hearings.
    3. Serving as the technical point of contact for the outsourcing laboratory to assist with technical questions and issues.
    4. Responsible for FSC DNA Quality Assurance and in that role works with the Director of Quality Assurance. The DNA technical leader has the authority to initiate, suspend and resume DNA analytical operations for an assigned laboratory or individual following notification of the FSC Commander and Director of Quality Assurance.
    5. Responsible for review and approval of technical specifications for outsourcing agreements.

B. Responsible for documenting an annual review of all procedures used by the FSC.

C. Responsible for proposing new or modified procedures to be included in the FSC DNA Procedures Manual:

1. The review and approval of all completed research projects that will affect casework.
2. The proposal of new methodologies or modified analytical procedures to be used by analysts.
3. The approval and use of validated non-routine procedures. The Commander will be notified when non-routine procedures are applied to casework samples.

D. Responsible for documenting an annual review of the DNA QA Manual and is responsible for the FSC DNA Quality Assurance Program to include:

1. The review of DNA quality issues and their corrective actions, as needed.
2. 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.
3. Assistance and resolution of DNA QA issues.
4. The resolution of outsourcing quality issues.
5. The maintenance of the DNA personnel database key.

E. Responsible for the documented review of each regional laboratory to include:

1. At least two on-site visits to each laboratory per year by a technical leader trained in the appropriate technology(s) being performed at that laboratory.
2. A review of each laboratory's annual DNA audit. The appropriate technical leader will provide appropriate input on technical matters which arise from audits and assist and approve responses to DNA audit findings and their corrective actions.

F. Has oversight responsibility for the DNA training and safety programs to include:

1. The oversight of training for DNA analysts and laboratory technicians.
2. The review and approval of educational qualifications for DNA analysts.
3. The review and approval of training records for DNA analysts and laboratory technicians prior to independent casework analysis.
4. The annual review of the training and safety programs.

G. Responsible for oversight of the FSC DNA proficiency testing program to include:

1. The oversight of proficiency testing performance of analysts via information provided by the Director of Quality Assurance and is responsible for any corrective action.
2. Documentation of the notification of the proficiency test results for each analyst.

H. Accessible to the laboratory to provide onsite, telephonic or electronic consultation as needed to include accurate and timely communication to the DNA analysts regarding Command decision affecting DNA.

I. 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.

III. The DNA Training Coordinator's duties are as follows:

A. Design and maintain the Command's DNA Training Program. Ensure review, input and approval by the technical leader(s).

B. Deliver training to new employees assigned to the Forensic Biology/DNA section and provide cross-training to existing employees when required.

C. Create, administer, and grade written and practical examinations.

D. Review all supervised casework generated by trainees.

E. Report to the appropriate technical leader and statewide training program management any contamination or extraneous DNA issue identified during supervised casework.

F. Ensure that all quality control requirements/quality assurance guidelines are followed by individuals in training.

G. Monitor court testimony of trainees by actual viewing of testimony.

H. Lend assistance to analysts in court preparation.

I. Give input on technical matters which arise from audits and ensure compliance with FBI Quality Assurance Standards.

J. Provide accurate and timely communications to DNA trainees regarding Command decisions affecting DNA analysis.

K. Work with the appropriate technical leader on any other appropriate training issues, such as difficulty with a given aspect of the training program, failure of a criterion test by a trainee, etc.

IV. The local CODIS Administrator/Manager must be a qualified or formerly qualified DNA casework analyst and must have an understanding of DNA profile interpretation. The CODIS Manager will meet all DNA analyst requirements if they will be performing casework. The Local CODIS Administrator/Manager must successfully complete the FBI DNA auditor training within six months of appointment (per NDIS) and CODIS software training within six months of appointment if either had not already been completed. The Local CODIS Administrator must also meet all requirements outlined by NDIS.

Each lab will have an Alternate Local CODIS Administrator. The Alternate Local CODIS Administrator is responsible for fulfilling the Local CODIS Administrator's duties when he/she is absent or unavailable. The Alternate Local CODIS Administrator must meet the same requirements as the Local CODIS Administrator.

Local CODIS Administrator Duties:

A. Administration of the laboratory's CODIS network.

B. Is responsible for backing up data and properly storing the backup media.

C. Assures the quality and security of data stored in CODIS is in accordance with state and federal law and NDIS operational procedures.

D. Is responsible for uploading appropriate profiles to SDIS.

E. Is responsible for ensuring that matches are dispositioned in accordance with NDIS operational procedures.

F. Is responsible for scheduling and documentation of CODIS computer training for casework analysts.

G. Requests verification of offender samples and confirmation of conviction matches from SDIS.

H. Provides the State CODIS Administrator when appropriate with:

1. Information on CODIS matches
2. User and laboratory changes
3. Requests for NDIS keyboard searches
4. Requests of non-NDIS participating laboratories in the state to have data searched at LDIS, SDIS or NDIS
5. Requests for searches of foreign databases
6. Requests for removal of data at SDIS

I. Has the authority to terminate the laboratory's or an individual analyst's participation in CODIS in the event of a problem until the reliability and security of the computer data can be assured.

J. Oversees the entry and evaluation of outsourcing data and related hits.

V. Laboratory Technicians

A. Will have documented training, education and experience commensurate with their responsibilities as outlined in job description.

B. Will perform duties commensurate with their level of training such as bleaching, performance checks, reagent preparation, maintenance of genetic analyzers, QA/QC testing and drying of DNA samples.

C. Will conduct analytical procedures with evidence samples upon qualification through the DNA training program and approval of the technical leader.

D. Will participate in the proficiency testing program to maintain their qualification with analytical procedures.

E. Will receive evidence for DNA analysis in accordance with the Evidence Control Policy and the DNA case acceptance policy outlined in the Command Directives.

F. Will return DNA evidence in accordance with the Evidence Control Policy.

## DNA QUALITY ASSURANCE
## SAMPLE HANDLING AND FACILITY REQUIREMENTS

I. All DNA analysts will follow clean technique as documented in the Forensic Biology/DNA Procedures Manual.

II. Laboratories conducting DNA analysis will conduct the following activities either in a separate space or at a separate time:

Evidence examination
DNA extractions
PCR set up

Note: Rapid DNA instruments/systems shall be maintained in rooms outside of evidence examination areas or those containing amplified DNA.

III. Processing of unknowns and reference standards from a case should be separated by time or space, as specified by the individual procedures.

IV. Amplified products will be generated and contained in a room separate from non-amplified products. Amplified product may be removed from the amplification area for disposal only and will be sealed in a closed container.

V. Cleaning and Sterilization Procedures

A. Glassware and plastic containers will be cleaned as appropriate. Before laboratory use, these items will be rinsed with distilled water or equivalent. Items that come in contact with DNA samples will be cleaned with detergent and 10% bleach solution, followed by a rinse with distilled water or equivalent.

B. The reagent preparation section details those reagents which require sterilization by autoclaving.

C. The laboratory will follow the decontamination procedures outlined in the Clean Technique section. The Laboratory Asset Manager (LAM) is used to monitor decontamination of facilities and equipment and will include the items below:

1. The Forensic Biology/DNA laboratory floor will be mopped using a freshly prepared 10% bleach solution once a week. The PCR room must be mopped last unless there is a dedicated mop in the PCR room. This must be documented in the LAM.

2. The entire Forensic Biology/DNA laboratory (computer tops, equipment, etc.), including the PCR room, must be bleached once a week with a freshly prepared 10% bleach solution. This must be documented in the LAM.

D. All waste from the PCR room will be sealed in a closed container before being removed from the PCR room.

## DNA QUALITY ASSURANCE
## EVIDENCE CONTROL

I. Illinois State Police laboratory system has a documented evidence control system to ensure the integrity of physical evidence. This is outlined in the following Command directives:

Evidence Receipt Forms
Submission of Physical Evidence by Mail
Submission of Forensic Biology Evidence
Blood Evidence
Submission of Evidence to the FBI
Collection of Biological Standards
Access to Physical Evidence
Evidence Packaging
Transferring Cases Between Laboratories
Case Tracking
Destruction of Physical Evidence
Documentation of Case Related Phone Calls or Conversations
Signature Requirements for Case Reports
Minimum Standards for Evidence Marking
Internal Evidence Chain
Case Acceptance Policy for DNA Analysis
Clean Technique
Uniform Guidelines for Mailing Evidence

II. Biological Evidence Retention and Return Policy

A. All evidence including parent items and sub-items will be returned after analysis is completed. Extracted DNA that is not work product will be dried prior to return to the agency.

B. Work product is material generated in analysis that does not require chain of custody and may be discarded upon completion of analysis.

Examples of work product include but are not limited to:

- Extracted DNA from items for which there is sufficient sample to repeat the analysis
- Extracted DNA from reference standards
- Dilutions of DNA when sufficient original extracted DNA remains to repeat the analysis
- Amplified DNA
- Extracted substrates
- Consumables (e.g. tubes, plates, filters, etc.)

C. Extracted DNA will be dried and returned to the submitting agency as appropriate.

D. Membranes used to obtain RFLP profiles are considered documents and will be retained frozen indefinitely.

E. The disposition of an item including retention of extracted DNA, packaging, sub-exhibit designation, etc. must be documented in the case notes. Work product will be discarded unless otherwise documented in the case notes.

## DNA QUALITY ASSURANCE
## INTERNAL VALIDATIONS AND SITE-SPECIFIC ASSESSMENTS

I. The laboratory will use validated methods and procedures as outlined in the Command Directives, which meet the requirements of the FBI DNA Quality Assurance Audit Document.

II. Internal validation study materials will be maintained at the FSC Research and Development Laboratory. Electronic copies will be distributed to each regional laboratory.

III. To meet the requirements of the FBI DNA Quality Assurance Standards each regional laboratory will conduct site-specific assessments for new methodologies and instrumentation to be used in casework. Use the required Site-Specific Assessment Form found in Appendix II. Before casework implementation, the technical leader will review and approve the site-specific assessment. This documentation will be maintained at their respective laboratories.

# DNA QUALITY ASSURANCE
# ANALYTICAL PROCEDURES

I. Procedures

The laboratory will have technical leader approved, written analytical procedures.

A. Procedures used in DNA analysis will be approved annually.

B. Procedures being developed as part of the R&D Program or an internal validation which are not in the current procedure manual (non-routine procedures) may be used in casework with the appropriate technical leader and Command approval.

C. The Laboratory Asset Manager (LAM) in LIMS will be used to document all manufactured reagents and chemicals, prepared reagents, instruments, QC records, calibrations, and maintenance.

II. Reagents

The laboratory will use reagents that are suitable for the methods employed.

A. The laboratory will enter all manufactured reagents and stock chemicals into the LAM.

Information contained in the LAM will include the date a chemical or reagent was received, the identity of the receiver, the lot number(s) received, and the quantity received. The expiration date will also be recorded. When the chemical does not expire, 01/01/9999 will be entered as the expiration date.

B. The laboratory will enter all prepared reagents into the LAM.

1. The formulas for all reagents are found in the Procedures Manual.

2. Information kept in the LAM must include the date the reagent was prepared, the lot numbers of chemicals used to prepare the reagent, the quantity prepared, the expiration date of the reagent, and the identity of the preparer. LAM requires a lot number be entered for the reagent being prepared. Use the MONTHDATEYEAR naming convention for assigning this lot number (e.g. 09192019).

C. Labeling requirements

1. Stock chemicals and manufactured reagents will be labeled with the identity of the receiver, the date of receipt and the expiration date. If the chemical does not expire, it must be labeled accordingly.

2. Prepared reagents will be labeled with the name of the reagent, the concentration, the expiration date, storage conditions, a hazard warning when appropriate, the identity of the preparer, and the date prepared.

D. Distilled, deionized water or its equivalent must be autoclaved for use in DNA analysis.

E. Expiration Dates

1. Stock chemicals will expire according to the manufacturer's listed expiration date, if any. If no date is specified, the chemical is considered stable and does not expire.

2. Reagents

a. Manufactured reagents will expire according to the manufacturer's listed expiration date. If there is no date specified, the expiration date will be one year from the date of receipt at the laboratory.

b. Reagents made in the laboratory will expire one year or less from the date of preparation, regardless of their storage conditions.

3. Autoclaved distilled deionized water or its equivalent will expire one year from the date autoclaved.

F. Critical Reagents
The following have been defined as critical reagents:

DNA IQ™ Resin
Maxwell® Casework Pro kits
Maxwell® FSC DNA IQ™ Casework kits
Quantification kits
DNA amplification kits
AppliedBiosystems™ RapidHIT™ ACE GlobalFiler™ Express (GFE) Sample Cartridges
BTmix

G. Critical Reagent Quality Control

1. Procedures for quality control of these reagents are found in this appendix.

2. Bulk ordered critical reagents may be quality checked through the Research and Development Laboratory.

3. Documentation of all quality control procedures performed on a particular lot of a reagent will be recorded in the LAM.

4. If a particular supply, chemical, reagent or material does not meet the required quality control standard(s), the manufacturer will be notified and the entire lot rejected.

5. Quality control records will be maintained indefinitely.

III. Standards and Controls

The laboratory will monitor the analytical procedures using appropriate controls and standards. Minimum standards and control requirements are defined in each analytical procedure. Please see the Clean Technique, Reagent Blanks and Controls for Extraction, and Amplification and Electrophoresis of STRs section of the Forensic Biology/DNA Procedures Manual for procedure-specific information.

The standards and controls used in DNA analysis are qualitative internal reference materials and do not require re-authentication.

Parameters for reagent blank use and consumption will meet conditions described in the FBI QAS Audit Document.

Reagent blanks from extraction batches where sample extracts are being preserved will be maintained by the laboratory indefinitely. When all sample extracts are consumed or no extracts are being retained, reagent blanks may be disposed.

Compromised results for PCR amplification controls and reagent blanks will be handled as follows:

A. If a DNA profile is confirmed in any reagent blank or negative control, the case must be brought to the attention of the technical leader.

B. If the DNA profile detected in the reagent blank or negative control matches the DNA profile of a laboratory employee, interpretation of probative samples will be conducted and reported. The same approach will be taken if the contaminant can be associated with another source (e.g., manufacturer).

IV. The laboratory will have written general guidelines for interpretation of data.

A. The laboratory will verify that all control results are typed correctly. Incomplete positive control results will be reviewed by the technical leader.

