Contents

Forensic Biology Unit Operating Procedure
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Page 1 of 168

Published: 03/18/2022
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Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 2 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 3 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

1.   Introduction

The goals and objectives of the SFPD Criminalistics Laboratory are detailed in the laboratory-wide Quality Manual.   These goals and objectives include providing professional, high-quality service along with consistent work product of superior quality.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 4 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

This manual outlines written analytical procedures approved by the DNA Technical Leader for each analytical technique used in the Forensic Biology Unit as well as procedures for quality assurance, quality control, validation, CODIS and outsourcing. The procedures specify reagents, sample preparation, extraction methods, equipment, and controls that are standard for DNA analysis and data interpretation. The terms male and female used during testing and analysis refer to the biological sex of individual.

This manual is intended to provide step-by-step processes to ensure operational uniformity and minimize analytical drift. These guidelines provide details regarding interpretation and reporting of results, and to help ensure objective quantitative and qualitative assessments in interpretation that are supported by empirical scientific data. The guidelines recognize that each case is unique, and it is impractical and infeasible to address every possible scenario that forensic data may present due to the multiplicity of sample types and results. Changes may occasionally be made to these methods in order to accommodate a particular sample; however, **deviations from procedures must be documented, authorized by the DNA Technical Leader and reported in the laboratory report when applicable and/or necessary for the interpretation of results.** These guidelines are based on a combination of scientific literature, validation studies, and professional experience. They are not intended to be applied retroactively and are expected to evolve over time.

All testing procedures are under the assumption that the given reagents and instruments are current in their quality assurance and quality control status. The Forensic Biology Unit will evaluate and implement, where applicable, the latest versions of the FBI QAS, NDIS Procedures, SWGDAM Guidelines, laboratory accreditation standards and OSAC Registry Approved Standards. Updates are made to procedures as soon as feasible to ensure consistent application.

2. **General**
   2.1. **Scope of lab**
   The Forensic Biology Unit within the San Francisco Police Department (SFPD) Crime Laboratory is responsible for testing of biological samples that are associated with a crime scene. Data generated in these tests are summarized in reports. Where possible, comparisons are made to collected known samples, and statistics are calculated to give weight to these comparisons. Criminalists within the unit are trained to provide expert testimony in relation to these findings. Eligible data is uploaded to the appropriate CODIS level, with this data being maintained by the CODIS team within the Unit.

   The Forensic Biology Unit employs a team approach with shared laboratory space between all analysts. There is no expectation of assigned laboratory space or equipment, or case ownership of the laboratory work. The workflow is dictated by the demands of the laboratory and can change under direction of laboratory management.

   2.2. **Sterile technique**
   Sterile technique is a specific set of practices and procedures performed to make equipment and work areas free of exogenous DNA and to maintain sterility.

   The Forensic Biology Unit is responsible for the handling and testing of items for various biological fluids including blood, semen and saliva. Any physiological stains, liquids, and tissues may contain infectious agents and caution should always be taken when handling

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 5 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

such items. This is done through the use of personal protective equipment (PPE) and cleaning practices.

Any spill of a biological material (DNA extract, amplification product, etc.) requires immediate attention. Spills are to be wiped up carefully with absorbent paper/wipes and disposed of in the biohazard bags. The affected area will be cleaned further with a 10% bleach solution which should stand for at least ten minutes prior to wiping. The DNA Technical Leader should be notified of any significant spills within the laboratory to ensure proper monitoring.

Chemical-resistant gloves, laboratory coat or disposable clothing, and a face mask should be worn when examining or handling biological evidence and chemicals. Safety goggles may be worn if handling chemicals outside a hood space. Laboratory coats should be laundered on a frequent basis and where appropriate separate coats used for discrete purposes. Appropriate face protection should be worn when handling open containers of liquid blood outside of a hood space. Appropriate filter masks should be worn when chemical vapors or aerosols are present.

Evidence is not allowed in areas where people eat and drink (including analyst desks). Food and drink are not permitted in laboratory analysis areas. When in the post-amplification lab, only gloves need to be worn. A lab coat and face mask can be worn, if deemed necessary by the criminalist.

2.3.   Preparing work area
All analysts will prepare their work areas according to the following procedures prior to examination of evidence and testing. This is considered standard laboratory practice and does not need to be specifically documented in notes.

Description	Discussion of activities	Special Considerations
PPE (gloves, face masks, lab coats)	Worn at all times as appropriate within the laboratory	Do not let face masks drop below the nose
Work surfaces	Wipe down individual work surfaces before and after each examination in the evidence examination areas with dilute (10%) bleach and place a clean sheet of white paper over the cleaned area prior to use. The paper is changed in between each examination and carefully discarded after use. Where appropriate, wipe down drawers and shelves as part of lab cleaning.	
Pipets and bench top tools/ equipment (including tube de-cappers, tube racks, micro-centrifuges, pens,	Wipe down with dilute bleach before and after use	Analysts should attempt to use single-use disposable implements whenever possible.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 6 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

label makers, cameras)		
Hoods	Cleaned before and after use with 10% bleach and ethanol; If available, a UV-light source can be turned on and pipettors/tips, de-cappers, racks, etc. may be left inside the hood	
Glassware	Washed with mild detergent in the laboratory dishwasher and allowed to dry. Heavily soiled items may be soaked for several hours; organic solvents (e.g., alcohols, acetone) may be used as needed and followed by the standard cleaning procedures.	Plastics prone to melting cannot be placed in the dishwasher

2.4. General lab duties

Laboratory chores (see "DNA Laboratory Monthly Chores" form in PDMS) will be assigned to analysts on a pre-scheduled rotation (see "DNA Laboratory QC Tasks and Maintenance Schedule" form in PDMS). All orders must be approved by a Supervisor prior to placing. In addition to general lab housekeeping, specific weekly and monthly instrumental duties will be performed on EZ1xl, Qiacube Connect, 7500 and 3500xl.

Laboratory cleaning (see "DNA Laboratory Monthly Cleaning" form in PDMS) will be performed on a monthly schedule and include cleaning common areas, cleaning extraction hoods, cleaning centrifuges with ethanol and bleach, discarding of post-amplification products older than 3 months, and visual inspection and basic maintenance of general service equipment as needed.

Quality assurance tasks (see "DNA Laboratory QC Tasks and Maintenance Schedules" in PDMS) include weekly, monthly, quarterly, semi-annual and annual checks on laboratory equipment; quality assurance checks on kits and reagents; and assisting in coordination of quality assurance and calibration processes.

2.5. Incompatible areas

Description	Definition of activities	Special Considerations
Pre-amplification area	All evidence examination, extraction and PCR set up (for both quantitation and STR typing)	

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 7 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

Post-amplification area	Confines all activities involving the processing and handling of amplified DNA product (including qPCR)	The door remains closed at all times except for passage. Dedicated lab coats (if needed) are worn which are not worn in any other areas of the laboratory. Gloves are disposed of before leaving. Equipment, reagent containers, etc. do not leave the room unless they have been thoroughly cleaned.

2.6. Autoclaving

Where appropriate, all disposables, reagents and containers which will hold samples/reagents should be autoclaved prior to use. Settings listed here are specific to the Tuttnauer 3870. Set cap or closure on top of the container (without engaging threads). Attach autoclave indicator tape to container and mark tape with initials and date.

Autoclaving consumables and glassware:
1) All items should be carefully cleaned before autoclaving
2) Fill containers, keep slightly ajar with autoclave tape. 96-well plates are placed in autoclave pouches.
3) Make sure there is enough water in system
4) For consumables: run on program 2 (icon looks like a dress), press start

Only autoclave appropriate types of plastics (e.g., polypropylene, polymethylpentene, etc.). Sterilization time is set at 20 minutes at 273°F and dry time set for 70 minutes. This setting is used for microcentrifuge tubes and 96-well plates used for amplification. Polycarbonate is autoclavable but temperature and time should be verified for the specific consumable. Small items may be autoclaved inside a beaker covered with foil.

Autoclaving liquids: Do not fill bottles more than approximately 50% to 75% capacity. Indicate liquid level on bottle prior to autoclaving and, if necessary, bring volume to original level (with sterile water) after autoclaving. For liquids, run on program with beaker icon (program 3).

2.7. Hazardous and chemical waste

Everyone must take responsibility for hazardous waste management in a manner that minimizes risks to all laboratory staff as well as the general public and does not generate unnecessary amounts of bio-hazardous materials. Glass bottles containing chemicals are to be disposed of off-site.

2.8. Sharps containers

These puncture-proof containers are designed to hold items that could cut or puncture the skin (e.g., scalpel blades, broken glass, used pipets, microscope slides). Items discarded in these containers may or may not be contaminated with potentially infectious material (e.g., blood). At least one inch of space should be left below the top of the container to ensure

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 8 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

that it can be closed safely. Once closed, containers can be placed inside a red biohazard bag.

2.9. Biohazard
These red marked bags are designed to hold items that may be contaminated with potentially infectious or toxic wastes. Red biohazard bags are not designed to hold items with sharp edges. These bags are also not to be used to hold regular trash. They will be disposed of by the appropriate waste disposal company. Appropriate waste for red biohazard bags includes the following:
- Blood-stained tissues and paper
- Used gloves or masks at the analyst's discretion
- Liquid blood/semen/biological material sealed in tubes
- Used plastic pipette tips

2.10. Glassware
Smaller glass pipets, test tubes, microscope slides and other glass with sharp edges will be placed in the red sharps container (puncture-proof containers). If the glass has bio-hazardous potential, the container will be sealed and placed in a red biohazard bag. If the glass does not have bio-hazardous potential, the container can be sealed and placed in regular trash. Empty glass bottles that are not broken should be rinsed thoroughly and recycled. Larger pieces of broken glass can be collected in a heavy cardboard box. When almost full, the box can be sealed with tape and disposed of with the biohazard waste or regular trash as appropriate.

3. Quality assurance/quality control procedures
The Forensic Biology Unit follows the laboratory-wide quality system outlined in the Quality Manual as well as Unit-specific policies and procedures. The Forensic Biology Unit applies the FBI QAS definitions for critical equipment/instruments/reagents to their procedures.

Critical Reagents	In-House Prepared	Commercially purchased
Sterile DI water	**X** (autoclaved aliquots from purchased lots)	
DTT	**X**	
TE buffer		**X**
PBS buffer		**X**
EZ1 DNA Investigator kits		**X**
MTL buffer		**X**
Trio quantification kits		**X**
Globalfiler amplification kits		**X**

Critical Equipment in Forensic Biology Unit	In-house scheduled quality assurance	Requires annual performance check	Requires performance check after repair and/or service
Hand-held mechanical pipettes	n/a	X (external)	n/a
NIST-traceable thermometer	n/a	Not required but sent out annually	n/a

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 9 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Heat blocks and thermomixers	n/a	X (in-house; temperature only)	n/a
EZ1-XL	Weekly, monthly, semi-annual	**X**	**X**
QIAcube Connect	Monthly	**X**	**X**
7500	Weekly, monthly, semi-annual	**X**	**X**
Thermal cyclers (9700 and Proflex)	Quarterly	X (same procedure as quarterly)	X
Thermal cycler temperature verification systems	n/a	**X**	n/a
TECAN liquid handling robots	n/a	**X**	**X**
3500xl genetic analyzers	Monthly, quarterly	**X**	**X**

Annual quality system review: The quality system as applicable to DNA is reviewed annually independent of other FBI QAS audits. This review is completed under the direction of the technical leader. The review shall be approved by the Technical Leader. The laboratory must retain documented completion and review by the DNA Technical Leader.

Annual casefile review: The laboratory will annually review casefiles determined by the Technical Leader to be a representative sample of the cases worked. This review of casefiles will be independent of an external FBI QAS Audit. The scope of the review shall be defined prior to each annual review and shall be approved by the Technical Leader. The scope must address both the representative sample and the time period of the records under review.

3.1. Accreditation
The Quality Manual describes accreditation for the laboratory.

FBI Quality Assurance Standards (QAS) audits
The Forensic Biology lab is audited every calendar year (at least 6 months and no more than 18 months apart). At least once every two years, an external audit is conducted by one or more FBI DNA auditor(s) from a second agency. Internal audits are conducted by an audit team that includes at least one FBI DNA auditor.

3.2. Training program
Training of new analysts within the Forensic Biology Unit is conducted according to the current training manual.

3.3. Continuing education and technical currency
Technical qualifications for members of the Forensic Biology Unit are maintained through mandatory continuing education. This includes a cumulative minimum of eight hours per calendar year of attendance at approved training sessions in subject areas relevant to the developments in DNA typing. Refer to current FBI QAS standards for the necessary documentation for internal, external and multimedia delivery/internet training. The

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 10 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

ongoing review of scientific literature is supported and encouraged in the Forensic Biology Unit. This is coordinated by the DNA Technical Leader and is documented on an annual basis using PowerDMS.

3.4.   Proficiency testing
Body fluid ID only analysts (i.e., non-DNA analysts who only perform biological fluid screening) are required to perform external proficiency tests once annually. Screening proficiency tests must be performed according to current Forensic Biology Unit Procedures and will cover all tests and methods used in the laboratory as they pertain to the test-taker to include:

1) Blood, semen and saliva chemical tests; ALS not mandatory
2) Interpretation
3) Report writing

DNA analysts are required to perform external proficiency tests twice annually. DNA proficiency tests must be performed according to current Forensic Biology Unit Procedures to include all tests and methods used in the laboratory as they pertain to the test-taker. The workflow differs slightly from casework as all tests are performed with every evidence sample (not scenario-dependent).

1) Biological screening: blood, semen and saliva chemical tests; ALS not mandatory
2) DNA extraction, quantitation, PCR amplification and STR analysis.
3) Interpretation and STRmix analysis: STRmix analysis for evidence samples will follow the same procedure as casework. When uploading evidence profiles to the CTS portal use the exact data used by STRmix for analysis, i.e., evidence data using "Globalfiler 150 RFU – STRmix" analysis method.
4) CODIS evaluation.
5) Report writing.
6) Reporting sheets will list the alleles in numerical order reporting homozygotes with a single numerical value.
7) The printed report from the proficiency test provider portal needs to have a case identifier on each page. This report will be included on the right side of the casefile, without pagination.
8) The case assignment manager will ensure upload of appropriate DNA profiles to the QA database upon closure of the final proficiency test in the cycle set.

3.5.   Casefile organization
The casefile refers to all relevant case information received and generated through testing. This may be in the form of a physical file or electronic file.

3.5.1.   Elements of a Casefile
Elements within a casefile may be in a physical or digital format given that documentation meets all accreditation and QAS requirements and is in accordance with lab's Quality Manual.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 11 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

Administrative: Case assignment forms, SFPD incident reports, 64 forms, QPRLs, CAL-EMA forms, CODIS forms, technical and administrative review forms, communications, etc. All documentation must have case identifier.

Lab Notes: These may be in physical or digital form. All documentation must have case identifier. Electronic documentation will be stored on S drive. Lab notes include the following, at a minimum:

1) Report(s) of Laboratory Examination
2) CTS data sheet, if applicable
3) Evidence examination, screening notes, sampling notes
4) Photographs: This includes general photos taken to document item (evidence that comes to lab as swabs) and photos that contain markups, either digitally or physically (i.e., underwear with testing notes and results).
5) DNA extraction worksheets (EZ1-XL, Qiacube Connect)
6) Quantitation worksheets: TECAN Trio set up and results table
7) PCR amplification worksheets: TECAN Globalfiler set up
8) Capillary electrophoresis: TECAN plate load sheet
9) Electropherograms: only to demonstrate quality issues including off-ladder alleles with OL calculations, unexpected peaks in reagent blanks
10) Evidence sample table
11) Statistical analysis worksheets: LR Assistant, ArmedXpert (legacy cases)
12) Any data sheets documenting edits, rejected analysis, amended data, etc. must be retained. The pages must contain the initials, date and a stated reason for the rejected data.

3.5.2. Casework documentation on server

Electronic data relating to a case is stored in the appropriate casework documentation folder (named by Lab Number) on the S drive. This will include:

1) STRmix data and run files
2) STR raw data files to include all samples (re-injections, re-amplifications, re-preparations, etc.), associated reagent blanks, controls and ladders
3) ArmedXpert projects (legacy cases)
4) Photographs documenting evidence taken during screening
5) Scanned copies of any chain of custody records with case relevant pages/items with signatures and dates
6) Scanned copy of mailed report
7) STRmix files associated to QA database searches for contamination evaluation

Note 1: After mailing a report, the casefile should be scanned on to the server. Use naming convention INCIDENT NUMBER_LAB NUMBER_portion of casefile scanned. Normally scanned in three parts: left side, right side, and report.

3.5.3. Documenting who performed work on notes and reports

Notes and other casefile elements used to generate a report will have necessary documentation of analyst's work in accordance with unit review checklist and lab's Quality Manual.

3.5.4. Page numbering of case notes

Page numbers follow the laboratory-wide Quality Manual policy.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 12 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

3.6. Quality reviews

Upon completion of the analyst's casework, the completed file is submitted for review by another qualified analyst or Supervisor. The Forensic Biology Unit conducts and documents technical and administrative reviews of all completed casefiles and reports. If any discrepancies result from the technical review or administrative review they must be resolved before final results are issued. The DNA Technical Leader is responsible for making a final decision about discrepancies with data and reporting. Refer to the Quality Manual for more details. The technical and administrative reviews must be conducted and documented for all casefiles and reports prior to releasing the report to the customer.

Following technical and administrative reviews, the "Review Tracking Form" noting suggested changes for the reporting analyst by their reviewers, will be saved electronically in the appropriate casework documentation folder.

Once the final report is printed, the technical reviewer and analyst must sign or initial the report to memorialize this as the final and official report. The final report is then released to the customer. The review procedures are laboratory-wide policies with Unit-specific requirements if appropriate; more details are found in the Quality Manual.

The Forensic Biology Unit has adopted a plan for random re-testing with the goal to monitor compliance with testing requirements and identify improvements that may be needed for training and procedures. Re-testing performed on cases will be done in a manner to minimize destruction of evidence. Cases will be intercepted after screening has been performed while the case is undergoing DNA testing by a DNA Case Assignment Supervisor. Either entire small cases or select items from larger cases will be re-tested. As well as verifying the testing performed, labels and seals will be checked. In cases where no serology tests were performed, labeling/sealing will be noted. Evidence will be requested by the DNA Case Assignment Supervisor and the re-testing will be assigned to a different analyst on the serology rotation.

By performing the re-testing before a report is issued, results can be verified without having to issue a supplemental report for further testing. Performing the re-testing right after serology is finished will diminish the influence on the turnaround time for that case. In the case of re-test results being different than the original:

a. If the original test was negative and the re-test test is positive, then the item can be sampled at the time of re-testing for DNA analysis.
b. If the original test was positive and the re-test is negative, the item would have been sampled already.

Any discrepancies arising from this program will follow the non-conforming work policy currently in place for the SFPD Crime laboratory.

3.6.1. Technical review

Technical review ("TR") is performed by an individual that is, or has been, a qualified analyst in the methodology being reviewed, and is not the author or co-author of the report contents and must be someone who has NOT performed any of the testing

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 13 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

being reviewed. The goal of the technical review process is to ensure conformance with Forensic Biology Unit policies and procedures.

Elements of Technical Review: Evaluation of reports, notes, data and other documents to ensure there is an appropriate and sufficient basis for the scientific conclusions. Review includes (not limited to): case notes, worksheets including extraction notes, quantitation notes, PCR-STR analysis notes, GMID-X data to include controls, internal size standards and allelic ladders to verify consistency with expected results; verification of conclusions for compliance with Forensic Biology Unit policies and procedures; verification/ justification for number of contributors and assumptions; STRmix mixture deconvolutions; STRmix LR From Previous reports; LR Assistant worksheets with correct verbal qualifiers ; ArmedXpert statistic evaluations (legacy cases); report addresses each tested item; verification of CODIS eligibility and applicable samples' CODIS status. Review of each item will be documented on the appropriate line on the Forensic Biology Unit-specific review checklist. Completion of the technical review will be documented on a unit review checklist.

3.6.2. CODIS review

After technical review and prior to entry into the CODIS database, a CODIS review ("CR") is performed by the CODIS Administrator, Alternate, or designee to verify:

1. CODIS Search Request sheet is filled out correctly
2. DNA types are consistent with data interpretation and report
3. MME meets statistical threshold (if applicable)
4. Profile eligibility/suitability according to current CODIS requirements (e.g., MME, minimum # of loci, conditions of case scenario) and proper documentation is in case folder.

Completion of the CODIS review is indicated by the reviewer's initials and date on the unit review checklist. Profiles are entered into the CODIS software during the CODIS review process. A Specimen Details Report will be printed for each entered profile and added to the casefile.

3.6.3. Administrative review

Administrative review ("AR") may be performed by the technical reviewer, or another individual who is not the author/co-author of the report contents and must be someone who has NOT performed any of the testing being reviewed. The administrative reviewer is not required to be a current or former qualified analyst.

Elements of Administrative Review: Evaluation of the report and supporting documentation for consistency with laboratory policies and for editorial correctness. Review includes (not limited to): case number, description of evidence, STR typing kit used, results/conclusions, interpretive statement, date (to reflect date of last edit), disposition, chain of custody and signature/title. Review of each item will be documented by initialing the appropriate line on the Forensic Biology Unit-specific "Case Review Checklist". Completion of the administrative review will be documented by the reviewer signing and dating the Forensic Biology Unit-specific unit review checklist.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 14 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

3.7.   Quality Assurance Database (QA Database)

The Forensic Biology unit maintains a "Quality Assurance, or QA Database". This database consists of the following:

Quality Assurance Database (QAdb)

Sample Type	Retention
Individuals who have direct contact with evidence • Lab personnel • CSI personnel (not limited to SFPD) • SART team	CSI and Lab personnel: Indefinitely SART can request removal if no longer collecting evidence/at their own discretion
Unattributed contamination profiles	Indefinitely
Laboratory positive controls	Indefinitely
Confirmed consumable contamination	Indefinitely
Individuals who may have indirect contact with evidence • Visitors* • Vendors*	Not exceeding 60 days
Evidence profiles^	Not exceeding 60 days

*Sign and receive information sheet
^ Fully determined genotype of ≥80% at 5 or more autosomal loci according to STRmix deconvolution report.

Note: References of any type (victim, suspect, witness, consensual partner, etc.) generated through DNA casework **shall not** be entered into the QA database. Reference processing will be physically separated during lab processing. (i.e., reference and evidence processing will have dedicated equipment and space).

The collection of elimination samples, while encouraged, is voluntary. Individuals (laboratory staff and members of CSI excluded) may request their samples be removed from the QA Database at any time. The form "Information Sheet for Providing Samples for SFPD Elimination Database" shall be filled out by the donor before a sample can be collected.

The purpose of the QA Database is to identify potential contamination of evidence by staff, visitors, law enforcement personnel or other evidence samples. The QA Database will be maintained by DNA Unit Supervisors who will track the entries by date (see "naming convention") and expunge on a weekly basis.

DNA Unit staff members have access to the Quality Database for searching during routine casework. Staff members have access to the QA Database in password protected computers and may enter, search and remove profiles. The server on which the QA Database resides is backed up nightly. Any changes in functionality of the database will result in a version change; versions can be tracked by creation date.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 15 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

The following naming convention will be used:

Date_SFPD INC #_Lab #_Sample Name
Date will follow naming convention: mmddyy

Use incident number associated with sample. i.e., Multiple incidents may be processed under one lab number. Use the incident number that is associated to the physical evidence the sampling was taken from.

Database Search
STRmix has a Database Search function that automatically searches evidence samples against the SFPD Quality Assurance database (QA db). The Database Search is set to occur immediately after each deconvolution run. A Database Search Report will detail the results of each search.

Every Database Search Report should be reviewed by the reporting analyst and the case reviewers. The report is divided into 6 sections.

- Details
- Database Search Setup
  - The Database Search Setup section lists search specifications and the testing parameters used.
  - Correct testing parameters are set to default and should not be changed without approval from the DNA Technical Leader.
    - Minimum LR: 10,000,000
    - Population: NIST1036_Cauc
    - Type of Search: Standard
    - FST: 0.0b (1.0, 1.0)
    - Assign sub-source LR: Y
    - Prior Method: Stratify
- Previous Interpretation Details
- Data Validation
  - The Data Validation section lists any QA db samples that have been entered incorrectly and cannot be searched. If any samples are listed here, find the entries in the QA db, correct them, and re-run the Non-Automated Database Search in STRmix.
- LR Results
  - The LR Results section lists any matches to the QA db found during the Database Search.
- Auditing Details

Non-automated Database Search
Note, these instructions should only be used when a Database Search Report lists Data that did not pass data validation.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 16 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

1) Open STRmix
2) Click on Investigation
3) Click on Database Search
4) Drag or browse the previous interpretation file into the Previous Interpretation field
5) Verify the Database File is the QA Database
6) Do not change any of the testing parameters defaulted
7) Click Start
8) Review Database Search Report results, verify all data passed data validation

Procedure for QAdb usage:

1) After editing case samples but before reporting and sample interpretation, enter any single source evidence samples into QAdb.
2) During STRmix deconvolution all samples will be run through the SFPD QAdb.
3) If a hit occurs, an evaluation regarding contamination will be completed by DNA unit supervisor/ or Technical Leader.
   a. If hit is confirmed to be contamination, reporting statement will be included in report.
   b. If hit is confirmed to be non-contamination, no further action will be taken. This type of hit falls outside of the scope of the QAdb. Hits of this category will be notified through CODIS if eligibility requirements are met.

Items to evaluate for contamination:
- *Proximity in time for DNA processing, extraction, screening*
- *Instruments used*
- *Accessory labware used, i.e., pipettes, hoods, thermomixers, centrifuges, screening benches*
- *Personnel in contact with evidence or lab space*

To enter a profile into the QA db, complete the following steps:

1) In the first available blank row, enter sample type
2) In the next cell over, enter the sample identifier for the relevant protocol; example: 031522_170123456_20-1234 2A12 C1.
3) Enter DNA profile along the row using the following rules:
   a. Each cell may only contain a single allele

14	15

   b. Homozygotes must be entered with both alleles. For example, genotype 13 is entered as shown

13	13

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 17 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

c. Both cells must have an allele value or they will not be searched by STRmix. If a locus is not fully resolved, the entire locus should remain blank. Obligate alleles should not be entered. Examples of <u>invalid </u>entries are shown

16	
16+	

d. If a locus has a tri-allele, leave the locus entry blank
e. Enter alleles in numerical order

10	13

6) Once all profiles have been added to the database, save and exit
7) Open the QA db as read-only

3.8. Assessing Contamination and Controls

Precautions to minimize the potential of contamination include: routine laboratory cleaning; monitoring using appropriate controls such as reagent blanks and negative controls during analysis; wearing gloves, lab coats, hair nets (where applicable) and face masks at all times and changing frequently; minimizing talking in the laboratory; opening one evidence item at a time and keeping tubes closed when not in use; minimizing the time evidence is exposed in the designated exam area; handling all laboratory tools with clean gloves to prevent cross-contamination; using fresh barrier pipette tips for each sample and careful pipetting technique; pulse-spinning tubes prior to opening to prevent aerosols and opening tubes carefully avoiding touching the interior of the tube cap.

Lab practice will make every attempt to process evidence and reference samples separately in time or space (ideally with evidence processed before references); however, some workflow exceptions are to be expected.

Following data generation on CE instruments, the GMIDX Profile Comparison Tool will be used to help identify contamination and other quality flags within batches. Please refer to Appendix B in the SOP for a guide regarding the use of the Profile Comparison Tool.

The standard operating procedures attempt to standardize how best to define and troubleshoot failed standards and contamination. However, there may be instances where these guides are not followed. Reasons may include conservation of evidence or other issues that may affect processing integrity.

Guides to assessing controls and contaminations
Select sample type. Allelic peaks for the purpose of troubleshooting will be defined as peaks within bins that cannot be attributed to amplification or CE artifacts.

a. NTC (negative quantitation control)
b. Negative amplification control
c. Positive Amplification control
d. Reagent blank
e. Reference samples

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 18 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

      f.   [Evidence samples](#)

Reprocessing Guide

Based on sample-type decision tree, proceed through steps until contamination is resolved or confirmed.

1. Re-inject sample.
2. Re-prep sample.
3. Re-quant sample and proceed through CE.

Using LRs to account for Contaminant Profile
The contaminant DNA profile may be used as a conditioned sample in a STRmix analysis to aid in the mixture deconvolution in accordance to the following procedure:

- A STRmix LR calculation for the contaminant DNA profile must be performed before conditioning.
- If the resulting LR > 1,000, the contaminant DNA profile can be used as a conditioned profile in a subsequent STRmix deconvolution.
- The original deconvolution should be noted on evidence sample table in the casefile. The results and conclusions must be reported and indicate that the contaminant DNA profile was used as a conditioned profile to obtain results.

Documentation of Contamination
If contamination has been attributed to an individual, a statement will be added to the report attributing the contamination to the source without using personally identifiable information. i.e., "Contamination was sourced to lab personnel…"

An Action Report will be completed any time unexpected peaks are observed in a negative control, reagent blank, or sample. Action Reports will record each occurrence and help to identify trends that may be causing these deviations.

Note: An action report is not necessary if an SFPD firearms examiner, who previously processed the evidence item, OR an SFPD CSI member is detected on an evidence item. The SFPD Firearms Unit does not wear gloves for safety reasons, therefore, it is not unexpected to find their DNA on an evidence item that a Firearms examiner processed prior to DNA testing. CSI evidence collection is outside of the lab's purview. It is necessary to notify the TL that a CSI member was detected as a possible contamination source so that information can be communicated to the CSI Unit.

3.9. **In-house prepared reagents**
Prepared in-house reagents shall be labeled with:
- Identity of the reagent
- Date of preparation and/or expiration (if applicable)
- Identity of individual preparing the reagent
- lot number if applicable (e.g., DTT)
- concentration (if applicable)
- hazard warnings and storage conditions where applicable.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 19 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

Complete any QC forms/log sheet associated with an in-house prepared reagent.

3.9.1. Bleach

Prepare a 10% diluted bleach solution by mixing bleach with deionized water in a 1:9 ratio. Expiration is two weeks from the date that the solution was prepared. Stored at room temperature.

3.9.2. DTT (1 M Dithiothreitol, 3.0 M Sodium acetate, pH 5.2, 15ml)

Dissolve 2.32 g dithiothreitol in 14.95 mL sterile deionized water. Add 50.3 µL 3 M sodium acetate (pH 5.2). Solution may be sterilized by filtration. Do not autoclave. Aliquot (recommended 250 µL) and store at -20°C. Quality control test before using in casework.

QC Method: Extract a known/control sample containing sperm alongside a reagent blank. Quantify, amplify, and run on CE to show that DTT lot properly lyses sperm cells and does not contain any exogenous DNA. Provide results to Technical Leader or designee to approve prior to release for use in casework.

3.10. Commercially purchased reagents

Commercial reagents shall be labeled with:
- Identity of the reagent
- Lot number if applicable
- Expiration date as provided by the manufacturer or as determined by the laboratory; if no expiration date is given by the manufacturer, one will only be assigned with adequate supporting justification
- Identity of individual preparing the reagent (if requires preparation step)

Complete any QC forms/log sheets associated with a purchased reagent.

Approval of QC of reagents may be performed by the DNA Technical Leader or designee.

QC testing of at least one kit per shipment of each manufacturer kit lot number is performed before use in DNA casework. The individual who received the reagent or consumable relevant to testing, date of receipt, storage conditions/location (as appropriate) should also be noted.

3.10.1. Phenolphthalein presumptive blood test kit

Kits for the phenolphthalein presumptive blood test will include: phenolphthalein reagent, ethyl alcohol, and hydrogen peroxide.

QC Method: New lots must be verified before being released for casework. This entails testing a known blood sample and a negative control. A color change to pink/purple within several seconds should occur when testing the known blood sample with the kit reagents. No color change should be observed within 60 seconds of the application of the kit reagents to the negative control. Provide results to Technical Leader or

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 20 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

designee to approve prior to release for use in casework. Reagents should be stored at room temperature upon receipt.

Note: It is an option to QC reagent grade (or equivalent) ethanol alongside the blood test kits. Ethanol can be the limiting reagent in some purchased blood test kits.

3.10.2.   Acid phosphatase

Make AP reagent fresh daily, as used, and perform QC accordingly. Powder is stored at at 2-8°C.

3.10.3.   RSID Saliva kits

Materials are supplied by Independent Forensics, the manufacturer of the RSID - Saliva Test and contain:

> 1) Human saliva test cassettes, stored at room temperature
> 2) TBS+ running buffer, stored at 4°C

QC Method: New lots must be verified before being released for casework. Test a positive control sample of known saliva and negative control sample of PBS-only using casework procedures. The known saliva sample shall produce a positive result and the PBS-only negative control shall produce a negative result to be acceptable for use in casework. Provide results to Technical Leader or designee to approve prior to release for use in casework.

3.10.4.   Christmas tree stain

SERI Christmas Tree Stain. Other vendor-supplied reagent may be used as appropriate.

Kit contains:

> Solution A: red (Kernechtrot Solution)
> Solution B: green (Picroindigocarmine Solution)

Solutions A and B are stored at room temperature.

QC Method: New lots must be verified before being released for casework. Test a positive control sample of known sperm-positive semen sample and negative control sample of PBS or sterile water using casework procedures for microscopy examination. The known sperm-positive semen sample shall produce a positive result for the presence of sperm and the negative control shall produce a negative result to be acceptable for use in casework. Provide results to Technical Leader or designee to approve prior to release for use in casework.

3.10.5.   EZ1 DNA Investigator kits

Commercially purchased from Qiagen. Kits contain pre-filled cartridges, proK, G2 buffer and carrier RNA. The MTL buffer is received separately. Upon receipt, these are stored at room temperature.

QC Method: Lots will be verified using 5 ul known liquid blood (or other known sample approved by the DNA Technical Leader) and a reagent blank. The known sample must provide measurable quantitiation results and the quality of the extract will be verified

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 21 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

through the amplification and typing of the sample. No DNA should be detected in the reagent blank. Provide results to Technical Leader or designee to approve prior to release for use in casework.

The carrier RNA is prepared by adding 310 μL of TE to the manufacturer-provided vial of 310 ug of cRNA; prepared cRNA is frozen in aliquots of 30 μL. Carrier RNA, MTL buffer and G2 buffer do not carry expiration dates; their quality is assessed with each QC (kit or separate) and effectiveness monitored on a continual basis. If any quality issues are observed or suspected, the analyst should report those to the DNA Technical Leader.

3.10.6. MTL buffer
Commercially purchased from Qiagen either as part of the EZ1 DNA investigator kits or separately.

QC Method: See QC method for EZ1 DNA investigator kits.

3.10.7. Quantifiler Trio kits
Commercially purchased from Life Technologies (Thermo Fisher Scientific). Kits contain the PCR reaction mix, primer mix, DNA standard and a dilution buffer. The reaction mix may be frozen upon receipt but after the first thaw should be kept in the refrigerator.

QC Method: The kit is verified using a male DNA sample of approximate known quantity and the negative control. The control standard from a previously QC'd Globalfiler kit can be used as the male DNA sample. The DNA quantity for the male sample should be reasonably close to the expected quantity. No DNA should be detected in the negative control sample. Provide results to Technical Leader or designee to approve prior to release for use in casework.

3.10.8. Globalfiler kits
Commercially purchased from Life Technologies (Thermo Fisher Scientific). Kits contain the PCR master mix and additive, primer set, allelic ladder and DNA Control 007. Upon receipt the exteriors of each packing layer will be wiped clean (outer box, outer PCR reagent box and outer ladder box). The contents are frozen upon receipt and at 2 to 8°C after initial use to avoid freeze and thaw cycles. The allelic ladder is removed from the kit only in the post-PCR room and placed in 2 to 8 °C storage in the post-amplification room. **Note:** fluorescent dyes are light sensitive and should be protected from light.

QC Method: The kit is verified through amplification and genetic analysis of a positive amplification kit control (007) previously QC'd, and a negative amplification control. The positive amplification control must yield the correct typing results. No typing results should be observed in the negative amplification control. The allelic ladder must fall within the current interpretation guidelines. Provide results to Technical Leader or designee to approve prior to release for use in casework.

3.10.9. Highly deionized formamide (HiDi formamide)

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 22 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Commercially purchased from Life Technologies (Thermo Fisher Scientific). No QC
testing required for formamide. Formamide is stored frozen.

3.10.10. Anode Buffer
Commercially purchased from Life Technologies (Thermo Fisher Scientific). No QC
testing required for anode buffer. Anode buffer containers are stored refrigerated in
the post amplification room.

3.10.11. Cathode Buffer
Commercially purchased from Life Technologies (Thermo Fisher Scientific). No QC
testing required for cathode buffer. Cathode buffer containers are stored refrigerated
in the post amplification room.

3.10.12. Internal lane size standard (LIZ)
Commercially purchased from Life Technologies (Thermo Fisher Scientific).

GeneScan 600 LIZ v2.0 is stored refrigerated in the post-amplification room. No QC
testing is required for LIZ.

3.10.13. Performance Optimized Polymer 4 (POP-4)
Commercially purchased from Life Technologies (Thermo Fisher Scientific).

POP-4 is stored refrigerated in the PCR lab. Make sure the polymer is at room
temperature and any particles are dissolved and well-mixed prior to use. Avoid
introducing bubbles. No QC testing is required for POP-4.

3.10.14. Tris EDTA (TE buffer; 10mM Tris, 0.1 mM EDTA, pH 8.0)
Commercially purchased from approved vendor. TE is stored at room temperature.

QC Method: Quantify, amplify, and CE a QC'd 007 amplification control and negative
control. Provide results to Technical Leader or designee to approve prior to release for
use in casework.

3.10.15. Phosphate buffer saline (PBS)
Commercially purchased from approved vendor. PBS procured must not contain
calcium or magnesium. PBS is stored at room temperature.

QC Method: Extract, quantify, amplify, and run on CE a known sperm-positive sample
(e.g. old CTS sample) and a reagent blank to show that the lot does not contain any
exogenous DNA. The known sperm-positive sample can be a differential sample from
a previously tested CTS case. Use the new lot of PBS as the PBS placed on the QIAcube
Connect for the differential extraction. Provide results to Technical Leader or designee
to approve prior to release for use in casework.

3.10.16. Sterile water
Commercially purchased from approved vendor. Autoclave aliquots of purchased
deionized water for benchtop use. Expiration of aliquots will follow the expiration

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 23 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

==date on the purchased box== . Quality control test before using in casework. Stored at room temperature.

QC Method: Extract, quantify, amplify, and run on CE a negative water control sample to show that the lot does not contain any exogenous DNA. Provide results to Technical Leader or designee to approve prior to release for use in casework.

3.11. Failed QC test

If quality control data for commercial kits, reagents, or in-house preparations, fall outside the current interpretation guidelines and/or fail to yield the anticipated typing results, the DNA Technical Leader must be notified. Data from each step involved in the typing procedure will be evaluated to determine what/where the problem manifested (e.g., extraction reagents, typing kit, etc.). It may also be useful to determine what components were changed since the last successful QC process to design the appropriate corrective measures. No kit, reagent or in-house preparation will be deemed suitable for casework until the correct quality control data is obtained.

3.12. NIST traceable testing

Newly validated DNA methods (from amplification through characterization), typing test kit, or platform instrument model are checked against an appropriate and available certified reference material, or sample made traceable to the certified reference material prior to implementation of the method.

A NIST traceable standard may be generated in-house by running an internal laboratory QC sample against a NIST standard. The internal laboratory QC to be designated as the "NIST-traceable" standard must be amplified on the same batch as at least one NIST standard with known/published DNA types. A successful amplification will be confirmed by comparing the DNA types of the NIST-standard on the amplification to the results published by NIST. After a successful amplification, the standard may be considered NIST-traceable. This sample will be tied to the amplification kit for which it was accepted as NIST-traceable by the laboratory; any introduction of new loci/kits necessitates re-qualifying the internal QC sample as NIST-traceable.

3.13. Equipment maintenance

The Forensic Biology Unit has a program (described in these procedures) and schedule ("DNA Laboratory QC Tasks and Maintenance Schedule" in PDMS) to ensure maintenance of equipment/instruments/reagents. Current equipment and operational manuals are relied upon and will be kept near the instrument, electronically or with the DNA Technical Leader.

If any instruments fail to meet the expected results from their calibration and maintenance, the DNA Technical Leader must be notified and troubleshooting and/or service conducted as appropriate. Work may need to be halted and corrective action taken at the discretion of the DNA Technical Leader.

The instrument will be clearly marked (e.g., "do not use", "out of service", etc.) until issues have been resolved. Documentation (written records or logs) of any maintenance, service and calibration is maintained by the Forensic Biology Unit.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 24 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

New critical instruments and equipment, or critical instruments and equipment that have undergone repair, service or calibration, undergo performance checks before use in casework. Any equipment that needs to be sent out will be packaged according to the manufacturer's recommendations whenever feasible. Critical parameters of equipment operation have been identified and are calibrated and documented in a manner necessary to maintain successful operation of the technique.

3.13.1. Autoclave
Autoclave maintenance is to be performed at least every 3 months.
1) Drain water in bucket
2) Fill with new deionized water.
3) Take out trays and clean with ethanol
4) Clean sensor in back with ethanol
5) Use two packets of Chamber Brite desalting powder – sprinkle along bottom of chamber
6) Run program 1 for maintenance. Close door
7) Press start
8) Repeat this step with water only, no Chamber Brite
9) Return trays to proper position
10) Complete autoclave log on front of autoclave with cleaned date and initials.

3.13.2. Thermometers and probes
Target/acceptable ranges:
> Freezers: -30 to 0°C
> Refrigerators: 0-10°C
> Heatblocks and Thermomixers: 51-61°C
> Off-set value: +/- 3°C

Thermometers and probes should be used and handled in a way to minimize mechanical damage. Thermometers should be visually inspected for mechanical damage and either repaired or discarded/replaced as appropriate.

Performance Check for NIST-traceable thermometer: Annually by an outside vendor using NIST traceable standards.   Additional in-house thermometers, probes or equipment, have annual temperature-calibration checks against the NIST-calibrated thermometer as evidence of correlation as described in the following section about the probes.

Annual in-house calibration check for refrigerator, freezer and heat block/thermomixer probes:
1) Record temperature of equipment after 15 minutes for each probe using a   NIST calibrated thermometer.
2) Calculate offset value by comparing each probe reading to the NIST thermometer reading.
3) To pass calibration check:
    a. Both temperatures within the target range. If the temperatures are beyond the target range, assess whether there is a reason (door open recently, etc.). If not

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 25 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

resolved, contact the Technical Leader as the equipment may need to be retired or repaired.

b. Target offset value +/- 3°C. If the offset is beyond the target range, contact the Technical Leader as the equipment may need to be retired or repaired.

3.13.3. Thermomixers

The thermomixer should be set to a temperature of 56°C. The thermomixer should not be used if the temperature reading is not within the acceptable range. If the temperature is out of range, the analyst should attempt to bring it within range. Thermomixers should be kept clean of debris and used in a way to best minimize structural and functional damage. They should be visually inspected by users for any obvious damage. The Technical Leader will be notified if thermomixer is not working optimally or as expected. Maintenance is performed as needed either in-house by or sent out to the appropriate outside vendor.

Annual in-house performance check for thermomixers: Place a tube of water in a thermomixer well and wait 15 minutes to overnight for the water to reach the thermomixer temperature. Place the NIST thermometer into the tube of water. Record temperatures of NIST thermometer and thermomixer digital temperature display after 15 minutes. Calculate offset value by comparing each thermomixer reading to the NIST thermometer reading. If both temperatures are within the target range, the equipment has passed its calibration. If the temperatures are beyond the target range, contact the Technical Leader as the equipment may need to be retired or sent out for calibration if applicable.

3.13.4. Refrigerators, freezers, and heat blocks

Temperatures of the freezers, refrigerators and heat blocks used for the storage of kits, reagents and evidence are monitored using a probe-based temperature monitoring system. If a temperature is recorded out of range for a set amount of time, an alarm notification will be sent to the designated personnel. The appropriate action will be taken to bring the temperature back into range. If the temperature cannot be brought into range, the Technical Leader should be consulted to discuss strategies for troubleshooting.

Heat blocks should be kept clean of debris and used in a way to best minimize structural and functional damage. They should be visually inspected by users for any obvious damage and trouble-shoot and/or notify the Technical Leader when a heat block is not working optimally or as expected. Maintenance is performed as needed either in house or by an appropriate outside vendor.

Annual in-house performance check for heat blocks: Place a tube of water in a heat block well and wait 15 minutes to overnight for the water to reach the heatblock temperature. Place the NIST thermometer into the tube of water. Record temperatures of NIST thermometer and heat block digital temperature display after 15 minutes. Calculate offset value by comparing each heat block reading to the NIST thermometer reading. If both temperatures are within the target range, the equipment has passed its calibration. If the temperatures are beyond the target range, contact the Technical Leader as the equipment may need to be retired or sent out for calibration if applicable.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 26 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Defrosting procedure for freezers and refrigerators: Where appropriate, a NIST or NIST traceable thermometer is used to monitor the temperatures. Defrost freezers whenever frost on the wall becomes ¼ inch thick. Never use a sharp or pointed instrument to remove frost as it may damage the freezer. Do not use any electrical device in defrosting freezers.

1) To defrost, remove all items from the freezer compartment and record new locations as necessary (for chain of custody purposes). Place the drip pan or tray in place.
2) Set the temperature control dial to "OFF". If necessary, disconnect power to the freezer.
3) Leave the freezer door open until defrosting is complete. To speed up defrosting, use warm to hot water (not boiling) by wiping or placing on shelves.
4) After defrosting, empty water from the drip tray and wipe the compartment down with a damp towel or sponge.
5) After desired temperature is reached, return items to the freezer and update any necessary chain of custody records.
6) In case an instrument/device does not meet the appropriate temperature specification, notify Technical Leader. If the Technical Leader is absent, notify any available supervisor.

3.13.5. Balances

Balances are tared at each use as follows: Press the control bar to engage the instrument. Allow the readout to stabilize. Place weigh paper or weigh boat on the balance pan. Allow the readout to stabilize. Press the control bar once to tare. Make the desired measurement. As needed, clean the weigh chamber with a small soft brush or damp towel.

Performance Check: Annually by an outside vendor. Weights used for calibration are NIST traceable.

3.13.6. Centrifuges

The centrifuges should be used and handled in a way to minimize mechanical damage to the instrument. Regular cleaning of the instruments should be performed.

Performance Check: Only centrifuges used for experiments with required speeds will need annual performance checks. These will be performed on-site annually by an outside vendor. ISO 17025 accredited centrifuge calibrations will be for speed and time.

3.13.7. Thermal cycler temperature verification systems

The temperature verification system should be used and handled in a way to minimize mechanical damage to the probe and body. The temperature verification system should be visually inspected for mechanical damage and either repaired (by an outside vendor) or replaced as appropriate.

Performance check: Annually by an outside vendor using NIST traceable standards.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 27 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

3.13.8. Qiagen BioRobot EZ1-XL

Daily cleaning (after each use):
1) Remove used disposable lab ware and dispose of used reagent cartridges.
2) Dispose of well #4 contents in biohazard container.
3) Clean racks and trays with ethanol followed by deionized water or with ethanol followed by bleach
4) Clean worktable with distilled water.
5) Clean the piercing unit.
   a. Select "2: MAN" on the main menu.
   b. Select "3: CLEAN and START". Wait for the display "Piercing unit can now be cleaned" (the unit will move forward and downward).
   c. Open the workstation door and clean the unit with ethanol followed by water.
   d. Press ENT to return unit to original position.
   e. When complete, close door, press ESC and turn off the instrument.

Weekly UV decontamination protocol:
1) Press "1: UV" in the main menu.
2) Select a minimum decontamination time of 20 minutes (time must be within 20 – 60 minutes) and press "ENT: NEXT to begin". After the run has finished, the lamp takes approximately 3 minutes to cool down and display the main menu and the instrument can be turned off.

Monthly O-ring maintenance:
To ensure the filter tips will properly adhere to the tip of the adapters, silicon grease must be applied to the end of the filter tip and the surface of the o-rings. To do this, apply a small amount of silicon grease to your fingers and apply the silicon grease to the surface of the o-rings. Use a Kimwipe to remove excess grease on the o-rings and tip adapter.

Semi-Annual Calibration check:
Temperature accuracy, pipetting accuracy and leakage tests are performed for each BioRobot instrument.

Preventative maintenance (PM): Annually by a Qiagen representative. This must be followed by a performance check. Additional maintenance outside a scheduled PM will typically be conducted on-site by a Qiagen representative or in-house designee, or sent out for service.

Performance check (PC):
Following any repair, service or calibration (including Preventative Maintenance by a Qiagen representative) other than routine calibration checks performed in-house. The performance check must involve the extraction of a known QC sample and reagent blank, followed by quantitation to assess the quantity and quality of the DNA extracted. This must be performed on each instrument that has undergone service and/or maintenance as appropriate. A successful performance check will be measured by obtaining the approximate expected quantitation results for known samples and negative controls in accordance with current interpretation guidelines. Documentation

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 28 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

of the performance check will be given to the Technical Leader (or designee) for review and approval prior to release into casework rotation.

Qiagen Biorobot EZ1-XL calibration check procedures
While EZ1 is off, remove the DNA Investigator card and insert the TEST card; proceed with instructions. Record calibration results and document in EZ1 log book. Upon completion of calibration checks, turn EZ1 unit off and remove the TEST card; insert the DNA Investigator card.

Temperature Accuracy: To evaluate the temperature accuracy of the EZ1-XL workstations. Measured temperature should be observed within 60°C ±3°C.

1) Switch on the EZ1-XL. Press "3" to display the tests menu.
2) Press "2" to choose the temperature accuracy test. Use the arrow keys to set the temperature to 60°C.
3) Pipette 200 µL deionized water into the testing hole in the heating block. Remove reagent cartridge rack.
4) Press "START" to begin the protocol. Wait 20 min for the heating block to heat to 60°C.
5) Record the temperature measured by the calibrated thermometer. Recorded temperature should be within 57-63°C to pass the test.
6) To run another temperature accuracy test, press "ESC" and follow the protocol above. Otherwise, close the workstation door, press "ESC", and switch off the EZ1-XL. Remove 200 µL volume.

Pipetting Accuracy: To evaluate the pipetting accuracy of the EZ1-XL workstation. This test also describes how to evaluate the tip adapter for liquid leaks.

1) Switch on the EZ1-XL.
2) Label micro-centrifuge tubes: fourteen 1.5 mL tubes/fourteen 2 mL tubes.
3) Press START to display the menu. Press "1" for the 100 µL protocol.
4) Press any key to proceed through the test displayed on the LCD and start worktable setup.
5) Remove the caps from the 1.5 mL tubes and load them into the first row.
6) Fill the 2 mL tubes (without caps) with approximately 1 mL water. Load the filled, opened tubes into the fourth row (back row).
7) Load tip holders (fourteen) containing filter-tips into the second row.
8) Close the workstation door. Press "START" to begin the protocol. When the protocol ends, the LCD displays "Protocol finished". Open the workstation door.
9) Remove the labeled tubes from the first (front row), and measure the volumes in each with a calibrated P200 micro-pipettor. The recorded volume should be within 92 and 108 microliters to pass the test.
10) Close the workstation door before pressing "ESC" to return to the main menu and switch off the EZ1-XL or to proceed with leakage test, press "3" and proceed.

Leakage Test: To evaluate the tip adapters for liquid leaks.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 29 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

1) Switch on the EZ1-XL. Press START to display the menu. Press "3" to choose the leakage test. Press any key to proceed through the text displayed on the LCD and start worktable setup.
2) Label micro-centrifuge tubes: fourteen 1.5 mL tubes/fourteen 2 mL tubes.
3) Remove the caps from the 1.5 mL tubes and load them into the first row.
4) Fill 2 mL tubes (without caps) with approximately 1 mL water. Load the filled, opened tubes into the fourth row (back row).
5) Load tip holders (fourteen) containing filter-tips into the second row.
6) Close the workstation door. Press START to begin protocol. NOTE: The automated procedure takes 12 minutes to complete. The instrument will wait for 10 minutes to check the tightness around the o-rings.
7) When the protocol ends, the LCD displays "Protocol finished". Open the workstation door. Check that there has been no dripping from the tips during the test. Record results; if any tips are leaking, inform the DNA Technical Leader and contact QIAGEN Service Representative if warranted.
8) Close the workstation door before pressing "ESC" to return to the main menu and switch off the EZ1-XL.

3.13.9. QIAcube Connect

Daily cleaning (after each use):
1) Remove and discard Master Mix tube and used rotor adapters from deck. Rotor adapters should be discarded into biohazard waste.
2) Wipe down with bleach, followed by 70% ethanol. Wipe down deck, interior lid of centrifuge, interior walls of centrifuge, and shaker plugs. Do not wipe door of QIAcube.
3) Empty waste and wipe down waste drawer. Note: If liquid is present on deck or in drawer, wipe with ethanol followed by bleach.

Monthly maintenance:
1) Perform Tightness Test to assess O-ring integrity:
   a. Load an empty 2ml flat-bottomed EZ1 tube into position 1 of the shaker.
   b. Remove the shaker plugs from positions 2-12
   c. Replace PBS reagent bottle rack from position 1 with a reagent bottle of 95% ethanol.
   d. Load 1000 ul tip (NOT wide-bore) into position 1 of the tip rack. A single tip is needed for the test.
   e. Select "Tools" in the main menu.
   f. Under "Maintenance" tab, select "Tightness Test". Read prompts to verify set-up.
   g. Press "Start". *The test takes ~5 minues. When protocol is complete, select "Next".*
   h. Inspect the 2ml flat-bottomed tube to see if the instrument has passed the tightness test.
      1. If the tube is empty and dry, select "Yes" and then "Done".
      2. If the tube has liquid, select "No" and then "Done".
         a. Notify QIAcube point person and the DNA Technical Leader or a DNA Unit Supervisor

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 30 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

        b. If the test fails, change o-ring and repeat test. If test fails again, instrument needs to be serviced.

    i. Note results on the monthly maintenance log sheet.

    j. Clean up.

        1. Discard 2 ml flat-bottomed tube.

        2. Add shaker plugs back into positions 2-12.

        3. Replace 95% ethanol reagent bottle with the PBS reagent bottle.

        4. Replace 1000ul tips with 1000ul wide-bore tips.

        5. Empty waste drawer.

        6. Wipe down deck with bleach, followed by ethanol.

*Preventative maintenance (PM)***:**
Annually by a Qiagen representative. At this time, the O-ring on the instrument will be assessed and changed. This must be followed by a performance check.

*Performance check (PC)***:**
To be conducted following any repair, service, or PM.

The performance check must involve the extraction of a known QC sample consisting of a mixture of male semen (with sperm cells) with a non-sperm fraction from a different donor, and reagent blank. Extraction will be followed by quantitation to assess the quantity and quality of the DNA extracted. This must be performed on each instrument that has undergone service and/or maintenance as appropriate.

A successful performance check will be measured by obtaining the approximate expected quantitation results for the known sample and negative controls in accordance with current interpretation guidelines. Documentation of the performance check will be given to the Technical Leader (or designee) for review and approval prior to release into casework rotation.

3.13.10. Applied Biosystems 7500 HID RT-PCR system

Preventative maintenance (PM): Annually by an Applied Biosystems representative and at a minimum must include the system's diagnostic programs and temperature verification process. This must be followed by a performance check.

Performance check (PC): Following any repair, maintenance, service or calibration (including preventative maintenance) other than routine calibration checks performed in-house. If not already included, an ROI calibration should follow any repair, maintenance, service or calibration.

7500 PC Procedure: Quantitation of a known QC sample (with approximate known DNA quantity) and at least one negative control using Quantifiler Trio   A successful performance check will be measured by obtaining the approximate expected quantitation result for the known QC sample with acceptable slopes and R2 values, and no quantitation results for the negative control(s) in accordance with the current interpretation guidelines. Documentation will be

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 31 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

given to the Technical Leader (or designee) for review and approval prior to release into casework rotation.

Operational calibration checks: Background fluorescence, optics check, calibration of regions of interest (ROI) and pure dye spectra, system hardware test, background assay, computer maintenance, and replacement of bulb are performed for the ABI PRISM 7500.

Weekly Maintenance: Check the available hard drive space weekly. If there is less then 20% move .eds files to an archive folder on the server.

Monthly maintenance: Check lamp status, background calibration, clean block, disk cleanup and defragmentation as needed.

Maintenance of computer hard drive
Defragment the hard drive monthly or when the Windows Operating System indicates that it is time to defrag the hard drive. In the desktop, select Start▶Help and Support to access the Help for the Windows operating system. Use the search function of the Help to find information on the "Disk Cleanup" and "Disk Defragment" utilities.

Background calibration
See also Installation and Maintenance guide but do not perform the optical calibration with the background; that will be performed semi-annually). The background calibration should be performed monthly and after replacing the lamp. The purpose of this calibration is to measure the level of background fluorescence in the instrument. This is performed using a pre-made plate provided in the Spectral Calibration Kit or by creating a background plate. The pre-made plate should be kept in the freezer in its foil sleeve and used for three calibrations.

1. To create a background calibration plate, place a 96-well optical plate in a plate holder taking care not to touch the plate or set it down on the bench top. Add 50 uL of DI water to each well. Seal plate with an optical adhesive cover. If using a pre-made plate, bring the plate to room temperature (about 5 minutes). Leave the plate in its packaging until ready to use. Remove plate from packaging and vortex (about 5 seconds) then centrifuge plate for 2 minutes. Ensure that the liquid in each well of the plate is at the bottom of the well. If not, centrifuge again at a higher rpm and for a longer period of time. Do not allow the bottom of the plate to become dirty. Fluids and other contaminants that adhere to the bottom of the plate can contaminate the sample block and cause an abnormally high background signal.
2. Load the plate onto the 7500. In the 7500 software, select Instrument▶Instrument Maintenance Manager. Select the Background tab. Click Start Calibration. Follow the wizard to complete the calibration.
3. The Background Calibration dialog box contains four tabs:
    • Overview – Displays information describing the calibration.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 32 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

- Setup – Displays instructions for setting up the background calibration. Clicking Next opens the Run tab.
- Run – Clicking START RUN starts the calibration process and displays the processing messages. Click Next opens the Analysis tab.
- Analysis – Indicates the calibration status (Passed/Failed). If the instrument does not pass calibration, see "Troubleshoot the Background Calibration" on page 30 of the AB 7500/7500 Fast Real-Time PCR System Maintenance Manual.

4. Remove the plate from the instrument, place it back in the packaging, and return to the spectral calibration kit in the freezer. If properly stored in the original sleeve and in the freezer, the background plate can be used up to three times after opening.

Semi-annual maintenance: Regions of interest calibration (ROI), optical and pure dye spectra calibration; these may be performed in-house or during the annual PM.

Regions of interest (ROI) calibration
To be completed every six months and after replacing the lamp. See also Installation and Maintenance guide). The purpose of this calibration is to map the position of the wells on the sample block to allow the software to associate an increase in fluorescence with specific wells of the plate. The 7500 uses a set of optical filters to distinguish emissions thus necessitating calibration for each filter to account for differences in the optical path. The pre-made ROI plate should be kept in the freezer in its foil sleeve and used for three calibrations.

1. Remove the ROI calibration plate from the spectral calibration kit in the freezer. Let the plate warm to room temperature (~5 min.). Do not remove the ROI plate from its packaging until ready for use. Prolonged exposure of the fluorescent dye in the wells of the plate to light can diminish the fluorescence from the plate.
2. Remove the plate from the packaging. Briefly vortex ~ 5 sec. Centrifuge for 2 min. The ROI plate must be well mixed and centrifuged before use. Ensure that the liquid in each well of the plate is at the bottom of the well. If not, centrifuge again at a higher rpm and for a longer period of time.
3. Place the ROI plate into the instrument and perform the automated calibration.
4. In the 7500 software, select Intrument►Instrument Maintenance Manager.
5. In the ROI screen of the Instrument Maintenance Manager, click Start Calibration.
6. Complete the calibration as instructed by the wizard. The ROI Calibration dialog box displays three tabs:
   - Setup – Displays instructions for setting up the ROI calibration. Clicking Next opens the Run tab.
   - Run – Clicking Start Run starts the calibration process and displays the processing messages. Clicking Next opens the Analysis tab.
   - Analysis – Indicates the calibration status (Pass/Fail). If the instrument does not pass calibration, see "Troubleshoot the ROI Calibration" on

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 33 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

page 17 of the AB 7500/7500 Fast Real-Time PCR System Maintenance Manual.

7. Remove the ROI plate from the AB 7500 and place back into the packaging sleeve.   If a background and optical calibration is to be performed on the same day, keep the ROI plate at room temperature. The optical calibration uses the ROI plate. Otherwise return the packaged ROI plate to the spectral calibration kit in the freezer.

8. After the ROI calibration has been performed a background, optical calibration, dye calibration and instrument verification must be performed.

Optical calibration
- See also the Installation and Maintenance guide
- The purpose of this calibration is to compensate for the physical effects of additional filters used with the 7500.

1. Remove the ROI calibration plate from the spectral calibration kit in the freezer. Let the plate warm to room temperature ~5 min. Do not remove the ROI plate from its packaging until ready for use. Prolonged exposure of the fluorescent dye in the wells of the plate to light can diminish the fluorescence from the plate. Remove the plate from the packaging. Briefly vortex ~ 5 sec. Centrifuge for 2 min. The ROI plate must be well mixed and centrifuged before use. Ensure that the liquid in each well of the plate is at the bottom of the well. If not, centrifuge again at a higher rpm and for a longer period of time.

2. Place the ROI plate into the instrument and perform the calibration.

3. In the 7500 software, select Instrument►Instrument Maintenance Manager. Select the Optical tab. Click Start Calibration. Complete the calibration as instructed by the wizard. The Optical Calibration dialog box displays four tabs:
   - Overview – Displays information describing the calibration.
   - Setup – Displays instructions for setting up the optical calibration. Clicking Next opens the Run tab.
   - Run – Clicking START RUN starts the calibration process and displays the processing messages. Clicking Next opens the Analysis tab.
   - Analysis – Indicates the calibration status (Pass/Fail). If the instrument does not pass calibration, see "Troubleshoot the Background Calibration" on page 30 of the AB 7500/7500 Fast Real-Time PCR System System Maintenance Manual.

4. Remove the plate from the instrument, place it back in the packaging, and return to the spectral calibration kit in the freezer.

Pure dye calibration
- See also Installation and Maintenance guide:
- The purpose of this calibration is to collect spectral data from a series of pure dye standards so software can use the data to characterize the dyes and distinguish the individual contribution. The raw spectral data is compared to the pure spectral data in the file. The Spectral Calibration Kit contains dye plates with ABY®, JUN® and Mustang Purple® Dyes. VIC and

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 34 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

FAM should also be performed for the Y markers. The pre-made pure dye spectra plate should be kept in the freezer in its foil sleeve and used for three calibrations.

1. In the 7500 software, select Instrument►Instrument Maintenance Manager. Select the Dye tab. Select System Dye Calibration. Click Start Calibration. Complete the calibration for each plate as instructed by the wizard. The wizard will guide the process for the calibration of each dye separately. Each dye plate must be set up, ran, and analyzed independently.
2. Remove the plates for the freezer and thaw at room temperature (~5 min). Remove the needed plate from its packaging and vortex for 5 sec. Centrifuge the plate for 2 min. Ensure that the liquid in each well has collected in the bottom of the well. Load the plate into the instrument.
3. The Dye Calibration dialog box displays four tabs:
    •   Overview – Displays information describing the calibration. When the software prompts, obtain the required materials, and select the dyes that are to be calibrated.
    •   Setup – Displays instructions for setting up the dye calibration. Clicking Next opens the Run tab. When prompted by the software, prepare and load the dye plates.
    •   Run – Click START RUN starts the calibration process and displays the processing messages. Clicking Next opens the Analysis tab.
    •   Analysis – Indicates the calibration status (Pass/Failed). When prompted, analyze the spectra collected from each dye plate.

Verify the status of the calibration as passed or failed. If passed go to the next step. If failed see "Troubleshoot the Dye Calibration" on page 43 of the AB 7500/7500 Fast Real-Time PCR System System Maintenance Manual.

Verify the grouping of the dye spectra. Select all the wells of the plate. Inspect the raw data. For each spectrum, verify that the peak is:
    •   Within the detectable range of the instrument.
    •   Free of irregular spectral peaks.
    •   Present in the correct channel for the dye

See diagram below. If all spectra are acceptable, click Next.

See pages 41 and 42 in the AB 7500/7500 Fast Real-Time PCR System System Maintenance Manual for all spectral profiles.

Remove the plate from the instrument and place back into its packaging. Return to freezer. After the plate has been removed, click Finish. Prepare and run the next dye plate. Repeat this process until all plates have been run.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 35 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021
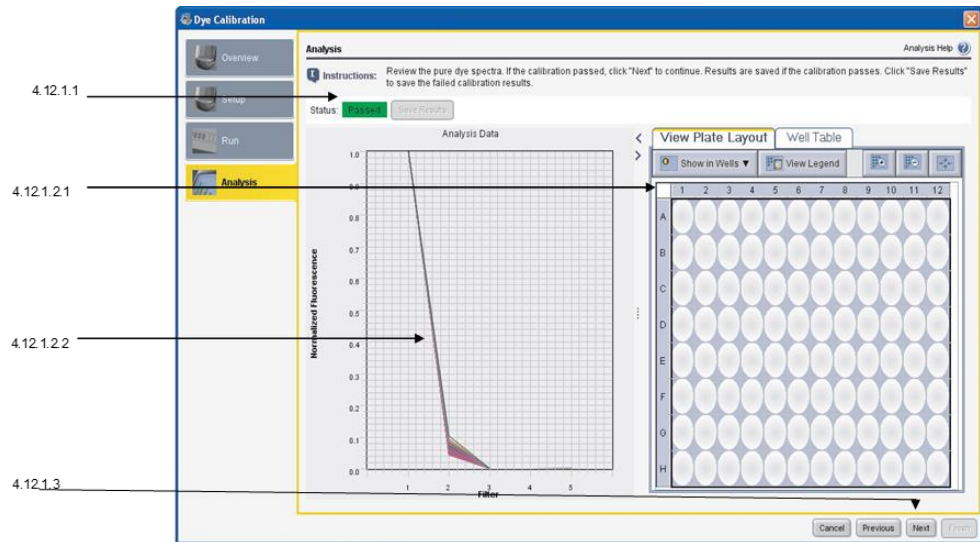
General maintenance: As needed either in-house by an Applied Biosystems representative or sent out to the appropriate outside vendor. If the bulb needs replacement, an ROI and background calibration should be performed (a new lamp should always be handled with gloves).