B. For a given population(s) and/or hypothesis of relatedness, the probability of observing a DNA profile will be estimated using a standard population genetic method(s) and/or directed method (as described in the Forensic Biology/DNA Procedures Manual). These calculations will be derived from a documented population database appropriate for the calculation.

## DNA QUALITY ASSURANCE
## INCIDENT REPORTS

I. An Incident Report is used to document quality issues that occur during the analytical procedures for DNA casework or proficiency testing. This includes, but is not limited to, issues that involve procedural non-conformance, loss of sample or data, contamination and sample handling. The form can be found in Appendix II.

II. The DNA analyst will notify their supervisor of the quality issue. The supervisor will need to determine if the appropriate documentation is an Incident Report. If necessary, the supervisor may need to consult with the Technical Leader to determine if this is a quality issue and/or the correct documentation is being used.

The issue must be clearly identified and understood. At that point, a course of action can be determined. The laboratory may identify a course of action, but the Technical Leader will need to review and approve the course of action. If necessary, the Technical Leader will determine the course of action. If the course of action is to reject the data, it will be documented in the form. The documentation in this form meets Standard 7.5.1.5 of the ANSI National Accreditation Board Supplemental document, AR 3125, as it provides the reason the data has been rejected, the identity of the individuals taking the action and the date of this action.

III. If any additional analytical work needs to be completed, the analyst will complete this course of action.

IV. If it is necessary to provide follow-up information of the course of action, it may be necessary to document in the form.

V. The Technical Leader will determine if the form has been satisfactorily completed. At that time, the form will be finalized by the Technical Leader and provided to the analyst, supervisor, Laboratory Quality Manager and the Director of Quality Assurance.

VI. The finalized Incident Report form will be maintained in the originating laboratory and be included in notes packets for all associated case(s). The Incident Report form is used to document issues that occur with specific samples in casework/proficiency testing. It is not used to document systemic issues with an analyst and/or laboratory. A systemic issue is documented as a Quality Issue Report (QIR).

VII. Management Responsibilities:

A. The Director of Quality Assurance, in conjunction with the appropriate Bureau Chief, will determine if a QIR is also necessary. The Technical Leader may also recommend a QIR. The Laboratory Quality Manager or Laboratory Management should also identify issues that may be systemic to the laboratory and/or analyst and consult with the Technical Leader, Director of Quality Assurance and Bureau Chief as to whether a QIR is warranted.

B. Incident reports and QIRs will be monitored by the DNA Technical Leader, Director of Quality Assurance, Laboratory Quality Manager and the Laboratory Director.

C. The FSC Quality Manual addresses Corrective Action policies in place for the laboratory system.

## DNA QUALITY ASSURANCE
## CRITICAL REAGENT QUALITY CONTROL OF BTMIX

I. Purpose
To demonstrate that BTmix components are free of extraneous DNA.

II. Procedure

A. Prepare BTmix as outlined in the Fired Cartridge Case Preprocessing for DNA IQ™ Extraction section.

B. Process two known samples and two reagent blanks following the Fired Cartridge Case Preprocessing for DNA IQ™ Extraction section and using the prepared BTmix.

C. Profile all samples and reagent blanks. The reagent blanks will be concentrated to 15uL for amplification.

D. Create a QC entry in the LAM and attach the supporting documentation as needed.

III. Assessment of Results

A. All samples and reagent blanks must be free of extraneous DNA.

B. If contamination is indicated in the samples or reagent blanks, repeat the procedure. If a second extraction produces extraneous DNA, refuse the lot and notify a Technical Leader.

## DNA QUALITY ASSURANCE
## CRITICAL REAGENT QUALITY CONTROL OF DNA IQ™ RESIN

I. Purpose

To evaluate a new lot of DNA IQ™ resin, either provided as a part of the DNA IQ™ System kit or in individual bulk reagent bottle as used in Tecan sample extractions.

II. Standard Preparation

Prepare this standard in large volume for use in the quality control procedure below. Store in the freezer. The standard does not expire; however, a new standard should be made when quantification results start to decline.

A. Dilute a liquid blood sample 1/10 with TNE.

B. Spot volumes of 2 µL, 5 µL and 10 µL, ten times each onto filter paper and let dry.

C. Extract the ten replicates of each volume following the magnetic separation stand procedure found in Non-Semen Preprocessing for DNA IQ Extraction and DNA IQ Extraction: Magnetic Separation Stand, using the current lot number of DNA IQ™ kit or system reagents, and eluting in 100 µL.

D. Quantify the extracts for total human DNA using Quantitative PCR (qPCR).

E. Review the mean quantity calculated in the qPCR report for each of the standard volumes. The volume whose mean quantity is closest to 0.1 ng/µL will be selected as the quality control standard.

F. Using the volume selected above, spot the remaining diluted blood onto filter paper and let dry. Store and label the standard appropriately. Use this dried standard to evaluate subsequent lot numbers of DNA IQ™ resin.

G. Calculate the standard deviation (SD) from the quantitation values for the accepted standard. The acceptance range for evaluating new lots of DNA IQ™ resin will be the mean quantity ± 3SD.

H. Documentation of qPCR results will include the qPCR report and standard curve. Create a QC entry in the LAM and attach the supporting documentation.

III. Quality Control Procedure

A. Extract the standard in triplicate and a single negative control following the magnetic separation stand procedures found in Non-Semen Preprocessing for DNA IQ Extraction and DNA IQ Extraction: Magnetic Separation Stand. Use the new lot of DNA IQ™ kit, or the new lot of DNA IQ™ resin and the other in-use lot numbers of lysis, wash, and elution buffers. Elute in 100 µL.

B. Quantify the extracts for total human DNA using qPCR. Assess the results against the acceptance range previously determined for the quality control standard.

C. Documentation of qPCR results will include the qPCR report and standard curve. Create a QC entry in the LAM and attach the supporting documentation.

IV. Assessment of Results

A. Review the mean quantity calculated in the qPCR report for the samples. The mean quantity must fall within the accepted range of the standard.

B. Course of Action

If the mean quantity falls outside the established range, or if quantifiable DNA is identified in the negative control, repeat the procedure. If a second extraction produces values outside the established range or if quantifiable DNA is reproducibly identified in the negative control, refuse the lot and notify a technical leader.

# DNA QUALITY ASSURANCE
# CRITICAL REAGENT QUALITY CONTROL OF THE DNA IQ™ CARTRIDGES FOR MAXWELL® INSTRUMENTS

I. Purpose

To evaluate a new lot of DNA IQ™ cartridges for Maxwell® instruments.

II. Standard Preparation

A. From an anticoagulant tube that has been mixed for ten minutes, dilute a liquid blood sample 1:1 with TNE buffer. Mix the diluted sample for ten minutes.

B. Prepare 20 µL stains on a sterile substrate, mixing between aliquots. Identify the sample by preparation date and store at -20°C. Results for this standard pertain to the sample collection date and not to the individual from whom the sample was collected.

C. Extract five replicates with the current lot number of DNA IQ™ cartridges and elute in 30 µL.

D. Quantify the extracts for total human DNA using quantitative PCR (qPCR).

E. Review the mean quantity calculated in the qPCR report. A minimum average yield of 1 ng/µL is required to accept the prepared sample as a QC standard.

F. Documentation of qPCR results will include the qPCR report and standard curve. Create a QC entry in the LAM and attach the supporting documentation.

III. Quality Control

A. Extract the standard in triplicate and a single negative control using the new lot number of DNA IQ™ cartridges. Elute in 30 µL.

B. Quantify the extracts for total human DNA using qPCR.

C. Documentation of qPCR results will include the qPCR report and standard curve. Create a QC entry in the LAM and attach the supporting documentation.

IV. Assessment of Results

A. Review the mean quantity calculated in the qPCR report for the samples. The mean quantity must be at least 1 ng/µL.

B. Course of Action

If the mean quantity falls below 1 ng/µL or if quantifiable DNA is identified in the negative control, repeat the procedure. If a second extraction produces values below 1 ng/µL or if quantifiable DNA is reproducibly identified in the negative control, refuse the lot and notify a technical leader.

## DNA QUALITY ASSURANCE
## CRITICAL REAGENT QUALITY CONTROL OF QUANTIFICATION KITS

I. Purpose

To evaluate a new lot of quantification kit.

II. Procedure

A. Prepare an eight point, 4X serial dilution of the NIST 2372A SRM.

B. Prepare an eight point, 4X serial dilution of the new PowerQuant kit standard.

C. Using the NIST SRM standard series and the reagents from the new lot of PowerQuant kit, quantify the PowerQuant kit standard series and a no-template control (NTC) in triplicate.

D. Calculate the average overall neat autosomal mean for each point in the PowerQuant kit standard series.

III. Assessment of Results

A. Evaluate the $R^2$ and slope of the standard curves. Results may be accepted when the standard curve $R^2$ values are equal to or greater than 0.98 and the slopes are -3.1 to -3.6.

B. Accept the results for the PowerQuant kit standard if the quantified values are within 10% of the expected values. If the mean neat concentration value is >55 ng/µL, calculate the dilution necessary to target 50 ng/µL.

The new lot will be refused if the quantified value for the neat standard is <45 ng/µL.

C. No-template controls may not have reproducible results and IPC $C_T$ flags may not be present for any sample tested during the QC run.

D. Create a QC entry in the LAM and attach the supporting documentation.

## DNA QUALITY ASSURANCE
## CRITICAL REAGENT QUALITY CONTROL OF STR TYPING KITS

I. Purpose

To demonstrate that all amplification components contained in the kit can produce accurate typing results.

II. Procedures

A. Prepare amplification reaction mixture using components from new lot number kit, as outlined in the appropriate Amplification and Electrophoresis section.

B. Amplify a positive and negative control. Use the control DNA provided in the kit and/or NIST traceable DNA as the positive control. For the negative control, amplification grade water or sterile ddi water may be used.

C. Profile the amplified controls.

D. Create a QC entry in the LAM and attach the supporting documentation.

III. Assessment of Results

A. All controls must type correctly.

B. If incorrect or incomplete typing results are obtained, repeat the procedure. If a second amplification produces unexpected results, reject the lot number and notify a technical leader.

# DNA QUALITY ASSURANCE

## CRITICAL REAGENT QUALITY CONTROL OF APPLIED BIOSYSTEMS™ RAPIDHIT™ ACE GLOBALFILER™ EXPRESS (GFE) SAMPLE CARTRIDGES

I. Purpose

To evaluate a new lot of Applied Biosystems™ RapidHIT™ ACE GFE Sample Cartridges.

II. Procedure

A RapidHIT™ ACE GFE Positive Control Cartridge and a RapidHIT™ACE GFE Negative Control Cartridge must be processed and analyzed for each new lot number.

III. Assessment of Results

A. All control cartridges must contain the appropriate results.

B. If either quality control sample fails, repeat the procedure with the appropriate control cartridge. If a second control cartridge does not pass, reject the lot number and notify the technical leader.

C. Document the quality control results in the LAM.

## DNA QUALITY ASSURANCE
## EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE

I. Equipment performance and maintenance checks will be completed for the entire DNA analysis system with a NIST traceable sample to verify that the entire PCR system is functioning within accepted criteria. Equipment performance checks and maintenance will be completed when the FSC obtains new genetic analyzers or when major equipment changes are necessary for current genetic analyzers. Annual verification checks will be completed for all genetic analyzers.

II. Equipment performance checks and maintenance will be required for the following pieces of equipment: balances, ovens, refrigerators, freezers, pipettes, microscopes, thermal cyclers, water baths, incubators/heat blocks, biohoods, robotics systems and qPCR instruments. Equipment listed below will be considered as critical equipment. Performance checks and maintenance procedures follow.

III. The following equipment/instruments have been identified as critical:

A. Handheld mechanical pipettes

B. Thermometers

C. Incubators/Heat blocks used in analytical procedures

D. Robotic systems

E. Thermal cyclers

F. Thermal cycler temperature verification system

G. Genetic analyzers

H. Rapid DNA instruments

IV. Critical Equipment Requiring Annual Calibration

A. Robotic systems

B. Mechanical pipettes

V. Critical Equipment Requiring Annual Performance Checks

A. Incubators/Heat blocks used in analytical procedures

B. Thermal cyclers

C. qPCR instruments

D. Robotic systems

E. Genetic analyzers

F. Rapid DNA instruments

VI. Calibration and Performance Checks

A. Records will be maintained for calibration and performance checks.

B. Tolerance window (where applicable) will be indicated in the LAM.

VII. Maintenance

A. If appropriate, maintenance procedures and schedules will be listed for equipment.

B. Maintenance records will be documented in LAM.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# PIPETTES

I. Calibration

A. Procedure:

1. New pipettes that arrive from the manufacturer with a certificate of performance may be immediately used in casework. These pipettes should be scheduled for calibration at the next annual date.

2. All pipettes will be calibrated by a company qualified to provide certification for their accuracy and precision.

3. Each pipette must be checked in at least two settings (one low and one high) in its range of use.

B. Frequency: Annually.

C. Results: Each setting tested must be within the contract specifications for each pipette. Document the calibrations in the LAM.

D. Course of Action: Any pipette whose measurements are outside of the contract specifications must be removed from casework. Submit the pipette for repair and recalibration.

II. Maintenance

No regular maintenance is required.

III. Use

Pipettes should not be used to collect or dispense any volume outside their calibration ranges.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# INCUBATOR/HEAT BLOCK TEMPERATURE CHECKS

I. Temperature Checks

A. Procedure: Record the temperature of the heat block using a NIST traceable thermometer. Adjust the temperature dial if necessary and recheck.

B. Frequency: Check monthly but observe temperature before each use. If not used regularly, check before use and at least annually.

C. Results: Record the results in the LAM.

D. Course of Action: If the temperature is not within the specified tolerance window, repeat the test. If it continues to fall outside the range, replace the unit or contact a repair company.

II. Maintenance

No regular maintenance is required.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# WATER BATHS

I. Temperature Checks

A. Procedure: Record the temperature of the water in the bath using a NIST traceable thermometer. Adjust the temperature dial if necessary and recheck.

B. Frequency: Check monthly.

C. Results: Record the results in the LAM.

D. Course of Action: If the temperature cannot be maintained within +/- 2°C of the required setting, contact a repair company.

II. Maintenance

Empty, clean, and refill with fresh water as needed.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# OVEN TEMPERATURE CHECKS

I. Temperature Checks

A. Procedure: Record the temperature of the oven using a NIST traceable thermometer. Adjust the temperature dial if necessary and recheck.

B. Frequency: Check annually, but observe temperature before each use.

C. Results: Record the results in the LAM.

D. Course of Action: If the temperature is not within the specified tolerance window, contact a repair company.

II. Maintenance

No regular maintenance is required.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# REFRIGERATORS AND FREEZERS

I. Temperature Checks

A. Procedure: Record the temperature of the refrigerator or freezer using a NIST traceable thermometer. Adjust the temperature dial if necessary and recheck.

B. Frequency: Check monthly.

C. Results: Record the results in the LAM.

D. Course of Action: If the temperatures cannot be maintained within the specified tolerance window, contact a repair company.

II. Maintenance

No regular maintenance is required. Defrost and clean as necessary. Contact the repair company for repairs.

## DNA QUALITY ASSURANCE
## EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
## THERMOMETERS

I. Only thermometers with ISO/IEC 17025 Certificate of Calibrations may be used. These thermometers must be traceable to national or international standards (e.g. NIST).

II. The calibration certificate for each thermometer will be entered in the LAM.

III. The vendor's calibration is the performance check. The thermometer may be placed into use immediately and used until the expiration date on its certificate.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# BIOHOOD CERTIFICATION

I. Certification

A. Procedure:

1. Biohoods will be maintained by a company qualified to provide a certification of the biohoods' specifications.

2. All biohoods used for DNA will be certified before use in casework.

3. Certification should include, but is not limited to, checking the HEPA filter for leaks, the velocity of the air movement, and electrical safety.

B. Frequency: Annually.

C. Results: Maintain the certification records in the LAM.

D. Course of Action: If the certification reveals parameters that do not meet specifications, contact a vendor or manufacturer for repairs and/or replacement parts.

II. Maintenance

Change fluorescent light bulbs when needed and UV light bulbs biennially, or as indicated by the annual certification.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# BALANCES

I. Performance Check

A. Procedure: Use NIST certified weights. Handle them with cotton gloves or forceps. Check a minimum of three weights spanning the range of the balance to include 0.1 g, 1 g, and 10 g.

B. Frequency: Check monthly, or if used frequently, prior to use.

C. Results: Record the results in the LAM.

D. Course of Action: If the balance is not within ±2 readability units for each checked weight, then contact a repair company.

II. Maintenance

No regular maintenance is recommended by the manufacturer.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# MICROSCOPES

I. Checks

A. Procedure: Microscopes will be cleaned and checked by a qualified professional. Optimum illumination will be established, where applicable.

B. Frequency:
1. Compound microscopes: At least every year, or as needed.
2. Stereomicroscopes: At least every three years, or as needed.

C. If a check by a qualified professional is delayed, an in-house performance check will be conducted by laboratory staff using a known standard or positive control as an interim check until the annual or triennial check can be conducted.

D. Results: Document the check in the LAM.

E. Course of Action: If problems are identified, contact a repair company.

II. Maintenance

A. Clean microscopes as necessary.

B. Cover all microscopes after use.

## DNA QUALITY ASSURANCE
## EVALUATION OF A NEW MAXWELL® INSTRUMENT

I. When a new Maxwell® instrument is received, a Contamination assessment that consists of an alternating pattern of known standards and reagent blanks must be conducted before it may be used in casework.

II. The total number of samples in the assessment will be dependent on the capacity of the respective Maxwell® instrument. The alternating pattern will consist of an equal number of known standards and reagent blanks. Previously characterized blood or buccal standards can be used as known standards.

III. Set up the cartridge rack or deck tray using an alternating pattern of DNA IQ™ reagent cartridges containing known standards and reagent blanks.

IV. Quantify all known standards and reagent blanks. All reagent blanks containing measurable amounts of DNA will be amplified at a level expected to produce the most informative DNA profile to potentially identify the source. If a DNA profile is identified in any of the reagent blanks, this assessment will be repeated. The technical leader will be consulted if a DNA profile is obtained.

V. The data from this study must be maintained in the LAM. Routine maintenance must also be recorded in the LAM.

VI. If major equipment repairs are necessary, the above study will be required.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# MAXWELL® INSTRUMENTS

I. Instrument Performance Checks

A. Procedure

1. Power on the instrument. If it is already on, power it off and then back on. Self-diagnostic tests will run automatically and the results may be displayed on the screen.

2. A standard that is traceable to a NIST Standard Reference Material (SRM) and a reagent blank will be extracted, quantified, and profiled with an autosomal chemistry.

B. Frequency: annually. These performance checks may be performed at the same time as the annual system verification

C. Results: All results will be maintained in the LAM. Please note the self-diagnostic tests do not generate electronic data.

D. Course of Action: If the instrument fails to power on successfully, contact Promega for troubleshooting and/or service. Review the data generated for the NIST traceable standard and reagent blank to ensure they both exhibit the expected results. The expected alleles for the NIST traceable standard can be found in the *Amplification and Electrophoresis of STRs: PowerPlex® Fusion* section of the DNA Procedures Manual. If the results are not as expected, the procedure will be repeated. If those results are not as expected, contact Promega for troubleshooting and/or service.

II. Routine Maintenance

A. Procedure

1. Linear Slides: If the linear slides become sticky, they may be lubricated with light machine oil. Apply to a cotton swab and apply using only as much as is needed to make the heads and plate slide easily. Do not get oil on the black drive belts.

2. Belts: Inspect the belts periodically.

B. Frequency: As needed.

C. Results: All routine maintenance must be recorded in the LAM.

D. Course of Action: Contact Promega to arrange for service if excessive wear or slack of the belts is noted.

## DNA QUALITY ASSURANCE
## EVALUATION OF A NEW MAXPREP™ LIQUID HANDLER

I. After the initial pre-amplification and post-amplification Maxprep™ Liquid Handler has been evaluated completing site-specific studies, any new Maxprep™ Liquid Handler that is placed in the pre-amplification or post-amplification area of the laboratory will need to be evaluated before implementation into casework. The evaluation will be dependent on whether it will be used in pre-amplification or post-amplification areas of DNA analysis.

**Note:** Per the QAS, the initial method which includes instrumentation for each laboratory in a multi-laboratory system will need to have completed applicable site-specific precision, sensitivity and contamination studies.

A. Perform the Maxprep™ Liquid Handler Weekly Maintenance Method.

B. Checkerboard Contamination Assessment

1. Pre-amplification Maxprep™ Liquid Handler

a. Using both deck trays prepare 24 buccal swab standards and 24 reagent blanks in an alternating pattern. This will be conducted separately two times.

b. Complete all the pre-amplification processes using the Maxprep™ Liquid Handler from sample preparation through amplification setup.

c. Profile all standards and reagent blanks using a genetic analyzer.

d. Examine all electropherograms to ensure the correct DNA profiles are identified and there is no extraneous DNA in the samples or reagent blanks.

e. The data will be maintained in the LAM.

2. Post-Amplification Maxprep™ Liquid Handler

a. Amplified samples that have been setup in a checkerboard pattern will be used to evaluate the post-amplification Maxprep™ Liquid Handler. This may be accomplished in coordination with a new or current pre-amplification Maxprep™ Liquid Handler or manual amplification setup.

b. Profile all standards and reagent blanks using a genetic analyzer.

c. Examine all electropherograms to ensure the correct DNA profiles are identified and there is no extraneous DNA in the samples or reagent blanks.

d. The data will be maintained in the LAM.

II. When major equipment repairs are necessary for the Maxprep™ Liquid Handler, the Weekly Maintenance Method and Checkerboard Contamination Assessment will be required.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# MAXPREP™ LIQUID HANDLER

I. Maintenance Checks

A. Promega or another qualified company will perform a preventative maintenance on the Maxprep™ Liquid Handler annually.

B. System checks to be completed following preventative maintenance:

1. Pre-amplification Maxprep™ Liquid Handler

a. Perform weekly maintenance.

b. Checkerboard Contamination Assessment: Using one deck tray prepare 12 buccal swab standards and 12 reagent blanks in an alternating pattern. Complete all the pre-amplification processes using the Maxprep™ Liquid Handler from sample preparation through amplification setup. Profile and examine all electropherograms to ensure the correct DNA profiles are identified and there is no extraneous DNA in the samples or reagent blanks.

2. Post-Amplification Maxprep™ Liquid Handler

a. Perform weekly maintenance.

b. Amplified samples that have been setup in checkerboard pattern will be used to evaluate the post-amplification Maxprep™ Liquid Handler. Profile and examine all electropherograms to ensure the correct DNA profiles are identified and there is no extraneous DNA in the samples or reagent blanks.

3. If there are multiple pre-amplification Maxprep™ Liquid Handlers, the amplification setup plates produced during the system checks may be combined at capillary electrophoresis setup by the post-amplification Maxprep™ Liquid Handler(s), as appropriate.

C. Documentation will be maintained in the LAM.

II. Calibration

A. Procedure:

1. The liquid handling arm will be checked for accuracy and precision by Promega or another qualified company. The company will provide certification as to the precision of the instrument.

2. The instrument will be calibrated according to the manufacturer's specifications.

B. Frequency: Annually.

C. Results: All results will be maintained in the LAM.

D. Course of Action: For hardware-related issues, contact Promega to ensure the instrument functions within the manufacturer's specifications. If adjustments to the pipettes are necessary, contact Promega for assistance and remove the Maxprep™ Liquid Handler from casework until necessary repairs and calibrations are completed.

III. Daily or Weekly Maintenance

A. Before setting up a run

1. Open the Maxprep™ software and on the home screen select "Maintenance".

2. Remove the tip eject bar from the Maxprep™ Liquid Handler prior to starting either maintenance method.

3. As appropriate select and run the Daily Maintenance or the Weekly Maintenance Method. Follow the prompts of the respective method. If the Weekly Maintenance Method has been run, the Daily Maintenance Method will not need to be run since it consists of the steps found within the Daily Maintenance Method.

**Note**:
If the Daily Maintenance method was run greater than 24 hours prior to the run, the Maxprep™ Liquid Handler will not allow an analytical method to be run.

-or-

If the Weekly Maintenance method was run greater than 7 days prior to the run, the Maxprep™ Liquid Handler will not allow an analytical method to be run.

4. Replace the tip eject bar. This **must be** replaced after the maintenance method and before starting an analytical method.

B. Upon completion of all runs for the day

1. Run the Sanitation method (UV), as needed.

2. Empty the waste container.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# qPCR INSTRUMENT

I. Instrument Performance Checks

If a vendor has performed any of the performance checks below as a part of scheduled maintenance, service, or repair, those checks will not need to be repeated by the laboratory. All remaining checks must be completed before the instrument may be used for casework.

A. Procedure

1. Perform the regions of interest (ROI), background calibration, optical calibration, and pure dye spectra checks.

2. Prepare a standard dilution series and set up a plate with a duplicate standard dilution series run on each side of the plate. Use two columns on the left half of the plate and two columns on the right half of the plate to ensure all four quadrants of the thermal cycling block are being checked.

3. Use each set of standard dilution series samples to generate a standard curve. Evaluate each standard curve to ensure the $R^2$ value and slope are within range. If each side of the plate produces an acceptable standard curve, the results of the performance check may be accepted.

   If the results are not acceptable, remake the standard dilution series and repeat the check. If the results are still not acceptable, remove the instrument from use and contact the technical leader and/or the vendor for further troubleshooting and/or service.

B. Frequency: To be completed upon implementation of a new instrument and/or chemistry, material modification to the procedure, or vendor service.

C. Documentation: Record the results in the LAM.

II. Performance Checks and Maintenance

A. Background Calibration Check

1. Procedure

   Perform the check according to manufacturer recommendations.

   If the background calibration check fails, repeat the test. If the issue cannot be resolved, remove the instrument from use and contact the company for repair.

2. Frequency: Monthly.

3. Documentation: Record the results in the LAM.

B. Lamp Intensity and Instrument Function Tests

1. Procedure
Check lamp intensity and perform a function test according to manufacturer recommendations.

If the lamp intensity test fails, replace the lamp. If the instrument function test fails, repeat the test. If it fails again, contact the manufacturer.

2. Frequency: Monthly.

3. Documentation: Record the results in the LAM.

C. ROI, Optical Calibration, Background Calibration and Pure Dye Spectra Checks

1. Procedure
Perform all checks according to manufacturer recommendations.

If one of the checks fails, repeat all of the semi-annual checks. If the issue cannot be resolved, remove the instrument from use and contact the company for repair.

2. Frequency: Semi-annually.

3. Documentation: Record the results in the LAM.

D. Vendor Maintenance

1. Procedure
Vendor provided maintenance will include temperature verification, temperature non-uniformity (TNU), cycle rate, temperature accuracy, heated cover temperature, and temperature uniformity.

2. Frequency: Annually or more often, as needed.

3. Documentation: All records will be maintained in the LAM.

E. Archive

1. Procedure: Archive or back up run files.

2. Frequency: As needed.

3. Documentation: No documentation required.

## DNA QUALITY ASSURANCE
## EVALUATION OF A NEW THERMAL CYCLER

I. When a new thermal cycler is received, it must be evaluated before it may be used for casework. The following studies must be conducted:

A. Instrument Programming
Program the instrument with the amplification parameters for the relevant STR kits. The programming will be verified by a second analyst.

B. Temperature checks
If a certificate of conformance is not provided by the manufacturer, perform the temperature verification and uniformity checks.

C. Reproducibility
Select four previously characterized samples and amplify them with each chemistry currently in use under the laboratory's scope at a level similar to that of the positive kit control. Include a positive and negative control. Profile all samples and controls on a genetic analyzer. Concordant profiles must be obtained for each sample and positive control. The negative control must have no labeled alleles.

**NOTE: The data from each of these studies must be maintained in the LAM. Routine maintenance must also be recorded in the LAM.**

II. These studies will be repeated when major equipment repairs are necessary.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# THERMAL CYCLERS

I. Temperature Verification and Temperature Uniformity Tests

A. Procedure: Using the Temperature Verification Kit, follow the procedure within the Thermal Cycler User's Guide for the test being conducted.

B. Frequency: Conduct Temperature Verification and Temperature Uniformity every six months.

**Note**: **If a Thermal Cycler is solely being used as a heat block, only the Temperature Verification Test is required; testing should be performed every six months. If not used regularly, check before use.**

C. Results: Record the results in the LAM.

D. Course of Action: If any temperature falls outside of the documented range, repeat the test. If temperatures are still outside the documented range, discontinue use of the thermal cycler and contact a repair company.