3.13.11.   **Tecan**

Preventative maintenance (PM): Annually by a Tecan representative and at a minimum must include the system's volume verification tests and standard curve. This must be followed by a performance check.

Performance check (PC): Following any repair, maintenance, service or calibration (including preventative maintenance) other than routine calibration checks performed in-house.

Tecan PC Procedure: Quantitation of a known QC sample (with approximate known DNA quantity e.g., Globalfiler kit 007) and at least one negative control using Quantifiler Trio with robotic set-up using the Tecan. A successful performance check will be measured by obtaining acceptable slopes and R^2 values, and no quantitation results for the negative control(s) in accordance with the current interpretation guidelines. Documentation will be given to the Technical Leader (or designee) for review and approval prior to release into casework rotation.

Tecan Maintenance:
1) Tip check – check the tightness of the tip screws once a week. Screws should be finger tight.
2) System liquid – refill at end of week or if low. Must degas (let sit out) for minimum of eight hours.
3) Waste liquid – empty as necessary
4) Clean deck and racks before each run with bleach, followed by 70% ethanol.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 36 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

5) Perform "flush" script at start of day 1 to 3 times (until bubbles are no longer observed in the lines).
6) Lines should be checked for air bubbles which may be introduced by reagents used in procedures (e.g. bubbles in a master mix).

3.13.12. 9700 and Proflexes

Visual inspection of sample blocks should be performed routinely by the analyst to assess any dirt or structural damage. Any maintenance will be performed as needed either in-house or sent out to the appropriate outside vendor.

Performance check: Annually (at a minimum), and always following any repair, service or calibration other than that performed in-house. The performance check must include the system's diagnostic programs and temperature verification system process. Note: the quarterly 'calibration check' satisfies the 'performance check' criteria. A successful performance check will be measured by obtaining the expected results for the system's diagnostic programs and temperature verification system. Documentation of the performance check will be given to the Technical Leader (or designee) for review and approval prior to release into casework rotation.

The following tests are performed on a quarterly basis (at a minimum):
1) Diagnostic File Checks (Display, Keypad, Rate, Cycle).
2) Temperature Calibration Verification Tests.
3) Temperature Non-Uniformity (TNU) Tests.
These tests are also performed before a new thermal cycler is introduced into casework and in accordance with manufacturer recommendations. In order to notify the user of the current calibration status, a tag is placed on the thermal cycler(s) that indicates the date of calibration and the due date of the next calibration.

9700 thermal cycler calibration check procedure
This procedure takes several hours to complete. Be sure that the instrument is not being used the day(s) that calibration checks occur. Warm up the instrument for 15 minutes. All results will be recorded on the 2-page "9700 Thermal Cycler Calibration Check" form. If tests do not yield expected results, notify the DNA Technical Leader.

Display Test: This test visually determines if the Liquid Crystal Display (LCD) screen is functioning properly.

Access the Utilities/Instrument Diagnostics/Hardware Diagnostics file from the initial display:
1) Press "Util", followed by "Diag", and then "Hard."
2) Access the DISPLAY TEST program by pressing "Disp".
3) Turn on all pixels by pressing "Run." To turn off all the pixels press "Stop" and to exit the program press "Stop" one more time. This will return you to the HARDWARE DIAGNOSTICS screen.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 37 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Keypad Test: This test verifies the operation of the keypad.

Access the Utilities/Instrument Diagnostics/Hardware Diagnostics file from the initial display:
1) Press "Util", followed by "Diag", and then "Hard."
2) Access the KEYBOARD TEST program by pressing "Keypad", followed by "Run" to start the program. A diagram mimicking the keyboard buttons will appear.
3) Press the keyboard key corresponding to the indicated flashing key displayed in the monitor. After the test is complete, you will return to the HARDWARE DIAGNOSTICS screen.

Rate Test:
1) Access the Utilities/Instrument Diagnostics/System Performance file from the initial display by pressing "Util" followed by "Diag" and then "System." Next, access the RATE TEST program by pressing "Rate."
2) After pressing "Rate" a "WARNING!!" screen will appear. Install the GeneAmp 0.2 mL Thermal Insulation Frame, slide the heated cover forward, and lock in place. Press "Cont."
3) At the conclusion of the test, the "Cool and Heat Rate Test" screen appears that displays the results to the test.
4) Acceptable results are as follows: Heating Rate > 1.5°C/sec; Cooling Rate > 1.5°C/sec
5) Press "Cancel" to return to the SYSTEM PERFORMANCE screen.

Cycle Test:
1) Access the Utilities/Instrument Diagnostics/System Performance file from the initial display by pressing "Util" followed by "Diag" and then "System." Next, access the CYCLE TEST program by pressing "Cycle."
2) After pressing "Cycle" a "WARNING!!" screen will appear. Install the GeneAmp 0.2 mL Thermal Insulation Frame, slide the heated cover forward, and lock in place. Press "Cont."
3) The cycle test executes a two-temperature PCR cycling protocol then measures and reports the average cycle time and cycle-to-cycle variation. When the test is completed, the cycle performance display will appear.
4) Acceptable results as follows: Average Cycle Time < 160 seconds; Cycle Time STD < 5 seconds.
5) Press "Cancel" to return to the SYSTEM PERFORMANCE screen.

Calibration Verification Test
1) Install the GeneAmp 0.2 mL Thermal Insulation Frame.
2) Use a cotton swab to coat well A6 with mineral oil.
3) Place the probe from the temperature verification system kit in sample well A6. Thread the probe through the channel in the Thermal Insulation Frame to prevent damage to the probe and lead wires.
4) Slide the heated cover forward and lock in place. Turn on digital thermometer.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 38 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

5) Access the Utilities/Instrument Diagnostics/Temperature Verification file from the initial display by pressing "Util" followed by "Diag" and then "TmpVer." Next, access the CALIBRATION VERIFICATION TEST program by pressing "Temp."

6) Press "Run" to start program. Read thermometer when the "Stabilizing at setpoint…" value decrements to zero and enter the value in the "Enter actual block temperature" field. (**Note**: round the four thermometer reading to three digits before typing it into the CALIBRATION VERIFICATION screen.).

7) Repeat steps with the second reading (the first reading is at 85°C the second is at 45°C). The results for the calibration of the sample block temperature for the set point values are displayed on the calibration verification screen.

8) Compare these results to the previously recorded results on Page 2 of the form. Press "Accept."

When the CALIBRATION VERIFICATION TEST is completed a screen will appear indicating whether the "Calibration is good," or "Instrument may require service." Mark "yes" or "no" on the form. If the tests passed, continue on to the next test. If the tests failed, repeat the procedure one more time. If the test still fails, document any resolution and notify the DNA Technical Leader.

Remove the probe assembly and clean off the oil with a dry cotton swab. **Note**: If not continuing on to the next test, make sure the sample block is cooled to room temperature before removing probe and temperature verification frame.

Temperature Non-Uniformity Test
1) Coat the following wells with mineral oil using a cotton swab: A1, A12, C4, C9, F4, F9, H1, H12.

2) Place the probe from the temperature verification system kit in sample well A1. Thread the probe through the channel in the Thermal Insulation Frame to prevent damage to the probe and lead wires.

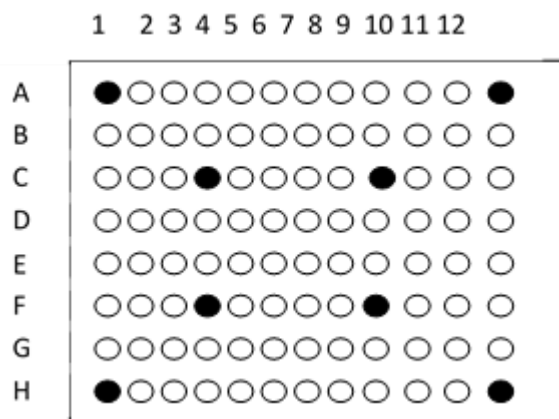3) Slide the heated cover forward and lock in place. Turn on digital thermometer.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 39 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

4) Access the Utilities/Instrument Diagnostics/Temperature Verification file from the initial display by pressing "Util" followed by "Diag" and then "TmpVer." Next, access the TEMPERATURE NON-UNIFORMITY TEST" by pressing "TNU."

5) When the heated cover and the sample block have reached the appropriate temperatures (this may take several minutes), press "Run."

6) The TNU PERFORMANCE screen counts down the time until the set point is stabilized. When the "Stabilizing at setpoint…" value reaches zero, read the digital thermometer and record value on the form. **Note**: Initially all test wells will be measured at 37°C. Once the 37°C measurements have been made the heated cover will ramp to 105°C and the test well readings will repeat at 94°C.

7) Enter the digital thermometer value in the "Enter actual block temperature" field on the TNU PERFORMANCE screen and press enter. **Note:** round the four-digit value from the digital thermometer to three digits before typing it in the TNU PERFORMANCE screen. After pressing "Enter" the TNU PERFORMANCE screen will display a prompt as to where to place the probe next.

8) Slide the heated cover back and place the probe in the appropriate well. Repeat steps for each test well. At the conclusion of the test, a summary screen will appear. Compare these values with the actual test results recorded on the Calibration Form. Press "Accept."

9) Once the TNU test is completed, the TNU PERFORMANCE screen will display a "PASS" or "FAIL" after each set point temperature. Record the results on the form. If the test has failed, repeat the procedure. If the test fails again, notify the DNA Technical Leader.

10) Remove the probe assembly and clean off the oil with a dry cotton swab. Clean all the wells with a cotton swab dampened with ethanol. **Note**: Make sure the sample block is cooled to room temperature before removing probe and Thermal Insulation Frame.

Proflex thermal cycler calibration check procedure

Heated Cover Test
This test determines if the heated cover holds temperature at 105+/- 3oC.

On screen:
>Settings>Maintenance and services>Block Verification Test>Verify Block Temperature>Heated Cover>Next

1) Connect flat probe to middle 2 zones (middle 4 columns). Turn on TVK. Make sure the cable is flat before closing the lid and connected to TVK probe. Connect USB cable to probe and to thermal cycler. Close heated cover.

2) Hit "Start". Follow prompt to make sure TVK probe is connected.

3) Wait for data collection. This test takes ~30 minutes.

4) After test:
   a. If failed: Rerun test. If fails again, bring to Technical Leader.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 40 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

b. If passed: Record temperature and pass/fail status, then close test.

Temperature Verification
This test determines the temperature accuracy of a thermal cycler by measuring sample well temperatures at two set points, 45oC and 85oC.

On screen:
>Settings>Maintenance and services>Block Verification Test>Verify Block Temperature>Temperature Verification>Next

1) Connect flat probe to left 2 zones (leftmost 4 columns). Turn on TVK. Make sure the cable is flat before closing the lid and connected to TVK probe. Connect USB cable to probe and to thermal cycler. Close heated cover.
2) Hit "Start". Follow prompt to make sure TVK probe is connected.
3) Wait for data collection. Each zone takes ~20 minutes to complete.
4) When the first data collection is complete, follow the on-screen prompt and move the probe to the middle 2 zones (middle 4 columns). Hit "next".
5) When the second data collection is complete, follow the on-screen prompt and move the probe to the right 2 zones (rightmost 4 columns). Hit "next".
6) After the final collection is complete, assess results. To pass the test, the six sample blocks must be within +/- 0.25 degrees Celsius of the set points.
    a. If failed: Rerun test. If fails again, bring to Technical Leader
    b. If passed: Record all temperatures and pass/fail status, then close test.

Temperature Non-Uniformity
This test determines the temperature uniformity of the thermal cycler by measuring temperatures at 24 well locations.

On screen:
>Settings>Maintenance and services>Block Verification Test>Verify Block Temperature>Temperature Non-Uniformity>Next

1) Connect flat probe to left 2 zones (leftmost 4 columns). Turn on TVK. Make sure the cable is flat before closing the lid and connected to TVK probe. Connect USB cable to probe and to thermal cycler. Close heated cover.
2) Hit "Start". Follow prompt to make sure TVK probe is connected.
3) Wait for data collection. Each zone takes ~20 minutes to complete.
4) When the first data collection is complete, follow the on-screen prompt and move the probe to the middle two zones (middle 4 columns). Hit "next".
5) When the second data collection is complete, follow the on-screen prompt and move the probe to the right two zones (rightmost 4 columns). Hit "next".
6) After the final collection is complete, assess results. To pass the test, the six sample blocks must be within +/- 0.5 degrees Celsius of the set points no later than 30 seconds after the set point temperature is changed.
    a. If failed: Rerun test. If fails again, bring to Technical Leader
    b. If passed: Record all temperatures and pass/fail status, then close test.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 41 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Cycle Performance

On screen:

>Settings>Maintenance and services>Block Verification Test>Verify Cycle Performance> Next

1) Place an empty plate into the thermal cycler.
2) Hit "Start"
3) Wait for data collection (approximately 10 minutes)
4) After the final collection is complete: Record all specs generated and pass/fail status. Passing parameters are listed on the log sheet. Close test. If failed: Rerun test. If fails again, bring to Technical Leader.

Self Verification Test (Remove blank plate)

This test checks the instrument hardware including the block, heated cover and other components.

On screen:

>Settings>Maintenance and services>Self Verification Test>Start Test

1) Hit "Start Test".
2) Wait for diagnostics to run (~10 minutes)
3) After test is complete: Record "Thermal Block Test Result" and any items listed in "Errors Encountered". Close test. If failed: Rerun test. If fails again, bring to Technical Leader.

3.13.13.   3500xL CE

Replacing the capillary array, running spatial and spectral calibrations will be performed as needed. General maintenance will be performed as needed either in-house or sent out to the appropriate external vendor.

Preventative maintenance (PM): Annually by an Applied Biosystems representative. This must be followed by a performance check.

Performance check (PC): Following any repair, service or calibration (including Preventative Maintenance by an Applied Biosystems representative) other than that performed in-house.

3500xL Performance check procedure: The performance check must include the typing of a positive control, negative control and ladder. This must be performed on each instrument that has undergone service and/or maintenance as appropriate. A successful performance check will be measured by obtaining the expected typing results for the positive and negative controls and the ladder in accordance with current interpretation guidelines. Documentation of the performance check will be given to the Technical Leader (or designee) for review and approval prior to release into casework rotation.

A spectral or spatial calibration need only be conducted post-PM if recommended by the AB representative, or as needed.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 42 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

3.13.14. **Pipettes**

Micropipettes should be used and handled in a way to minimize structural and functional damage. They should be visually inspected by users for any obvious damage. Analysts should refrain from using any micropipette that is not working optimally or as expected. Maintenance will be conducted as needed by the appropriate outside vendor.

Performance check: Annually (at a minimum) by an outside vendor. Valid certificates of calibration from external vendors may be used to satisfy proof of a performance check.

3.13.15. **Ovens**

Lab ovens are currently used only for the general purpose of drying glass microscope slides to affix the material. The ovens should be used in a way to minimize structural and functional damage. They should be visually inspected by users for any obvious damage. Analysts should refrain from using any laboratory oven that is not working optimally or as expected. Maintenance will be conducted as needed either in-house or sent out to the appropriate outside vendor.

3.13.16. **Microscopes**

The microscopes should be used following manufacturer's recommendations and kept clean of debris and mounting solution residue. Microscopes should be visually inspected by users for any obvious damage and the Technical Leader notified of any microscopes not working optimally or as expected. Microscopes are serviced as needed by an outside contractor.

3.13.17. **LSV (Leeds Spectral Vision) Alternate Light Source**

The LSV instruments should be used following manufacturer's recommendations and kept clean of debris. Instruments should be visually inspected by users for any obvious damage and the Technical Leader notified of any units not working optimally or as expected. \Instruments are serviced as needed by an outside contractor.

4. **General laboratory procedures**
    4.1. **Handling of evidence and associated work product**
       4.1.1. **Preservation, storage, and disposition**

While in the laboratory (both prior to and post-analysis), items are stored in secured, limited-access refrigerators, freezers or room temperature storage. During the course of analysis, an item may be refrigerated to avoid multiple freeze/thaw cycles. Items that may produce condensation when stored in the freezer should be stored at room temperature (i.e., metal tools, firearms, cell phones, etc.). Analysts also have the option of using secured room temperature temporary storage for any work-in-progress; however, examination-in-process evidence must be secured in limited-access areas and include tamper-proof temporary seals. Any seal, including temporary seals, must ensure that contents cannot readily escape and must allow

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 43 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

detection of alteration to the container or seal and be labeled with the individual responsible for sealing the evidence.

In order to ensure that evidence samples and extracts are stored in a manner that minimizes loss, contamination, and/or deleterious change, items of evidence and extracted DNA samples are kept in secured cold storage and removed only long enough to examine, document, sample, quantitate or amplify.

Custody and storage of evidence items and associated DNA extracts, including transfer dates, initials/signatures and item numbers, is documented and stored within each casefile. Evidence items from the SFPD Property Control Division (PCD) should already have unique "item number" identifiers for the main item; work product envelopes and item numbers are generated by PCD upon return by the Forensic Biology laboratory.

4.1.2. Sample consumption

Efforts are made to avoid unnecessary consumption of evidence stains, and to retain or return a portion of the sample (including extracts and other work product) for re-analysis, if consistent with also obtaining optimum results from the examination. For cases where DNA testing has been conducted, and subsequent biological screening is requested, efforts are made to not consume remaining evidence e.g. if half a swab is used for initial DNA testing, a quarter of the remaining swab will be used for subsequent screening.

If the entire sample must be consumed in order to obtain an interpretable result, then the analyst will confer with the DNA Technical Leader. Where appropriate, the submitting agency will be consulted so that legal implications of proceeding with analysis may be properly considered; the outcome of this consultation should be retained in written or electronic form within the casefile. If a sample has been totally consumed, this must be noted in the "Disposition" section of the laboratory report.

4.1.3. Work product

Work product is defined as material that is generated as a function of analysis. It is protected in secured storage, and except for DNA extracts, is not typically tracked with a separate internal chain of custody as evidence (unless required to maintain a chain of custody). In the Forensic Biology Unit, this includes microscope slides, supernatants (if item was consumed), quantification reaction plates, PCR product, CE setup plates, and post-extraction cuttings. The latter are discarded after analysis. A new work product envelope should be created for each new request/case assignment (e.g. A supplemental request will have a new work product envelope made. This is the case even if the original request's work product envelope is still in lab storage).

4.1.4. Short-term storage

Cuttings removed from evidence items, extracted DNA samples and other types of work product such as microscope slides will be stored in laboratory refrigerators or freezers while work for each case is in progress. Cuttings pending extraction will be stored in a freezer pending DNA assignment. They will be placed in sample storage racks within a designated refrigerator pending completion of testing.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 44 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

4.1.5. Long-term storage

Evidence is not stored long-term in the Forensic Biology Unit. Upon completion of analysis, the evidence (e.g. original item and extra cuttings/swabs from the item), along with work product (e.g. extracts, microscope slides, etc.) is returned to PCD or other appropriate submitting agency. Work product may be sealed inside original evidence containers/packaging or may be packaged separately and entered as a new item number by PCD as appropriate. Work product will be returned to PCD once the report has been mailed to the customer.

4.1.6. Disposal of non-probative materials

Items used to assist forensic examinations (e.g., RSID cassettes, used microcentrifuge tubes and spin baskets, supernatants, filter paper and/or swabs for presumptive testing, etc.) are not considered work product and are discarded following analysis. Documentation of the associated testing results is retained for review purposes.

4.1.7. PCR product retention and storage

Amplified DNA is stored only in the post-amplification room. PCR product is considered work product but will not be returned to PCD. Once interpretation of the evidentiary PCR samples (and corresponding controls) has been completed and reviewed, the PCR product is discarded after being stored for 3 months. The product is discarded in regular trash by carrying directly to the trash receptacle to minimize contamination potential. If the evidentiary stain and/or DNA extract is/are consumed during testing, the PCR product (and associated controls) may be retained for long-term storage purposes. PCR product plates may be stored at $2 – 8°C$ if stored for less than 2 weeks, and $- 15°C – 25°C$ protected from light for more than 2 weeks.

4.2. Evidence receipt and return

Prior to assigning an examination request to an analyst in the Forensic Biology Unit, elements of the case and the potential evidence are evaluated (i.e. police reports, medical reports, discussions with investigators, prosecution and/or defense attorneys as needed) and the appropriate items for testing are selected. After a request is assigned, items are retrieved, routinely from SFPD PCD, though other routes of evidence submission may be necessitated on occasion. Once property arrives, analyst verifies contents of form against property delivered. If something is crossed out on the request form, the analyst/property officer must write a note on the form explaining the edit. The item(s) is then placed in appropriate storage pending examination. Forms are scanned and stored in the "Case Documentation" folder on the S: drive.

After examinations have been completed, analyst completes "Evidence Return" or "Work Product Return" form and submits to Property for pick-up. Analyst verifies form against property being returned. If something is crossed out on the request form, the analyst/property must write a note on the form explaining the edit. Forms are scanned and stored in the "Case Documentation" folder on the S: drive.

The laboratory uses a "Direct to DNA" workflow for rape kits. Upon receipt, kits are inventoried, items sampled, and samples entered directly into the DNA testing process. Biological screening of rape kits is typically performed upon request by the submitter, District Attorney or other external agency.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 45 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

4.3.   Sexual Assault Forensic Evidence Tracking (SAFE-T)
SAFE-T is managed by the California Department of Justice. It is a web-based database for tracking all victim sexual assault evidence kits received. Users of the CODIS Hit Outcome Project (CHOP) also have access to SAFE-T. All sexual assault evidence kits received by the laboratory are entered into SAFE-T. This is generally performed after the kits are sampled for DNA analysis by the individual who screened the kit.

Adding a New Kit in SAFE-T
1. Log onto SAFE-T.
2. Perform a search for the kit using the SFPD incident number (with spaces) to check if the kit has not already been entered.
    a. If the search returns with a record, open the record to verify that the information is correct. Click "Save" and close the record. You don't need to proceed any further.
    b. If the search returns with "No records to display," continue to enter the kit by clicking "Add New Kit."
3. If adding a new kit, complete the highlighted fields. Click 'Save'. 'Close the Record'.

4.4.   Evidence sample types
The following are sample types commonly encountered in the Forensic Biology Unit. Other sample types should be treated as appropriate by the experience of the analyst and the forensic science community. The following amounts represent ideal target sampling amounts, but oftentimes evidence material may be present in limited supply. Analysts should use as much of the evidence as they deem appropriate to maximize the likelihood of obtaining a DNA typing result without consuming. Volumes of reagents may be altered proportionally to accommodate sample size. The analyst may retain or discard the substrate at their discretion and given the case circumstances.

Swabs: Samples swabbed by CSI then processed by the lab will be cut according to the following chart. A representative portion of each swab will be cut and combined into a single tube for downstream processing. Analyst may deem it appropriate to sample evidence swabs differently than dictated by chart (i.e. multiple apparent blood swabs may not need a cutting taken from each swab).

Swabs submitted (per item)	Representative portion of each swab
1	~1/2
2	~1/2
3	~1/3
4	~1/4

Touch DNA: This includes possible wearer or handler DNA recovered from clothing, tools, or any other potentially handled items. Generally, items being examined for touch DNA will be sampled using the wet/dry swabbing technique. Sterile water is used to wet the swab. Following wet/dry swabbing, ~½ of each swab head will be cut and placed in a tube. If item is small (i.e. single bullet or casing), one swab can be used to wet/dry swab item. Only ~½ of swab head will be cut and placed in a tube with this scenario. Wet and dry swabs used by analyst, will be placed in a labeled envelope, sealed, then placed in the work product envelope of the associated case.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 46 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Biological material (with/without semen): This includes suspected blood, semen, saliva, mixed stains, etc. Target sample size is approximately 0.5 to 1 cm^2 cutting of the stain area, ¼ to ½ of a swab head, or a swabbing/scraping of an appropriately-sized area given the evidence. If a wet and dry swab combination is used, the analyst should combine half of each swab into one tube; if more than one wet/dry swabs are present, the analyst should attempt to take the best representative sampling by either combining half or a quarter of each swab type into one tube. The analyst may choose to cut into smaller pieces. The remainder of the wet/dry swabs will be packaged with the evidence work product and returned to property control division (PCD).

Tissue: Target sample size is approximately 0.5 to 1 cm^2 tissue (or less if tissue is very fresh). The analyst may choose to cut into smaller pieces.

Whole blood: Target sample size is approximately 5 µL to 50 µL whole blood.

Purported/apparent hair:
Loose: Examine under the stereoscopic microscope and determine whether potential root material is visible. If potential evidentiary biological material is observed on the shaft, an attempt should be made to collect it by swabbing or collection of a wash step with sterile DI water. Holding carefully with forceps, use a sterile razor blade or scalpel to cut the potential root and place in a sterile tube (note: item may also be cut then washed). In some cases with old/possibly degraded hair, the analyst may choose to cut and separately process ~ 1 cm of the remaining shaft as a control and in an attempt to monitor any DNA on the hair not originating from the root end (i.e. contaminating DNA).

Slide-mounted: After locating the apparent root end under a microscope, cut or break the cover slip directly over the apparent root end using a diamond tip scribe. Remove glass fragments. If mounting media is present, fill the hole with solvent (i.e. xylene or toluene) to dissolve it. Another option would be to heat the slide to slide cover slip off, then wash hair in xylene. Continue with sampling and washing as described above for "loose purported/apparent hair".

Purported/apparent teeth: The surface should be cleaned with sterile water prior to sampling; the analyst should attempt to remove the inside material (purported pulp). DNA extraction from teeth are not standard practice for the DNA lab.

Purported/apparent bone: The surface should be cleaned using sterile water. Weigh the "bone" prior to (and after) sampling. Approximately 2 grams of material should be used for the extraction procedure but more/less may be used based on the age and appearance of the sample. Prepare the sample by crushing with a hammer with the intention of obtaining as many surfaces as possible to allow contact with the chemicals. DNA extraction from bones are not standard practice for the DNA lab.

Blank Water Controls: Collection of water controls is not standard practice within the DNA laboratory. The processing of water controls prepared by CSI is not standard practice, but may be done by request.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 47 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Reference samples:

Blood: Whole blood preserved in EDTA tubes (lavender-top), or ACD tubes (yellow-top containing acid citrate dextrose) are preferred for collection of reference blood samples. Samples collected in tubes with other preservatives (e.g., sodium fluoride), or without preservative, may yield reduced DNA quantities. Liquid blood samples should be refrigerated upon receipt. To preserve the sample, an analyst may make a dried whole blood stain and store frozen and/or return the liquid sample for refrigeration at the Medical Examiner's Office or PCD. If a blood sample is collected post-transfusion, then a pre-transfusion hospital sample is desirable.

Secondary/alternate reference samples: If no reference sample can be collected from an individual, an alternate reference source may be used. Alternate reference samples must be described by the investigator as having come from the individual, or reasonably assumed to have come from the individual. Typically alternate references are processed with non-diff evidence samples, unless indicated otherwise by the DNA Technical Leader.

Saliva: May be collected by a cotton swab (to ensure that cellular material is collected) on sterile gauze, cloth, filter paper, etc., and air-dried. Saliva samples should be stored frozen. Miscellaneous "evidence" swabs (e.g. from SAEKs) may be used as "reference samples" as appropriate and authorized by a Forensic Biology Unit Supervisor. Normally ½ of a swab head from a buccal reference is cut, then divided into half. Each portion is then placed into two separate tubes and processed in parallel.

Personal Articles: When necessary, various personal articles such as a hairbrush, toothbrush, clothing, dentures, etc., may be used as secondary reference material. These may be stored frozen depending on the item.

4.5. Evidence examination and documentation

Upon examination of a piece of evidence, the analyst is responsible for marking the item for identification with a unique case identifier (further than what has already been assigned by PCD as necessary), the analyst's initials and the date.

The analyst is responsible for documenting the condition of the item as received and before sampling. This may include the condition of packaging, seals, labels, handwriting, etc. and may be recorded in case notes and/or with photography. If there are any discrepancies in condition or content, the analyst should consult the customer for further clarification and document the outcome.

Each evidence item is examined separately on a clean work surface. Items should be examined as appropriate to the case scenario and documented in the case notes and/or with photography. The evidence should be surveyed for general characteristics and condition such as color, size, brand name, apparent wear, indications of apparent prior analysis, etc. as applicable. Where appropriate, trace evidence (e.g. possible hairs) should be collected, labeled and packaged with the original item. Analysts in other forensic disciplines may be consulted regarding prioritization of tests and examinations.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 48 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

The analyst should document approximate size, appearance and location/orientation of stains. Photos of items can serve as documentation of these observations. If multiple stains or areas of interest are identified/tested on an item, the analyst may need to designate the discrete areas with unique identifiers to distinguish them through processing and allow sample tracking in subsequent analysis. This may be done on the item directly (e.g. circled in ink, indicated with a sticker label, etc.) and/or photographed or diagrammed. Photographs shall be taken of post cutting from non-swab items. Swabs do not need a photograph post-cutting. Photographs will be taken of all evidence items. If a sub item of evidence is being retested within a larger evidence item, take photos of all outer packaging and contents of package (do not unnecessarily open items for sake of photography). Only sub item of evidence being retested will be opened and photographed further. Case notes and diagrams can supplement photography. When photographing any evidence use a scale, case identifier, item identifier, analyst initials, and the date is present.

Reagent lot numbers and expiration dates shall be recorded for any reagents used during the course of screening (i.e. serology tests).

To better troubleshoot contamination, where possible lab equipment will be documented on relevant forms and worksheets.

4.6. General screening workflows

4.6.1. Swabs

Swabs submitted to the Forensic Biology Unit for processing will be tested according to 64 form submission request and direction from supervisor. In general, the only serology test done on submitted swabs would be KM if possible blood staining is observed. AP, RSID, or micro will only be done when advised by supervisor and noted on case assignment worksheet. When sampling swabs, do not take more than ~1/2 of swab head from each swab and try to have total sampling be the equivalent of one swab.

Example, for one swab submitted only cut ~1/2 of swab head. If two, swabs are submitted cut ~1/2 of each swab head and combine into tube. If four swabs are submitted, the analyst can either take ~1/2 of two swabs heads and combine or take ~1/4 of all four swabs and combine.

4.6.2. Sexual assault kits

Swabs submitted in the kit will be sampled as described above. Underwear will be AP mapped for the front exterior, front interior, back exterior, back interior. All uniquely AP positive stains will be sampled for DNA (i.e. if there are stain groupings, only take one sampling of grouping). Test any possible blood stains for KM only taking KM positive stains to DNA if deemed probative based on case scenario. If underwear is AP negative, any white/yellowish stains may be taken forward to DNA. Tampons and pads processed like kit underwear; AP-map entire tampon and string. If pad is adhering to underwear, analyst will AP map top and underside of pad, and AP map panties underneath pad once removed (inner/outer crotch).

All samplings taken from a sexual assault kit will be sent to differential extractions, unless case scenario clearly states that touch DNA, saliva, or blood are only types of

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 49 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

evidence for consideration. If liquid blood or urine is contained in the kit, these must be separated out from the kit and placed in refrigerator storage to be booked back into evidence under a new/separate item number.

4.6.3. Items

Entire items (clothing, tools, etc.) submitted to the Forensic Biology Unit for sampling will be processed based on case assignment details provided by supervisor.

Note: When CSI collects casings at the scene, their judgment based on the scene dictates how they package the casings (i.e. group together from a cluster, or individually). If they have packaged multiple casings in the same envelope, the lab can swab them together and re-package them together. If they come to the lab packaged together, there is no need for the examining analyst to re-package them individually or based on casing type/brand.

4.6.4. Sampling labeling

The analyst should clearly label the cuttings and/or swabs in a way that can be followed through the testing procedures to avoid physical confusion with other items. Multiple stains from the same item should be uniquely identified. If an item does not lend itself to marking, the proximal container or identifying tag must be marked to be uniquely identifiable.

The Tecan workbook and programming does not allow spaces. The basic system of nomenclature is sequential alternating of letters and numbers e.g. Item 1→Item 1A→1A1→1A1A. Multiple swabs from the same origin can be named the same (i.e. two vaginal swabs from Item 1 would each be named 1A). Multiple swabs from different origins may be packaged in individual envelopes within a larger outer packaging. Labeling schematic does not change in this case. (i.e. vaginal swabs, anal swabs, and buccal swabs may all be packaged separately within item 1. Interior envelopes containing vaginal, anal, and buccal swabs would be labeled 1A, 1B, and 1C.) Also, there does not need to be an identifier distinguishing between ASAP and SAK portion of a sexual assault kit (i.e. Three sets of swabs are in the ASAP portion and six sets are in the SAK portion of item 1. These can be named 1A through 1I, rather than 1A1 through 1A3 in the ASAP and 1B1 through 1B6 in the SAK). This is subject to variation depending on the workflow needs of the unit. Additionally, an "_O" will be added to the end of any itemization of a sampling taken from an orifice swab from a sexual assault kit. This designation is used to help identify samples that can be terminated following quantification. Samples can be added to an extraction queue following sampling/screening. These entries should be checked by a second analyst to ensure accurate entry.