II. Temperature Verification Kit

A. Procedure: Send to a company qualified to provide certification for calibration.

B. Frequency: Calibrate annually.

C. Results: Record the results in the LAM.

D. Course of Action: If the kit fails calibration, discontinue its use.

III. Maintenance

No regular maintenance is required.

## DNA QUALITY ASSURANCE
## EVALUATION OF A NEW GENETIC ANALYZER

I. When a new genetic analyzer is received, it must be evaluated before it may be used for casework. The following studies must be conducted:

A. Spectral Calibration
Perform a spectral calibration using the appropriate Spectral (Matrix) Calibration Standard, according to the manufacturer's instructions.

B. Instrument Run Time
Determine the appropriate run time before proceeding with the remaining studies. Run five allelic ladders with the appropriate sizing standard. Ensure each ladder contains all the peaks required for the sizing method. Repeat the process, if necessary, increasing or decreasing the run time until a suitable run time is established.

C. Precision and Reproducibility
Choose three samples that have been previously characterized. Each sample will be run twenty times and analyzed according to ISP protocol. Precision will be evaluated by determining the average, standard deviation, and minimum and maximum base pair sizes for the alleles at each locus for each sample. Verification of concordance to samples that have been previously genotyped will serve as the reproducibility study.

D. Sensitivity
Choose three previously characterized samples and make a dilution series for each sample to include a range of values covering the limit of linearity (LOL) of the instrument, as suggested by the internal validation study. These samples should contain alleles that represent both high and low alleles across all loci. Inject these samples in duplicate to determine the instrument's sensitivity. The instrument must be able to detect a full profile with 0.6 ng of DNA at the analytical threshold.

**NOTE: The data from each of these studies must be maintained in the LAM. Routine instrument maintenance must also be recorded in the LAM.**

II. When major equipment repairs are necessary to a genetic analyzer, one or more of the above studies may be required. The type of studies required will be dependent on the type of repairs conducted.

## DNA QUALITY ASSURANCE
## EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
## GENETIC ANALYZERS

When a genetic analyzer is being used for casework, the maintenance tasks below should be performed at the suggested frequency. For instruments that have been out of service or in shutdown, all appropriate maintenance tasks must be performed before utilizing the instrument for casework samples.

All maintenance records will be maintained in the LAM.

Applied Biosystems® 3500/3500xL Genetic Analyzer

A. Before each casework run

1. Check the Dashboard for the status of the buffer containers and polymer and replace them as necessary.

2. Visually inspect the fluid levels in the buffer containers. The fluid must line up with the fill line.

3. Check for bubbles in the block and channels.

4. Check the capillary tips for damage.

5. Check for leaks around the array Buffer-Pin Valve, check valve, and array locking lever.

B. Weekly
Run the Wash Pump and Channels Wizard.

C. Monthly (or sooner, if necessary)

1. Flush the pump trap.

2. Empty the condensation container and the water trap waste container.

3. Replace the cathode buffer container septa.

D. As-Needed

The capillary array will be replaced when its performance begins to decline, or earlier at the analyst's discretion. Decreased resolution, peak broadening, decreased sensitivity, and excessive artifacts may be indications of an array that needs to be replaced. A spatial calibration must be performed when the array is replaced.

E. Short- and long-term shutdown
Use the Instrument Shutdown Wizard to prepare the instrument for periods of inactivity longer than one week.

## DNA QUALITY ASSURANCE
## ANNUAL VERIFICATION OF THE 3500/3500XL GENETIC ANALYZER

I. Spectral Calibration
Perform a spectral calibration using the appropriate spectral calibration standard, according to the manufacturer's instructions.

II. Sensitivity

A. Quantify the PowerPlex Fusion 2800M standard in triplicate. Calculate the mean concentration for the standard. The value provided by the manufacturer should not be used.

B. Dilute the PowerPlex Fusion 2800M standard in $TE^{-4}$ buffer, utilizing at least 2 µL of the neat standard for the initial dilution, to create a 2X serial dilution ranging from 2 ng to 0.03 ng.

This sensitivity series may be used for multiple genetic analyzers in a laboratory. The series will not be stored for future use.

If an instrument's sensitivity decreases by more than a factor of two, remove the instrument from use for casework. Troubleshoot the instrument and if necessary, contact the company for repair.

III. Documentation of these checks and calculations will be recorded in the LAM.

## DNA QUALITY ASSURANCE
## EVALUATION OF A TECAN FREEDOM EVO® 150 WORKSTATION

I. When a new automated workstation is installed and set up in the laboratory by the manufacturer it must be evaluated before it may be used for casework. The following system checks and test plates must be conducted:

A. System Checks

1. Make sure all diluter connections and DiTi cones are finger-tight.

2. Flush the system with at least 30 mL of system fluid.

3. If bubbles are still observed within the diluters or tubing, continue flushing the system.

4. Run the extraction method with water only for one column of "samples". Watch the instrument as it runs and visually verify the method runs as expected.

B. Checkerboard Contamination Plates
Complete three checkerboard contamination plates as follows:

1. Perform an automated extraction on a total of 40 buccal standards and 40 blank controls alternating by well across a 96-well plate.

2. Complete the extraction, quantification, and amplification steps on the Tecan.

3. Profile all standards and blanks using a genetic analyzer.

4. Examine all electropherograms to ensure the proper profiles are identified and no blanks are contaminated.

**NOTE: The data from each of the test plates must be maintained in the LAM.**

II. When major equipment repairs are necessary for the Automated Workstation, the above system checks and test plates may be required.

# DNA QUALITY ASSURANCE
# EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE
# TECAN FREEDOM EVO® 150

I. Maintenance Checks

A. Tecan or another qualified company will perform a preventative maintenance on the Tecan Freedom EVO® 150 Automatic Workstation annually.

B. System checks to be completed following preventative maintenance:

1. Make sure all diluter connections and DiTi cones are finger-tight.

2. Flush the system with at least 30 mL of system fluid.

3. If bubbles are still observed within the diluters or tubing, continue flushing the system.

4. Run the RoMa verification script ensuring the plates are gripped securely and placed properly at each deck position. Ensure the Te-Shake clip is fully engaged each time a plate is placed at this position.

5. Run the extraction method with water only for ten columns of "samples". Watch the instrument as it runs paying close attention to the LiHa positioning across the entire plate and visually verify the method runs as expected.

6. Perform an automated extraction on a total of eight buccal standards and eight blank controls alternating by well across the first two columns of a 96-well plate. Complete the extraction, quantification, and amplification steps using the Tecan. Profile all standards and blanks using a genetic analyzer. Examine all electropherograms to ensure the proper profiles are identified and no blanks are contaminated.

C. All maintenance records will be maintained in the LAM.

II. Calibration

A. Procedure:

1. The liquid handling arm will be checked for accuracy and precision by Tecan or another qualified company. The company will provide certification as to the precision of the instrument.

2. The instrument will be calibrated according to the manufacturer's specifications.

B. Frequency: Annually.

C. Results: All results will be maintained in the LAM.

D. Course of Action: For hardware-related issues, contact Tecan to ensure the instrument functions within the manufacturer's specifications. If adjustments to LiHa positioning or RoMa vectors are necessary, contact Promega for assistance and remove the Tecan Freedom EVO® 150 from casework until necessary repairs and calibrations are completed.

III. Daily Maintenance

A. Before setting up a run

1. Check all syringe and diluter connections. Tighten, if necessary.

2. Check DiTi cones. Clean and tighten, if necessary, using the DiTi cone wrench.

3. Check for bubbles. Flush the system until all bubbles are removed.

B. Upon completion of all runs for the day

1. Refill the system liquid.

2. Empty the waste container.

**DNA QUALITY ASSURANCE**
**EQUIPMENT PERFORMANCE CHECKS AND MAINTENANCE**
**APPLIED BIOSYSTEMS™ RAPIDHIT™ DNA SYSTEM**

I. Weekly
If the instrument will not be used for casework in a given week, a known reference standard will be run on the instrument

II. After vendor service

A. Run an ACE GFE Positive Control Cartridge.

B. The ACE GFE Positive Control Cartridge must type correctly. Record the results in the LAM.

C. If a passing result is not obtained, repeat the procedure with a new cartridge. If a second cartridge does not pass, contact the manufacturer for service and notify the technical leader.

III. Annual System Verification

A. A NIST SRM or NIST Traceable Standard will be used to verify the procedures are able to generate typing results.

B. Compare the results to the known results for the standard. If there are any discrepancies, repeat the procedure. If the discrepancies are not resolved, contact the technical leader. Record the results in the LAM.

IV. As needed

A. If the instrument remains idle for 15 days, a prompt that maintenance needs to be conducted will appear. Insert a RapidHIT™ ID Utility Cartridge into the instrument. The cartridge can remain on the instrument for approximately two 15-day cycles (approximately 30 days).

B. Primary cartridge: The primary cartridge lasts for approximately 100 to 150 sample runs or 6 months once on the instrument. When replacing the primary cartridge, a RapidHIT™ ID Utility Cartridge, GFE Control (Ladder) Cartridge, RapidHIT™ ACE GFE Positive Control Cartridge, and a RAPIDHIT™ ACE GFE Negative Control Cartridge will be run. The RapidHIT™ ACE GFE Positive Control and the RAPIDHIT™ ACE GFE Negative Control Cartridges must pass.

# DNA QUALITY ASSURANCE
# ANNUAL SYSTEM VERIFICATION

I. Purpose

A standard that is traceable to a NIST Standard Reference Material (SRM) will be used to annually verify the DNA procedures are able to generate typing results for each technology utilized (e.g. STR, Y-STR). The NIST SRM is a certified standard reference material issued under the NIST trademark. The standard and documentation of the traceability of the standard to the NIST SRM may be obtained from the ISP Indexing Laboratory. The NIST traceable standard will be assigned a lot number designated by the date the traceability was established.

This system verification must be completed at a minimum from amplification to characterization for each automated and manual method, instrumentation and chemistry in use at the laboratory or following any substantial change in test kit, platform or chemistry.

II. Procedure

Extract the standard, if necessary. Extraction of the standard traceable to the NIST SRM will be required when no previous extract remains or when the lot of NIST traceable standard changes. Amplify using the appropriate instrumentation and chemistries. Analyze the samples using the appropriate genetic analyzers.

III. Assess Results

Compare the results to the known results for the standard. Any discrepancies will be reanalyzed and resolved. Create a QC entry in the LAM and attach the supporting documentation.

## DNA QUALITY ASSURANCE
## PROFICIENCY TESTING

I. Proficiency testing is performed in accordance with the Federal Bureau of Investigation Quality Assurance Standards Audit Document.

II. All comparisons reported as inconclusive on a proficiency test will be reviewed by the technical leader for compliance with laboratory guidelines prior to submission to the vendor.

III. Upon return of the results from the vendor, the quality review coordinator (QRC) will review the proficiency tests. The documentation of the QRC's review along with the vendor results will be forwarded to the technical leader for review. Issues of non-concordance identified by the vendor will be documented as outlined in QM-6 Competency and Proficiency Tests in the Quality Manual.

## DNA QUALITY ASSURANCE
## REINTERPRETATION OF LEGACY DATA

I. A DNA analyst who has previously qualified through the Illinois State Police Training Program in a legacy technology, chemistry, and platform may reestablish their qualification for the purpose of reinterpreting legacy data.

II. To reestablish their qualification, an analyst must review the Illinois State Police validation studies and the most recent archived procedures for the legacy technology, chemistry, and platform.

III. Laboratory management will provide documentation of this review to the DNA technical leader.

IV. The DNA technical leader will provide documentation of their approval to laboratory management authorizing the analyst to reinterpret legacy data for two years.

V. Once authorized, the DNA analyst may use the legacy procedure for re-interpretation. Any statistics reported will apply current population frequency data and formulas. Combined Probability of Inclusion will not be used.

## DNA QUALITY ASSURANCE
## AUDITS

I. All laboratories will be internally audited once a year according to guidelines established in QM-9 of the Command Quality Manual.

II. In addition to the Command's inspection program, each DNA laboratory will be audited annually in accordance with the FBI DNA Quality Assurance Audit Document. A record of the audit report will be maintained in the laboratory.

## DNA QUALITY ASSURANCE
## REPORTS (NOTES PACKETS)

I. All pages of the notes packet will be automatically appended with page numbers and initials of the analyst who authors the report.

II. Notes packets should contain notations of the following, when applicable:

A. Documentation of evidence seals both at the time of evidence receipt and completion of analysis.

B. The description of the item and the amount of the stain used for DNA analysis. Include a visual representation, if desired.

C. The order the samples are extracted for DNA analysis must be clearly documented in the case notes. This must show the separate handling of the unknowns and their reagent blanks from the standards and their reagent blank(s).

D. Disposition of evidence items and/or remaining stain specimen, including repackaging and any sub-items produced. Samples identified as work product that have been discarded will be documented.

E. If assistance with analytical work is received from an evidence technician or another analyst, the technician or analyst's name must be added to the worksheets or forms produced.

F. All testing performed and the results.

G. When a notes packet contains multiple electropherograms for the same sample, the electropherogram(s) used for interpretation will be clearly identified.

# DNA QUALITY ASSURANCE
# REVIEWS

I. **Author Review**

Prior to submission for administrative and technical review, all cases must be reviewed by the author of the report for clerical accuracy, technical accuracy and completeness. Submission of the assignment to technical review indicates the author's review has been completed.

II. **Administrative and Technical Review**

All aspects of an assignment will be reviewed by a current or previously qualified analyst in the methodologies being reviewed. The technical reviewer must have participated in proficiency testing to the full extent in which the reviewer participated in casework. The minimum scope of this review will be to determine whether the analytical results support the interpretations and conclusions stated in the report.

Concurrence of the technical reviewer with the analyst's conclusions will be indicated by completion and approval of the appropriate technical review checklist in LIMS.

Discussions between the analyst and technical reviewer will be documented in the routing history. Questions or concerns identified by the technical reviewer should be brought to the attention of the forensic scientist for resolution. If a difference of opinion occurs between the forensic scientist and the technical reviewer regarding this concern or question, the issue should be brought to the attention of the technical leader and will be resolved in accordance with the appropriate policies in the Command Directives and the Quality Manual.

III. **Supervisory Review**

These are outlined in the Command Directives and in the Command Quality Manual.

IV. **Court Monitoring**

This is covered in the Command Quality Manual under Administrative Reviews and Courtroom Testimony Reviews.

## DNA QUALITY ASSURANCE
## SAFETY

I. The safety program is found in the Command Safety Manual and laboratory's Facility Operations Manual.

II. This Procedures Manual and manufacturer's documentation also contain information of safety.

III. MSDS must be available in the laboratory and/or on-line.

## DNA QUALITY ASSURANCE
## OUTSOURCING

I. Vendor laboratories providing outsourcing services for the FSC will demonstrate compliance with all current FBI QAS Audit Document Standards. The technical leader responsible for the laboratory which may outsource samples/cases must review and approve all technical specifications for the outsourcing agreement before the contract is awarded. To demonstrate the laboratory's ownership of the resulting DNA results for CODIS purposes, the State CODIS Administrator's and the appropriate technical leader's approval of the agreement and/or analysis must be obtained and documented before initiation of the DNA analysis by the vendor laboratory. The documentation of the state CODIS administrator's and the technical leader's approval for a statewide contractual outsourcing agreement will be maintained at Command Headquarters. If the outsourcing agreement is site specific then the documentation of the state CODIS administrator's and the technical leader's approval will be maintained at that particular laboratory where the CODIS upload or search may occur. See Command Directive TCH 20 for additional information.

II. An initial on-site visit to the vendor laboratory will be required before accepting the technical specifications of an outsourcing agreement. The initial on-site visits will include:

1. An evaluation of facility requirements outlined in QAS Standard 6
2. An evaluation of clean techniques utilized in the laboratory
3. An evaluation of critical reagent quality records
4. An evaluation of critical equipment quality records
5. An evaluation of laboratory corrective actions
6. An evaluation of case file documentation
7. An evaluation of electronic data back up and security

Should the contract extend beyond one year an additional site visit will be completed or appropriate documentation of a site visit will be obtained from another NDIS participating laboratory.

III. Review of data obtained from outsourcing laboratories will include:

1. All DNA types identified for each sample
2. Acceptance of positive, negative and reagent blank controls
3. Acceptance of allelic ladder and internal lane standard results
4. Verification of DNA types reported, profile eligibility and specimen category assignment for CODIS
5. The final report listing results for each tested item and conclusions

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

## APPENDIX V: CODIS

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**Reviewed by:**

_____
Forensic Scientist Heather May, Chairperson
Forensic Biology/DNA Command Advisory Board

**Approved by:**

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Forensic Scientist Jeanne M. Richeal
DNA Technical Leader

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Brenda Danosky
FB/DNA Program Manager

## INTRODUCTION

CODIS (Combined DNA Index System) is the FBI's software system that is composed of LDIS (Local DNA Index System), SDIS (State DNA Index System) and NDIS (National DNA Index System). This software allows DNA profiles entered from evidentiary samples to be compared to DNA profiles that reside at the local, state and national levels to provide investigative information such as the identity of a potential suspect or the involvement of this individual in another crime.

## GENERAL INFORMATION ON CODIS

I. The original CODIS core STR loci are D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX, and vWA.

   The expanded CODIS core STR loci are D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, and D22S1045.

II. Alleles, not genotypes, are entered into CODIS and can be referred to as a specimen or a CODIS profile.

III. Each DNA analyst is responsible for creating the proper upload files from their casework in LIMS. These files will be uploaded by the local CODIS administrators or their designees.

IV. Entries into CODIS may not be the same as the interpretation results of the item to allow for the different indexes' stringency requirements.

V. Alleles derived from evidentiary profiles that are unambiguously attributed to a source other than the perpetrator shall not be entered into CODIS (i.e. alleles known to be contributed solely by the victim).

VI. If there are multiple exhibits within a case that could have originated from the same source, the exhibit with the most complete information at the CODIS core loci will be selected for entry.

VII. Victim and elimination reference profiles and non-probative profiles cannot be entered into CODIS. The exception per Illinois statute is an evidentiary reference standard collected from a victim of a homicide.

VIII. An unsourced open profile can be entered into an acceptable specimen category of NDIS without being compared to an elimination reference standard **if** there is a documented request to the agency for this standard.

IX. A search of CODIS can result in a candidate match, which is a computer-generated link between samples. These candidate matches may or may not be valid associations and require review by a DNA analyst.

X. The following are the minimum requirements for upload and search of the respective index systems:

A. LDIS: A minimum of six autosomal STR loci are required for entry into LDIS. Profiles must satisfy a moderate match estimate (MME) threshold of no more than ten candidate matches in 700,000 at the original core loci with moderate stringency and no mismatches. LDIS contains the laboratory personnel DNA database.

B. SDIS: A minimum of six autosomal STR loci are required for search/upload into SDIS. Profiles must satisfy a moderate match estimate (MME) threshold of no more than ten candidate matches in 700,000 at the original core loci with moderate stringency and no mismatches.

1. Binary profiles: When a profile does not satisfy the MME threshold, the calculation must be included in the notes packet to ensure correct entry of the profile and evaluation of the MME.

2. STRmix™ profiles: The Excel-based program POPSTR is used to evaluate profile MME. The notes packet will contain a POPSTR evaluation for each CODIS-eligible contributor. Exceptions may include profiles assessed as common donors using the Excel-based program FORESTR or profiles associated to victim or elimination profiles.

C. NDIS: Refer to the NDIS procedures for the minimum number of CODIS core loci and match rarity estimate (MRE) threshold required for a search of the different specimen categories.

XI. Any profile determined to be eligible for entry into CODIS must have a technical review by a qualified DNA analyst prior to uploading or searching at SDIS or before an agency is notified of the search results. The technical review will include the following:

A. Verify that all DNA types are supported by raw and/or analyzed data (e.g., electropherograms).

B. Verify that expected results were obtained in all associated positive and negative controls, reagent blanks, ladders and internal lane standards.

C. Verify that the specimen category is correct, and that the "Source ID" and "Partial Profile" fields have been completed correctly.

D. Verify the eligibility of the entry.

## STR DATA ENTRY IN CODIS

I. Enter the Specimen ID for the profile. This will generally contain the laboratory case number and exhibit number from which the profile originated. Append the following suffixes to the specimen ID, when appropriate:

| | | |
|---|---|---|
| -CP | (Criminal Parentage) | Must be used when an obligate parental allele profile is entered in any specimen category. |
| -HV | (Homicide Victim) | Must be used when an evidentiary reference standard is entered in the Legal specimen category in accordance with Illinois Statute 55 ILCS 5/3-3013. |
| -SA | (Sexual Assault) | May be used when the profile is associated with an offense of sexual assault or sexual abuse. Must appear last when used in combination with other suffixes. |

II. Select the appropriate category for the specimen.

A. The following are the commonly used categories for evidentiary profiles interpreted as single source:

1. Select the **Forensic Unknown** specimen category if the profile is complete at the original CODIS core loci. This profile can have three alleles at one locus to account for a tri-allelic pattern but may not have more than two alleles at any other locus. This includes profiles at the original CODIS core loci that are fully deduced from mixtures where there are no loci with multiple genotype pairings.

2. Select the **Forensic Partial** specimen category if the single-source profile has allele and/or locus dropout at any of the original CODIS core loci. This profile can have three alleles at one locus to account for a tri-allelic pattern but may not have more than two alleles at any other locus. This profile must be fully deduced where there are no loci with multiple genotype pairings.

3. Select the **Forensic Mixture** specimen category if the profile is assumed to have originated from only one contributor but consists of multiple genotype pairings.

4. Select the **Forensic Limited** specimen category if the profile has a low Moderate Match Estimate (MME) and is expected to generate a large number of adventitious hits with a one-mismatch search configuration. Samples in this category will be uploaded to SDIS and will not be searched against the Forensic Partial or Forensic Mixture specimen categories.

5. The **Forensic Targeted** specimen category may be selected by the local CODIS administrator or their designee for profiles originally entered into the **Forensic Partial** or **Forensic Mixture** categories that do not meet the NDIS MME threshold of 1 in 10 million. The local CODIS administrator may modify the search stringency from moderate to high at one or more loci to determine if the required MRE threshold of 1 in 10 million can be met.

6. The **Other** specimen category may be selected for profiles that would otherwise be categorized as Forensic Unknown, Forensic Partial, or Forensic Mixture but are not eligible for NDIS.

B. The obligate alleles for a perpetrator of an alleged sexual assault that is deduced by assuming the known parent's DNA profile from the DNA profile of their offspring can be searched/uploaded into CODIS. The categories to be used for obligate allele profiles are found below. The –CP suffix will be included in the specimen ID. Contact the appropriate local CODIS administrator for further information.

1. Obligate parental alleles deduced using the DNA profile of a male offspring

   a. Select the **Forensic Partial** specimen category and enter only the loci with a definitive obligate allele. If the resulting MME is at least 1.000E+007, the profile will remain in this specimen category.

   b. If the MME is less than 1.000E+007, the specimen category will be changed to **Criminal Parentage** and the loci with ambiguous obligate alleles will be added.

   c. Y-STR analysis will be used to evaluate any potential candidate matches that cannot be excluded by the autosomal STR results. This includes full profile candidate matches, as well as those with a one-locus inconsistency.

      Potential candidate matches with inconsistencies at the rapidly mutating loci, DYS570 and DYS576, will not be excluded without first being reviewed by the technical leader. A one-locus inconsistency at any of the other loci will result in the comparison being inconclusive and analysis of additional loci may be warranted.

2. Obligate parental alleles deduced using the DNA profile of a female offspring

a. Select the **Forensic Partial** specimen category and enter only the loci with definitive obligate alleles. If the resulting MME is at least 1.000E+007, the profile will remain in this specimen category.

b. If the MME is less than 1.000E+007 but greater than or equal to 7.000E+005, the specimen category will be changed to **Criminal Parentage** and the loci with ambiguous obligate alleles will be added.

c. If the MME is less than 7.000E+005, the obligate profile must be removed from CODIS.

d. Potential candidate matches with a one-locus inconsistency will result in the comparison being inconclusive and analysis of additional loci may be warranted.

C. A mixture of two people may be entered into CODIS. In general, a mixture of three or more people should not be entered into CODIS. Select the **Forensic Mixture** specimen category if the specimen is a mixture.

D. The following are the commonly used categories for reference standards that have been submitted to the casework laboratory:

1. Select the **Suspect, Known** specimen category when the suspect reference profile is more complete than or is excluded from the probative evidentiary profile(s).

   Only suspect reference standards submitted for comparison with evidentiary items may be entered into or searched in CODIS. Suspect reference standards cannot be submitted for the sole purpose of searching the database.

2. Select the **Legal** specimen category when uploading an evidentiary reference standard collected from the victim of a homicide. The Specimen ID of these CODIS profiles must end with the designation "-HV," (e.g. J08-00001-10-HV).

3. Contact your Local CODIS Administrator for information on how to proceed with profiles related to the **Missing Persons, Relatives of Missing Person, or Unidentified Human (Remains)** Indexes.

III. The following questions will be answered as "Yes" or "No":

A. Source Identified?

1. Determine whether the source of the specimen has been identified via an association to a reference standard or an adjudication. Select "Yes" or "No".

2. Select "No" for partial or mixture DNA profiles if it is possible that future reference standards would not be excluded from the DNA profile.

B. Partial Profile (for the overall profile)?

1. Select "Yes" for Partial Profile, if the profile entered is incomplete at any of the CODIS core loci amplified.

2. Select "No" if the profile is complete at the original CODIS core loci.

IV. Entering of alleles

A. If the interpretation results in a profile that has loci with multiple genotype pairings, enter all alleles and designate any allele that is specific to the probative profile (ex. non-victim allele) as an obligate allele using a "+" or enter only the obligate allele. For example, the genotype pairings (*A,B*), (*A,C*), (*A,D*) can be entered into CODIS as *A or A+,B,C,D*. It is important to consider the different stringency requirements of CODIS as to which option will provide the best opportunity for a valid association. If there are any questions, please contact the Local CODIS Administrator of the respective laboratory.

B. A CODIS profile entered into the Forensic Unknown or Forensic Partial specimen categories cannot have an obligate allele marked with a "+". If an obligate allele is marked with a "+" in either one of these categories this will result in the specimen not meeting the NDIS definition of either specimen category.

C. Select "Yes" under the Partial Profile column for any loci that are incomplete.

V. A DNA record is the result of the completion of steps I through IV.

## SEARCHING THE INDEXES OF CODIS

I. An LDIS/SDIS search of specimen profiles will be performed in accordance with current CODIS guidelines. Profiles in the **Forensic Unknown** specimen category will be searched allowing one mismatch. All other profile categories will be searched allowing zero mismatches.

II. An open single source profile of six loci or more that is not eligible for inclusion in CODIS will be searched as a quality assurance measure against the laboratory personnel DNA database.

If no associations are returned, the Local Match Detail Report from the keyboard search will be included in the notes packet to document the correct entry of the profile.

If an association is excluded, a technical review for correct entry and the exclusion will be conducted and documented. The Local Match Detail Report will not be included in the notes packet.

If an association cannot be excluded, the Technical Leader will be notified. Refer to Command Directive TCH 21. If further evaluation does not support exclusion, the Local Match Detail Report from the keyboard search will be included in the notes packet.

III. Profiles related to the Missing Persons and Unidentified Human (Remains) Indexes will be searched in the same manner as an evidentiary profile.

IV. Profiles in the Criminal Paternity and Relatives of Missing Person Indexes will only need to be searched against the laboratory personnel DNA database. A low stringency search will be conducted once the CODIS profile is uploaded to SDIS. These profiles must be uploaded as soon as possible to ensure a timely search at SDIS.

V. Any search requests that are outside the established criteria for LDIS and SDIS will be directed to the DNA technical leader and to the state CODIS administrator or to their respective designees.

VI. For additional information on permissible searches, contact your Local CODIS Administrator.

## MATCH REVIEW AND DISPOSITION

I. GENERAL INFORMATION

A. Evaluation of Candidate Matches

1. Binary profiles: The candidate match will be evaluated to determine if it is a valid or non-valid association. This may include review of the data in the casefile of the candidate match to ensure the CODIS profile was correctly entered as well as any other information necessary to evaluate the candidate match. Once a candidate match is determined to be a valid association, then this may also be referred to as a hit.

2. STRmix™ profiles: Candidate matches involving STRmix™ profiles may be evaluated manually or using likelihood ratios through the Excel-

based software tool DATABSR. The software tool will be used to manage and evaluate associations. Once a candidate match is determined to be a valid association, then this may also be referred to as a hit.

The likelihood ratio of a valid association will be included in the notes packet.