5. Screening and serology

5.1. Stereomicroscope

The stereomicroscope can be used when examining shoes, knives, hairs, fingernail clippings/scrapings, etc. to aid in visualizing small stains or particles.

5.2. Alternate Light Source (ALS)

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 50 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

An analyst can use alternate light sources to help visually locate semen, blood or other biological fluids that may not be readily visible to the naked eye. Certain biological fluids, including semen, and to a weaker extent saliva, are known to fluoresce using light in the approximate 450 nm range with orange filters. Others, such as blood, absorb light which can improve contrast and detection compared to ambient lighting conditions. In addition to fluorescence and absorption, bright lights, a lighted magnifier and oblique lighting may help differentiate staining patterns on fabric or items. Members of the Forensic Biology Unit can use the handheld light or the Leeds Spectral Vision System (LSV) for ALS needs.

5.3. Blood testing

The phenolphthalein (Kastle-Meyer) chemical test is a presumptive test for the presence of blood. For color changing tests like the phenolphthalein (Kastle-Meyer) chemical test results should be recorded in the following manner:

Controls: Note *color change @ time* and outcome (positive or negative).
Evidence (positive reactions): Positive @ *time. (color change does not need to be noted as it is assumed based on control results)*
Evidence (negative reactions): Test only needs to be noted as "Negative".
Additional information can be added but is not necessary.

5.3.1. Phenolphthalein (Kastle-Meyer) test

The phenolphthalein (Kastle-Meyer) chemical test is a presumptive test for the presence of blood. This test relies on the presence of peroxidase-like activity of hemoglobin in red blood cells, which catalyze the reduction of hydrogen peroxide and results in a color change. While sensitive, it is recognized that catalytic tests are not highly specific and may yield false positive results from plant sources, animal blood, iron-containing materials, microorganisms such as algae, fungi, mold, bacteria which may be present in soil and chemical oxidants and catalysts such as rust.

The Forensic Biology Unit uses prepackaged phenolphthalein presumptive blood test kits. Only one lot will be active at any one time.

Kit components:
Bottle #1: Alcohol (methanol)
Bottle #2: Phenolphthalein reagent (contains sodium hydroxide and zinc)
Bottle #3: Hydrogen peroxide 3%

Method:
1) QC reagents with a blank control and blood stain control prior to testing evidence samples.
   a. A pink color change in less than 1 minute indicates a positive reaction.
   b. No color change at 1 minute indicates a negative reaction. Record the time (seconds) for reaction up to 1 minute. Time does not need to be recorded for negative reactions.
   c. If controls yield the anticipated results, proceed with testing evidence items.
   d. If the controls do not yield the anticipated results, a new lot of chemicals may need to be used/prepared and the Technical Leader should be consulted for possible troubleshooting.
2) To test evidence:

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 51 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

    a. Gently rub the area with a folded piece of filter paper, a swab, or remove a small cutting and test directly.

    b. Add one drop alcohol

    c. Add one drop of Phenolphthalein reagent and observe for reactivity. Color development after the addition of reagent A could indicate a false positive result and invalidate the test result. This should be recorded in case notes.

3) Add one drop of hydrogen peroxide 3% and observe for reactivity. Record the time (seconds) for reaction up to 1 minute.

4) A pink color change at < 1 minute indicates a positive reaction.

5) No color change at 1 minute indicates a negative reaction. Record the time (seconds) for reaction up to 1 minute.

6) A positive presumptive test alone should not be interpreted as positive identification of blood; however, a negative result is proof of the absence of detectable quantities of blood using this method.

7) If subsequent DNA analysis will be performed on stains that tested positive, the analyst will remove a cutting of the stain of interest. If a significant amount of the stain is removed for testing, this should be documented.

5.4. Semen testing

Suspected semen stains may be tested with a presumptive chemical test ("AP spot test") for seminal fluid and/or confirmatory microscopic examination for spermatozoa. For color changing tests like the acid phosphatase (AP) chemical test results should be recorded in the following manner:

<u>Controls</u>: Note *color change @ time* and outcome (positive or negative).

<u>Evidence (positive reactions)</u>: Positive *@ time. (color change does not need to be noted as it is assumed based on control results)*

<u>Evidence (negative reactions)</u>: Test only needs to be noted as "Negative".

Additional information can be added but is not necessary.

5.4.1. Acid Phosphatase

This test relies upon sodium alpha-naphthyl phosphate being acted upon by the enzyme acid phosphatase to produce sodium phosphate and napthol. In a coupling reaction, this then combines with diazo blue B dye to give a pink/purple colored complex. It is recognized that the AP spot test is not highly specific and may yield false positive results from other body fluids such as vaginal secretions and fecal material, as well as bacteria, fungi, and many plants. Slow or weak/faint color reaction, or reactions of a different color, may not be seminal fluid.

Make AP reagent fresh daily as used.

SERI AP Spot Test: this is a premixed powder which contains the necessary chemicals for the reaction. Water is mixed with the powder and the prepared reagent is stable and sensitive for one day's use. Discard daily after use. Other vendor-supplied reagent may be used as appropriate. Dissolve 0.26 g AP Spot Test PMR (SERI) in 10 mL dH2O.

Method:

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 52 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

1) QC reagents with a blank control and semen stain control prior to testing evidence samples. A pink/purple color development at < 1 minute indicates a positive reaction. No color change at 1 minute indicates a negative reaction. Record the time (seconds) for reaction up to 1 minute. The time does not need to be recorded for negative reactions.
   a. If controls yield the anticipated results, proceed with testing evidence items.
   b. If the controls do not yield the anticipated results, a new lot of chemicals may need to be used/prepared and the Technical Leader should be consulted for possible troubleshooting.

2) To test:
   a. Moisten a piece of filter paper with sterile DI water and press on the suspected semen stain, or remove a small cutting and test directly. For AP mapping, use large or multiple filter papers (with orientation clearly marked) in order to appropriately test the entire area and press firmly to cover the entire area uniformly.
   b. Add a drop (or spray for mapping) of AP spot test reagent and observe for reactivity.

3) Results:
   a. A positive presumptive AP spot test alone should not be interpreted as positive identification of semen.
      Note: a strong, dark purple color in less than 30 seconds indicates a strong reaction for the presence of acid phosphatase. A light pink/purple color in the reaction time of 30 to 60 seconds indicates a weak reaction for the presence of acid phosphatase and could be due to a weak stain or a body fluid other than semen.
   b. A negative AP test does not necessarily mean that a stain does not contain semen or spermatozoa. This assay is dependent upon the amount of enzyme reactivity which is impacted by time and quantity. Thus, spermatozoa may be observed in stains which yield a negative AP spot test result. Typically tests yielding a negative AP spot test will warrant subsequent microscopic analysis (unless other case information justifies not proceeding with microscopic examination).

4) If subsequent DNA analysis will be performed, the analyst will remove a cutting of the stain/swab of interest.

5.5. Microscopy of sperm cell visualization

The presence of intact sperm cells displaying a head, midpiece and tail, or sperm heads showing an acrosomal cap identifies spermatozoa.

Materials:
SERI Christmas Tree Stain or other vendor-supplied reagent may be used as appropriate.
Kit contains:
   1. Solution A: red (Kernechtrot Solution)
   2. Solution B: green (Picroindigocarmine Solution)

Method for Preparation of Microscope Slides:
   1) Place an appropriate-sized cutting of sample in a sterile microcentrifuge tube.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 53 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

2) Suspend the sample in ~200 – 300 µL of sterile PBS or sterile DI water to cover the evidence cutting. If RSID saliva testing is to be done concurrently, PBS must be used.

3) Incubate for approximately 15 minutes at room temperature. Incubation may be increased to ~1 hour if RSID saliva testing is done concurrently. Cellular material may be loosened from the substrate by periodic agitation during and after incubation with a sterile toothpick, pipette tip, forceps, or vortexing for 15 - 30 seconds.

4) Piggy-back the substrate in a sterile spin basket and centrifuge for 2–5 minutes. Retain the substrate in a separate tube as needed.

5) Without disturbing the pellet, remove all but ~20 to 50 µL of the supernatant. The supernatant will typically be discarded; if the evidence has been consumed during preparation the analyst may choose to retain the supernatant labeled and placed with extracts.

6) Re-suspend the cell pellet (non-sperm cells and spermatozoa) in the remaining supernatant and remove a representative sample of the pellet (approximately 5 – 10%) to a microscope slide.

7) Fix the specimen on the slide (e.g. 60°C heat block, hot plate. or oven for ~30 minutes to 1 hour).

8) Cover the slide preparation with Solution A (nuclear fast red stain). Stain for 15 to 30 minutes. Wash Solution A off the slide with a gentle stream of dH2O.

9) Cover the slide preparation with Solution B (green stain). Stain for 15 to 30 seconds. Wash Solution B off the slide with a gentle stream of absolute ethanol.

10) Dry slide and mount by placing a drop of mounting medium over the stained area and carefully adding a cover slip avoiding bubbles.

11) Examine the slide for spermatozoa and epithelial cells at 400X. Photomicrographs may be taken at the analyst's discretion or at the direction of the reviewer.

12) Verification by second analyst must be done for all prepared slides, with the exception of those made as part of a proficiency test.

The following is a general guide to scoring sperm cells at 400X magnification. This guide is to aid the analyst in note-taking and possible decision-making regarding re-sampling. Sperm cell counts will not be reported in issued reports unless approved by the DNA Technical Leader.

Score	Cell Counts Observed and Comments
0	No cells observed
1	One sperm head observed.
+1	2 to 10 cells per slide
+2	$\geq$ 11 cells and $\leq$ to 50 cells per slide
+3	>51 cells per slide

Other structures may be observed on a microscope slide such as epithelial cells, yeast cells (uniform red stain with polyp-like structures), white blood cells (stain red-purple) and bacteria (Gram positive bacteria stain deep violet; Gram negative bacteria are not stained). These structures will not be recorded in case notes and will not be reported in issued laboratory reports. Only sperm counts will be recorded in notes.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 54 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

All microscope slides made for the visualization of sperm will need a second read to confirm results, with the exception of slides prepared as part of a proficiency test. Criminalists who perform the second read of a slide may not do the technical or administrative review of the case.

5.6. Saliva testing

The indication of possible saliva in a sample may provide additional information for evidence items examined within the Forensic Biology Unit. Evidence types commonly encountered which may contain saliva include bite marks, oral copulation samples, cigarette butts, cups, etc. Suspected saliva stains may be tested with the RSID-Saliva immunoassay test which is considered a presumptive test for saliva.

5.6.1. Rapid Stain Identification of Saliva (RSID-Saliva)

Materials are supplied by Independent Forensics, the manufacturer of the RSID - Saliva Test and contain:

1) Human saliva test cassettes, stored at room temperature
2) TBS+ running buffer, stored at 4°C

Method:

Note: a positive and negative control sample cassette must be included with each set of forensic samples assayed. If subsequent DNA analysis will be performed, the analyst will remove a cutting of the stain/swab of interest.

Forensic samples:

1) Add portions of swabs/cuttings to approximately 200-300 µL of PBS and incubate at room temperature for ~1 hour.
2) Pellet cells by piggy-backing substrate into spin basket and centrifuge for 2–5 minutes. Retain the substrate and supernatant as needed.
3) Take 20 µL of aqueous extract and add to 80 µL TBS+ running buffer for a total of 100 µL.
4) Add 100 µL prepared sample/control extract into the sample well on the test cassette.

Positive control sample (known saliva):

1) Add 50 µL of neat saliva to 1 mL PBS.
2) Take 20 µL of the extract and add to 80 µL of TBS+ running buffer for a total of 100 µL.
3) Add 100 µL prepared sample/control extract into the sample well on the test cassette.

Negative control sample (PBS only):

1) Add 20 µL PBS to 80 µL of TBS+ running buffer for a total of 100 µL.
2) Add 100 µL prepared sample/control extract into the sample well on the test cassette.

Time reaction for 10 minutes. Record results at 10 minutes using the following table as an interpretation guide. If the controls do not yield the anticipated results, a new cassette may need to be used and the DNA Technical Leader should be consulted for possible troubleshooting. Discard cassettes when finished.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 55 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Control (C) Position Activity	Test (T) Position Activity	Result
Visible red line	Visible red line	Positive
Visible red line	No red line	Negative
No red line	Visible red line	Failed test
No red line	No red line	Failed test
Light gray line	Light gray line	Possible non-specific reaction, no conclusion

6. DNA extraction

DNA extraction and purification separates the DNA molecules from other cellular material such as proteins which could interfere with the subsequent analysis of the DNA. Extracted DNA may contain PCR inhibitors following lysis. For bloodstain evidence this can be caused by red blood cell components, including heme compounds. A generally predictive indicator of potential PCR inhibitor(s) is discoloration of the DNA extract (e.g. with fabric dye). Inhibition can often be overcome by purification with the Qiagen EZ1 BioRobot procedure.

All DNA extraction procedures must follow outlined quality control practices.

1) Sample transfer steps will be witnessed as part of standard practice in the laboratory. Tube transfers will be witnessed by another authorized analyst in the laboratory, and dated and initialed in the appropriate location on the workbook forms.
2) Known samples will be sampled in duplicate, processed and reported.
3) Analysts should minimize the number of samples within a work area. e.g. if analyst has prepared multiple racks of samples, only the rack being actively worked should be inside the hood; the remaining racks should be placed outside the hood on a bench top.
4) A reagent blank contains no template DNA and is used to monitor contamination from extraction to DNA typing. The reagent blank is treated the same as, and parallel to, the casework sample being processed (i.e. extracted concurrently; amplified using same primers, instrument model and concentration conditions as required by the sample(s) containing the least amount of DNA; and typed utilizing the same instrument model, injection conditions and most sensitive volume conditions of the extraction set). The blank is typically located at the end of the sample order, and will contain all reagents added to the associated samples. Concentration conditions for the reagent blank (i.e. most sensitive concentration conditions) will be used accordingly within each extraction set.
5) Each extraction set shall have at least one associated reagent blank. When multiple reagent blanks are prepared, all will be quantitated but only one (the blank with the greatest signal strength upon quantitation) needs to be amplified. If, after quantitation, a sample requires additional processing (e.g. additional cleanup due to inhibition) the reagent blank associated with that extraction set will need to follow through the same process.
6) Nomenclature for reagent blanks: "RB" followed by date, initials and if multiple reagent blanks are prepared same person/same day a number or letter (e.g. RB010120AB1 or RB010120ABA).
7) The appropriate extraction worksheet template will be used to generate an extraction set of samples. There are templates for non-differential, differential, hair, and reference samples.

6.1. Non-differential evidence samples

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 56 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

For biological material being processed for possible semen, see the "differential" section below; otherwise, proceed as follows:

1) Prepare a lysis master mix, accordingly. To each sample add 500 µl G2 buffer, 10 µl Proteinase K, and 1 µl of carrier (cRNA). Mix gently.
2) Digest samples at 56°C for a minimum 2 hours. Digestion may be performed overnight, but should not exceed 24 hours.
3) Following digestion, agitate substrate and piggy-back to a clean, sterile spin basket. Spin sample on centrifuge at max speed for 2-5 minutes.
4) Spin basket containing substrate can be removed and discarded. If evidence item has been consumed, substrate may be saved for possible re-processing.
5) Proceed to automated DNA purification with the Qiagen EZ1-XL Biorobot.

6.2. Differential evidence samples

The intent of the differential extraction is to preferentially lyse epithelial cells (non-sperm fraction) prior to sperm cells (sperm fraction) with a set of chemicals, producing separate fractions for each cell type. A clear and distinct separation of DNA is not always achieved; the term sperm fraction is not conclusive to spermatozoa cells being present which would be confirmed through microscopy. The Forensic Biology Unit utilizes the QIAcube robots for automated differential extractions.

6.2.1. QIAcube Connect

Preparation of QIAcube Connect Instrument

1) Open the Samples for Extraction workbook and select the sheet labeled "Differentials". Copy and paste (do not cut and paste) differential samples to be extracted into the QIAcube loading sheet master underneath "Samples". Once successfully copied, delete the transferred samples from the Samples for Extraction workbook.
2) Complete the rest of the worksheet by adding in a reagent blank (non-sperm and sperm fractions) to the samples list, and selecting the instrument and reagents to be used from the drop-down menus to the left. Save the worksheet.
3) Items to remember:
   - One reagent blank must be used per QIAcube.
   - Samples must be ordered with the NS fraction followed by the SP fraction.
   - 2 or 22 samples (1 or 11 differentials) cannot be run.
   - Verify all reagents and equipment selected at throughout the extraction. Make corrections as needed by hand.

   Tube Pull Verification step: Check sample names on tubes against the QIAcube loading sheet. Samples will not have "SP" on the tube labels at this time.
4) Prepare the First (non-sperm) Master Mix as designated on the QIAcube loading sheet. The Master Mix has 500uL G2 Digest Buffer, 10uL ProK, and 1ul cRNA for each sample. Mix gently after preparing.
5) Add to 511uL of Master Mix to each sample and then digest samples at 56°C for 45 minutes to one hour.
6) Following digestion, agitate substrate and piggy-back to a clean, sterile spin basket. Spin sample on centrifuge at max speed for 2-5 minutes.
7) Remove and discard the spin basket containing substrate. Close the tubes. In some instances the originating evidence item has been consumed and the substrate may be saved for possible re-processing.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 57 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

8) Prepare the Second (sperm) Master Mix as designated on the QIAcube loading sheet. The Master Mix should be prepared in an EZ1 2ml flat-bottomed screw cap tube. The Master Mix has 137uL G2 Digest Buffer, 2.7uL ProK, 5.5uL DTT and 0.27ul cRNA for each sample. Mix gently after preparing.

9) Prepare labeled EZ1 2ml flat-bottomed screw-cap tubes for each non-sperm fraction. Place labels on flat area of tubes (below ridges) as tube height is critical in the instrument.

10) Bring your digested samples, prepared non-sperm fractions, prepared MasterMix, and QIAcube loading sheet to the QIAcube Connect instrument to prepare to load.

QIAcube Connect Instrument Run Protocol

1) Press the grey power button to turn on the QIACube connect instrument.

2) Log on to the instrument.
   a. Username: SFPD (all caps)
   b. Password: dnaanalyst (all lowercase)

3) On the touch screen, select DNA > Pipetting > Epithelial & Sperm Cells > Separation & Lysis 12

4) Load non-sperm fraction tubes into the shaker according to the placement detailed on the QIAcube loading sheet.
   a. Make sure there is a shaker plug next to each non-sperm fraction present. Remove shaker plugs from spaces where no tube is present. Shaker plugs are what the QIAcube Connect uses to determine sample number.

5) Label the tops of the sperm fraction tubes with the position number according to the placement detailed on the QIAcube loading sheet. Load the sperm fraction tubes into rotor adaptors and place the tube/rotor adaptor unit into the correct position in the centrifuge.

6) Load the prepared Second (sperm) Master Mix into Position A.

7) Fill the reagent bottle with PBS to the line and place in Position 1. Do not overfill.
   Notes:
   • The QIAcube Connect touch screen prompts say to fill the reagent bottle with G2. The SFPD procedures have been validated with PBS, do not use G2.
   • After discarding the insert, the tube caps are loose. Push up or down on the tube cap hinge to create tension on the seal and help ensure the cap remains closed.
   • Bubbles may form when discarding the insert. A quick spin can be useful to remove bubbles.

8) Fill all three tip racks with grey, 1000uL WIDE BORE pipette tips and proceed with the verification.
   QIAcube Deck Verification step:
   1. Check labels, position number on tube cap, and placement of sperm fractions against worksheet.
   2. Check labels and placement of non-sperm fractions against worksheet.
   3. Check the placement of reagents and that the tips are filled.
      Once the verification is complete, the analyst will uncap the non-sperm fractions, sperm fractions, and reagent tubes. For the sperm fractions, the analyst will, one at a time, open the tube, flip the lid back into the longer of the two rotor adapter spots, and then return the rotor adapter to the correct position in the centrifuge. See photo.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 58 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

9) Uncap the non-sperm fraction tubes, the Master Mix tube, and the reagent bottle.
   a. Tubes must be pushed down and fully seated prior to starting the run or pipetting may be disrupted. Labels on tubes should be placed with care so as not to interfere with proper seating.
10) The touch screen will run through a series of loading prompts regarding samples, reagents and tips. Once complete, Press "Start" to begin.
11) Stay with the QIAcube Connect until the loading check is complete. Run times vary according to sample number.
12) Once the run is complete:
   a. Remove and recap the non-sperm samples
   b. Remove the sperm fraction tubes from the rotor adaptor and recap the samples.
   c. Refill and recap the reagent bottle.
   d. Discard the Master Mix tube.
   e. Empty the waste drawer.
   f. Clean deck with bleach followed by ethanol. Clean waste drawer with ethanol followed by bleach.
   g. Turn off instrument.
13) Place the sperm fraction samples on the thermomixer at 56°C for 10 min at a moderate speed; recommended speed setting is "850rpm". Spin the tubes briefly. The non-sperm samples do not need additional digest post Qiacube run.
14) Prepare labeled EZ1 2ml flat-bottomed screw-cap tubes for each sperm fraction.
   SP transfer Verification step: Check sample names on tubes against corresponding EZ1 flat bottomed screw cap tubes prior to transfer. Initial sample tubes will still not have "SP" on the tube labels.
15) Transfer sperm fractions into corresponding EZ1 2ML flat bottomed screw cap tubes.
16) Proceed with the protocol for Automated DNA Purification with the Qiagen Biorobot (EZ1-XL).

QIAcube Connect Error prompts
- If there is an error prompt during load check: Wait until the instrument quiets, then open the door. Address what the error is, but do not touch any other item on the deck. The load check will resume from where it paused, it will not restart unless the protocol is exited and re-started.
- If there is an error prompt at any other time during the run, there is no option to address the problem and continue. Document the error prompt – both number and description - and consult a Supervisor.

Daily Maintenance (after each use):
- Remove and discard all used disposables from deck.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 59 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

- Recap lid of reagent bottle securely.
- Wipe down platform and shaker plugs with bleach followed by ethanol. Do not wipe door of QIAcube Connect.
- Empty waste and wipe down waste drawer.

6.3. Hair and bone samples

1) Prepare a lysis master mix, accordingly. To each sample add 500 µl G2 buffer, 20 µl dithiothreitol (DTT), 10 µl Proteinase K and 1 µl cRNA. Mix gently.
2) Digest samples at 56°C overnight. Additional aliquots of ProK and cRNA may be added after 2-4 hours of incubation time.
3) Following digestion, no piggy-back spin step is required. The insoluble material from bone and teeth should be spun down and removed and retained.
4) Proceed to automated DNA purification with the Qiagen EZ1-XL Biorobot.

6.4. Reference samples

1) Prepare a lysis master mix, accordingly. To each sample add 500 µl G2 buffer and 10 µl Proteinase K. Mix gently.
2) Digest samples at 56°C for a minimum of 1 hour. Digestion may be performed overnight, but should not exceed 24 hours.
3) Following digestion, no piggy-back spin step is required.
4) Reference substrates can be discarded unless item has been consumed.
5) Proceed to automated DNA purification with the Qiagen EZ1-XL Biorobot.

6.5. Automated DNA purification with Qiagen EZ1-XL Biorobot

Automated DNA purification using the BioRobot is used on evidence and reference samples. Nucleic acid purification is achieved through magnetic particle technology. Nucleic acids from sample lysates are isolated by binding the DNA to the silica surface of magnetic particles under proper pH conditions. The carrier RNA is added to enhance the binding of DNA to the silica surface of the magnetic particles. While bound to the magnetically-coated silica beads, the nucleic acids are able to be purified with various chemical buffers. The EZ1-XL Advanced BioRobot holds up to 14 samples in a single run.

The Qiagen DNA Investigator Kit is compatible for automated nucleic acid purification on the Qiagen BioRobot. The following procedure is intended for use with the DNA Investigator Kit with the Qiagen EZ1-XL instruments.

CAUTION: The pre-filled reagent cartridges contain ethanol, guanidine hydrochloride and guanidine thiocyanate, which can form highly reactive compounds when combined with bleach and are harmful irritants. Wear chemical resistant gloves, lab coat and other appropriate protective gear when handling the reagent cartridges.

All substrates should be removed at this point. A single "extraction set" must have the same lot numbers for the extraction reagents; if this is the case, the samples may be run on separate robots and still be covered by one associated reagent blank(s) prepared at the same time as the samples.

1) Turn the instrument on.
2) If a weekly reminder appears, Press 1: Done.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 60 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

3) Press START to display the PROTOCOLS menu.
4) Press ESC: NO to create report file.
5) Select the "Large Volume" protocol.
6) Select TE buffer as elution buffer and select the appropriate elution volume from the menu:
   a. When low quantity evidentiary DNA samples are processed, the elution volume will be 40 µL.
   b. For known reference samples and high quantity evidentiary samples, the elution volume will be 200 µL.
   c. Press any key to proceed through the text that is displayed on the screen.
7) Open the workstation door and remove the tip and cartridge racks.
8) Invert the appropriate number of reagent cartridges several times to mix, and tap the cartridges to deposit the reagents to the bottom of their wells. Do not shake cartridges. Ensure the liquid is at the bottom of the cartridge with minimal bubbles; this can help prevent low-elution volume issues.
9) Insert the reagent cartridges in the cartridge rack and load the rack onto the BioRobot. Ensure that the reagent cartridges are seated properly in the cartridge rack (a click should be heard when properly placed) and the samples are in the correct positions.
10) Place the empty tip rack back on the instrument and load the appropriate number of tip holders containing filter tips in the second row of the tip rack.
11) Add 400 µL Qiagen MTL buffer to each labeled BioRobot sample tube (flat bottom tubes). Transfer the 500 µL volume of lysed and digested cells to the labeled tubes containing the MTL buffer.

Verification step: Tube order prior to the transfer from sample tubes to EZ1 processing tubes must be verified by a qualified analyst.

12) Load the uncapped labeled sample tubes into the back (fourth) row of the tip rack.
13) Load the appropriate number of empty, uncapped labeled elution tubes (tapered bottom tubes) into the first row of the tip rack (the third row should be left empty).

Verification step: Tube placement for EZ1 processing must be verified by a qualified analyst.

14) Close the workstation door and press "START" to begin the purification protocol. When the instrument has stopped (approximately 15-20 minutes), open the workstation door. Remove the elution tubes for long-term storage. If final elution volumes fall below selected elution volume (<40 µL or <200 µL), TE may be added to bring volume up. Notify a Supervisor in case service is needed. Close the workstation door and turn off the instrument. Store samples at 2 to 8°C or freeze until ready to quantitate.
15) Upon completion of a run, a screen will display asking if you want to perform the UV RUN. This should be completed weekly; the analyst has the option to press ESC and NO if the UV RUN will not be completed at this time.
16) Perform "Piercing Unit Clean" (PUC) step and discard contents of well #4 from DNA Investigator cartridge in biohazard waste, then discard used cartridges, tips, and tip holders. Clean using ethanol. Fill out the EZ1 Component Usage Log sheet located near the instrument.

7. DNA quantitation

The presence or absence of detectable human DNA must be assessed prior to amplification with regard to the unknown evidence samples. The Quantifiler Trio amplification kit quantitates total amplifiable human (large and small target) and male (higher primate) DNA using quantitative

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 61 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

fluorogenic 5' nuclease polymerase chain reaction (qPCR). qPCR is PCR performed in a thermal cycler configured to measure the amount of PCR product in "real time" as it increases with each cycle.

7.1.   Quantifiler Trio

Real-time PCR products are detected once per cycle using the HID Real-Time PCR Analysis software v1.2. The amount of fluorescent signal detected is directly related to the target sequence copy number. With each cycle, the fluorescent signal increases until it crosses a threshold ("cycle threshold" or "CT value"). A sample with a higher starting template copy number (more DNA) reaches the threshold earlier than a sample with lower starting template copy number (less DNA). By plotting the CT values of known standards against the log of their DNA concentrations ("Log CO") the quantity of the unknown samples can be extrapolated by the comparison of fluorescent activity of each unknown sample to the known human DNA standard curve (i.e. 50 ng/μL - 5 pg/μL).

Quantifiler Trio Targets	Amplicon length	Ploidy	Copy Number	Dye/Quencher
Human Target, small autosomal	80 bases	Diploid	multicopy	VIC® dye with MGB quencher
Human Target, large autosomal	214 bases	Diploid	multicopy	ABY® dye with QSY® quencher
Human Male Target	75 bases	Haploid	multicopy	FAM™ dye with MGB quencher
Internal PCR Control	130 bases	N/A	Synthetic IPC template is included in the primer mix	JUN® dye with QSY® quencher

Sample	Detector	Reporter	Quencher	Task	Quantity
DNA Standard	Quantifiler Trio Large Autosomal	ABY	QSY	Standard	50†
	Quantifiler Trio Small Autosomal	VIC	MGB	Standard	50†
	Quantifiler Trio Male	FAM	MGB	Standard	50†
	IPC Quantifiler Trio	JUN	QSY	Unknown	NA
Unknown	Quantifiler Trio Large Autosomal	ABY	QSY	Unknown	NA
	Quantifiler Trio Small Autosomal	VIC	MGB	Unknown	NA
	Quantifiler Trio Male	FAM	MGB	Unknown	NA
	IPC Quantifiler Trio	JUN	QSY	Unknown	NA

† = a different numerical value will be displayed depending on the DNA standard (i.e. 50 ng/μL - 5 pg/μL)

DNA Standard Concentrations:

DNA Standard	Concentration (ng/μL)
Std 1	50.0
Std 2	5.0

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 62 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

Std 3	0.5
Std 4	0.05
Std 5	0.005

7.2. Tecan workbook and quantitation setup for Quantifiler Trio

1) Open "TECAN Workbook Master" file.
2) Make sure Macros are enabled.
3) Immediately resave macro enabled workbook with new run name as follows: Date/Initials/RunNumber (Ex: 100215MP1). This run name will be maintained throughout the entire process with additions for Quant (Q) and Amp (A) when needed.
4) Copy and paste the extraction samples into "Paste in Extraction samples" tab. DO NOT skip any spaces on the log sheet or use spaces in sample names (underscores only). Ensure that no two samples have the same sample name. Save workbook when complete.
5) Move to "Tecan Quant Tube Order" tab and delete any 0 leftover. Choose reagent lot numbers from drop down menus. Print sheet for casefile and have order/sample names verified by another analyst. Save workbook when complete.
6) On "Tecan Quant Sample File" tab, delete all "delete row" sample rows by highlighting rows, right clicking and choosing delete. Save workbook when complete.
7) Save the file on this tab as a .csv file to the S drive (S:\Tecan Workflow\Quant CSV sample files). Name the file as follows: Q/Date/Initials/RunNumber (Ex: Q100215MP1). Once save as has been clicked, click ok on the first prompt, and then yes on the second prompt.
8) The CSV file is now created. Close out of Excel. When prompted do not save the workbook in CSV format.
9) Move the created Quant CSV file from the S drive to the "Sample files" folder on the Tecan computer desktop.
10) Connect system liquid and waste.
11) Log on to Evoware. Log-ins can be found on desktop of Tecan computers.
12) Ensure syringes are finger tight and carriers are all the way in.
13) Choose "DailyFlush" in maintenance section, hit start and then run. Daily flush needs to be performed at least once per day, before the first run; if flush has been performed, then you may skip and proceed to casework script. Multiple flushes may need to be performed to remove all air bubbles.
14) After flush is complete, click cancel.
15) Choose "Run existing script", then start.
16) Choose "QuantTrio_SFPD" script, click start, then run.
17) Follow prompts. Note this script has prompts to either make new standards or use existing standards. Unless quality issues requires new standards be prepared, they should be depleted prior to making new due to the limited amount of THP DNA Standard. The prepared standards may be stored for up to 2 weeks at 2 to 8°C.
18) Select sample file (.csv) from the desktop.
19) Confirm sample file is correct and number of samples is correct.
20) Choose fresh standards or existing standards.
21) Prepare master mix as directed by the TECAN in 5 mL tube (note: TECAN will add overages of primer and reaction mix to ensure enough reagent for the reaction) and

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 63 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

vortex gently. Mixing by pipetting can also be done. Make sure there are not excessive bubbles in the tube and no master mix is stuck to the lid before placing on the deck.

22) Place sample tubes as per sample file (tubes load on rack from back to front).

23) Place standards and reagents in correct positions. Make sure there is at least 200 μL in the dilution buffer tube.

24) Check system liquid, waste and tips.

25) Place a fresh, labeled plate in the "Reaction plate" spot on the deck.