B. If an offender sample profiled in a legacy amplification chemistry is involved in a candidate match with an evidentiary profile that contains:

1. Less than twelve loci matching at high stringency, the analyst will request PowerPlex® Fusion. The candidate match will need to be updated with the additional loci in Match Manager by the Indexing Laboratory, so the analyst can evaluate this information and determine the appropriate disposition.

2. Twelve or more loci matching at high stringency, the analyst may disposition the match as an offender hit.

   Upon receipt of the offender hit letter, the analyst will review the candidate match in Match Manger to confirm any loci added to the offender profile by the Indexing laboratory still support the disposition of offender hit.

C. Y-STR loci may be necessary to determine the proper disposition of the candidate match (i.e. Criminal Parentage).

D. Each candidate match that is excluded from an evidentiary or databasing reference standard will be evaluated as a partial match according to the following minimum requirements:

1. The evidentiary autosomal STR profile(s) must be male, unsourced, single contributor or a major with a single genotype at all interpreted loci, and results for at least ten of the thirteen original CODIS core loci or fifteen of the twenty expanded CODIS core loci.

2. The autosomal STR and evidentiary or databasing reference profiles must share a minimum of 70% (rounded down to the nearest whole number) of the alleles at the CODIS core loci. Homozygote genotypes will count as two alleles.

   Additionally, if the DYS391 locus is detected the results must match.

   If the two profiles do not meet the minimum percentage of shared alleles and the analyst determines the partial match warrants additional

consideration, an exception may be made upon consultation with a technical leader.

3. If the minimum percentage of shared alleles between the profiles has been met, Y-STR analysis must be conducted on both exhibits. If either one of the autosomal STR profiles is female, then no further DNA analysis will be conducted by ISP and the partial match will not be reported. A candidate match that involves a Relative of Missing Persons specimen is exempt from this procedure. For additional information, please consult the Command Directives.

   If there is insufficient sample or reagent blank to perform Y-STR analysis of either exhibit, the partial match will not be reported.

   If the partial match is between two evidentiary profiles, the case analyst(s) will determine if there is an evidentiary reference standard matching one of the profiles that has not been uploaded to CODIS. If there is no evidentiary reference standard on which to conduct Y-STR analysis, the partial match will not be reported.

II. All candidate matches require a disposition in CODIS. Before the profile is entered into CODIS, it must be evaluated to determine if it has been sourced since this information affects the disposition. A list of dispositions can be found within CODIS. If there are questions as to the appropriate disposition, the DNA analyst should contact their Local CODIS Administrator.

   A. Sourced profiles are those from which a reference standard cannot be excluded. These also include profiles to which a hit was previously made to a convicted offender, arrestee, detainee, legal, or other databasing reference standard.

      1. If a specimen is sourced by an evidentiary reference standard and hits to:

         a. A reference standard, evidentiary or databasing, the local CODIS administrator or DNA analyst will verify the names associated with both specimens are the same. If the names are concordant, a disposition of **Investigative Information** for hits to evidentiary reference standards or **Conviction Match** for hits to databasing reference standards will be used and no verification or laboratory report is required. If the names are not concordant, proceed as for an unsourced/open profile, below. The results of this check will be documented.

         b. Another sourced specimen, a disposition of **Investigative Information** will be used and a laboratory report will be issued.

2. The Local CODIS Administrator or DNA analyst may contact the appropriate agency to determine whether the case has been adjudicated. If there has been a conviction or a plea, obtain the convicted person's name and proceed as above.

3. If a specimen is sourced by a previous hit to a databasing reference standard, was not compared to an evidentiary reference standard, and at a later date the specimen hits to a reference standard analyzed by an Illinois State Police (ISP) casework laboratory (e.g. suspect known, legal) or another sourced specimen, the local CODIS administrator or DNA analyst will verify the names associated with both specimens are the same. If the names are concordant, a disposition of **Investigative Information** will be used and a laboratory report will be issued.

B. Unsourced or open profiles

1. If the hit is to an Illinois Indexing reference standard (e.g. convicted offender, arrestee, detainee), the local CODIS administrator or the DNA analyst will request verification of that standard as follows:

   a. The casework laboratory will disposition the hit as appropriate (e.g. **Offender Hit**, **Arrestee Hit**).

   b. To ensure that the Indexing Laboratory has received the hit disposition, confirm that the Indexing Laboratory's disposition has been changed to match the local casework laboratory's disposition within the next two working days.

   c. Documentation that the Indexing reference standard has been verified must be received before the hit is reported to the agency. The Local CODIS Administrator or DNA analyst will issue a laboratory report and request an evidentiary reference standard from the subject for comparison.

2. If the hit is to a non-Illinois databasing reference standard, please refer to the NDIS procedure "6.1 NDIS Offender Candidate Match".

3. If the hit is to a reference standard analyzed by an Illinois casework laboratory (e.g. suspect known, legal), the casework laboratory will disposition the hit as appropriate (either **State Defined #1** or **Legal Index Hit**) and a laboratory report will be issued. If the reference standard was analyzed by an Illinois State Police (ISP) casework laboratory, data may be obtained and the inclusion with statistics may be reported at the agency's request. Submission of a new reference standard is not required. If the reference standard was analyzed at a non-ISP casework

laboratory, submission of a new reference standard is required prior to reporting the inclusion with statistics.

4. If the hit is to a non-ISP casework laboratory sample and a reference standard cannot be excluded, a disposition of **Forensic Hit** will be used. A laboratory report will be issued, which will include a request for submission of a reference standard from the subject for comparison.

5. If the hit is to a non-Illinois evidentiary profile, please refer to the NDIS procedure "6.2 NDIS Forensic Candidate Match".

C. If a candidate match between two specimens contains limited information, such that there is insufficient overlapping data to conclusively evaluate the candidate match, it will be dispositioned as **Insufficient Data**. This may occur in situations where there is limited available data or low likelihood ratio. A comment within CODIS may be used to document the disposition.

In accordance with the NDIS Operational Procedures, the information from a candidate match that has been dispositioned as **Insufficient Data** will not be released.

D. An effort will be made to disposition the candidate match in CODIS and inform the agency of the hit within 30 working days of receiving the Match Detail Reports. Additional information on dispositioning can be found in the NDIS Procedures. If the candidate match is between a casework laboratory's specimen and a databasing reference standard, it is the responsibility of the casework laboratory to determine the resolution. If the candidate match is between casework laboratories, the respective DNA analysts or local CODIS administrators of the appropriate laboratories will ensure any issues are resolved and the dispositions of the profiles are congruent.

## DOCUMENTATION AND REPORTING

I. To ensure all appropriate profiles have been entered into CODIS a LIMS Crystal Report of exported profiles will be generated and compared against the list of imported profiles from CODIS. This reconciliation will be conducted by the local CODIS administrator or their designee at least once per month.

II. Documentation of hits

A. The Match Detail Report will be attached to the assignment.

B. If the search of an unsourced profile results in a hit, the Local CODIS Administrator or the DNA analyst will issue a laboratory report with the information on the hit. Information for the other case (e.g. agency name, case number, contact information) or Indexing reference standard should be

included, as applicable. Therefore, if both cases of an in-state hit have unsourced profiles, a laboratory report will be issued for both cases.

III. Documentation of non-valid associations (i.e. **No Match** dispositions)
In accordance with the NDIS Operational Procedures, the information from a **No Match** will not be released.

IV. Documentation and reporting of a potential-relative match

A. Refer to the Command Directives for additional information.

B. If Y-STR analysis excludes the evidentiary or databasing reference standard as a paternal relative of the evidentiary profile, a laboratory report will be issued indicating Y-STR analysis was performed on the relevant exhibits.

## UPLOAD OF DATA

I. The Local CODIS Administrator will upload data from LDIS to SDIS via the FBI's Criminal Justice Information Service-Wide Area Network (CJIS-WAN) or by other FBI-approved devices.

A. Uploads can be set to occur automatically by the LDIS laboratory.

B. Normally, incremental uploads will be performed.

C. Uploads must be performed by the LDIS laboratory at least once per week.

II. In the event that the Local CODIS Administrator position is unoccupied, the laboratory will not upload DNA profiles to NDIS.

## MODIFICATIONS OF DNA RECORDS IN CODIS

I. CODIS profiles may be modified (e.g. alleles of the specimen, Source ID changed to "Yes") by the Local CODIS Administrator or the DNA analyst.

II. Any revisions to the CODIS profile resulting from an error in the original upload file will be documented in an amended notes packet. Modifications due to a change in the specimen status (e.g. source ID or specimen category) will be recorded in the CODIS audit trail.

## REMOVAL OF DNA RECORDS FROM CODIS

I. Ineligible CODIS profiles

A. A DNA record may be removed by the Local CODIS Administrator or the DNA analyst.

B. The following are examples of an ineligible CODIS profile:

1. The profile is determined to have hit to an individual's consensual partner or in the situation that the individual is known by the victim and is not suspected to be the perpetrator.

2. The profile is not discriminating enough to remain in CODIS.

3. The laboratory receives information from an agency that a profile was not connected to a criminal investigation.

C. The following are the steps for removal of a DNA record:

1. Before the profile is deleted, edit the Specimen Detail Report by adding the reason for the deletion in the comments box with the analyst's initials who is deleting the profile.

2. Document the removal of the DNA record by attaching the Specimen Deletion Report to the assignment. If a laboratory report has already been issued stating that the DNA profile was included in CODIS, a CODIS Removal Report shall be issued stating the DNA profile has been removed and will no longer be searched.

3. An incremental upload will be performed immediately after any deletion to ensure timely removal of the DNA record from all levels of CODIS.

4. The Local CODIS Administrator or the DNA analyst will confirm that the DNA record has been deleted from SDIS and NDIS by examining the Reconciliation Reports.

II. Expungement

The request for an expungement of a DNA record will be handled in accordance with the laws, statutes, acts and administrative rules that are in place at the time of the request. The Local CODIS Administrator or the DNA analyst will notify the Laboratory Director or designee of the expungement order. Written documentation that the expungement was completed in accordance with the court order will be provided to the court (e.g. a copy of the supplemental laboratory report).

Follow the steps for removal of the DNA record as outlined above under I.C.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

## APPENDIX VI: INTERPRETATION OF OUTSOURCING VENDOR DATA AND CODIS HITS

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Approved by:

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FB/DNA Program Manager

## INTRODUCTION

The following information will be used whenever determining if there is a "true" CODIS hit involving Outsourcing Vendor data. Do not hesitate to remove Outsourcing Vendor case profiles from CODIS if the data given is too incomplete or too difficult to make any decisions with regards to CODIS hits. If necessary, request more work to be done on the sample. Remember that the purpose of entering data into CODIS is to have enough information to exclude rather than include.

### General Considerations for Potential Hits

I. Remember the Outsourcing Vendor most likely uses different reaction conditions and interpretation guidelines than ISP. Outsourcing Vendor interpretation guidelines may be very subjective and interpretation for each case may be different. Common sense and caution are necessary when reviewing Outsourcing Vendor data to determine a hit. CODIS data from the Outsourcing Vendor will either be a clean profile or a mixture where the Outsourcing Vendor has "deduced" a male profile. Mixtures in which the Outsourcing Vendor did not deduce may be deduced and entered into CODIS with caution.

II. Keep in mind the degree of degradation, inhibition, etc. in the electropherograms.

III. Try to determine if the listed alleles constitute a genotype or if it is just a list of alleles with other possible genotypes (Is it a deduced profile? What do the foot notes say?).

IV. If you cannot confirm or refute the hit with the information provided, consider pulling the profile from CODIS. Profiles can be re-entered later. If the profile is incomplete and more sample is available (extracted or unextracted), ask the FSC outsourcing project manager for more work to be done on the sample or for another stain to be typed if available. Call the Outsourcing Vendor and talk to the analyst, if you believe they can provide additional information that will assist you. If the ambiguity can not be resolved, contact the Technical Leader.

V. When in doubt, take the conservative route (remove the profile from CODIS, exclude rather than include).

### Specific Situations

I. *Single Source Profiles* - barring any other reason (degradation, etc.), these can be used as reported.

II. *Deduced Profile, Called "Genotype" by Outsourcing Vendor* - barring any other reason (degradation, etc.), these can be used as reported.

III. *Deduced Profile, "Other Genotypes Possible"*

A. 1 Allele Listed: Examine electropherogram to determine if the genotype is A, A; A, INC or all possible combinations of A and the alleles seen in the mixture.

B. 2 Alleles Listed: Examine the electropherogram to determine if you think it is a genotype or a "Could be A, A or A, B" call.

C. More Than 2 Alleles Listed: Examine the electropherograms and genotypes of all the samples, to see if there is an obligate allele (that is, an allele that the genotype MUST include). For example, with a mixture involving the victim who is 11,12 and the "deduced male profile" is 11,12,14, the 14 allele cannot be from the victim and must be from someone else. If so, consider all combinations of that obligate allele with the other alleles listed (from the previous example, this would include 14, 14 11, 14 and 12, 14). If not, use all combinations of the listed alleles as possibilities. Also keep in mind that profiles with 3 alleles exist.

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

## APPENDIX VII: FORENSIC BIOLOGY/DNA OUT-SOURCED CASE REVIEW

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## APPENDIX VII
## OUT-SOURCED CASE REVIEW

Case sample DNA data and the Common Message Format (CMF) files from contractual vendors will be reviewed prior to upload to the State DNA Index System (SDIS). All documentation used in the review will be added to the case file. The review of all suitable DNA profiles with CMF files and all appropriate controls must be completed no later than 30 days after the data has been received from the vendor, unless an extension is granted by the Laboratory Director. All case notes and written reports will be reviewed and any suitable profiles without CMF files will be uploaded into CODIS within 60 days unless an extension is granted by the Laboratory Director.

1. The Local CODIS Administrator (LCA) or designee will enter the contractual data into CODIS by importing the CMF file and immediately will **unmark for upload** all of the specimens in the import file. Suggested methods of retrieving all samples in an import file:

   A. Select the specimens in the import file by configuring a view in Specimen Manager/Advanced/Import File for the particular file or
   B. Configure a view by "Assigned to" name and the date the file was imported.

2. The LCA will print the Specimen Detail Report for each profile from CODIS Specimen Manager.

3. The analyst/reviewer will review the appropriate data for each specimen.

   A. The review will be documented by completing the Out-Sourced Case Review Checklist.
   B. Specifically, the analyst must review the eligibility, specimen category, the controls and allelic ladder associated with the specimen, and confirm the allele values on the Specimen Detail Report with the electropherograms.
   C. A profile is unacceptable if any of these categories are marked "NO".