26) If you refill tips, hit yes to reset diti (reset where tips start). If continuing a previous run, the Tecan will begin from the last tip used on that run. It will continue down to the next rack when one is empty.

    a.   Note starting grid (2 for left column of tips or 8 for right column of tips)
    b.   Set rack set (1 for back, 2 for middle or 3 for front)
    c.   Set tip number (A1 =1, H12 = 96)

Verification step: Sample order and Tecan deck must be verified for proper setup by a qualified analyst.

27) Start run. Each reaction contains 2 ul of sample, 8 μL of Primer/Probe mix and 10 μL of PCR Reaction mix.

28) After all samples and standards have been aliquoted by the Tecan in the 96-well reaction plate, place an optical adhesive cover over the plate and seal with a rubber spatula. Avoid touching the optical cover and plate wells. Spin the 96-well plate in a centrifuge to remove bubbles and ensure all liquid is at the bottom.

If the same analyst will be performing the amplification on the same Tecan, tubes may remain uncapped pending amplification so long as there are no tube changes.

29) Fill out the Tecan Evo 150 Usage Log sheet located near the instrument.

30) 7500 export file is stored in 7500 setup file folder on Tecan computer. Transfer 7500 setup file from Tecan computer to S drive (S/Tecan Workflow/7500 Setup). Continue to PCR room for 7500 run.

7.3.   7500 Real-time PCR system

1)   In the post-amp laboratory, turn on (or wake from sleep) the laptop connected to the 7500 instrument. Turn on the 7500 instrument and allow it to finish its starting cycle (steady green power light) before launching the 7500 HID software v 1.2. Log in as "guest".

2)   From Home Screen (or File menu) choose experiment to run. Using the HID software, confirm the following are correct: 7500 (96 wells), Quantitation (HID standard curve) and Taqman reagents.

3)   After the template is launched, select Import and Browse sample setup from the File menu. Go to the folder named "Tecan Workflow/7500 Setup Files" and highlight the file to import. Type in the run name in the Experiment Name line: Q[date][analyst initials]. Click on the Plate Setup button and Assign Targets and Samples tab. Verify the plate map is correct and targets have been assigned to all standards and samples.
Note: the Quant run name is normally the date the quant is performed and can serve as the date requirement for the page. If the run name is not the same as the date performed, the true run date must be noted on the note page**.**

4)   When the template has been successfully imported, verify that the thermal cycler parameters in Run Method are as follows for Quantifiler Trio; the cycling stage has 40 cycles:

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 64 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

Holding Stage: 95C for 2 min
Cycling Stage 1:   95C for 9 sec
Cycling Stage 2:   60C for 30 sec

5) Open the door of the 7500 instrument by gently pushing on the depression on the front of the instrument. Place the plate in the 7500 ensuring the orientation is correct (notched corner of plate in upper right corner of tray). Close tray by gently pushing on the depression on the front.

6) In the top right of the software screen, click green "Start Run" box. A dialog box opens and asks you to save. Click "save" and a "Run status" window will open displaying a status of "running". Once the instrument is running the orange "in use" light will flash.

7) Look at the QC summary for any flags. Results can be viewed under the Results Tab. The Plate tab will display the quantitation results and can be used to omit wells by double clicking on the well and selecting "Omit Well". The other tabs (Spectra, Component, Amplification Plot and Standard Curve) provide additional data that may be useful in troubleshooting background or amplification problems.

8) The Component tab displays the response to each detector during the course of the run. The detectors displayed are as follows for Quantifiler Trio:

Mustang Purple as passive reference
JUN for IPC
ABY for Large Autosomal
VIC for Small Autosomal
FAM for male DNA

9) Check the Standard Curve by selecting Small Autosomal, Large Autosomal or Male in the Detector drop-down list to evaluate the quality of the results according to the values below. If the values fall outside these ranges, the analyst will decide whether the plate should be re-prepared and re-quantitated (if sufficient material remains), or appropriate adjustments made to subsequent amplification input amounts:

R^2 value: this measures the closeness of fit between the standard curve regression line and the individual CT data points of the standards. A value of 1.00 indicates a perfect fit; for analysis, the R^2 value must be > 0.98. If the R^2 value falls outside this range, examine the curve points to see if one of the standards should be omitted as outliers could result from pipetting errors, etc.

If more than one well needs to be removed for the slope to fall within the acceptable ranges, a DNA Supervisor will be consulted prior to continuing.

Slope: this indicates the PCR amplification efficiency for the assay. A slope close to - 3.3 indicates optimal efficiency. The range and average of standard-curve slope values should be:

Small Autosomal (SA):   typical slope range - 3.0 to - 3.6 (average -3.3)
Large Autosomal (LA):   typical slope range - 3.1 to - 3.7 (average -3.4)
Y target: typical slope range - 3.0 to - 3.6 (average -3.3)

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 65 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

10) If the slope falls outside these ranges, examine the curve points to see if one of the standards should be omitted as outliers could result from pipetting errors, etc. When viewing results in the "view plate layout" view, right click on the well to omit. Select "well" or specific target within well (if metrics passed for other targets within well). Data will need to be reanalyzed.

If more than one well needs to be removed for the slope to fall within the acceptable ranges, a DNA Supervisor should be consulted prior to continuing.

Print the standard curves for the SA target, LA target and Y target showing the R^2 and slopes for the casefile. Only the curves for standards need to be displayed. This is done by highlighting only the standards before printing.

11) Save the results to the Quantifiler Data folder and save the results folder using the following naming convention: Q[date][analyst initials].eds. Export the results in a .txt file to "Tecan Workflow/ 7500 Results".

12) On "Tecan Amp Tube Order" worksheet, make sure Quant Controls says "passed". If it is red and says "check controls", see a DNA unit supervisor or Technical Leader.
13) On "Tecan Amp Tube Order" worksheet, fill in the number of dilution plates used (0, 1, 2)
14) On "3500xL Amp Plate Map" worksheet, have a second criminalist check the controls for the run and verify they are as expected.

7.4. Reading and interpreting quantitation results

If the value "#######" is displayed on the results page, the table column needs to be expanded to display the numerical results.

Negative Control: Large autosomal, small autosomal, and Y target must have a quantification below 0.0002ng/ul

Large Autosomal:
  Slope: must fall between -3.1 to -3.7
  Y-Intercept: must be greater than 24.428
  R^2: must be greater than 0.98

Small Autosomal:
  Slope: must fall between -3.0 to -3.6
  Y-Intercept: must be greater than 25.498
  R^2: must be greater than 0.98

Y:
  Slope: must fall between -3.0 to -3.6
  Y-Intercept: must be greater than 25.053
  R^2: must be greater than 0.98

7.4.1. Positive results

Positive amplification occurs when the CT value for the target is <40. Because samples contain unknown amounts of DNA and inhibitors, a large range of CT values is

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 66 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

possible. The IPC system template DNA is present at a consistent concentration across all reactions on a plate. Therefore, the IPC (JUN dye) CT should be relatively constant in typical reactions. However, the presence of PCR inhibitors and/or higher concentrations of DNA can increase the IPC CT relative to the average IPC CT of the quantification standards on the same plate.

7.4.2. Negative results
No human DNA is detected when:
1) No signal for the Small Autosomal, Large Autosomal and Y targets (VIC, ABY and FAM dyes, respectively) is detected, indicating that the human and/or male-specific targets did not amplify.
2) The IPC target (JUN dye) amplifies and amplification does not appear reduced relative to the average IPC CT value for quantification standards.

7.4.3. Complete amplification failure
Undetected results for all assay targets, including human and male-specific targets and the IPC target, indicates a complete failure of PCR amplification for the reaction. This could be caused by conditions such as incorrect thermal cycling or incorrect formulation of PCR reagent mix (which would affect multiple reactions or possibly the entire plate), or by severe PCR inhibition affecting individual samples. It will be indicated as "UNDETER" in the TECAN workbook. This type of result is invalid and a DNA Supervisor will need to consider the best approach for the sample(s).

7.4.4. PCR inhibition
No amplification or weak amplification of the IPC may indicate PCR inhibition (partial or complete) in the sample. In addition, suppressed amplification of the human and/or male-specific targets can occur due to PCR inhibition. This is typically more pronounced in the large autosomal target than the small autosomal target since the large autosomal target is more susceptible to inhibitory effects.

7.4.5. Inconclusive IPC results
With increasing concentrations of human genomic DNA (>5 ng/µL), competition between the human and/or male-specific and IPC PCR reactions may suppress IPC amplification for that sample. This can occur independently or in combination with the effect of PCR inhibitors, yielding inconclusive results. However, samples with high DNA concentration will be diluted during STR reaction setup to meet the optimal target input amount of DNA in the STR reaction. Therefore, the effect of most inhibitors, if present in the sample, on STR kit performance is likely to be minimized.

7.4.6. Termination at quantitation
Termination thresholds may be applied by the lab using the following criteria.

7.4.6.1. Termination limit
Overall rules:
1. Sample name starts with "RB", no termination
2. Sample name starts with "CSI", no termination

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 67 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

In-house validation data demonstrated the following concentrations yielded profiles deemed too low for interpretation (i.e. generally 4 or less alleles in a profile). Serology samples get labeled according to sample type with "O" (orifice) determining what samples are terminated at quant. Evidence samples at these levels will not proceed with STR analysis.

Termination values:
0.00024 ng/µL for small autosomal value
0.00025 ng/µL for male quant value

7.4.6.2.   Male to female ratio
The Male DNA to Female DNA Ratio is automatically calculated by Tecan Workbook using the following formula and can serve as an indication of male DNA to female DNA ratio and potential profiling success (or consideration for Y-STR testing) or can help the analyst decide to target more DNA for amplification:

Male DNA: Female DNA Ratio = Quantity of Male DNA/(Quantity of Human DNA - Quantity of Male DNA)

In-house validation data demonstrated that generally no more than 3 unshared alleles from the male profile were obtained in a **male: female ratio at or below 1:100**. Therefore, evidence samples with male: female ratios at or below 1:100 will not proceed to STR analysis. Even if the foreign DNA is female in an orifice swab, the non-sperm fraction of an orifice swab is unlikely to yield interpretable results due to being overwhelmed by DNA belonging to the individual.

Non-Differential samples
1. If small human (0.0002ng/uL) **AND** male (0.0003ng/uL) quant values are below stated threshold (terminate threshold)

Differential samples
OSP and SP Fraction (applies to both orifice and non-orifice samples):
1. If small human (0.0002ng/uL) **AND** male (0.0003ng/uL) quant values are below stated threshold (terminate threshold)
2. If small human/male is greater than 100 (terminate ratio)
Note: if male is 0.0000 ng/ul, then small human must be greater than 0.0100 ng/ul (thus becoming greater than 100 for small human/male)

NS Fraction (non-orifice samples):
1. If small human (0.0002ng/uL) **AND** male (0.0003ng/uL) quant values are below stated threshold (terminate threshold)

ONS Fraction (orifice samples):
1. If small human (0.0002ng/uL) **AND** male (0.0003ng/uL) quant values are below stated threshold (terminate threshold)
2. If small human/male is greater than 100 (terminate ratio)
Note: if male is 0.0000 ng/ul, then small human must be greater than 0.0100 ng/ul (thus becoming greater than 100 for small human/male)

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 68 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

7.4.7. Degradation Index

The Degradation Index is automatically calculated by Tecan Workbook using the following formula and can serve as an indication of level of degradation to help guide troubleshooting decisions on amplification input amount; the small autosomal (SA) target is the primary quantification target for total human genomic DNA and the large autosomal (LA) target is used mainly as an indicator of DNA degradation:

SA target DNA (ng/µL)/LA target DNA (ng/µL)

<1: Typically indicates that DNA is not degraded or inhibited.

1 to 10: Typically indicates that DNA is slightly to moderately degraded. PCR inhibition is also possible, however not enough to significantly suppress IPC amplification. Analyst may choose a target amplification amount closer to 1.0 ng (versus 0.5) to maximize allele recovery

>10 or blank: Typically indicates that DNA is significantly degraded. PCR inhibition is also possible, however not enough to significantly suppress IPC amplification.

8. DNA amplification

8.1. Globalfiler

The Forensic Biology Unit uses the Globalfiler amplification kit. The Globalfiler kit has 24 total loci, which includes the 20 required CODIS loci, 3 highly recommended loci, and a male-specific Y insertion/deletion marker, per the CODIS Core Loci Working Group's recommendation. The thermal cycling program used with Globalfiler has 29 PCR cycles.

The target range for amplification for samples run using the 3500xl is approximately **0.125 to 0.5 ng** of human template DNA with an ideal target o**f 0.4 ng**; amplification of less or more DNA template may also yield interpretable results. Because of slight underestimations of DNA concentrations approaching the highest point on the standard curve in Quantifiler Trio, targeting **0.2 to 0.4 ng** of template may help reduce the observation of pull-up for high quantity samples such as reference samples. The target input amount should be adjusted based on the outcome of the quantitation results and knowledge of the sample.

If a sample is expected to have a mixture, more DNA may be targeted in attempts to obtain data for all possible contributors; however, this may not always be known from the outset of testing. If the sample contains **degraded DNA**, amplification of a higher concentration may be beneficial. If the sample contains **inhibited DNA**, amplification of a lower concentration may be beneficial. Samples with a template DNA input too high (> 2ng) may exhibit instrument saturation and increased artifacts. Samples with a template DNA input too low (<0.1ng) may exhibit imbalances of alleles and loci, allele drop out and allele drop in.

Positive and **negative** amplification **controls** are introduced at the amplification step to monitor the success of the reaction. These controls are amplified concurrently on the same instrument with the evidence samples, with the same primers (at all loci), and carried through the remainder of the typing process. Prepare fresh master mix daily and do not

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 69 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

save and re-use. The 9700 and Proflex thermal cyclers are used by the Forensic Biology Unit for amplification.

Guidelines for DNA input are as follows:

Type	Add to PCR reaction
DNA Sample	Add 0.125 to 0.5 ng total DNA to reaction
Reagent blank	Same volume as the largest DNA sample volume (to mimic the lowest concentration sample).
DNA Control 007 (positive amplification control)	Typically add 0.4 ng total DNA to reaction. May adjust, accordingly. Contains approximately 0.1ng/ul per manufacturer but may vary slightly in concentration.
Negative amplification control	Add 15 µL TE buffer

8.2.   Tecan amplification setup
8.2.1.   Workbook setup

1) Open saved workbook and make sure macros are enabled.
2) On "Quant Results" tab, click on well A1 so that it is chosen. Click the "Import Quant Results" button and navigate to your text file. Import the file and then click "Delete Blank Rows Results".
3) Go to the "Amp Set-up" tab. The results from the quant should be populated. Delete any extra blank sample rows. If desired, change target value and volumes will be recalculated. If target is changed for all samples in the amp then the target will be updated on the 'Tecan Amp Sample File' tab. If less than all the sample target values are updated then the target values must be updated on the 'Tecan Amp Sample File' tab as well. Save workbook and print this sheet for the casefile.
4) On the "Tecan Amp Tube Order" tab, check to ensure "Quant Controls" reads "Passed". If it is red and reads "Check Controls", see a supervisor. Choose reagent lot numbers from drop down menus and print this sheet for the casefile and tube order verification. Save workbook and print this sheet for the casefile.
5) On "Tecan Amp Sample File" tab, delete all rows marked as "delete rows" by highlighting rows, right clicking and choosing delete. Update target values where required. Save workbook when complete.
6) If you are not amping a quantitated sample then add _DNU to end of name in the sample file but make sure you retain the space in the tube racks. Save workbook when complete. Conversely, if there are samples you want amplified that are terminated, go to the worksheet titled "Tecan Amp Sample File" and find the sample you are interested in, then remove the "_DNU" after the sample name. On the "Amp Setup" worksheet, delete the contents of the cell under the column "Terminate" for that same sample.
7) Save the file on this tab as a .csv file to the S drive (S:\Tecan Workflow\Amp CSV sample files). Name the file as follows: A/Date/Initials/RunNumber (Ex: A100215MP1). Once save as has been clicked, click ok on the first prompt and then yes on the second prompt.
8) The CSV file is now created. Close out of Excel. When prompted do not save the workbook in CSV format.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 70 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

8.2.2. Tecan Globalfiler amplification procedure

1) Move the quant results text file to the "7500 results file" folder and the created amp CSV file from S/Tecan Workflow/Amp csv sample files to the "Sample files" folder on the Tecan computer desktop.
2) Connect system liquid and waste.
3) Log on to Evoware. Log-ins are saved on desktop of Tecan computers.
4) Choose "DailyFlush" in maintenance section, hit start and then run. Daily flush needs to be performed at least once per day, before the first run; if flush has been performed, then you may skip and proceed to casework script. Multiple flushes may need to be performed to remove all air bubbles.
5) After flush is complete, click cancel.
6) Choose "Run an existing script", then start.
7) Choose "3500_GlobalFiler_SFPD" script, click start, then run.
8) Load sample file and results file.
9) Confirm number of samples and file name.
10) Load dilution plates as directed in the appropriate spots on the deck. Mark number of dilution plates used on "Tecan Amp Tube Order" worksheet. Note: Samples over 500 quant will require a manual dilution.
11) Load TE-4 in trough at TE position on deck.
12) Prepare Master Mix in 5 mL tube as directed by the screen prompts. Mix by pipetting or under medium power on vortex. Do not vortex primers. Note: TECAN will add overages of primer and reaction mix to ensure enough reagent for the reaction. The TECAN script will prepare a total of 25 µl per sample: 15 µl of sample (or TE) is added to 7.5 µl of PCR Reaction Mix and 2.5 µl of GlobalFiler Primer Mix. Make sure there are not excessive bubbles in the tube and no master mix is stuck to the lid before placing on the deck.
13) Place sample tubes as per sample file (tubes load on rack from back to front).
14) Place standards and reagents in correct positions.
15) Check system liquid, waste and tips.
16) Place a fresh and labeled plate in the "Reaction plate" spot on the deck.
17) If you refill tips then hit yes to reset diti (reset where tips start). If continuing a previous run, the Tecan will begin from the last tip used on that run. It will continue down to the next rack when one is empty.
    a. Note starting grid (2 for left column of tips or 8 for right column of tips)
    b. Set rack set (1 for back, 2 for middle or 3 for front)
    c. Set tip number (A1 =1, H12 = 96)

Verification step: Sample order and Tecan deck must be verified for proper setup by a qualified analyst.

18) Start run.

Following completion of TECAN run:

19) Cover dilution plate with foil film cover. Transfer 3500xL file to S drive (S:\Tecan Workflow\3500xL set-up files).
20) Fill out the Tecan Evo 150 Usage Log sheet located near the instrument.
21) Cap or seal all columns containing sample, carry the PCR samples into the post-PCR room.
22) Spin the 96-well plate in a centrifuge to remove bubbles and ensure all liquid is at the bottom. Place the samples securely into the thermal cycler block and close the lid.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 71 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

23) Start the appropriate program on the thermal cycler for GlobalFiler:

Initial incubation (enzyme activation): 95°C for 1 min

29 cycles,
Denature: 94°C, 10 sec
Anneal/extend: 59°C, 90 sec
Final extension: 10 minutes at 60°C
After PCR, may 'soak' at ~4°C

Verify run has started; if it has not, notify a Supervisor for troubleshooting.

Upon completion of the amplification process, store the PCR samples in the post-PCR room refrigerator or freezer until ready for fluorescent detection.

8.2.3. Generate amp/CE plate map

Open saved workbook and make sure macros are enabled. Open "Tecan Plate Map Import" tab, then click the "Import Plate Map" button. Choose sample file that was generated by Tecan. This file is both the amp plate sheet and the CE load sheet.

9. Capillary Electrophoresis

Precautions: Formamide is a teratogen and is harmful by inhalation, skin contact, and ingestion. Use in a well-ventilated area. Formamide should always be deionized and aliquotted in small volumes to minimize using the same tube; subsequent multiple freeze-thaw cycles may cause the formamide to break down and form ionic bioproducts that could interfere with the electrokinetic injection. Formamide waste tubes and pipette tips and prepared plates will be disposed of in the hazardous waste bucket. When rinsing components, they should be allowed to air dry and canned air should not be used as it could introduce contaminants.

9.1. ABI Prism 3500xl Genetic Analyzer

Detailed documentation may be found in the 3500xL Genetic Analyzer Data Collection Software version 3.1 User's Guide and in the 3500 Series Data Collection Software 4 for HID. The current Data Collection used for Human Identification is Data Collection 4.0 for use on the 3500xL instruments. Fill out the Genetic Analyzer Component Usage Log sheet located near the instrument.

9.2. Using the ABI 3500xl Genetic Analyzer

The order in which you turn on the computer and instrument is critical for proper communication between the instrument and the computer. Follow the sequence of steps given in this section (power on computer but do not log in, power on instrument, log in to Windows operating system).

1) Power on the computer and monitor, but do not log in to the Windows® operating system.
2) Verify that the instrument is connected to the appropriate power supply. Do not unpack or plug in any components until a service representative has configured the system for the proper operating voltage. Do not rename the computer after the 3500 Series Data Collection Software 4 is installed. The instrument computer has been assigned a unique name. Changing the name may cause the software to malfunction.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 72 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

3) Inspect the instrument interior. Ensure that:
   a. The oven door is closed.
   b. No objects are left inside the instrument. Misplaced objects left inside the instrument can cause damage.
4) Close the instrument door.
5) Power on the instrument:
   a. Press the power on/off button on the front of the instrument and wait for the green status light to turn on. Note: If the door is open during power on, the yellow light will continue to flash until you close the door. See indicator descriptions in "Instrument front panel indicators" of the User Guide.
   b. If desired, press the Light button to turn on the interior light.
   c. Check the instrument status. Ensure the green status light is on and not flashing before proceeding. See indicator descriptions in "Instrument front panel indicators" of the User Guide.
6) Log on to the Windows operating system.

9.2.1.   Starting the software for the 3500xl

1) After you log on to the Windows operating system, wait ~1 to 2 minutes.
2) Look in the Windows taskbar at the bottom right of the desktop.
3) If the Server Monitor icon is displayed, go to "Step two: Start the 3500 Series Data Collection Software 3" of the User Guide. The Server Monitor icon is displayed in the taskbar, then a status bubble is displayed. It takes ~1 minute for the Server Monitor to start up.
4) When the Server Monitor icon is displayed, go to "Step two: Start the 3500 Series Data Collection Software".
5) If the Server Monitor icon does not change to a green check mark, you cannot start the software. See "Software troubleshooting — general" of the User Guide for more information.
6) The 3500 Series Data Collection Software 4 splash screen is displayed for a few seconds, then the 3500 Series Data Collection Software 3 Login dialog box opens. The Login dialog box is displayed if your system includes a license for the Security, Audit, and E-Signature module.

9.2.2.   Instrument preparation for the 3500xl

1) Check calendar reminders and consumables status in the Dashboard.
2) The Calendar Reminders section displays reminders for the tasks listed in the schedule (see "As-Needed instrument maintenance tasks" of the 3500/3500xL genetic analyzer with 3500 series data collection software 3.1 user guide. You can set the time to trigger calendar reminders in Preferences. Review the Calendar Reminders pane.
3) Perform any scheduled tasks, then click to mark it as complete, (or click to mark it as dismissed if you do not perform the task). Actions are recorded in the notifications log (for more information, see "Review the Notifications Log" of the User Guide.
4) Perform any daily, monthly, or quarterly tasks that are not listed in the Calendar reminders pane (see "Maintenance schedule" of the 3500/3500xL genetic analyzer with 3500 series data collection software 3.1.) Click Refresh to update

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 73 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

consumable status. The Consumables pane displays expiration dates and lot numbers (determined from the RFID tags on the consumable containers).

5) Check the consumables gauges for the number of injections, samples, or days remaining for a consumable. See "Instrument consumables handling, usage limits, and expiration" of the User Guide for information. When <10% of the allowed use of the consumable remains, the gauge moves into the red warning range. The consumable also displays in red in the consumables pane. The Polymer Sample Counter decrements only for wells that contain sample, but the Polymer Injection Counter decrements for each injection, regardless of whether all wells contain sample. The sample limit and the corresponding injection limit may not coincide. The first limit that is reached depends on whether you perform partial or full injections.

6) Inspect the instrument interior. Wipe any spills. Check for leaks around the Buffer-Pin Valve, check valve, and array locking lever.

7) Remove dried residue and ensure that the array locking lever is pushed securely in place. Check the fill levels on buffers. Verify that the buffer level is at the top of the fill line and check that the seal is intact. The meniscus must line up at or above the fill line. Ensure that the septa on the CBC are properly seated.

8) If any consumables need to be replenished see: "Replenish polymer or change polymer type" of the User Guide.
   a. "Install the anode buffer container (ABC)" in the User Guide.
   b. "Install the cathode buffer container (CBC)" in the User Guide.
   c. "Fill capillary array with fresh polymer" in the User Guide.
   d. "Install or change the capillary array" in the User Guide.

Install the anode buffer container (ABC)
1) Check the expiration date on the label to ensure it is not expired and will not expire during use. Life may be extended past expiration date if quality of data has not shown a noticeable decrease from previous runs, consult with Technical Leader.

2) Allow the refrigerated ABC to equilibrate to room temperature prior to first use. Do not remove the seal until you have verified that the buffer is at the fill line. Invert the ABC, then tilt it slightly to move most of the buffer to the larger side of the container. The smaller side of the container should contain <1 mL of the buffer.

3) Verify that the buffer is at the fill line.
4) Peel off the seal at the top of the ABC.
5) With the RFID label toward instrument, place the ABC into the anode-end of the instrument, below the pump. Position the anode in the large chamber of the ABC, then push the ABC up and back to install. IMPORTANT! The RFID label must be facing the instrument (away from you) to ensure that the RFID information is read accurately by the instrument.
6) Close the instrument door to re-initialize.
7) In the Dashboard, click Refresh, then check the Quick View section for updated status.

Install the Cathode buffer container (CBC)

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 74 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

1) Check the expiration date on the label to ensure it is not expired and will not expire during use. Life may be extended past expiration date if quality of data has not shown a noticeable decrease, consult with Technical Leader.
2) Allow refrigerated CBC to equilibrate to ambient temperature.
3) Wipe away condensation on the CBC exterior with a lint-free tissue.
4) Condensation can cause arcing and termination of the run. Check that seal is intact. Do not use if buffer level is too low or seal has been compromised. A fill tolerance of ±0.5 mm is acceptable.
5) Tilt the CBC back and forth gently and carefully to ensure that the buffer is evenly distributed across the top of the baffles. If you do not tilt the CBC back and forth, the buffer sticks to the baffles because of surface tension.
6) Verify that the buffer is at or above the fill line.
7) When ready to install CBC, place the container on a flat surface (such as a lab bench) and peel off the seal.
8) Wipe off any buffer on top of the CBC with a lint-free tissue. Ensure that the top of the container is dry. Moisture can cause arcing and termination of a run.
9) Place the appropriate septum on each side of the CBC: Align the buffer septum (the part that is symmetrical) over the 24 holes of the CBC.
10) Push the septum lightly into the holes to start and then push firmly to seat it.
11) Align the capillary washing septum over the other chamber of the CBC. Push the septum lightly into the holes to start and then push firmly to seat it. IMPORTANT! Look at the CBC from the side and ensure there is no gap between the container and the lip of the septum.
12) IMPORTANT! Ensure that the washing septum is securely seated to prevent displacement of the septum during operation.
13) Click the Tray button on the front panel to move the autosampler to the front position.
14) With the tab facing you and the RFID tag to the right, install the CBC on the autosampler. When properly installed, the CBC tabs will click as you snap them into place on the autosampler.
15) Click the Tray button to retract the autosampler, then close the instrument door to initialize.
16) In the Dashboard, click Refresh, then check the Quick View section for updated status.

Replenish polymer or change polymer type
1) Check the expiration date on the label to ensure that the polymer is not expired and will not expire during intended use. Polymer use can be extended past expiration date pending quality of data of CE runs.
2) Do not use if the product is expired, if the pouch or label is damaged, or if the top seal is missing or damaged. Polymer use can be extended past expiration date pending quality of data of CE runs.
3) Allow the refrigerated polymer to equilibrate to ambient temperature (15–30 C) before use.
4) In the Dashboard, click Wizards, then click Replenish Polymer (requires 10 to 20 minutes) or Change Polymer Type (requires 60 to 70 minutes).
5) Follow the prompts in the Wizard window. When instructed to install the polymer, peel off the seal at the top of the pouch fitment.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 75 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

6) Note: You may notice a tiny droplet of polymer inside the fitment (residual from the pouch filling process). This is not expected to cause any performance issues.

7) With the RFID label facing the instrument, slide the pouch fitment onto the slot of the lever assembly. Push the lever up to snap the pouch into the connector end of the instrument pump. Note: The RFID label must face the instrument (away from you) to ensure that the RFID information is read accurately by the instrument.

8) In the Dashboard, click Refresh, then check the Quick View section for the updated polymer status.

9) Refer to the instrument user guide for instructions on initiating the runs.

10) Remove bubbles from the polymer pump

11) Remove bubbles from the polymer pump fluid path before each run. See "Daily instrument maintenance tasks" for more information.

12) Note: The Bubble Remove wizard takes 5 to 15 minutes to complete. In the Dashboard, click Wizards. In the Maintenance wizards screen, click Remove Bubbles. Follow the prompts in the Bubble Remove wizard window. Check the Quick View section of the Dashboard for updated status of the polymer pouch after removing bubbles from the polymer pump fluid path.

13) Reference the User Guide for information on washing the pump chamber and channels and flushing the water trap (pump trap).

9.2.3. Sample preparation for the 3500xl

1) For each sample to be typed, including allelic ladder and controls, prepare the loading solution as follows:
(# samples + 2) x 9.6 ul deionized formamide
(# samples +2) x 0.4 ul Internal Size Standard GS600 LIZ

2) Mix loading solution thoroughly and spin briefly. Aliquot 10.0 ul of master mix into the appropriate number of wells of a genetic analyzer 96-well plate.

3) Add 1.0 ul of PCR product to the corresponding well located in the genetic analyzer 96-well plate. Add 1.0 ul of allelic ladder to the corresponding well located in the genetic analyzer 96-well plate. There must be at least one ladder per injection (i.e. per 24 samples). Each well will have 11 ul total volume.

4) Every 24 wells must contain volume. For columns injected that contain wells without sample add 10 ul of formamide; this will help keep air out of the system and better preserve the array.

5) Place a septa mat over the plate making sure that the mat is seated securely onto the plate.

6) Briefly spin the plate in a centrifuge to ensure that the contents of each well are mixed and collected at the bottom.

7) Incubate the plate at 95C for 3 minutes. Chill the plate for 3 minutes.

8) Place the plate (with septa) onto a plate base and snap the plate retainer over the plate and plate base.

9) Verify that the holes of the plate retainer and the septa strip holes are aligned. If not, reassemble the plate assembly. Damage to the array tips will occur if the plate retainer and septa strip holes do not align correctly.

10) Present the autosampler and place the plate assembly on one of the two plate positions. Ensure that the notch end of the base plate is facing towards the back

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 76 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

of the instrument and that the plate assembly lies flat on the autosampler. Return the autosampler and close the instrument doors.

9.2.4. Sample electrophoresis for the 3500xl

Creating a 3500xL Plate Record. This file associates sample information with the location of the sample on the 96-well plate.

1) From the dashboard click the button in the lower right corner to start pre-heat.
2) Click on "Create plate from template"
3) Select '6dye_36_POP4xL' and click open.
4) From the Workflow tab enter the following information:

   Name: [plate name] (e.g. A091819CB1)
   Number of wells: 96
   Plate type: HID
   Polymer Type: Pop 4

5) Click "Assign Plate Contents"
6) Click "Import" and select the run sheet of interest from the run sheet export file located at S:\Tecan Workflow\3500xL Set-Up files". Click open. The assay name should read J6_GF_SFPD_24cap; the file name convention should read SFPD and the Results group should read SFPD_GF. If not auto-populated then go to 'Actions' and select from library.
7) After making the plate record, click the tray button on the front panel to move the autosampler to the front position, then open the instrument door.
8) Place the plate in the autosampler with the labels facing you (or the instrument door) and the notched corner of the plate in the notched corner of the autosampler.
9) Click "Link plate for run' and it prompts you to save changes. Click yes. This links a plate record to the plate located on one of the two positions on the autosampler tray. When you click this button it automatically takes you to 'Load plates for run'
10) Once a plate record and a plate are linked, the link plate on the 'Load plate for Run' will be greyed out.
11) Click the blue arrow button at the bottom of the screen to start the run.

    Injection: 3500xL GlobalFiler default parameter of 15kV for 24 seconds
    Data collection software is 3500xL Data Collection Software Version 4.0

12) Re-injection: A re-injection physically re-injects all samples in the capillary array. You can specify whether to collect data for all or only selected samples in the array. By default, you can specify a re-injection before the run completes. To allow reinjections after a run is complete, set the 'Pause After Last Injection' preference (see "User Preferences").
13) From the Dashboard click on 'Edit Existing Plate" and select the plate name and click open.
14) Highlight the samples you do not need to reinject and uncheck the Assay; file name convention and results group for those selected samples.
15) Click 'Save As' and rename the plate with the existing amp name_reinj
16) Click ok and link plate for run

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 77 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

17) In the workflow table under 'load plates for run' click 'create injection list' and highlight the instrument protocol and verify samples to be rejected. A '1' will be added to the wells to indicate which samples will be reinjected.