4. If all of the data is acceptable:

   A. The analyst/reviewer will mark the specimen for upload in CODIS and perform a local and/or remote state search, as appropriate.
   B. Use Searcher to search the newly marked profile. **Do not use Autosearcher** until all samples have been reviewed.

5. If any of the data is not acceptable:

   A. The analyst/reviewer will complete an incident report and follow the incident report procedure appropriately, to include informing the appropriate Technical Leader.
   B. The analyst/reviewer will request the LCA or designee to delete the problem profile from CODIS.

C. **An unacceptable profile will not be searched.**

6. One person may perform all tasks if given authority by the LCA in CODIS Preferences.

# OUT-SOURCED CASE REVIEW CHECKLIST

Case/Specimen Number _____

Reviewer Name/Initials _____

Review Date _____

| | | Acceptable (circle one) | | Comments |
|---|---|---|---|---|
| 1. | Controls | | | |
| | Allelic Ladder | Yes | No | _____ |
| | Positive Amp Control | Yes | No | _____ |
| | Internal Lane Standard | Yes | No | _____ |
| | Negative Amp Control | Yes | No | _____ |
| | Extraction Blank | Yes | No | _____ |

| | | Acceptable (circle one) | | Comments |
|---|---|---|---|---|
| 2. | Case File | Yes | No | _____ |
| | Report | Yes | No | _____ |

| | | Acceptable (circle one) | | Comments |
|---|---|---|---|---|
| 3. | CODIS Eligibility | Yes | No | _____ |
| | | | | _____ |

| | | Correct (circle one) | | Comments |
|---|---|---|---|---|
| 4. | Specimen Category | Yes | No | _____ |

If incorrect, changed to: _____ Initials _____ Date _____

5. Specimen Detail Report (from CMF) Allele Designations

| | Agree with Electropherogram (circle one) | | Comments |
|---|---|---|---|
| | Yes | No | _____ |

6. Date marked for upload _____ Initials _____

7. Unacceptable data reported to: _____

Initials _____ Date _____

Profile deleted from CODIS Initials _____ Date _____

# ILLINOIS STATE POLICE

# FORENSIC BIOLOGY/DNA PROCEDURES MANUAL

## APPENDIX IX: CASE APPROACH REQUIREMENTS

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Approved by:

_____

Brenda Danosky
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## CASE APPROACH REQUIREMENTS
## GENERAL INFORMATION

This appendix contains requirements to ensure that the evidentiary items selected for analysis have the potential to provide results that are informative to a criminal investigation. It is important that presumptive and/or confirmatory testing be conducted as it may be informative to the investigation as well as aid sampling for downstream DNA analysis. In addition, requirements for case approach will provide consistency between analysts and laboratories within the Illinois State Police. Alternative approaches may be warranted based on case circumstances, priority status, and analyst assessment. Any situation that warrants an alternative approach will require documentation by the analyst.

Information provided by the agency will be assessed in order to determine the analysis of the evidentiary items. The information provided at case submission or through additional correspondence should be task-relevant so the analyst may determine what evidentiary items and analysis would be beneficial to the investigation. Should there be ambiguity regarding the case questions to be addressed, the laboratory will seek clarification prior to beginning examination. Essential information includes the alleged offense, number of alleged offenders, the location and/or who the item was recovered from.

The swabs from sexual assault kits will have DNA analysis performed without biological screening. However, if more than seven days have passed between the alleged assault and the collection of kit swabs, analysis of these kits swabs is not required, except in cases involving deceased victims. Analysis of other evidentiary items, such as clothing or bedding, may still be warranted.

Biological testing may not be appropriate for certain samples due to factors like sample size or age. When appropriate, these samples may be forwarded for DNA analysis with no biological testing. The reason for not performing testing will be documented in the case notes.

The biological examination of evidentiary items for a case will be based on a tiered approach. Analysis will begin with items that have been assessed as being potentially the most probative in order to aid the investigation. For details on the item submission policy, review Command Directive ESH Appendix 1. While the tiered approach limits initial examination to a specific number of items to provide the agency with results more expediently, it does not prevent additional analysis on an evidentiary item and/or other evidence to obtain probative results. If necessary, the laboratory will communicate with the agency to ensure that analysis is providing the investigative information that the agency is requesting.

If DNA analysis has answered the investigative question, additional evidence may be deferred. However, the agency can request analysis of deferred items if new information warrants further testing.

# CASE APPROACH REQUIREMENTS
# FORENSIC BIOLOGY TESTING AND COLLECTION

I. Techniques for locating body fluid stains

A. Semen or Saliva

- Initial examination for potential semen or saliva stains for subsequent testing may be achieved visually using the unaided eye in ambient room lighting.
- Tactile examination may also be useful in locating semen stains. Semen stains may appear off-white with a stiff texture.
- An alternate light source (ALS) will be used to facilitate the visualization and detection of fluorescing stains for subsequent analysis. Please note urine and sweat may also fluoresce using an ALS.
- Caution should be used on substrates with patterns or materials (i.e. menstrual pads, diapers) that have innate fluorescence.
- Use a marker to circle potential stains. Ensure the marker is non-contaminating and easily distinguishable from the evidence.
- Assign a question (Q) number to serve as a unique identifier for each stain and document clearly in case notes. This will allow tracking of the Q stain.

B. Blood

- Initial examination for potential bloodstains for subsequent testing may be achieved visually using the unaided eye in ambient room lighting and/or assistance from a bright magnifying light or stereoscope.
- Tactile examination may be beneficial, especially on dark or black substrates.
- Blood testing may not be warranted based on case information. For instance, the agency may have performed a presumptive test such as Bluestar®, age, storage conditions of item, etc. The reason for not performing blood testing will be documented.
- Use a marker to circle potential stains. Ensure the marker is non-contaminating and easily distinguishable from the evidence.
- Assign a question (Q) number to serve as a unique identifier for each stain and document clearly in case notes. This will allow for tracking of the Q stain.

II. Documentation within the Case Notes

Documentation of evidence examination is a critical aspect of biology examination. It is imperative all results are thoroughly recorded in the casefile as it reflects the quality of the analyst's examination. It is better to be overly detailed than to not provide enough information.

A. Clearly document the location and the biological testing for each questioned stain. When possible, use a photograph to supplement documentation to include the location of the Q testing area. It may be helpful to create a table with the testing results for each Q stain.

B. When presumptive and/or confirmatory testing is positive, documentation with a photograph is suggested. For evidentiary items with minimal staining and/or testing, a photograph will not be required as long as the documentation provides details of its coloring, size and location on the item. Photographs are not required for swabs. The photographs will clearly depict the tested area. With large items, it may be beneficial to include a full-scale photo and a photo scaled to the relevant questioned stain. Include a ruler to record the scale of the item and/or stain. It is not required to photograph stains that are negative, but it may be beneficial if questions arise as to locations tested on the item.

C. Measurements are approximate and the use of "~" is encouraged. When measuring a stain that is irregular, use the longest width and length.

D. Record information about an evidentiary item that may impact downstream DNA analysis (i.e. apparent mold).

E. Describe any defects to the item that may be of evidentiary value (i.e. missing buttons, apparent tears or burned areas, zipper functionality). Note the presence of any apparent stab or bullet holes. Fabric defects should only be documented when they are potential alternations to the garment compared to if it was new.

F. Use caution when examining pockets. Gently patting down the pocket and turning inside out with forceps is recommended. Record contents of pockets, collecting any recovered contents in separate packing. Further sub-itemization of pocket contents is not required.

G. If there are obvious differences in staining on an evidentiary item, it should be documented (i.e. smears).

III. Semen Testing on Clothing/Textiles

Once a potential semen stain has been located on clothing/textiles, it will be analyzed using presumptive (indication) and/or confirmatory (identification) testing for semen.

The following are the three tests available for semen testing:

- **Acid Phosphatase (AP)**: A presumptive color test used to check for the presence of this enzyme found in high quantities in semen.
- **P30:** A presumptive test used in the detection of the prostate specific antigen (PSA).
- **Kernechtrot-Picroindigocarmine Stain (KPIC):** A confirmatory test that includes using two color stains to visualize sperm cells microscopically.

In certain situations, all three tests may be necessary to ensure the quality and completeness of the analysis, as each test examines a different component of semen.

A. AP testing will be required to assist in screening and stain selection for clothing/textiles. However, it may be deferred on smaller questioned stains to conserve sample. If AP testing is not conducted, the reason must be clearly documented.

   Smaller cuttings should be taken from larger stains to map potential variations within the stain. Sampling should be conducted in a way to determine the extent of the stain that is positive but not so excessive that it prohibits subsequent semen testing (P30 and/or KPIC) or DNA analysis. Use caution with certain fabric types and colors; checking the filter paper for bleed through by a particular fabric may provide a better indication of test results.

   **Note:** If AP is negative, no further testing is required.

B. Inconclusive and positive AP results require further testing with P30 and/or KPIC to assist in sample selection for DNA analysis. Additional small cuttings should be removed from areas exhibiting inconclusive or positive AP results. Cuttings may be grouped based on stain location and AP results with clear documentation in the case notes.

C. Verify the P30 and/or KPIC test results correlate to the AP results. In situations with conflicting results, an analyst may choose to repeat testing, use a different semen test, or forward the sample for DNA if there are no other positive stains preserved from the item.

D. Negative semen testing results will only be reported in the following situations:
   - AP: negative
   - AP: +/-
     P30 and/or KPIC: negative
   - AP: +1, +2, +3, or +4
     P30: negative
     KPIC: negative

E. Results of semen tests will be used to determine the amount of stain selected and preserved for DNA analysis. An appropriate amount of the representative stain should be collected based on the combination of test results as well as consideration as to the age of the stain.

The preservation of the stain based on biology testing is a critical decision point in obtaining the most discriminating and informative DNA results. It is the responsibility of the analyst collecting the stain to determine if permission to consume for DNA analysis should be obtained. Obtaining permission to consume at the preservation stage allows DNA analysis to be performed more efficiently and effectively.

If the DNA analyst determines at DNA quantification that the questioned stain was under sampled and the stain was not consumed, then additional material will be collected for DNA analysis.

F. Document the questioned stain or the portion of the stain that is being preserved with a scaled photograph or written notes. Be mindful of the amount of sample placed in the microfuge tube(s) for DNA analysis. An excessive amount of substrate may hinder the extraction process, therefore multiple microfuge tubes may be necessary.

G. If semen is not indicated/identified and the case scenario alleges a digital or oral assault occurred, a swab should be collected from specific areas of the evidentiary item based on case information.

H. If reddish-brown staining is observed during semen examination, KM testing is not required unless the result would be probative.

## IV. Semen Testing on Condoms

A. Document the general condition of the condom (i.e. wadded up, tied off) and whether fluids are present (liquid or dried). If wet, the item should be allowed to air dry after samples have been collected.

B. Using swabs moistened with sterile water, collect one swab from "inside as received" and one swab from "outside as received" since orientation cannot be conclusively determined.

C. The collected swabs from each side of the condom can be forwarded for DNA analysis without semen testing. If no semen testing is performed, then a differential extraction will be conducted. However, if semen testing is performed with negative results, then a non-semen extraction will be conducted.

D. If reddish-brown staining is observed during examination, KM testing is not required unless the result would be probative.

V. Blood Testing on Evidentiary Items

A. Potential bloodstains on clothing or porous substrates may have soaked into the item making it difficult to readily observe, whereas potential bloodstains on non-porous substrates tend to dry on the surface.

B. Bloodstains on dark colored substrates may be difficult to locate. Use of a bright magnifying light or stereomicroscope may assist in stain visualization. Tactile feel may also be utilized.

C. Some evidentiary items have crevices (i.e. shoes or pocket knives) which could retain material that is probative and should be examined carefully. In some situations, dismantling the item may be necessary for a comprehensive evaluation. It is recommended that the item be photographed prior to its dismantling.

D. When examining the outside of a shoe, it is important to look carefully in the groove between the sole and upper shoe, as well as the shoelaces and their eyelets. When examining the inside of a shoe, it is important to search below the insert.

E. Reddish-brown staining will be presumptively tested for the presence of blood. A small sample will be collected for testing by cutting, swabbing, or rubbing with filter paper.

F. It may not be appropriate to perform KM testing on certain samples due to factors such as sample size or age. If it is warranted, these samples may be forwarded for DNA analysis without prior KM testing. Additionally, a questioned stain that is negative for KM may be forwarded for DNA analysis if the negative KM result is reasonable due to circumstances such as age of the stain. Documentation will be included in case notes.

G. If KM testing was positive, the stain intensity and/or coloring will determine the amount of stain collected for DNA analysis. An appropriate amount of the representative stain should be collected based on the test results as well as consideration as to the age of the stain.

The preservation of the stain based on biology testing is a critical decision point in obtaining the most discriminating and informative DNA results. It is the responsibility of the analyst collecting the stain to determine if permission to consume for DNA analysis should be obtained. Obtaining permission to consume at the preservation stage allows DNA analysis to be performed more efficiently and effectively.

If the DNA analyst determines at DNA quantification that the questioned stain was under sampled and the stain was not consumed, then additional material will be collected for DNA analysis.

H. Document the portion of stain preserved either by scaled photograph or written notes. Be mindful of the amount of sample placed in the microfuge tube(s) for DNA analysis. An excessive amount of substrate may hinder the extraction process, therefore multiple microfuge tubes may be necessary.

I. Be aware of the potential probative value of the item to other forensic disciplines such as latent prints, footwear, and gunshot residue.

## VI. Debris Collection

In most situations, documentation of the observation of debris is sufficient. Potentially probative debris observed on examination paper after testing should be collected in a druggist fold or similar packaging.

If debris collection is necessary, the following methods will be used dependent on the case scenario:

A. Picking of particles
This method is best for removal of large particles. Examples include glass, plastic, and vegetation. Pick with forceps and preserve in druggist fold or coin envelope.

B. Scraping
This method may be useful for soil or glass in large amounts or removal of hairs on a sheet. Use a spatula or similar device to remove particles by physical force.

C. Taping of debris
Adhesive tape is used to collect surface debris from an item. It can be used in strips or by a roller device. Label tapings at the top or in a fashion so markings do not obstruct view of debris. The taping method is not commonly used in order to preserve potential touch DNA.