18) Click 'start run'

19) Additional options for reinjecting samples: Select the injections or wells to re-inject: Note: Re-inject is dimmed if you select an injection that contains more than one results group, or if you select flags in the flags table that correspond to samples with different results groups. To enable Re-inject, select samples that specify the same results group. Select the injections or wells to inject:

a. To collect data for all wells in an injection
   i. Select the injection in the injection list.
   ii. Click "Re-inject" icon.

b. To collect data for only specific wells. (Note: You can also re-inject specific samples in "Review Results".)
   i. Select the injection.
   ii. Select in the array view the capillary that corresponds to the well or sample of interest (see "Array view" in the User Guide).

c. To collect data for only samples that contain flags
   i. Select the samples in the flag table (see "Check sequence or sample quality" in the User Guide.
   ii. Click "Re-inject" icon.

d. In the "Re-injection" dialog box, select options, then click OK. Sample data file for each re-injection can be saved in a separate folder in the results group folder if specified in the results group.

Note: If you are running an HID plate, see "Re-inject HID allelic ladder samples" on in the User Guide.

Note: If the "Injection Number" attribute is selected for display in the plate view, the number of the original injection and the re-injection are shown.

If you select only specific wells for the re-injection (which physically re-injects all samples for the capillary array but collects data only for the selected wells), the reinjection number is displayed for all samples in the re-injection, not just the samples selected for data collection. If you select to re-inject a sample that includes an allelic ladder in its results group but the allelic ladder is not part of the injection, the software prompts you to select one or more allelic ladder samples to re-inject. The software prompts you to select one or more allelic ladder samples to re-inject. The allelic ladders available to select are from the same plate and within the same results group as the original injection. If the results group does not contain an allelic ladder sample, the software does not prompt you to select one for reinjection.

Reinjections are marked with a ".2" at the end of the sample name for a re-injection during the same run, or "reinject" at the end of the sample name for a separate run of the same plate. Re-prepared samples will have "reprep" added to the end of the sample name. The completed run folder will automatically be transferred to the 3500xL folder located on the "S" drive.

9.2.5.   Instrument maintenance for the 3500xl

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 78 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

9.2.5.1. Daily maintenance (with each use):
Clean the assemblies, anode buffer container, and cathode buffer container, and ensure that the outside of the assemblies is dry.

Task	Frequency	For information, reference following section in User Guide
Click Refresh, then check consumables on the Dashboard – Refer to the gauges on the Dashboard to see the status for anode buffer container, cathode buffer container, and polymer.	Before each run	"Check system status in the Dashboard"
Visually inspect the level of fluid inside the anode buffer container and the cathode buffer container. The fluid must line up with the fill line.		"Install the anode buffer container (ABC)" "Install the cathode buffer container (CBC)"
Ensure that the CBC septa are properly seated on the container.		"Ensure proper installation of CBC septa"
Ensure that the plate assemblies are properly assembled. Align the holes in the plate retainer with the holes in the septa to avoid damaging capillary tips.		"Prepare the plate assembly"
Ensure that the plate assemblies and the cathode buffer container are positioned on the plate deck properly. They should sit securely on the deck.		"Load the plate in the instrument"
Ensure the array locking lever on the capillary array is secured.		"Instrument components" figures
Check for bubbles in the pump block and channels. Use the Remove Bubble wizard to remove bubbles.	Before each run	"Remove bubbles from the polymer pump"
Check the loading-end header to ensure that the capillary tips are not crushed or damaged.		"Install or change the capillary array"
Clean the instrument surfaces of dried residue, spilled buffer, or dirt.	As needed	"Clean the instrument" on page 231

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 79 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Check for leaks and dried residue around the buffer-pin valve, check valve, and array locking lever. If leaks persist, contact Life Technologies.		"Check calendar reminders"

9.2.5.2.   Monthly maintenance for the 3500xl

Task	Frequency	For information, see ...
Check the storage conditions of the used arrays to ensure the array tip is covered in the reservoir.	Monthly or as needed	"Store a capillary array"
Run the **Wash Pump and Channels wizard**.		"Wash the pump chamber and channels"
Use a lab wipe to clean the anode buffer container valve pin assembly on the polymer delivery pump.		"Instrument components" figures
Restart the computer and instrument.		"Restart the instrument and the computer" on page 253

Task	Frequency	For information, see ...
Run install check.	Monthly or as needed	"Run an install check"
Flush the pump trap.		"Flush the water trap (pump trap)"
Empty the oven condensation reservoir.		"Instrument components" figures
Replace cathode buffer container septa.		"Install the cathode buffer container (CBC)"
Clean the autosampler.		"Clean the instrument"
Clean the drip tray.		
Check disk space.		"Monitor disk space"
If Security, Audit, and E-sig is enabled, archive and purge audit records.		"Archive and purge"
Defragment the hard drive.	Monthly, or before fragmentation reaches 10%	"Defragment the computer hard drive"

9.2.5.3.   Quarterly maintenance for the 3500xl

Task	Frequency	For information, see ...
Archive and purge audit records	Every three months	"Archive and purge"

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 80 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

9.2.5.4. As-needed maintenance for the 3500xl

Task	Frequency	For information, see ...
Change the tray.		"Clean the instrument"
Remove dried polymer from the capillary tips with a lint- free tissue moistened with deionized water.	As needed	
Archive and purge library objects.		"Manage library resources"

9.2.5.5. Spatial and spectral calibrations for the 3500xl

Spatial Calibration:
1) A spatial calibration must be performed when the detector door is open or the instrument is moved.
2) To perform a spatial calibration, click Maintenance in the Dashboard, then select Spatial Calibration in the navigation pane.
3) If the capillaries contain fresh polymer, select No Fill. Otherwise, select Fill. Click Start Calibration.
4) When the run is complete, evaluate the spatial calibration profile using the following criteria:
   a. Height: similar heights for all peaks
   b. Apex markers: one cross (+) marker at the top of every peak. No misplaced markers.
   c. Shape: one sharp peak for each capillary. Small shoulders are acceptable.
5) If the results meet the above criteria, click Accept Results.
6) If the calibration failed, click Reject Results, then see the "Spatial calibration troubleshooting" section in the "Applied Biosystems 3500/3500xL Genetic Analyzer User Guide 3.1".

Spectral Calibration:
1) A spectral calibration must be performed when a new dye set is introduced to the instrument, when an array is replaced, after optical service (such as CCD camera, mirrors or laser realignment/replacement), when the instrument is moved, or when a decrease in spectral separation (pull-up and/or pull-down peaks) in the raw or analyzed data is observed.
   a. Remove matrix standard tube from the refrigerator and spin briefly. Do NOT vortex standard unless this is the first time the box is being opened.

      NOTE: If this is the first time the box is opened, only vortex the standard briefly for ~5 seconds. Excessive vortexing will cause the dyes to sheer off and the spectral will not pass.

   b. Mix 6 ul of the matrix standard and 294 ul of HI-DI formamide by inverting (Do NOT vortex). Briefly centrifuge the mixture.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 81 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

    c. Add 10 ul of the mixture into each well in columns 1, 2, and 3 using a 96 well optical plate (24 wells total); if saturated, adjust volume to 5 to 7ul.

    d. Cover the plate with a septum and briefly spin the plate in a centrifuge.

    e. Heat the plate in the themalcycler at 95°C for 3-5 minutes to denature the DNA, then snap cool it in the freezer for 3-5 minutes. Ensure there are no bubbles at the bottom of the wells.

    f. Place the plate in a plate base, cover with plate retainer, and verify the holes of the plate retainer and septa strip are aligned. Place it in the autosampler with the labels facing the instrument door and close the door.

2) To run the spectral calibration, select Maintenance, then select Spectral Calibration in the navigation pane. Select the number of well, standard and dye set.

3) Select the plate position for the plate loaded in the instrument.

4) Specify the starting well for the injection position in which you loaded the standard in the plate.

5) For chemistry standard select: DS-36 Matrix Standard; for dye set select J6; and for application select Fragment analysis. Prepare standards as follows:

6) Select the number of wells (96) and specify the plate location in the instrument. Select Allow Borrowing. Click Start Run. The run should be done in 30 minutes.

7) The Capillary Run Data displays the results of each capillary. Click a capillary in the table to display the spectral profile and raw data for each capillary. Below the run results table, verify the Quality Value is within the range of 0.95-1.0, and Condition Number 1.0-8.0 for J6 dye set. Verify the order of the peaks in the spectral profile from left to right: purple-blue-green-yellow-red-orange for 6-dye. Verify that the peaks in the spectral profile do not contain gross overlaps, dips or other irregularities and that peaks separate distinctively.

8) If the data for all capillaries meet the criteria above, click Accept Results. Otherwise, click Reject Results, then go to "Spectral calibration troubleshooting" section in the "Applied Biosystems 3500/3500xL Genetic Analyzer User Guide 3.1".

9.2.5.6. Shutting down the 3500xl

See "Shutdown move and reactivate the instrument" section in User Guide.

See "Restart the instrument and the computer" in the User Guide for information on restarting the instrument and the computer.

When to use this procedure:

1) If communication errors are displayed;
2) If the front panel indicator is blinking red;
3) At the end of spatial calibration, if Accept/Reject buttons are dimmed;
4) If maintenance wizards are taking longer than expected
5) If software operations are taking longer than expected.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 82 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

9.2.5.7. Instrument consumables handling, usage limits, and expiration

Buffers

Cat. no.	Description	Storage conditions
4393927	Anode Buffer Container (ABC) 1X running buffer, 4 containers	Store at 2–8°C. The 1X running buffer has been qualified to ship at ambient conditions.
4408256	Cathode Buffer Container (CBC) 1X running buffer, 4 containers	

Instrument	On-instrument supported limits Lower of:	Guidelines
24-capillary	14 days, 100 injections, or expiry date. Life may extend past expiration date and will be evaluated on quality of run data.	The buffer has been verified for use for up to 14 days on the instrument. The software displays a warning message when a usage limit is met and allows you to continue running.

Pouch size	Instrument	On-instrument supported limits Lower of:	Guidelines
384 samples	24-capillary	14 days, 384 samples, 20 injections, or expiry date. Life may extend past expiration date and will be evaluated on quality of run data.	The polymer has been verified for use for up to 14 days on the instrument. The software displays a warning message when a usage limit is met and allows you to continue running.

The pouch has adequate polymer to support the stated number of samples or injections, plus additional volume to accommodate installation and wizard operations. Multiple pouch installations and/or excessive use of wizards reduce the number of remaining samples and injections. For example, if you run the total bubble remove option in the Remove Bubbles wizard more than four times, the number of remaining samples and injections is reduced.

Conditioning reagents

Cat. no.	Description	Storage conditions
4393718	Conditioning reagent, 1 pouch	Store at 2 to 8°C. After removing from storage, use the pouch within 24 hours.

On-instrument supported limits	Guidelines
Once installed on the instrument, the pouch is good for a one-time use.	Refer to the expiration date on the label. Life may extend past expiration date.

Capillary arrays

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 83 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Cat. no.	Description	Storage conditions
4404685	24-Capillary, 36 cm	Ambient temperature

On-instrument limits	Guidelines
160 injections or expiration date listed on packaging and RFID label. Life may extend past expiration date and will be evaluated on quality of run data.	Capillary arrays have been verified for use for 160 injections. The software displays a warning message when a usage limit is met and allows you to continue running. Store capillary arrays with the loading-end of the capillary array in distilled water to prevent the polymer from drying in the capillaries.

10. Data analysis

10.1. Starting a new project using data from 3500xl and GMID-X 1.6

1) Open the GeneMapper ID-X program by double clicking on the GeneMapper ID-X icon.
2) Log into the Genemapper ID-X database host (PT-CRML-12) using first initial/ last name (i.e. jsmith) and password for initial log in; you will be prompted to change password on first log in.
3) Select "Add samples to project" under the Edit menu. Navigate to the appropriate folder on the "S" drive and select the run folder containing the .HID files that are to be analyzed. Click the "Add to List" button then click the "Add" button to start a master project.
   Re-preparations may be added to the same project as the names should be distinguishable in the "run" column.
4) Select the appropriate Analysis Method ("GlobalFiler 150 RFU" or "GlobalFiler 150 RFU - STRmix", Panel (GlobalFiler _Panel_v1.1.1X or GlobalFiler_Panel_v1.1.1X - STRmix) and Size Standard (GS600_LIZ (60-460)) from their respective drop down menus.
5) Save project name to include the instrument being used and the run number associated with its use (i.e. 2CE_A052720AIL1). Under Security Group, select "Casework Security Group" (if prompted) from dropdown list and Click OK.
6) The analysis methods are locked and the settings should not be changed.
   a. Analysis range: Full range
   b. Sizing Range: Partial sizes
      i. Start Size: 60
      ii. Stop Size: 600
   c. Smoothing and Baselining: Light
   d. Size Calling Method: Local Southern Method
   e. Peak Detection
      i. Peak Amplitude Thresholds
         1. B, G, Y, R, P, O: 150 RFU (all)
         2. Min. Peak Half Width: 2
         3. Polynomial degree: 3
         4. Peak window size: 11

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 84 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

5.   Baseline window: 33
   f.   Slope Threshold
      i.   Peak Start: 0
      ii.   Peak End: 0
   g.   Normalization: select "Use normalization, if applicable".
7)   Click the "Analyze" icon (green left pointing arrow located on tool bar) to begin analysis of the selected .fsa files.

10.1.1.   Checking the internal lane size standard (ILS)

Highlight the samples in the project, click on the Display Plots icon and select Check LIZ Size Standard plot from the drop down menu. LIZ data needs to be verified but does not need to be edited.

Examine each sample to confirm that the base pair size of each ILS peak has been labeled properly as follows: 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440 and 460 base pairs.

10.1.2.   Allele designations
1)   To view allele designations select the desired sample(s) and click on the "Display Plots" icon and select "Sample Viewing and Editing" from the drop down menu at the top.
2)   Visually inspect the ladder(s) and controls to ensure that alleles are above the analytical threshold and are called correctly. The analyst may choose to use one allelic ladder or have the GMID-X software average multiple ladders to make a virtual ladder. If all of the allelic ladders fail, the samples will need to be re-injected to ensure accurate sizing of peaks. Examine the negative amplification control to verify the absence of DNA peaks.
3)   Visually inspect each sample to assess allele and artifact calls:
   a.   Verify/trouble shoot any off-ladder (OL) peaks, micro-variants or artifacts.
   b.   Make note of any samples that require reinjection and/or reprocessing.
   c.   Review all peaks labeled as "OMR" and "Spike" by the software to ensure no true alleles were incorrectly labeled.
   d.   The quality flags may be used as indicators of data quality but should always be followed up with the analyst's manual review.
   e.   The stutter filter may need to be disabled to allow calling of a peak in stutter position.
4)   Edits will be made with the reason for change including:
   a.   Forward and back stutter with % calculated for elevated stutter, etc.
   b.   ST+PU for artifacts including those which cannot be differentiated between stutter and pull-up.
   c.   Observations requiring further detail/explanation should be printed on a separate page to adequately illustrate the data and may require zooming in on the baseline with appropriate colors included (e.g. all dyes affected by a spike, the ladder and sample to illustrate an off-ladder allele, or the appropriate plots to illustrate pull up).
5)   Under the genotypes tab, verify the allele calls prior to exporting the table.   In the table below are the expected results for the DNA positive amplification control 007

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 85 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

6) The analysis summary tab can be used to verify the correct allele assignments for allelic ladders, positive and negative controls.

Green square: correct alleles have been assigned and the size standard has called correctly

Yellow triangle: ladder requires manual comparison to expected types and may not be suitable for use in genotyping. Samples should be re-prepared and re-injected on a case-by-case basis if it appears information is being lost or will affect modeling in STRmix.

Red octagon: controls and ladders require manual comparison to expected types

Locus designation	Chromosome Location	Alleles included in Allelic Ladder	Dye Label	Control 007
D3S1358	3p21.31	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	6-FAM	15, 16
vWA	12p13.31	11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24		14, 16
D16S539	16q24.1	5, 8, 9, 10, 11, 12,13, 14, 15		9, 10
CSF1PO	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		11, 12
TPOX	2p23-2per	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15		8, 8
Y indel	Yq11.221	1, 2	VIC	2
Amelogenin	X:p22.1-22.3	X, Y		X, Y
	Y:p11.2			
D8S1179	8q24.13	5, 6, 7, 8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19		12, 13
D21S11	21q11.2-q21	24, 24.2, 25, 26, 27, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 37, 38		28, 31
D18S51	18q21.33	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27		12, 15
DYS391	Yq11.21	7,8, 9, 10, 11, 12, 13		11
D2S441	2p14	8,9, 10, 11, 11.3, 12, 13, 14, 15, 16, 17	NED	14, 15
D19S433	19q12	6, 7, 8, 9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2		14, 15
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3		7, 9.3
FGA	4q28	13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2		24, 26
D22S1045	22q12.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	TAZ	11, 16
D5S818	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18		11, 11
D13S317	13q22-31	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16		11, 11
D7S820	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		7, 12
SE33	6q14	4.2, 6.3, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19,		17, 25.2

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 86 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

		20, 20.2, 21, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35, 35.2, 36, 37		
D10S1248	10q26.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	SID	12, 15
D1S1656	1q42.2	9, 10, 11, 12, 13, 14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18.3, 19.3, 20.3		13, 16
D12S391	12p13.2	14, 15, 16, 17, 18, 19, 19.3, 20, 21, 22, 23, 24, 25, 26, 27		18, 19
D2S1338	2q35	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28		20, 23

10.1.3. Electronic file and folder naming convention and storage

- The instrument run number is used to facilitate electronic data tracking. The instrument run number identifies the instrument being used and the run number associated with its use (i.e. "3CE_A101515MCP" refers to the instrument 3 and amplification run).
- Some alphanumeric characters are not valid for user or file names. Do not use commas, spaces, or symbols such as \ / : * ? " as they could interfere with analysis and/or data imports/exports.
- Post-run documents: GMID-X Project: (run number) Master (run date) (analyst initials)
- All electronic files related to a particular case will be stored on the server in a case specific folder. Each folder will contain the raw data, and export of analyzed data. Raw data is left in the CE folder until the final report and technical review have been completed.

10.2. Evaluation of data using 3500xl and GMID-X 1.6

10.2.1. Analytical threshold

The value below which observed allelic peaks cannot be reliably distinguished from instrument noise.

The analytical threshold in the SFPD DNA Unit is based on internally derived empirical data. It is not established for purposes of avoiding artifact labeling with potential loss of allelic data, and as such non-allelic peaks above the AT will need to be addressed within an interpretation.

The analytical threshold in the SFPD DNA Unit is 150 RFU; all data will be analyzed using this threshold and allele calls assessed and reported for peaks at/above 150 RFU.

10.2.2. Stochastic threshold

A stochastic threshold refers to the peak height above which it is reasonable to assume that dropout of a sister allele of a heterozygote pair has not occurred. The use of STRmix reduces the need for a defined stochastic threshold for interpretation; however, in the case of single source samples (e.g . reference samples for comparison)

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 87 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

and number of contributor estimation, stochastic potential may still need to be considered. Internal validation studies for Globalfiler and the 3500xl showed stochastic potential (heterozygous locus exhibiting considerable peak imbalance and/or allelic drop-out from random, disproportionate amplification of alleles) in peaks below **1000 RFU.**

10.3. Evaluation of controls

All controls are evaluated to ensure they yield results within their prescribed specifications. If the expected results are not obtained from a control sample, the analyst and DNA Technical Leader will confer and determine whether the control and/or associated samples should be re-processed or if DNA interpretation may proceed within prescribed limitations. A verification of controls should be done by a second analyst and marked on the "3500xL Amp Plate Map" worksheet. This would include checking the positive and negative amp controls, reagent blanks, ladders, and ILS within samples.

10.3.1. Internal lane size standard (ILS)

The internal lane size standard (ILS) will be examined for each sample and control to ensure that all peaks used to build the sizing curve were assigned accurately.

The LIZ 600 size standard is used for GlobalFiler and uses peaks from 60 to 460 base pairs in its sizing algorithm. Size standard peaks should be greater than the analytical threshold. If there are issues with peak heights for ILS peaks, this will be documented in the casefile. If an ILS fails, the sample should typically be re-prepared and/or re-injected.

10.3.2. Allelic ladder

The allelic ladder that is used for genotyping will be examined by the analyst to ensure that all allele designations were assigned correctly.

Normal variations in fragment migration speed could occasionally result in allelic ladder peaks differing by more than 0.5 bases causing the software to assign alleles as "off-ladder". Genotyping the affected sample (or subset of samples), with a different injection of the allelic ladder may alleviate this situation. If a different ladder doesn't remedy the issue, the sample may need to be re-injected to ensure it falls within the ±0.5 base range for sizing; an exception would be if there were no peaks in the sample to size. Temperature fluctuations within the room could affect run migrations; however, monitoring the room temperature is not necessary as the data will demonstrate the quality of the run.

Allelic ladder peaks should be greater than the analytical threshold; if there are issues with peak heights, this will be documented in the casefile. At least two ladders are included on every run to accommodate the potential of temperature fluctuations and poor injection quality. All allelic ladders are imported into the project and the GMID-X software creates an averaged "virtual" ladder. NOTE: even ladders that have failed could be included so the analyst should be aware of this.

10.3.3. Positive amplification control

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 88 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

The purpose of the positive control is to monitor the success of the amplification reaction with a DNA sample of a known genotype.

The AmpFlSTR Control DNA (007) reaction must produce the known typing results (above the analytical threshold) reported by the manufacturer to be considered successful. In the event of a positive control failure (i.e. types are not correct, analyst failed to add sample, thermal cycler failure, etc.) the analyst should review data in order to aid in troubleshooting. Consult with a DNA supervisor/Technical leader on how best to troubleshoot based on data. Reprocessing of associated samples may be necessary pending results. Where amplification results are concordant with quantification results in samples, reprocessing may not be necessary.

10.3.4. Negative amplification control

The purpose of a negative control is to monitor possible contamination in reaction by amplifying a sample with no added DNA to control for the amplification reagents and set-up.

The negative control should yield no DNA typing results greater than the analytical threshold (within the allele size ranges to be analyzed) to be considered successful.

If the negative amplification control exhibits DNA peaks above the analytical threshold, the analyst must attempt to identify the cause and document this in the casefile and associated report. In some cases, samples set-up and amplified with this reaction will be considered inconclusive and will be re-amplified, if possible and appropriate.

If suspected DNA peaks are observed below the analytical threshold, the analyst may analyze below the standard analytical threshold to aid in troubleshooting, but this data will not be included in the report.

If the analyst and DNA Technical Leader determine that the typing results are inconsequential compared to the test sample results, or not observed in any other sample, the analyst may be able to use the typing data for interpretation.

Instances of failed negative amplification controls will be documented in an Action Report.

10.3.5. Reagent blank control

The purpose of a reagent blank is to monitor any possible reagent contamination. The reagent blank should yield no DNA peaks greater than the analytical threshold (within the allele size ranges to be analyzed) to be considered successful.

If the quantitation value for a reagent blank exhibits a positive quant value, the sample will still be amplified. If positive quant/no DNA peaks are detected (see below), the positive quant value does NOT need to be reported.

If the reagent blank exhibits DNA peaks above the analytical threshold, the analyst must attempt to identify the cause and document this in the casefile and associated

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 89 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

report. Please refer to section regarding contamination to best troubleshoot and document a reagent blank with unexpected peaks present.

If controls (positive, negative, reagent blank) are run for any reason, they do not need to be added to the GMID project for the case unless they indicate contamination and need to be reported in the Case Report.

Instances of failed reagent blanks will be documented in an Action Report.

10.3.6. Locus designation

Alleles at each STR locus span a range of base pair sizes including the ladder range as well as other known or reported "off-ladder" microvariants in between virtual bins and outside the ladder range.

Microvariants that fall within the allelic ladder to size and call may not be resolved due to the inability of the software to discern the valley between two peaks. Analysts will need to document this appearance in case notes. Loci will not be included in a statistic if there is uncertainty regarding the resolution of alleles.

Alleles are assigned to a locus by virtue of their sizing within the allele size range of a locus. If an area of overlap occurs between the allele size ranges of adjacent loci, then an allele peak may be assigned to a locus by virtue of available information such as sample zygosity at the flanking loci.

10.3.7. Allele designation

Numerical allelic nomenclature is designated in accordance with the DNA Commission of the International Society of Forensic Haemogenetics (now ISFG), CODIS recommendations, and AmpFlSTR User's Manuals.

Whenever possible, STR allele designations are determined operationally by comparison to 0.5 base pair bins built around known allelic ladder alleles. Allelic nomenclature is based on the number of repeat motif units contained within the allele.

Alleles at the amelogenin marker are designated X or Y, a description of their residence on either the X or the Y chromosome. GlobalFiler also contains two male specific targets (DYS391 and Y indel). These male markers are found on the Y-chromosome and are haploid.

10.3.8. Off-ladder allele estimation

Microvariant alleles are easily observed in heterozygous samples when one allele at a locus sizes according to the ladder and the second is in between virtual bins on the allelic ladder, or above the largest or below the smallest allele of the ladder.

All off-ladder alleles need to be re-injected to confirm the sizing if they are not confirmed by another sample in the case. This also applies to samples that will not be interpreted in the final report (i.e. inconclusive samples). Confirmation is based on allelic size, therefore other samples in the case can be used to confirm that OL in

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 90 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

another sample within the case. Off-ladder alleles in reference samples cannot be used to confirm off-ladder alleles in evidence samples. If an off-ladder allele is near the analytical threshold but is below the threshold upon re-injection it can still be used as confirmation of initial sizing.

This includes off ladder alleles in stutter positions in either STRmix or non-STRmix analysis profiles.

To calculate an off-ladder allele that falls within the indicated markers designating a locus, use a ladder from the run, preferably the ladder closest to the same injection number, to calculate the size difference between the off-ladder allele using the ladder alleles both immediately above and below the off-ladder allele. Both calculations should agree for sizing of the off ladder allele. The microvariant is labeled according to the number of complete repeats and, separated by a decimal point, the number of base pairs in the incomplete repeat motif(s) (e.g. 28.1).

Example: OL called at 221.44 bp @ D12S391. 15 ladder is 220.60 bp. 16 ladder is 224.62 bp.

221.44 - 220.60 = 0.84. (~1 bp ahead of 15 = 15.1)

221.44 – 224.60 = -3.16 (~3 bp behind 16 = 15.1)

Note: only one calculation for one microvariant is needed in evidence samples if there is consistent sizing between injections/samples.

If an allele is located above or below the defined allelic ladder (the ladder doesn't have alleles both immediately above and below the off-ladder allele), the microvariant is designated as either less than (<) or greater than (>) the nearest allele (or virtual bin) in the ladder.

10.4. Artifacts and non-allelic peaks

It is necessary to identify any potential non-allelic peaks prior to the interpretation and comparison of DNA data. Edits not made correctly in data can affect downstream interpretations including number of contributor estimates.

Non-allelic peaks may include PCR products (stutter, minus-A), artifacts (spikes, raised baseline), instrument limitations (pull up) or introduced (disassociated primer dye). Generally, artifacts will not be reproducible and may be observed at any locus of a DNA profile. If an artifact is suspected, the analyst needs to look at suspect peaks within the context of the entire profile.

The Globalfiler User guide discusses common amplification artifacts. In addition, particular sample types (e.g. anal/rectal swabs) may be susceptible to artifact peaks.

10.4.1. Stutter

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 91 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Stutter peaks are a product of the PCR process which result when STR loci are copied by a DNA polymerase. Stutter is likely to be present to some extent at all loci the majority of the time.

The primary proposed mechanism is "slipped-strand mispairing" which results in a smaller peak typically one repeat unit less (i.e. four-base pairs for tetranucleotides or "N – 4") than the main peak.

Observed stutter values are locus specific and their detection may be affected by off-scale data and artifacts such as raised baseline. Stutter will typically be reproducible between injections but may not be reproducible between separate amplifications.

The peak morphologies of stutter are the same as allelic peaks because they are true PCR products; however, they are typically differentiated from alleles based on the size relative to the allelic peak and their peak heights.

Generally when the height of a peak in the stutter position is at or below the stutter percentage expectation, the peak is subtracted out as a stutter peak. If the peak in the stutter position exceeds the stutter percentage expectation, it is typically consistent with being of allelic origin (unless elevated stutter is justified) and will be given an allele assignment.

In the case of a DNA mixture, the analyst will need to consider that the peak in the stutter position may be shared by stutter and allele. For interpretations, the analyst will need to estimate possible stutter contribution (i.e. subtract out expected stutter contribution) when assessing the stochastic potential of a peak (i.e. whether a paired peak could have dropped out).

The percent stutter generally increases with allele length. Smaller alleles display a lower level of stutter relative to the longer alleles within each locus.

Expected stutter values for Globalfiler can be found in the table below:

Locus	D3S1358	vWA	D16S539	CSF1PO	TPOX	
Stutter %	10.98	10.73	9.48	8.77	5.55	
Locus	Y Indel	Amelogenin	D8S1179	D21S11	D18S51	DYS391
Stutter %	---	---	9.60	10.45	12.42	7.43
Locus	D2S441	D19S433	TH01	FGA		
Stutter %	8.10	9.97	4.45	11.55		
Locus	D22S1045	D5S818	D13S317	D7S820	SE33	
Stutter %	16.26 (n-3) 6.69 (n+3)	9.16	9.19	8.32	14.49 (n-4) 3.97 (n-2)	
Locus	D10S1248	D1S1656	D12S391	D2S1338		
Stutter %	11.46	12.21 (n-4) 2.45 (n-2)	13.66	11.73		

Important notes:

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 92 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

- N-2 stutter is observed in SE33 and D1S1656 which include regions of dinucleotide repeats; these loci have filters to prevent calling these peaks.
- D22S1045 is a trinucleotide repeat that shows an elevated level of plus stutter.
- The majority of plus stutter (N+4) peaks are observed below 2%; SFPD Globalfiler validation data showed only 6.6% of plus stutter peaks were observed above 2%. Plus stutter peaks at/less than 2% of the parent peak should generally be removed and labeled as plus stutter.

10.4.2. Incomplete terminal nucleotide addition (-A peaks)
Minus-A peaks are caused by incomplete 3' (A) nucleotide addition. It may result with amplifications containing too much DNA template or sub-optimal thermal cycling parameters. Incomplete adenylation will result in split peaks differing by 1 base pair (n − 1).

The presence of minus-A peaks can be resolved if the analyst amplifies the sample with less DNA. Minus-A peaks are amplification products that will typically be reproducible between injections but may not be reproducible between separate amplifications.

The peak morphologies of minus-A peaks are the same as allelic peaks because they are true PCR products; however, they are typically differentiated from alleles based on the size relative to the allelic peak and their peak heights.

10.4.3. Spikes
Spikes may be caused by random electrical fluctuations in the run typically distinguished from allelic peaks by their sharp and narrow peak morphology and lack of reproducibility between injections. Spikes are typically seen at the same base pair size in multiple colors.

10.4.4. Elevated Baseline
Elevated baseline is identified by broad spectral elevation in the raw and analyzed data and could have the effect of elevating or masking true peaks. Elevated baseline is typically not reproducible between injections.

10.4.5. Pull-up and pull-down
Pull-up occurs when the spectral calibration matrix fails to completely separate signal between overlapping emission spectra of fluorescent dyes. Pull-up relates to multi-component analysis accuracy and not to the quality of the DNA sample. Pull-down is observed in raw data.

Pull-up peaks appear in different dye channels at approximately the same base pair size as the source peak. Often reproducible between injections on the same run but may not be between runs, specifically if a new spectral matrix is applied to the sample. Complex pull-up can occur when source peaks are one repeat unit apart creating a single bridge-like artifact.

In the SFPD validation of the 3500xl, the majority of pull-up peaks were observed at/below 3.0% of the source peak. Pull-up greater than 3% would likely be caused by

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 93 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

saturated data, a bad/old spectral or instrumental issues. If a Criminalist observes suspected pull-up above 3%, they should discuss with a Supervisor to evaluate troubleshooting action (re-injection on different instrument, re-amplification) prior to editing the peak out.

As a Criminalist assesses pull-up peaks, they should look at the entire profile: across dye channels at the same base pair locations, and within dye channels for consistency of pull-up peaks from source peaks of similar heights.

10.4.6. Dissociated primer dye

Disassociated dye peaks, often called "dye blobs", are caused by dye molecules becoming detached from the primer. They are typically distinguished from allelic peaks by their broad peak morphology. Dye blobs are typically reproducible between injections of the same amplification, but may be resolved by re-amplification with new reagents.

10.4.7. Oversaturation due to increased template DNA input

The limit of linearity is the value above which signal exceeds the linear dynamic range of the detection platform. This is often referred to as "saturated" or "off-scale" data and must be interpreted with caution.

Because off-scale peaks may be assigned artificial height values that do not represent a peak's true amplitude, the data becomes unreliable for peak height ratio and stutter assessments. As such, off-scale peaks should not be used in quantitative aspects of interpretation.

The effects of oversaturated signal may result in excessive pull-up between spectra, baseline artifacts, split peaks, inter-locus peak height imbalance due to inhibition from amplifying excessive DNA, and elevated stutter peaks.

The analyst may prepare a dilution of the amplified product but caution should be used as PCR artifacts from saturation will still exist in the dilution. Alternatively, the analyst can amplify with less template DNA.

10.5. Evaluation of data

10.5.1. Partial profiles

A partial DNA profile occurs when typing results are not obtained at all tested loci. This could be due to reasons such as low quantity template, DNA degradation or inhibition. Samples that contain low-level data for which the analyst has decreased confidence in an allele call can be re-injected/re-amplified to confirm the reproducibility of the peak(s).The determination of which alleles and/or loci are suitable for comparison and statistical evaluation is made prior to comparison to known samples and documented in notes.

10.5.2. Inhibition

Inhibition occurs when something (dyes, humic acid, etc.) is present in the DNA sample that may affect the amplification reaction. Dilution or additional purification prior to proceeding with amplification may help.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 94 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

10.5.3. Degradation

When the DNA molecule has been broken into small pieces due to environmental or other factors resulting in a gradual loss of signal affecting the high molecular weight loci first. This may be observed as a full DNA profile with a "ski-sloped pattern", a partial DNA profile, or no DNA data generated due to the inability of the extract to amplify.

Of particular importance with mixture interpretation is differential degradation where contributors to a DNA mixture are subject to different levels of degradation which could impact mixture ratios across the profile.

Troubleshooting degradation is not feasible as it is an inherent property of the DNA extract affected by it.

10.5.4. Poor injection

Electrokinetic injection is dependent on the levels of salt in a sample. As salt levels increase in a sample, competition between the salt ions and DNA molecules may occur, causing less DNA sample to be injected in the capillary. As a result, samples may show low peak heights in one injection and higher peaks on a subsequent injection of the same sample.

Care should be taken to avoid dried capillaries; buffer crystals may collect around the capillary opening forming a physical obstruction that results in low current or no current. Buffer should be replaced regularly to avoid buffer depletion and/or imbalance of ions which could result in poor separation of fragments and a drop in current.

A loss in resolution in a sample, defined by broad peaks, across multiple injections may suggest the capillary/array should be replaced; this is due to the dynamic coating on the inside capillary wall failing to work properly.

Sample reinjections or re-prepping of the CE load may be done to resolve poor injection issues.

10.5.5. Multiple amplifications/injections

Associated DNA processing worksheets from duplicate amplifications and injections will be printed and kept in the casefile. The exception is when an entire run fails; in this case, a note will be made on the appropriate worksheet explaining why data is not being used. Data not used ("DNU") for analysis and/or interpretation will be noted.

In the event that an evidence sample has undergone multiple amplifications and/or injections, the analyst should use the results with the most genetic information for comparisons and statistical calculations; this decision will be made on a case-by-case basis.

10.5.6. Composite profiles

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 95 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Composite profiles are defined as DNA typing results obtained from different amplifications of the same extract where different loci are combined into a single composite DNA profile and used for interpretation and comparison.

The DNA unit does not use composite profiles in any evidence samples where STRmix analysis is being performed. A composite profile may be warranted for reference samples. If so, the technical leader will be consulted, and a note will be included in the casefile.

10.6. Troubleshooting

In the event that a sample fails to produce an optimal or expected result, the analyst may need to perform troubleshooting on the sample. This may involve re-sampling of a different area, re-extracting and/or re-purifying a sample that shows inhibition, amplifying more template if the sample yields a partial/incomplete or inconclusive profile (i.e. if increases the chance for a CODIS entry), amplifying less template if the sample shows signs of oversaturated data or inhibition, or re-injecting a sample that displays an artifact or inconclusive data. Any processing and results will be documented in the notes.

10.7. Importing GeneMapper ID-X v1.5 Projects into GeneMapper ID-X v1.6
1) Open GeneMapper ID-X v1.6.
2) Go to Tools > GeneMapper ID-X Manager.
3) Click Import.
4) Navigate to S:\Equipment and Software\GeneMapper\GMIDX Projects
    a. Case-specific projects will be in folders labeled "*Year* case projects".
    b. Batch projects will be in folders labeled "*Year* projects".
5) After project has been imported, go to File > Open Project.
    a. Do not click green arrow once project is open.
    b. Apply Globalfiler panel, if needed, to project so that peaks will be labeled.

10.8. Archiving and Deleting GeneMapper ID-X Projects
1) Open GeneMapper ID-X v1.6.
2) Go to Tools > GeneMapper ID-X Manager.
3) Select project.
4) Click Export.
5) Navigate to S:\Equipment and Software\GeneMapper\GMIDX Projects
    a. Case-specific projects will be in folders labeled "*Year* case projects".
    b. Batch projects will be in folders labeled "*Year* projects".
6) Save project using original project name.
    a. Confirm project has been saved in appropriate GMIDX Projects folder
7) Within Genemapper ID-X Manager, select the exported project > Click Delete.
8) Click Yes to prompt.
Note: Projects can be sorted by holding SHIFT and clicking on column header. This helpful to sort projects from oldest to newest for archiving purposes.

11. Data interpretation

The interpretation of any DNA profile is predicated on the identification of unambiguous DNA alleles versus possible artifacts, and observations about the quality and quantity of the DNA. Once the analyst is confident in the allele calls within a profile, alleles may then be considered within the

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 96 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

context of the entire profile, sample type and case. Analysts must note analysis and interpretation observations within the case notes. This documentation must be sufficiently understandable to reconstruct the interpretation.

Mixture deconvolutions require documentation of all assumptions used, including but not limited to (where applicable) the assumed number of contributors, any known contributors, and assumed portions. This includes all assumptions used in formulating conclusions and impacting comparisons. Assumptions must be made within a framework of reason supported by empirical data, validation studies, peer reviewed journals and experience; they should be continually evaluated throughout the course of the interpretation. Attempts will be made to derive independent interpretations without the possibility of introducing bias by prior knowledge of reference samples by using a workflow that shields the analyst from task-irrelevant information. The NOC Interpretation Flowchart is used by the unit as a guide to determine the number of contributors present in mixture samples and whether enough data is present in the profile to make conclusions on.

Occasionally, a DNA typing result that was developed using a protocol that is no longer in use may need to be further evaluated for comparison to newly submitted samples or to assess a candidate match and may require documented re-qualification for legacy kits (see Forensic Biology Unit Training manual). Elements that do not require documented requalification include: 1) Evaluating a candidate match within CODIS from a high stringency search that just involves a comparison between two single source CODIS entries that match at high stringency and 2) Comparing a casework (forensic) DNA profile interpretation that previously had the genotypes of the possible contributors documented in the mixture interpretation worksheets compared to the results of moderate stringency search candidate matches.

11.1. Male marker interpretation
Interpretation of these markers is limited to qualitative observation of number of potential male contributors. Barring a duplication event, haploid loci will only contain one allele for a single source male profile, two alleles indicate at least two males, three alleles indicates at least three males, etc. These markers will not be included in statistical calculations. The male markers used included in the Globalfiler kit are Y Indel and DYS391.

11.2. Single source
Generally a sample is considered to be single-source if only one or two alleles are observed at all typed loci and peak height ratios for all heterozygous loci are within the expected ranges. Tri-allelic patterns and peak height discrepancies from primer binding site variations have been documented; however, due to the rarity of these events it would not be likely to observe these effects at more than one locus in a profile. Typically, any trialleles or genetic variations would also be observed in known samples.

Samples that are low in DNA quantity but showing a pattern of being a single-source may be assumed to be from a single source. STRmix analysis will account for any possible stochastic effects.

11.3. Mixtures
Generally a sample is considered to be a mixture of more than one individual if three or more alleles are present at more than one locus and/or peak height ratios fall outside of the

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 97 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

expected ranges which could suggest allele sharing. The minimum number of contributors can typically be estimated based on the locus with the greatest number of alleles as follows:

> 3 to 4 alleles at a locus = ≥ 2 contributors
> 5 to 6 alleles at a locus = ≥ 3 contributors
> 7 to 8 alleles at a locus = ≥ 4 contributors
> \> 8 alleles at a locus = > 4 contributors, not interpretable (must be deemed inconclusive)

Minimum number of contributors does not always equal the actual number of contributors being assumed in a mixture. Peak height ratios and allele sharing also need to be accounted for when trying to determine a specific number of contributors.

11.3.1. Peak height ratios

Intra-locus peak height ratios are calculated by dividing the lower peak height value by the higher peak height value and multiplying by 100. With single source samples, they represent percentages above which two heterozygous alleles can be grouped as a possible genotype.

Peak height ratios become lower with lower DNA amounts. When template DNA copy number is low, stochastic PCR effects (i.e., unequal signal from alleles in a heterozygote due to chance sampling) may result in substantial imbalance between alleles. Possible causes for peak height imbalance in single-source samples are degraded DNA, presence of inhibitors, low template, a mutation and preferential allele amplification. If the analyst suspects a mixture, this will be noted in their notes; if the imbalance is only observed at a few loci with no other indication of a mixture, the analyst's interpretation can be assumed to be a single source and no further documentation is needed.

While STRmix uses biological models to calculate expected and acceptable peak height ratios, general trends observed in internal validation studies (single source/Globalfiler/ 3500xl) may aid in estimating the number of contributors present in an unknown sample. Samples may fall outside of these ranges so should be relied upon within a framework of reason.

RFU range in sample	Observed peak height ratio
1 to 999	May be 40% or lower; this range may include complete drop-out of a sister allele in a true heterozygote pair
1000 to 9999	May be as low as 50%
10,000 and above	May be as low as 60%

With the use of STRmix major/minor contributors are no longer determined. However, the general concepts used during these interpretations can be helpful in determining number of contributors and evaluating genotype weights. Previously, mixture proportions of 80% or greater were deemed to be a major contributor. These peak height ratios are calculated by adding the peak heights of the contributor in question, then dividing by the total of all peak heights calling at a locus.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 98 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

11.3.2. Allele sharing

In interpreting a mixture of multiple individuals, the possibility of additive allele sharing must be considered. Allele sharing occurs when one peak may be from multiple copies of a single allele originating from different donors. It is more common at less polymorphic loci and with relatives. Allele sharing and stutter contribution may cause peak height imbalances within a mixture samples.

The probability of a higher order mixture (5 or 6 contributors) appearing as having originated from 1 or 2 contributors (based on allele count) is very small. However, the probability of a higher order mixture appearing as having originated from one fewer contributor (based on allele count) gets higher as the number of contributors increases. The risk of underestimating the number of contributors increases with stutter masking and the possibility of allelic drop out.
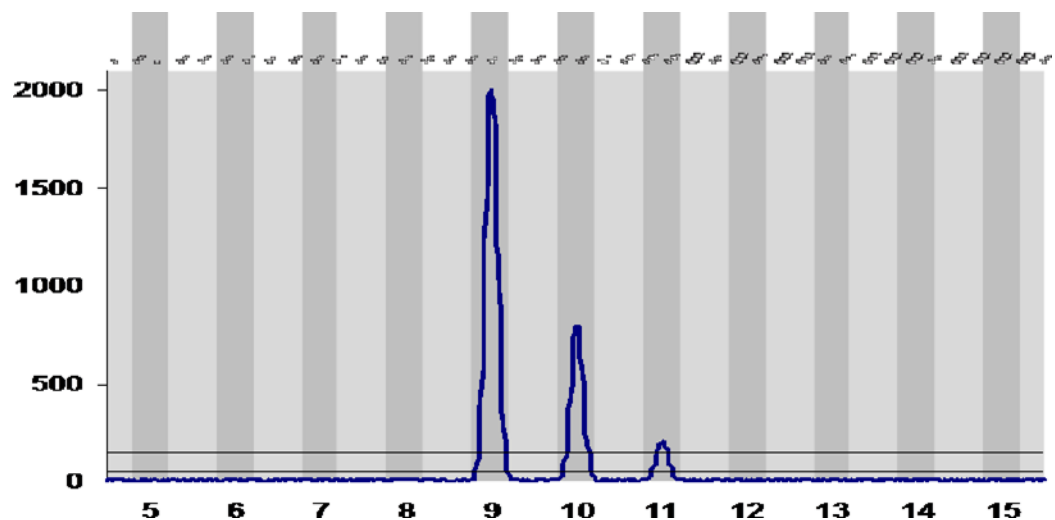
Figure 6.4.2 A: This represents a mixture with only three alleles detected, but where none of the alleles can pair exclusively with each other thus supporting a mixture of more than two individuals with allele sharing likely.

11.3.3. Stutter in mixtures

If a trace donor is evident in a mixed sample, a peak in the stutter position may either be a stutter peak, an allele peak, or indistinguishable as either; this does not mean the peak is "inconclusive" as it may still be used in reporting and interpretation. The analyst may need to analyze a sample with stutter filters on and off in order to properly account for these peaks when attempting to determine the number of contributors in an evidence sample. Peaks in the forward and back stutter positions (not associated with artifacts, i.e. pull-up) should never be edited out of the profile as STRmix uses these peaks when modeling the expected genotypes during analysis.

In samples where a peak in a stutter position is consistent with (or greater than) peak heights attributable to a minor donor, the peak in the stutter position will be evaluated as a potential allele peak. While STRmix is able to model stutter peaks, the analyst must still try to determine if peaks are masked in stutter positions in order to properly determine the number of contributors.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 99 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

11.3.4. **Low-level data**

Peaks below the analytical threshold may be observed and considered in the interpretation as information regarding possible number of contributors or support for troubleshooting options such as re-sampling. STRmix is able to model for drop-out and drop-in events based on validation data. However, it is important for the interpreting analyst to consider these types of events when determining the number of contributors in a profile. The assignment of the number of contributors has a direct effect on the genotype pairings done during STRmix analysis. The number of contributors must be supported by the empirical data.

11.3.5. **Assumed donors**

Greater resolution of mixture deconvolution is possible when an assumed contributor can be accounted for in a mixture. Assumptions of a specific contributor(s) to a DNA profile can be made under several different circumstances:

1) Intimate samples are biological samples from an evidence item obtained directly from an individual's body that can reasonably be expected to yield DNA types from the donor individual (e.g. vaginal swabs, oral swabs, body swabs, fingernails, etc.).
   Note: When comparing contributors from the separate fractions of a differential extraction, one fraction (e.g. a single source or major contributor) can be used as a "known" contributor to deconvolute a mixture in the other associated fraction. However, a statistic must always be provided for the probative portion of the sample.
2) Personal items such as clothing, bedding, toothbrushes, etc. may also have a reasonable expectation of donor contribution based on investigative information; however, these items should be used with the caution that multiple people, such as family members, may have come into contact with the item and cannot always be assumed to have a single contributor. For example, a victim may be assumed to be on her own clothing but the analyst must also consider other people could be on the same item.

12. STRmix analysis

This Standard Operating Procedure describes the use of STRmix™ for the interpretation of Globalfiler DNA profiles run on the 3500 within the SFPD Forensic Biology Unit. Refer to applicable version validation summaries and STRmix Users and Operation manuals for further information.

STRmix is a fully continuous software program for the interpretation of autosomal STR DNA profiling results. STRmix can deconvolute mixtures (break down into individual contributors/components) and attach statistical weighting to comparisons of reference profile(s) to evidence sample profile(s). This deconvolution of a DNA mixture into its component parts (i.e. contributors) is reported as a mixture ratio in the DNA report, detailing the proportion of which each component part contributes to the overall mixture. When comparing a reference DNA profile to the deconvolution report, STRmix generates a statistic reflecting the likelihood of that reference being a contributor to the evidence profile. This statistic is in the form of a likelihood ratio (LR) and applies the weights of the genotype combinations determined during the deconvolution. A high LR suggests inclusion of the reference profile while a low LR favors exclusion. A verbal scale is used to add context to the reported LR value. Because STRmix breaks down mixture profiles into its contributor parts, major

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 100 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

and minor profiles are no longer noted as part of DNA interpretation in the DNA unit at the SFPD Crime Lab.

12.1. General workflow

The general approach to profile interpretation in the SFPD Forensic Biology Unit is as follows:

1) The project-specific folder containing 3500xL CE run data (.HID files) is copied and pasted into the same folder. This will add "- Copy" to the end of the folder name. Rename it "STRmix". This only needs to be done the first time a case is written from the data set. Delete non-case related samples.

2) Open a new GeneMapper ID-X (GMID-X) project. Click on Edit > Add samples to project.

3) Import only the samples for the case from the appropriate run folder. Include reference samples and evidence samples; for evidence samples requiring STRmix analysis, also import a copy of the evidence sample from the STRmix run folder – this will be analyzed in GMIDX with the stutter filters off, using the appropriate STRmix settings. Alternatively, all evidence samples from a case can be imported to GMIDX from the STRmix run folder and deleted if STRmix analysis will not be performed on some or all of the evidence samples for a case.

4) Analyze references and original evidence data with stutter filters on to help estimate NOC.
   • Stutter filters on: Analysis Method "Globalfiler 150 RFU", Panel "Globalfiler_Panel_v1.1.1X)
   • GS600_LIZ (60-460) size standard

5) Analyze "STRmix" data with stutter filters off to allow STRmix modelling.
   • Stutter filters off: Analysis Method "Globalfiler 150 RFU-STRmix", Panel "Globalfiler_Panel_v1.1.1X-STRmix)
   • GS600_LIZ (60-460) size standard

6) Only data files that are being reported require edits (for artifacts such as pull-up, elevated baseline, spikes, etc.). This would include one of the two reference duplicates and either the evidence sample with stutter filter on or off.
   a. If STRmix is being run, then the evidence sample with stutter filters off will require editing.
   b. If STRmix is not being run (single source samples matching the assumed contributor, inconclusive samples), then only the sample with stutter filters on requires editing. It is okay to edit a sample with and without stutter filters on.

7) Electropherograms are not printed for the casefile and are reviewed using GMID-X. PDF versions of the printouts will be provided for discovery.

8) Samples not being used for analysis will have "_DNU" added to the sample name column in GMID-X. This will avoid duplicate names of samples when trying to input .txt file data into STRmix.

9) After analysis, export the evidence data by clicking the "Genotypes" tab, selecting the STRmix panel (Globalfiler_Panel_v1.1.1X-STRmix), then File>Export Table…, then save as a .txt file in the appropriate location within S:\Casework Documentation with the case number. A separate table for samples analyzed with and without stutter filters will need to be exported (references will not have stutter peaks when imported into STRmix).

10) To export the reference data for STRmix analysis and comparisons, select the reference run folder; go to the genotypes tab; highlight the Globalifler_Panel_v1.1.1X, click on

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 101 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

'File' in the upper right hand corner select 'export table'. Save the .txt file in the casework documentation folder with the case number.

11) The number of contributors is assigned and documented. All assumptions should be documented on the Evidence Sample Table. Any other notes that the analyst deems necessary for interpretation can also be added to Evidence Sample Table. Save the Evidence Sample Sheet to the appropriate folder in Case Documentation, and print for the casefile.

12) Suitable profiles will be interpreted using STRmix.

13) STRmix deconvolution reports are reviewed and results reported. This includes evaluating genotype weights, diagnostics, mixture proportions, and RFU values.

14) Likelihood ratios are calculated using STRmix and reported for references in the case. If inclusion for a single sample is supported for two or more references, then a separate LR will be calculated with both references as contributors to the same profile. The LR worksheet associated with this calculation will be included in the casefile, but not reported if inclusion is still favored. If exclusion is favored when the references are run together, a reporting statement will be included.

12.2. Analysis before running in STRmix

12.2.1. Analysis in Genemapper ID-X

At analysis, artifacts must be removed from the profiles. These include:

1) Dye blobs or spikes.
2) Pull-up or bleed through peaks.

Note: n-2, forward and back stutter will be modeled in STRmix and should be retained for STRmix analysis.

Note: Spikes may be auto-edited by GMID-X software and will not be included in the exported genotypes text file used for STRmix analysis. If this is the case, spikes do not need to be edited manually in GMID-X.

Note: Two base pair or half back stutter is two base pairs smaller than its 'parent' allele (N). For GlobalFiler™ this means N-2, and is only modelled in STRmix at SE33 and D1S1656.

Note regarding duplicate references: both references must be analyzed to verify alleles are consistent with one another; if one amplification shows enough data to confirm the other sample it does not need to be re-run if minor quality issues are observed. Both references need to be edited for STRmix.

12.2.2. Assigning number of contributors (NOC)

The assignment of the number of contributors to a profile is made by the analyst prior to the comparison of the profile to any reference samples. The potential of peaks to be stutter should be considered. The number of contributors chosen for the analysis should be the most likely number required to reasonably explain the observed profile.

Sometimes the number of contributors may be unclear. This could be because the profile is complex and may contain indications of additional contributors, allele sharing, or because case circumstance suggests that the possibility of further

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 102 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

contributors exists. Reporting analysts should use their professional judgement when assessing the number of contributors and may account for sub-analytical threshold (AT) peaks and high stutter. In circumstances when the number of contributors cannot be determined, the profile is reported as uninterpretable.

It is also possible for an analyst to make an assignment of the number of contributors and run STRmix™, then after analysis of the STRmix™ output – but prior to comparison with the reference profiles - determine that the deconvolution does not conform to scientific expectation. In this case, it may be re-run under a different number of contributors and the course of action should be detailed on the "Evidence Sample Table". The electronic file of the first deconvolution is kept in the casework folder.

The methods used by SFPD in assigning NOC are:
1. Maximum allele count
2. Peak height information
3. Visible peaks below analytical threshold

The following steps should be followed in assigning the number of contributors to a profile:

Review the profile as a whole, assessing the level of degradation, presence of low level peaks, noisy or clean baseline and general quality (template) of the profile.

Assess likely stutter peaks (both forward and back) by reference to SFPD laboratory's per allele stutter ratio expectations (plots of SR ~Allele). Find the locus with the highest number of unambiguously allelic peaks. If this number is odd, add 1. A/2 is the initial postulate of the number of contributors to the profile. i.e. THO1 has 7 alleles, (5+1)/2 = 3; three contributors would be the initial postulate.

Review peak height imbalances at the most informative locus (greatest number of alleles). Taking into account allele sharing or 'stacking', visually try to 'pair' alleles and assign to contributors. If there is too much imbalance between alleles this may mean the presence of an additional contributor above that indicated by allele count alone.

If one or more contributor at this locus is either minor or a clear major, check that this pattern is represented at other loci.

Apply the general pattern of contributors (number and proportion) to other loci in the profile. If it holds, assign this number of contributors to the profile, otherwise consider the addition or subtraction of one.

Justification for the chosen number of contributors must be documented in the Evidence Sample Table if reasoning goes beyond maximum allele count (i.e. peak height ratios, etc.).

Discriminating loci such as FGA, D21 and SE33 are likely to be the most informative when determining the number of likely contributors to a profile.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 103 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

The presence of one or two minor peaks may indicate the presence of drop-in and not an additional contributor.

NOTE: Peaks below the AT, but not in artifact positions, may be useful to inform the presence of low level or trace additional contributors. These are unlikely to affect the interpretation of the other contributors but could affect the analyst's ability to call a NOC.

12.2.3. Assessing suitability for STRmix analysis
STRmix should be used to interpret all qualifying profiles. A qualifying profile is defined as a single source profile or two, three and four person mixtures.

Exceptions to this include:
- If the evidence profile has failed SFPD QA standards.
- If the number of contributors cannot be reasonably assigned, document on the Evidence Sample Table.
- Greater than four contributors.
- If the inclusion does not provide probative value in the context of the case (for example an individual's own DNA on their intimate swabs or clothing).
  NOTE: For intimate samples that match the victim, non-STRmix GMID-X analysis parameters may be used.
- Samples with an assumed donor with only 1-2 autosomal alleles that do not belong to the assumed donor. There may be 1-3 male markers which would not be considered for STRmix analysis anyway. These cases will not be run in STRmix because they will never need an LR for the 1-2 alleles foreign to the assumed donor.

12.3. Setting up propositions/hypotheses
Many cases encountered do not have a reference from a "Person of Interest" (POI). In these situations, the deconvolution is still run to separate the mixtures into individual contributors, with assumed contributors added where possible. When references of POIs are available, propositions can be set up for obtaining an LR. The deconvolution should first be run with only contributors assumed in both hypotheses (example: victim on her own body swab) and then an "LR from previous" calculated for POIs.

The likelihood ratio assesses the probability of the evidence given two alternate propositions or hypotheses; one that aligns with the prosecution (Hp) and one that aligns with the defense (Hd). Hp is typically inclusionary of the person of interest. The propositions are set typically to minimize the LR. This can be undertaken by minimizing the number of unknowns within the different propositions.

The first hypothesis (Hp in STRmix) should be set up comprising the person of interest and known or unknown individuals up to the number of contributors (i.e. for a three person mixture Hp would be POI and two unknowns). The second hypothesis (Hd in STRmix) should consist of any assumed (or conditioned) and then all unknown individuals up to the number of contributors (i.e. for a three person mixture with no assumed contributors, Hd would be three unknowns).

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 104 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

Examples:
Two person mixture from intimate swab collected from C. DNA profile corresponds to POI and C.

Hp: DNA originates from C and POI
Hd: DNA originates from C and unknown

Three person mixture from firearm located at scene. One component corresponds to POI.

Hp: DNA originates from POI and two unknowns
Hd: DNA originates from three unknowns

Three person mixture from firearm located at scene. One component corresponds to POI1 and another to POI2. Three LRs may be calculated:

Hp1: DNA originates from POI1 and two unknowns
Hp2: DNA originates from POI2 and two unknowns
Hp3: DNA originates from POI1 and POI2 and one unknown
Hd: DNA originates from three unknowns

When setting up LRs, first run each reference individually. Any references that give an exclusion do not need further hypothesis testing combined with other references in the case. For example, if POI1 gives an inclusionary LR and POI2 gives an exclusionary LR, an additional LR does not need to be calculated for POI1 + POI2 in Hp. Inclusionary LRs for multiple POIs will be run and the electronic data maintained but not included in the report. For cases with multiple elimination and/or victim references that require numerous combined LRs, please consult with the DNA Technical Leader and/or supervisor before proceeding to run the combined LRs. Exclusionary LRs resulting from multiple POIs who are individually included may need to be further investigated (e.g. review of loci with LR =0) to see if the new information supports adjusting the number of contributors. If investigation supports an NOC adjustment, both interpretations and accompanying LR results will be reported.

In the case of a consensual partner in a sexual assault case, or another scenario where an assumed contributor and a second individual can reasonably be assumed to be present, perform STRmix analysis as follows:

Perform the STRmix deconvolution conditioned with the assumed contributor in Hp and Hd. Perform an LR from previous with the consensual partner (or other reference deemed reasonably expected to be present) compared. If the LR is greater than 1000 for all population groups supporting inclusion, rerun the analysis on the original sample and condition on the presence of both individuals in Hp and Hd, if necessary i.e. for CODIS deductions. If the LR supports exclusion or is below 1000 supporting inclusion, there is no need to rerun anything, report as appropriate.

Conditioning of a non-intimate sample with a victim/elimination reference may be done for CODIS purposes only. There are instances where a victim's profile is one of the contributors

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 105 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

of a mixture profile eligible for CODIS upload. For CODIS purposes, it is ideal to avoid uploading profiles associated with a victim/elimination reference to CODIS. Where an LR has been calculated that associates a victim/elimination reference to a CODIS eligible mixture profile with a likelihood ratio of 1 million or greater, the victim/elimination reference can be conditioned on to prevent its upload to CODIS.

12.4. Generating data in STRmix

12.4.1. Deconvolution of an evidence sample

1) Open STRmix™ software program.
2) At the home screen, select "Interpretation" to interpret a DNA Profile.
   a. Type in the case number, Sample ID and any case notes.
   b. The case number is the lab number (e.g. 18-0001) and the sample ID is the item number (e.g. 1A1AB_NS).
   c. If you have hypotheses for POIs, indicate those used in the "Case Notes" section.
   d. Select the number of contributors that has been assigned. Click "Next".
3) Add your evidence profile. If the profile has a tri-allelic pattern, exclude that locus.
   a. Profiles can be added by dragging and dropping the GMID-X export table text file in the "Evidence Profile Data" window and selecting your sample(s). Select a sample by clicking in the grey box next to the sample (a check mark should appear) and click "confirm".
   b. Alternatively, you can perform the following:
      i. Click on the green plus (+) symbol" under the 'Evidence Profile Data'
      ii. Click "Select" > Navigate to the edited GMID-X export table file (stored in the casework documentation case folder)
      iii. Double click on the text file
      iv. Select a sample by clicking in the grey box next to the sample (a check mark should appear) and click "confirm".
      v. Click "Start".
4) Add any reference profiles assumed present in the sample.
   a. Profiles can be added by dragging and dropping the GMID-X export table text file in the "Reference Profile Data" window and selecting your sample(s).

   Alternatively, you can perform the following:
   i. Click on the green plus (+) symbol under the reference profile data.
   ii. Click "Select" > Navigate to the edited GMID-X export table file (stored in the casework documentation case folder)
   iii. Double click on the text file
   iv. Select a sample by clicking in the grey box next to the sample (a check mark should appear) and click "confirm".
   v. Click "confirm".
   vi. Click "Add"
   vii. Click on the HD grey box and a check mark should appear.
   viii. Click "Start".
   ix. After the deconvolution is finished and you need to compare a POI.
   x. Click on "Investigate" and select "LR from Previous" and confirm sample information then click "next".
   xi. Click on the green plus (+) symbol" under the 'reference profile data'.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 106 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

xii. Click on the green plus (+) again.
xiii. Click "Select" > Navigate to the reference GMID-X export table file (stored in the casework documentation case folder).
xiv. Double click on the text file and select a sample by clicking in the grey box next to the sample (a check mark should appear) and click "confirm".
xv. Click 'Start'.

5) Once STRmix is done running, the interpretation results PDF will be created. Check the run diagnostics are within the expected ranges. Results are saved on the C drive of your computer in <C:\ProgramData\STRmix\Results>. Each results file needs to be moved to the appropriate folder for the case in <S:/Casework Documentation>.
6) Click "Finish" to return to home screen.

OR

7) If there is a person(s) of interest for comparison click on 'Investigate'.
   a. Click on 'LR from Previous'.
   b. Review the sample information and click on 'next'.
   c. Click on the green plus (+) symbol" under the 'Reference Profile data".
   d. Then click the green plus (+) symbol again under the 'Create Reference' box
   e. Click "Select" > Navigate to the edited GMID-X export table file (stored in the casework documentation case folder).
   f. Double click on the text file.
   g. Select the reference sample of interest by clicking in the grey box next to the sample (a check mark should appear) and click "confirm".
   h. Click "confirm".
   i. Click "Add".
   j. Click "Start".
8) Once STRmix is done running, the results pdf(s) will be created.
9) Results are saved on the C drive of your computer in <C:\ProgramData\STRmix\Results>.
10) The name of the sample used for comparison will be added to the end of the folder name automatically.
11) All folders need to be moved to the appropriate folder for the case in <S:/Case.

12.4.2. Perform an 'LR from a previous interpretation'
1) From the main STRmix menu, click on "Investigation".
2) Click on 'LR from Previous' and select 'Browse'.
3) Go to the casework doc folder to the case folder and select the deconvolution folder for the sample of interest.
4) Open the deconvolution folder and either select the 'Config' XML document or drag the entire folder into the 'reference profile data' box and click open.
5) Review the sample information and click on 'next'.
   a. Click on the green plus (+) symbol" under the 'Reference Profile data".
   b. Then click the green plus (+) symbol again under the 'Create Reference' box.
   c. Click "Select" > Navigate to the edited GMID-X export table file (stored in the casework documentation case folder).
   d. Double click on the text file.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 107 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

    e. Select the reference sample of interest by clicking in the grey box next to the sample (a check mark should appear).

    f. Click "confirm".

    g. Click "Add".

    h. Click "Start".

6) Once STRmix is done running, the results pdf(s) will be created.

7) Results are saved on the C drive of your computer in <C:\ProgramData\STRmix\Results>.

8) The name of the sample used for comparison will be added to the end of the folder name automatically.

9) All folders need to be moved to the appropriate folder for the case in <S:/Casework Documentation>.

10) Click "Back to Main Menu" in STRmix to return to the home screen.

12.4.3. Perform a deconvolution batch

1) From the main STRmix menu, click on "Batch Mode".

2) Click on the add to batch icon and select 'Add interpretation'.

3) Type in the case number, Sample ID and any case notes.

    a. The case number is the lab number (e.g. 18-0001) and the sample ID is the item number (e.g. 1A1AB_NS).

    b. If you have hypotheses for POIs, indicate those used in the "Case Notes" section.

    c. Select the number of contributors that has been assigned.

    d. Click "Next".

4) Add your evidence profile. If the profile has tri-allelic patterns, exclude those loci.

    a. Profiles can be added by dragging and dropping the GMID-X export table text file in the "Evidence Profile Data" window and selecting your sample(s).

    b. Alternatively, you can perform the following:

        i. Click on the green plus (+) symbol" under the 'Evidence Profile Data"

        ii. Click "Select" > Navigate to the edited GMID-X export table file (stored in the casework documentation case folder).

        iii. Double click on the text file.

        iv. Select a sample by clicking in the grey box next to the sample (a check mark should appear) and click "confirm".