## VII. Reference Standards

If questioned stains are being forwarded for DNA analysis, all appropriate reference standards will be forwarded as well. Unsubmitted reference standards from assumed or expected contributors that will aid the DNA analysis will be requested.

## VIII. Collection Techniques for Questioned Samples/Stains

The collection method selected for each sample and the amount of sample collected will be based on factors such as the type of substrate, stain intensity, sample age, results of any biological testing, etc. The collected samples/stains will be placed in microfuge tubes for DNA analysis or packaged in envelopes for possible analysis at a later date. The following methods are commonly used for collection:

A. Swabbing

This method is used on most non-porous and porous items. Moisten a portion of sterile cotton swab with a small amount of sterile water and swab off the desired portion of the stain; then go back over the same area with the remaining dry portion of the same swab. Use as few swabs as possible to collect the sample/stain. Dry the swab(s) before placing them in microfuge tubes or packaging them for future analysis.

B. Cutting

This method is often used for clothing items, paper, etc. Use sterile, disposable, or bleach-cleaned tools to remove the desired portion of the sample/stain. This method should also be considered if an item of clothing was swabbed for wearer's DNA and a limited amount of DNA was obtained.

C. Scraping/Shaving

This method is often used for porous items where stain cannot be effectively collected by swabbing. Use sterile, disposable, or bleach-cleaned tools to remove the desired portion of the sample/stain.

## CASE APPROACH REQUIREMENTS
## SEXUAL ASSAULT KIT PROCESSING

Sexual assault kits submitted to the laboratory will be processed in DNA without biological screening. Screening may be conducted after DNA testing or as requested by the submitting agency.

I. Processing Approach

A. Sample selection

Analysts will review the case scenario and determine which samples to prioritize in the first round of testing. Only swabs will be tested in the first round. If the first round of testing answers the case question, additional work will be deferred.

Subsequent rounds of testing are expected if the initial round does not answer the case question. The analyst is expected to evaluate the case and determine how to utilize additional rounds of testing to complete the case. At the analyst's discretion, more samples may be included in the initial round of testing to prevent the need for subsequent rounds of testing.

1. Analysts may choose to prioritize samples for the first round of testing based on certain factors such as:

a. Case scenario

Example: victim alleges oral assault only. Testing of the vaginal and anal swabs is not required in any round of testing.

Example: victim alleges the suspect licked her breast with no vaginal, anal, or oral penetration. Testing of the vaginal, anal, and oral swabs is not required in any round of testing.

b. Time between alleged assault and evidence collection

Example: sexual assault kit is collected after 5 days and the victim alleges vaginal assault only. The anal swabs may be deferred in the initial round of testing.

Example: sexual assault kit is collected within 7 days and the victim alleges the suspect ejaculated on her shirt. The shirt is available for testing. Analysis of the kit may be deferred pending results of the shirt.

c. The victim alleges ejaculation occurred on a specific clothing item or a condom was used. If these items are available for testing, they may be prioritized over the sexual assault kit swabs.

d. Post-assault activities such as showering, bathing, defecation, etc.

Example: sexual assault kit is collected after 3 days and the victim alleges vaginal assault only but showering afterward. External body swabs such as breast swabs and neck swabs may be deferred in the initial round of testing.

Example: sexual assault kit is collected after 3 days and the victim alleges vaginal and anal assault with defecation afterward. The anal swabs may be deferred in the initial round of testing.

e. In the absence of a scenario or if the victim is unable to give an account due to age or another mitigating factor, all relevant swabs will be tested.

f. In cases with multiple assailants and/or consensual sex partners, deferral of items may not be possible.

g. Oral swabs may be deferred if collected more than 24 hours post-assault. If ejaculation occurred in the oral cavity, testing of oral swabs collected more than 24 hours post-assault may be warranted.

2. If analysis of the selected priority samples does not answer the case question, subsequent analysis will be performed on relevant items deferred in the initial round of testing. Information to assist in this evaluation is listed below:

a. Anal swabs were deferred in the initial round of testing. If the DNA results for that round are negative, the anal swabs will be worked if anal penetration is alleged. Anal swabs should also be worked when vaginal penetration is alleged as drainage is expected with intimate collections.

Example: victim alleges vaginal assault only. Vaginal swabs are analyzed in the first round and are negative. The anal swabs will be analyzed.

Example: victim alleges vaginal and anal penetration. Vaginal swabs are analyzed in the first round of testing. The vaginal swabs are positive. The anal swabs may be deferred.

Example: victim alleges vaginal penetration and ejaculation on her pants. The vaginal swabs are analyzed in the first round and are negative. The pants are screened in biology and are negative. The anal swabs should be analyzed before analysis of the kit is considered complete.

b. Samples collected for touch DNA may be low level or may have extreme male to female ratios. Consumption of these samples may be appropriate. These items may be placed on a new assignment with a notification for consumption approval sent to the agency.

Example: neck swabs in manual strangulation case.

Example: fingernail specimens in a manual strangulation case or if a victim scratched the assailant.

Example: wrist swabs if the victim was physically restrained.

c. Samples collected from injuries to the body to include bruises, scratches, and abrasions will generally be deferred.

d. Samples collected from unknown locations may be submitted. Due to the lack of information, these samples will generally be deferred. The report will notify the agency testing was deferred due to lack of information by editing the item description or adding a remarks statement. The following statement is available in the LIMS:

*No information was provided regarding the collection of Item <item>. If your agency is in possession of information as to the location or reason for collection, this sample may be suitable for analysis.*

e. The outside of the white clothing bags provided with the sexual assault kit contains a question as to whether the contents were worn during or immediately after the alleged assault. A check box is present to mark yes or no. Given sexual assault kits can be submitted when collected seven days post assault, a check box marked "no" will not be used to defer analysis of these items if the case question has not been answered.

f. Deferral of sexual assault samples collected beyond seven days may not be warranted for deceased or immobile victims.

B. Representative sampling of swabs

Once an item is selected for analysis, half of the swabs will be tested. This eliminates the need to receive consumption approval prior to testing. Whole swabs will be tested when possible. If an item contains four swabs, two whole swabs will be selected for processing. If an item contains one swab, half of the swab will be tested.

1. Additional information may be available to assist the analyst in representative sampling of swabs (e.g. color variation, collection information, and packaging). A non-exhaustive list of examples follow.

Vaginal swab collection contains two red-brown swabs and two yellow swabs. One swab of each color should be selected.

Anal swab collection contains one brown swab and one unstained swab. Half of each swab should be selected.

Breast swab collection contains three swabs. 1.5 swabs should be selected.

Oral swab envelope contains six swabs in three smaller envelopes with distinct collection areas (i.e. along gum line, tongue, and oropharyngeal). One swab from each location should be selected.

Envelope contains two swabs "loose" in the envelope and two swabs in a swab box. One swab from the envelope and one swab from the swab box should be selected.

Four swabs are in a box, with two heads facing opposing directions. One swab facing each direction should be selected.

Two of the four swabs have markings on the sticks. One swab with markings and one swab without markings should be selected.

2. Analysts may make informed decisions regarding sample selection based on unique case circumstances.

   Example: vaginal swab collection contains ten swabs. Fewer than five swabs may be considered a representative sample.

C. Male to Female Ratio (MTFR)

The MTFR is commonly used in sexual assault cases to determine a suitable amplification chemistry. Quantitation data up to approximately 1:50 is generally suitable for a Fusion amplification. This information along with total male quantity, extraction volume, degradation index value, and case circumstance may be used to determine if a sample is suitable for Fusion or Y23 amplification. Higher ratios may be amplified in Fusion at the analyst's discretion.

**Note**: autosomal testing should be exhausted on all relevant items before Y-STR testing is conducted.

II. Reporting

Analysis of sexual assault kits may result in the issuance of multiple reports. Once a probative profile is identified additional work is typically deferred. The number of reports issued on a case and the order in which they are issued is dependent on case circumstances.

A. Report sequence

The reports for a sexual assault case requiring multiple rounds of testing will commonly follow the sequence below.

1. First report

   This report typically contains DNA results of kit swabs only and does not contain results of Y-STR testing, biological screening, or analysis of items requiring consumption approval.

2. Second report

   This report typically contains the results of biological screening of clothing items and other non-swab evidence.

3. Third report

   This report typically contains DNA results of the items in the second report, items that required consumptive testing (e.g. remaining half of selected swabs, fingernail scrapings), and/or other relevant items deferred in the initial round of testing.

4. Fourth report

   This report typically contains DNA results of Y-STR analysis.

B. A number of statements are available in the LIMS to provide information to the agency about the level of testing conducted on the case and opportunities for additional testing. Examples of statements that may be used specific situations are below.

1. No probative information is provided in the first analytical report and additional testing is possible with consumption approval and submission of relevant standards. This commonly applies to cases that stop at quantification and cases amplified with no DNA results; these samples may be suitable for Y-STRs.

   *A portion of the evidence was tested. Additional analysis may be possible upon receipt of consumption approval and submission of male reference standards. Please notify the laboratory if additional analysis would aid in your investigation.*

The analyst may choose to move forward with consumptive testing without male reference standards based on case circumstance. The wording above may be modified to remove the request for the submission of male reference standards.

2. No probative information is provided in the first analytical report, additional testing is possible with consumption approval, and MTFRs for item(s) are approximately 1:50-1:300. A new assignment will be created and a permission to consume notification sent to the agency. The LIMS notification will be the primary mechanism to notify the agency.

   *A portion of the evidence was tested. Additional analysis can be conducted upon receipt of consumption approval; a notification has been sent to your agency. If this analysis would aid in your investigation, please provide consumption approval and if necessary, re-submit the appropriate evidence to the laboratory.*

   Recommended workflow in LIMS:

   Create multiple assignments to manage testing. A biology assignment may be needed for clothing items. A DNA assignment can also be made with the items that need the kit swabs consumed. The laboratory can use a priority status of "hold" in the LIMS to place the DNA assignment on hold while the biology work is being completed. The DNA items can be combined into one assignment once biology work is completed. If the biology items are negative, the hold should be removed from the DNA assignment.

3. Items are deferred in the first analytical report. This statement may be used as warranted by case circumstance or at analyst discretion.

   *Several items were not analyzed at this time. Please notify the laboratory if additional analysis would aid in your investigation.*

4. The laboratory identifies additional items for testing. This may include clothing items or non-swab evidence (e.g. hairs, fingernail scrapings, or combings).

   *Item being retained for additional testing: Item <item>.*

5. When samples are deferred for amplification, items may be reported as a whole or individually. Analysts may use the reporting method that best balances comprehension and candor.

   a. Report the fractions with the parent item or report the fractions with the individual sub-items (e.g. 1B-F1 and 1B-F2 vs. 1B1-F1, 1B1-F2, 1B2-F1, and 1B1-F2).

b. Report on an item as a whole or report the item/sub-item's individual fractions (e.g. 1C vs. 1C-F1 and 1C-F2; or 1C1-F1, 1C1-F2, 1C2-F1, and 1C2-F2).

c. Report on the parent item or each tube separately (e.g. 1D vs. 1D1 and 1D2).

6. The following appendix statements are available in the LIMS when amplification is deferred:

a. No male DNA detected

*Quantitative PCR is used prior to amplification to determine the quantity of DNA present. Male DNA was not detected in this sample.*

b. No human DNA detected

*Quantitative PCR is used prior to amplification to determine the quantity of DNA present. Human DNA was not detected in this sample.*

c. MTFR

*Quantitative PCR is used prior to amplification to determine the quantity of DNA present. Male DNA was detected in this sample. However, the quantity of total human DNA in relation to male DNA indicates this sample is not suitable for autosomal amplification. This sample may be suitable for Y-STR DNA analysis upon submission of male standards; please contact the laboratory for information regarding Y-STR DNA analysis.*

d. General deferral

*This sample was not amplified at this time. Additional analysis of this sample may be conducted upon request.*

## CASE APPROACH REQUIREMENTS
## ABBREVIATIONS

Forensic Biology and DNA working files may contain the following abbreviations:

| | |
|---|---|
| A | Anal |
| ALS | Alternate light source |
| AMP | Amplification |
| AMP'D | Amplified |
| AP | Acid phosphatase test |
| APP/APPT | Apparent |
| AT | Analytical threshold |
| BET | Blue evidence tape |
| BIO | Biology |
| BLS | Blood-like stain |
| BP | Base pair |
| BPB | Brown paper bag |
| BSCK | Buccal swab collection kit |
| C | Contains/containing |
| CE | Capillary electrophoresis |
| CET | Clear evidence tape |
| CIDI | Case number, item number, date, initials |
| CONT | Contains/containing |
| CPB | Clear plastic bag |
| CSA | Criminal sexual assault/abuse |
| DEG | Degradation/degraded |
| DIFF | Differential |
| Ejac | Ejaculation |
| ELIM | Elimination |
| ENV | Envelope |
| EPG | Electropherogram |
| EXT | Extract/extracted/extraction |
| F | Female |
| FPSC | Filter paper stain card |
| GET | Green evidence tape |
| GPP | Glassine paper packet |
| INC | Inconclusive |
| INJ | Injection |
| KM | Kastle-Meyer test |
| LP | Latent print/Latent print section |
| M | Male |
| MAN ENV | Manila envelope |
| MAN TAG | Manufacturer's tag |

| | |
|---|---|
| ME | Manila envelope |
| MR | Mixture ratio |
| N | Neat |
| NAC | No analysis conducted |
| ND | Not detected |
| NE | Not examined |
| NOC | Number of contributors |
| NON-DIFF | Non-differential |
| NR | Not reproducible |
| NS | Non-Semen |
| NT | Not tested |
| NTS | Not to scale |
| O | Oral |
| OBS | Observed |
| ORIG | Original |
| PET | Pink evidence tape |
| PHR | Peak height ratio |
| PKG | Package |
| PLBG | Plastic bag |
| PRES | Preserved |
| PTC | Permission to consume |
| QUANT | Quantification/qPCR |
| RBS | Red-brown stain |
| RET | Red evidence tape |
| RXN | Reaction |
| S | Suspect |
| SAK | Sexual assault kit |
| SI | Sub-itemed/Sub-itemized |
| SLD | Sealed |
| STD | Standard |
| ST | Stochastic threshold |
| TOT | Turned over to |
| Unk | Unknown |
| V | Victim |
| Vag | Vaginal |
| WCB | White cardboard box |
| WPB | White paper bag |
| WPE | White paper envelope |
| WPP | White paper packet |
| YET | Yellow evidence tape |