5) Add any reference profiles assumed present in the sample.

    a. Profiles can be added by dragging and dropping the GMID-X export table text file in the "Reference Profile Data" window and selecting your sample(s).

    b. Alternatively, you can perform the following:

        i. Click on the green plus (+) symbol" under the reference profile data.

        ii. Click "Select" > Navigate to the edited GMID-X export table file (stored in the casework documentation case folder).

        iii. Double click on the text file.

        iv. Select a sample by clicking in the grey box next to the sample (a check mark should appear) and click "confirm".

6) Then click 'Queue'.

7) Repeat the above steps for all the sample(s).

8) Click 'Start Batch'.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 108 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

12.4.4. Perform an LR from previous batch
1) From the main STRmix menu, click on "Batch Mode".
2) Click on add to batch icon and select 'Add LR from previous batch'.
3) Click browse.
4) Drag and drop the deconvolution folder into the 'choose previous interpretations for LR' dialog box.
5) Review case information and click 'next'.
6) Double click on the text file.
   a. Click on the green plus (+) symbol" under the 'Reference Profile data".
   b. Then click the green plus (+) symbol again under the 'Create Reference' box.
   c. Click "Select" > Navigate to the edited GMID-X export table file (stored in the casework documentation case folder).
   d. Double click on the text file.
   e. Select the reference sample of interest by clicking in the grey box next to the sample (a check mark should appear).
   f. Click "confirm".
   g. Click 'Add'.
   h. Then click 'Queue'.
7) Repeat for additional deconvolutions and references.
8) Click 'start batch'.

12.4.5. Perform an investigation batch
Results for each deconvolution comparison with reference will include Comments detailing the samples being compared. Interpretations can be set up to run against one or more references for individual deconvolutions.

1) From the main STRmix user interface, click on "Batch Mode".
2) Click on add to batch icon and select 'Investigation batch'.
3) Click on the green plus (+) symbol" under the 'Interpretations' dialog box on the left side.
4) Navigate to the appropriate casework documentation folder and drag and drop all the deconvolutions of interest.
5) Click on the green plus (+) symbol" under the 'Investigations' dialog box on the right side.
6) Click on the green plus (+) symbol under the 'reference profiles' dialog box on the left side and click 'select'.
7) Navigate to the edited GMID-X export table file (stored in the casework documentation case folder).
8) Double click the text file.
9) Select the reference sample of interest by clicking in the grey box next to the sample (a check mark should appear).
10) Click Save.
11) Click Queue.
12) Click Start Batch.

OR

1) From the main STRmix user interface, click on "Investigation".

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 109 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

2) Click on "Investigation Batch"

3) On the left side under "Interpretations", drag and drop in deconvolution folders (as many as needed).

4) On the right side under "Investigations", click on the (+) sign. On the next page under "Reference Profiles", drag and drop in your Genotypes Table for references. Choose as many reference samples as needed. Hit "Confirm".

5) Once your reference samples are listed under "Reference Profiles", hit "Save". Those reference samples will be grouped together as "LRPrev_1". (Multiple groups can be created if needed. Further reference samples added will be grouped as "LRPrev_2" and so on.)

6) Once all selections have been made, hit "Queue".

7) This will bring you to the "Batch Mode" screen, which lists all combinations of samples that will be compared. The name of the file will have both the evidence sample and the reference sample listed. Check that all LRPrev combinations are listed. Those that are not needed can be removed.

8) Hit "Start Batch".

9) Once all batch calculations are complete, open up the results folder on the C-drive. Alternatively, you can also click on the folder icon in the upper right corner to open up the results folder. The LRPrev results will be listed with the same naming convention.

12.4.6. Generating LR worksheet from macro

1) Open "STRmix LRs 3.0" in STRmix folder on S drive.

2) Click "Click Me" button.

3) Select "Sample ID_Results".txt file from "LRPrev" results folder generated after STRmix LR from previous analysis.

4) The lowest value for 99.0% 1-sided lower HPD interval will be highlighted in teal and a result is selected (i.e. favor Hp, favor Hd, exclusion or uninformative).

5) Print for casefile. Save in appropriate Casework Documentation folder.

12.5.   STRmix settings
12.5.1.   Ignoring a locus in STRmix

The basis of STRmix™ is that given a set of mass parameters (DNA amount, degradation, and amplification efficiency) we can determine the expected height of a peak of a certain size, at a given locus. If there are reasons for the expected peak height to be incorrect or for issues with allele designation STRmix™ is unable to handle loci with these effects. Examples include:

1. Primer binding site mutations
2. Trisomies (trialleles)
3. Somatic mutations
4. Null alleles
5. Technical issues (loci compromised by potential pull-up or poor resolution such as a 9.3/10 at TH01 that is not resolved)

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 110 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

The "Ignore Locus" function should be used to ignore the affected locus from the deconvolution or during comparison (LRprev calculation); do not alter the GMID-X file. When using the ignore locus all reference profiles must be manually compared to the omitted loci. This is done within an interpretation at the "Add profile data" screen.

Click "Kit Settings" > "Ignore Loci" > Highlight the locus to ignore and move it to the right side with the ">" button > Click "Save" > Click"Save" and continue on with the run.

A record should be made within the comments box of the STRmix™ run specifying the locus omitted and the reason for the omission (ex: Run without TPOX due to presence of triallele).

12.5.2. Rare alleles

Non numeric values such as OL and < or > are not permitted within the STRmix™ input files. Unambiguous alleles including those that are rare should appear in the corresponding input file as their actual allelic size designation, for example D21 30.1, rather than OL. If alleles fall more than two repeat units off the end of the ladder they cannot be sized accurately or labeled correctly. These loci should be removed from the deconvolution using the Ignore Locus option. If an LR is calculated the POI should be manually compared at this locus to ensure this is not an exclusion. The electropherogram with calculation should be printed and placed within casefile.

12.5.3. Compatibility between different versions of STRmix

You are able to open files in v2.5 generated from analysis undertaken in previous versions of STRmix™. For example if you had undertaken a mixture deconvolution in v2.5 you are able to carry out a 'LR from Previous Analysis' in later versions by navigating to the relevant Settings file within the Results folder. This is relevant to reporting cases where the reference data is generated on 3500xls but evidence data may have been previously generated on 3130xl's and analyzed with STRmix v.2.5.

1) Launch STRmix
2) Click 'Investigation'.
3) Click 'LR from Previous'.
4) Click 'Browse'.
5) Navigate to the previous STRmix v2.5 deconvolution and select the "config_input" file. Click 'Select'.
6) Verify the case number, sample ID, case notes, and number of contributors. Click 'Next'.
7) Under the reference profile data, click the "+" sign, followed by the "+" sign again. Click 'Select'.
8) Navigate to the reference genotypes table generated from STRmix and select the .txt file.
9) Select the reference for comparison by clicking in the box to the left of the sample name.
10) Click 'Confirm'.
11) Cick 'Add'.
12) Cick 'Start'.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 111 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

a. The 'LR from Previous' report will be generated and stored on the C: drive.

13) Click 'Finish'.

12.5.4. Evidence and references analyzed in different kits

STRmix™ allows the user to calculate a likelihood ratio when the evidence (questioned) and reference samples are analyzed in different autosomal profiling kits. LRs will only be provided for those loci in common between the two kits. For example if you had a questioned sample analyzed with Globalfiler™ and a reference analyzed in Identifiler Plus™ then you would be able to provide a LR using the DNA sites in common. For this analysis both the questioned sample and the reference sample have to input from .txt files. This function also works with LR from Previous Analysis, provided .txt files are input. When selecting the kit in the STRmix™ entry screen, select the kit of the evidence sample. STRmix™ allows users to edit reference samples generated in different autosomal STR kits so that that may be 'stitched' together. During this 'stitching' process STRmix™ carries out a concordance check of designated allele(s) at those loci in common.

12.5.5. Mx priors for 2-person mixtures with low-level 2nd donor

If the STRmix mixture proportions do not reflect the observed data (i.e. electropherogram), the analyst may manually configure the mixture proportions using the "Mx Priors" feature which configures the mean and variance for each contributor and re-run the deconvolution. The mean contributor order should range from largest contributor to smallest unless conditioning on an individual in which case they are Contributor 1.

1) Review data to manually calculate an approximate mixture proportion
2) Select "Interpretation" from the home screen
3) Enter case number, sample ID and number of contributors.
4) Select "Run Settings"
5) Check the box under "MX PRIORS" that reads 'Use Mx Priors'. This will bring you to a screen called "Configure Mx Priors".
6) Select Contributor 1 from drop-down box.

7) Slide the "mean" bar to select the mean of the Mx range you manually estimated from the data. E.g. if initial STRmix report shows a proportion of 70:30% but manual estimation is 90:10%, slide the mean bar to the right to 0.9.
8) Slide the "variance" bar to 0.0078125.   Click 'Confirm' then 'Apply'
9) In the MX PRIORS dialog box click the 'Edit Mx Priors' and repeat configuration of mean and variance for Contributor 2 and click 'Confirm and 'Apply'

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Published: 03/18/2022
Page 112 of 168
Effective date: 03/18/2022
Replaces published version: 12/20/2021

10) Ideally there should be no overlap between contributors.
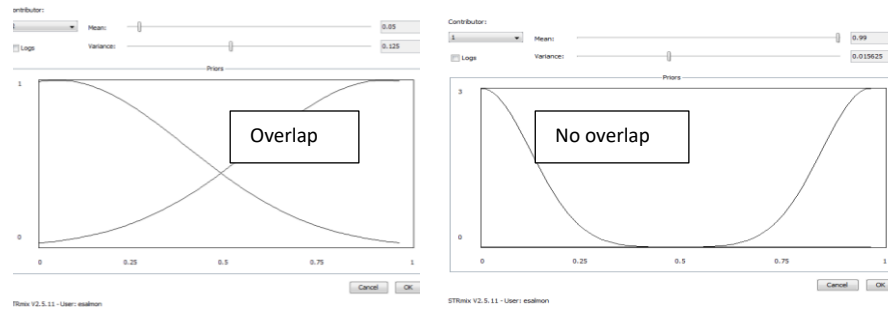
12.5.6. STRmix run settings and parameters

All settings and parameters will be set according to the STRmix validation studies performed by the Forensic Biology Unit of the SFPD Crime Lab. These include DNA profiling kit settings, population settings, and STRmix configuration default settings. These will not be adjusted during routine analysis and can only be accessed by system administrators.

12.6. Review of STRmix output

12.6.1. STRmix results

On completion of the interpretation, a configured Run Report will be generated and saved in pdf format.

12.6.2. Review of run diagnostics

In the output report from STRmix™ there are numerous diagnostics that may indicate that a deconvolution has not completed as expected: Primary diagnostics can be intuitively approximated by an experienced analyst. Secondary diagnostics diagnostics need to be evaluated based on STRmix output rather than intuitive knowledge.

Diagnostic	Type	Description	Comment/Troubleshooting
Weights	Primary	A probability that reflects how well a particular genotype combination explains the evidence profile.	
Mixture proportions	Primary	The mean of all accepted template amounts throughout the post-burn-in MCMC.	If approximate percentage of each contributor to the sample does not fit data, assess with Mx priors.
Per locus LRs	Primary	For each locus, the probability for the evidence given H1 (H_p) and H2 (H_d) are individually listed, as well as the ratio of the two probabilities (LR).	
Allele and stutter variance	Secondary	Describe how variable the allele and stutter	Can be used as a guide to level of stochastic variation in peak heights present in the profile. Used in

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 113 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

		peak heights are within the run	conjunction with low average log(likelihood), a large variance or stutter variance constant can indicate poor PCR. Check sample quality, NOC, editing (alleles/stutter removed; artifacts left in).
Gelman-Rubin convergence	Secondary	Informs user whether MCMC chains have converged; calculated by comparing the within-chain and between-chain variances of the MCMC chains.	For a fully converged analysis, the GR will be 1. If the GR is above 1.2 the analysis may not have converged and should be scrutinized. Note on the evidence sample table the reason the analyst is accepting the GR >1.2 e.g. complex mixture of 4, low level 2nd contributor, etc.
Average log(likelihood)	Secondary	Average $\log_{10}$ (likelihood) for the entire post burn-in MCMC.	A negative value suggests that STRmix™ has not been able to describe the data very well given the information provided. Low average log(likelihood) values alone are not necessarily an indicator of an issue especially if the profile is single source or low template. Check NOC, editing (alleles/stutter removed; artifacts left in).
Total iterations	Secondary	Total number of proposed genotype combinations made during the MCMC process.	See "Acceptance rate".
Acceptance rate	Secondary	Total number of accepts during the MCMC process, divided by total iterations.	A very low acceptance rate (e.g. 1 in thousands to millions) may, in combination with the other diagnostics, indicate that the analysis needs to be run for additional iterations.
Effective sample size	Secondary	Number of independent samples that have been taken from the posterior distribution of the MCMC likelihood.	A low absolute value of ESS (e.g. 10s or 100s) will mean that there is potential for a large difference in weights if the analysis was run again. A low ESS on its own is not an indication that rework is required.

Note: These and other term associated with STRmix analyses can be found in the STRmix glossary.

12.6.3. Review of genotype weights

Where possible, the weights generated by STRmix™ should be assessed at each locus for their intuitiveness.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 114 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Guidance on assessing intuitiveness:

1) Do the mixture proportions fit with the observed data? For example, in a mixture with one donor here the major is very high and only a few minor alleles are present, the mixture proportions would be ~99:1 or 95:5, depending on the data.

2) Are the top choices what you would expect given the observed data? While assessing this, keep in mind potential stutter, the drop-in cap of 300 rfu and other mass parameters incorporated into the process.

3) As you evaluate the profile and weights together, determine which combinations you would expect as contributor 1, 2, etc. A review of the STRmix weights should reveal a similar choice. Depending on the proportion of the contributor peak heights, along with STRmix estimates of stutter and drop in/out, there might be slight differences in the order of the combinations, however, the STRmix weight assignments should be logical.

4) Do the low level combinations with Q alleles make sense with the data? For example, when a low level minor is being deduced where dropout of both alleles could be possible, is Q,Q an option and does the weight make sense based on the proportion of that contributor and it's expected RFUs? Note: Q = refers to an allele other than the called alleles. Example: locus with alleles 11, 12, 13, and 15 may have a "15, Q" genotype which means a 15 with any allele other than 11, 12, 13, or 15.

5) If positive LRs are obtained for all loci except one where the LR is 0, the analyst should scrutinize the input file and weights to ensure no STRmix input errors were made. If an error is identified, the deconvolution/LR from previous should be re-run as needed. If no error is identified, the analyst must review the interpretation of the data and assumptions to see if the discrepancy can be reasonably attributed to an amplification or genetic anomaly. In these cases the analyst must clearly document this observation and omit the locus from the STRmix interpretation.

12.7. **Technical review of STRmix results**
As part of the technical review of the case, the STRmix™ Run Report must be reviewed.

This includes:
- Ensuring that the settings used were correct.
- The population(s) and kit types selected were the same as the evidence input,
- The assumed number of contributors and any other assumptions made are documented and supported.
- The run diagnostics are checked.
- The profile and STRmix weights are evaluated and the reviewer should be in agreement with the reporting analyst. Any changes recommended and made should be documented and visible within the case notes.

13. Statistics
Any inclusionary statements on probative samples, irrespective of the number of alleles or loci used, must be accompanied by a statistical evaluation of the weight of the inclusion as relevant in the context of the case. Profiles with a reasonably assumed contributor do not require a statistical

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 115 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

evaluation. The assumption of a specific donor must be stated in the report unless the sample is an intimate sample taken directly from an individual. A statistical evaluation may be performed at a later date if situations surrounding the evidence change (e.g. case information changes, a request is made, etc.).

Population frequency estimates from published NIST data will be used for profile frequencies and will be documented in the notes section of the report. Profile frequency estimates are calculated (but not always reported) for the following population groups: Caucasian, African American, Southwest Hispanics and General Asian. Given the number of different reported ethnic population groups, it is not feasible to report frequency estimates for every population group.

13.1. Random match probability (RMP) for legacy cases

The probability of randomly selecting an individual from an unrelated population who could be a potential contributor to the evidentiary profile. Random match probability and modified random match probability will only be calculated in supplemental reports where a DNA profile was interpreted under previously published non-STRmix guidelines and a reference sample has been submitted for comparisons.

The RMP inherently includes an assumption of the number of contributors. This calculation may be applied to single-source profiles (as typed or as deduced) or to mixtures as a "modified RMP" that considers alternate genotypes at a locus. When an interpretation is based on the assumption of one donor (typed or deduced), the formulae used for the RMP are those described in NRC 4.1, 4.2 and 4.4. An RMP analysis is performed by calculating the heterozygous or homozygous frequency at each locus then multiplying the resulting frequencies together using the product rule. The final number is expressed as a probability and is reported as "1 in" the number calculated for each ethnic population.

The modified RMP follows the same statistical formulae and process described for the RMP, but first sums the individual frequencies for the possible genotypes at each locus prior to applying the product rule. When an interpretation results in a single possible genotype at one locus, and multiple possible genotypes at another locus, these frequencies will be multiplied together according to the product rule. Due to the application of theta for homozygotes, avoidance of double-counting of genotype frequencies should be made; however, a calculated RMP may occasionally exceed 1.0. In these instances, the analyst will report the value as 1.0.

13.1.1. Formulae for RMP statistical analyses

1) **Heterozygotes:**
   Genotype frequency for a heterozygote is estimated, as suggested by NRCII 4.1b, using twice the product of the estimated frequencies of the two alleles. The formula for heterozygote genotypes is **2pq**.

   Example: Genotype frequency of the alleles (22, 24) in population X at locus Y
   Locus Y allele 22 frequency in population X = .2250
   Locus Y allele 24 frequency in population X = .1861
   Locus Y (22, 24) genotype frequency = 2(0.2250) (0.1861) = 0.0837 = 8.4%

2) **Homozygotes:**

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 116 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

Genotype frequency for a homozygote is estimated, as suggested by NRCII 4.4a, using the formula $p^2 + p(1-p)\theta$ where p represents the estimated allele frequency and theta is a measure of potential population subdivision. A theta value of 0.01 is typical for most situations. A theta value of 0.03 is reserved for smaller, isolated populations (such as the Native American population).

For loci where a single allele is present and zygosity is in question because of stochastic effects, the analyst must consider drop-out of a possible sister allele and account for that in their statistical analysis. Acceptable equations to account for possible drop-out of a sister allele are **2p** or **2p - p²**; the latter calculation utilizes the 2p calculation described in NRCII 4.1 and is further refined by subtracting out a double-counted homozygote. Alternatively, the analyst may use the equation **p² + p(1-p) θ + 2p(1-p)**.

3) **Minimum allele frequency:**
   A lower limit for the frequency of rare STR alleles (i.e., alleles with fewer than five observations in the population database) produces a conservative frequency. This 'minimum allele frequency' alleviates potential concerns of underestimation. The minimum allele frequency is calculated using the following expression: **5/2n** where n represents the number of individuals in the population database.

4) **Multilocus profiles:**
   The multilocus genotype is calculated by multiplying the frequency estimates of the individual (or summed per locus) STR genotypes; this is referred to as the "product rule". Independence, or equilibrium, is expected for genetic markers located on different chromosomes or far apart on one chromosome, in accordance with Mendel's Law of Independent Assortment.

5) **Example calculations:**
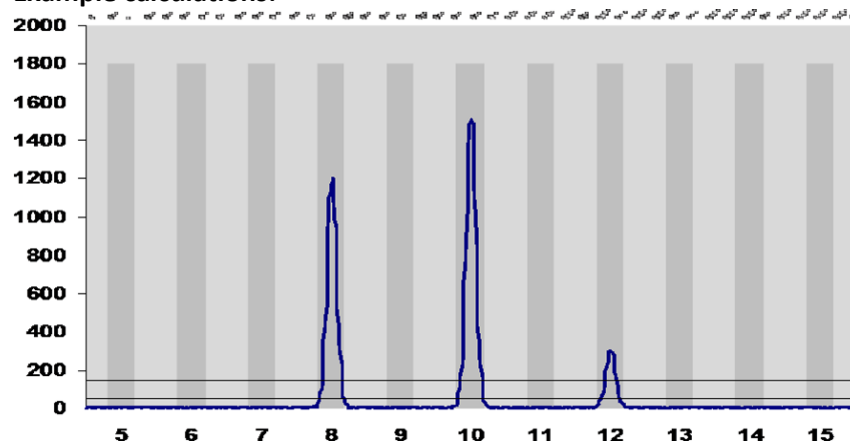
This profile represents a sperm fraction mixture. Assuming two contributors, one of whom is the victim 8, 10, the sperm contributor genotype could be 8, 12 *OR* 10, 12 *OR* 12, 12. In this case, the modified RMP for the sperm contributor would be calculated as **2pr + 2qr + [r² + r (1-r) theta]** where "p" is the frequency of the 8 allele, "q" is the frequency of the 10 allele and "r" is the frequency of the 12 allele.
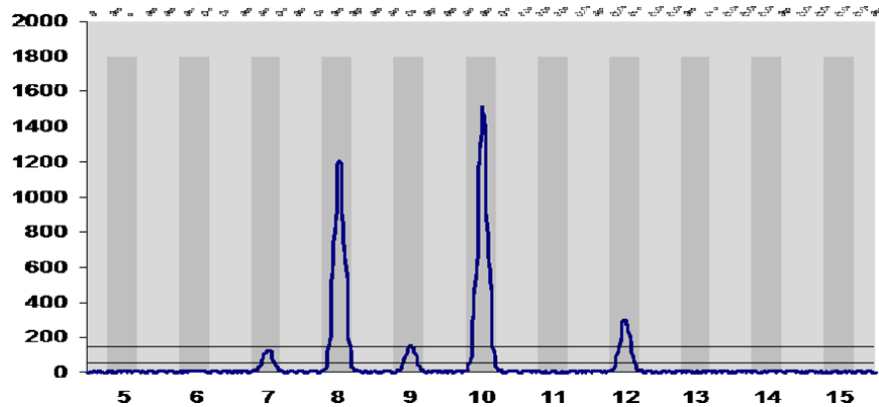
Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 117 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

This profile represents a sperm fraction mixture. Assuming two contributors, one of whom is the victim 8, 10, the sperm contributor genotype could be 8, 12 *OR* 10, 12 *OR* 12, 12. However, in addition to these genotypes, possible sharing with a peak in the stutter position should also be considered. In this case, the modified RMP for the sperm contributor would be calculated as **2pr + 2qr + [r² + r (1-r) theta] + 2sr + 2tr** where "s" is the frequency of the 7 allele and "t" is the frequency of the 9 allele.
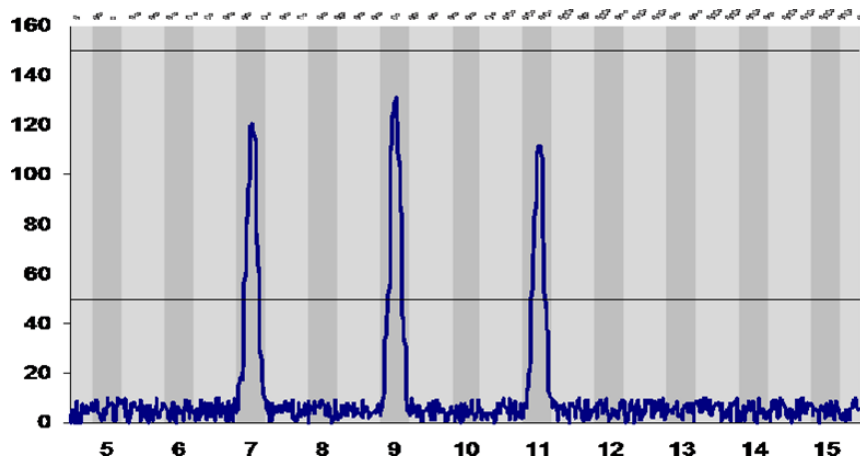
This profile represents a mixture of at least two individuals where all three alleles are below a theoretical stochastic threshold of 150 rfu. Assuming only two contributors, the interpretation must include: heterozygote/heterozygote pairs with and without possible drop-out of a fourth allele, as well as heterozygote/homozygote pairs with no drop-out. Simplified, the formula would be **(2p - p²) + (2q - q²) + (2r - r²) − 2pq − 2pr − 2qr** where "p" is the frequency of the 7 allele, "q" is the frequency of the 9 allele and "r" is the frequency of the 11 allele and double counting is subtracted. This could be reported as "7, any", "9, any" and "11, any" meaning that the alleles could be paired with "any" other allele.

13.1.2. Armed Xpert calculation of RMP and mRMP (Legacy cases)

ArmedXpert will be used only for supplemental reports where a DNA evidence profile has been interpreted using ArmedXpert at a previous date and a reference has been submitted for comparisons. In these cases, a random match probability (RMP) or

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 118 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

modified random match probability (mRMP) will be calculated using the previously interpreted evidence profile. If a reference cannot be excluded an RMP or mRMP calculation will be done in ArmedXpert as follows:

1) Open the ArmedXpert RMP input template.
2) Enter in all alleles present at each locus for the interpreted evidence sample that is being used for comparisons. This will be based off of allele table in original report that contains the interpreted results. i.e. if locus is an 11,11 or 11,12 or 12,13; enter 11, 12, 13 at that locus in the template.
3) Once full profile has been entered into template, "save as" a text file with a case specific identifier. This will be used as the ArmedXpert input.
4) Open ArmedXpert software.
5) Import saved text file. (ArmedXpert icon > Import > Other > Select saved text file.
6) Under interpretation tab, click on RMP.
7) Select allele calls for each locus based off of the allele table in the original report.

After all data has been properly entered, the ArmedXpert calculation is saved and the accompanying worksheets are printed off. The calculation is reported for each population group in the supplemental report. This calculation can only be performed and technically reviewed by individuals qualified/previously qualified in the use of ArmedXpert and random match probabilities.

13.2. Likelihood ratios

The SFPD provides weight for all comparisons using a likelihood ratio. This likelihood ratio is calculated by the STRmix software. This likelihood ratio is a calculation dependent upon the evidence profile and comparison reference profile(s), and compares the probability of observing data under two or more alternative hypotheses.

It is often expressed as $\frac{P\ (E|H1)}{P\ (E|H2)}$ where E = evidence, H1 = hypothesis 1, and H2 = hypothesis 2. It is verbally expressed as the probability of the evidence given hypothesis 1 [divided by] the probability of the evidence given hypothesis 2.

The LR for the profile is produced by multiplying the LR values from each locus. The resultant value is the ratio of the probabilities of the evidence profile if the inclusionary hypothesis is true as opposed to if the exclusionary hypothesis is true. The LR for mixtures is conditioned on an assumed number of contributors and when possible uses peak height information when including genotype combinations for proposed contributors. The LR requires that hypotheses be formulated based on relevant case information (i.e. case circumstances, individuals involved, evidence type or source). These hypotheses may involve different assumptions and should be evaluated and documented in the casefile. The LR calculated with STRmix utilizes the weights of a given genotype to calculate a weighted LR. This type of LR best utilizes the available data for a given evidence sample.

The formulae for homozygote and heterozygote loci calculations were developed using the population genetic model described in Balding and Nichols (equations 4.10a and b from

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 119 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

NRCII). θ is the F_{ST} value. Minimum allele probabilities are calculated using the third calculation below.

Heterozygote loci	$\dfrac{2[\theta+(1-\theta)p_i][\theta+(1-\theta)p_j]}{(1+\theta)(1+2\theta)}$
Homozygote loci	$\dfrac{[3\theta+(1-\theta)p_i][2\theta+(1-\theta)p_i]}{(1+\theta)(1+\theta)}$
Minimum allele frequency	$\dfrac{x_i+\frac{1}{k}}{N_a+1}$

13.3. Parentage and Kinship Index

The parentage/kinship index is a likelihood ratio to determine how likely it is that the alleged parent/relative is the true parent/relative versus an unknown individual selected at random and is calculated as follows:

The formulas used by the SFPD Forensic Biology Unit are contained within FBI's Popstats software program. All pertinent information can be found in the CODIS 9.0 Functional Specification: Popstats manual. Paternity and sibship formulas can also be found at http://dna-view.com/patform.htm and http://dna-view.com/sibfmla.htm.

14. Reporting guidelines

14.1. Standard casework

Typically, the following information is described when reporting profiling results:
1) A general description of the DNA profile (single source/mixed).
2) Assumptions used if any, including the minimum number of contributors.
3) The hypothesis that is supported when an LR has been calculated.
4) The likelihood ratio, using the lowest (most common) 99% HPD LR. In some rare cases, the point LR will be reported if the HPD LRs are different by more than one million (after reviewing data for the likely cause).

Mixture proportion	Reported value
<0.1	Report number as is
0.1 - 0.49	To the nearest tenth
0.5 and above	To the nearest whole number

Example Mixture proportion	Reported
0.02	0.02
0.25	0.3
0.49	0.5
0.51	1
2.32	2

Only report 0 if reported by STRmix as 0. Numbers should not be rounded to 0.

5) For samples where multiple NOC's are considered for interpretation and all STRmix runs meet quality control standards, report the lowest LR and add a note that more than one number of contributors was evaluated in the analysis.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 120 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

6) For samples where one of the NOC's considered is run but does not pass quality control standards, add a note explaining why data won't be used. Report the LR for the run that passes quality control standards.

7) All samples are analyzed using STRmix with the exception of the following profiles:
   • Single source profiles of assumed contributors.
   • Profiles with one or two additional alleles not consistent with the assumed contributor.
   • Profiles that are either too complex or too limited to confidently determine number of contributors.

General Closeout Procedures

After report has finished the review process, it can be provided to the investigator. This is most easily done via email. Once the report has been provided, the analyst will sign and date the review checklist section acknowledging that the report was provided to the investigator. Casefile can then be given to a supervisor so that appropriate data can be logged in the various tracking systems (Justice Trax, SAFE-T, SAEK tracking site, WP return, etc.) before the casefile can be filed.

14.2. Supplemental casework

Cases where new evidence is submitted for testing, STRmix will be used for interpretation and the STRmix supplemental report template will be used for reporting.

Cases where a reference is submitted for comparisons, the supplemental report template used to issue the report and the associated statistics will depend on which template was used for the original report. For example, if the original case was interpreted using legacy guidelines in ArmedXpert, then the supplemental will be issued using the appropriate template and RMP or mRMP statistics.

For supplemental reports, if a second reference sample is submitted, the new reference item will be compared to evidence items from the first round of testing and the results of the new comparison reported in a supplemental report. If mixtures are reported/associated in first report, comparisons for supplemental requests are reported by continuing the LR table from the first report.

Discordant loci (not applicable with parentage index cases)

If a locus was determined to be interpretable (for example a major profile was determined, deduced profile determined, or all alleles were determined to be present) and subsequently during comparison with a reference sample (either a known sample or a convicted offender sample), a discordance is observed at one locus, the locus may be deemed inconclusive and removed from the interpretation and/or statistical calculations. For this to be allowable, there must be a scientific reason for the discordance. Reasons that may cause such a discordance may include (but are not limited to) unexpected preferential amplification leading to peak imbalance, a peak in stutter position, allelic dropout where it was not expected, inability of analysis software to distinguish separation of peaks 1 base pair apart, or shared alleles. Any post-comparison revisions to the interpretation must be clearly documented in the case record and shall include detailed scientific reasoning for the revision, and the date of the change. The post-comparison revisions must be reviewed by a

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 121 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

second qualified scientist and must be approved by the Technical Leader (or designee). These records and reviews will be made a part of the permanent case record.

If there is a discordance at more than one locus, the profiles shall be reported as an exclusion.

See "Standard casework" section for rounding rules.

14.3. **Parentage and Kinship cases**

The SFPD Forensic Biology Unit will perform parentage/kinship comparison and statistical analysis cases for the SFPD and SF Office of the Medical Examiner upon request. Kinship cases will not be eligible for CODIS upload.

If a relative of a suspect is offered as the possible source of an unknown profile that matches the suspect, every effort should be made to obtain a known sample from the relative for a direct comparison.

Samples submitted may include blood stains (e.g. from the decedent), products of conception and buccal swabs from a close biological relative(s) and/or parent/alleged parent. Calculations are performed using CODIS PopStats at the CODIS terminal. Formulas used are generally accepted for paternity and kinship; mutation rates are from the American Association of Blood Banks. Allele frequencies used are NIST 2017 (default set in Popstats). Tested relationships will be limited to those available in PopStats; pedigree analysis will not be performed.

Samples will be analyzed following current procedures in the SFPD Forensic Biology Section. Samples submitted for parentage/kinship that yield mixtures may be ineligible for comparison unless assumptions can be made to deduce a single source/obligate alleles (e.g. product of conception with DNA from mother present). If a mixture is obtained from a product of conception confer with the Technical Leader. See "Standard casework" section for rounding rules.

"Invalid Alleles" – As soon as you enter an invalid allele, a red circle with a white X inside will appear next to the allele value. If you hover your mouse over the locus name, you will see information regarding the locus including a list of valid alleles from smallest to largest. Most likely, the allele you've entered is beyond this listed range and a < or > symbol will be used. These upper and lower limits, with < and > signage, are listed in the valid allele list. If your allele is within the bounds but not listed, confer with the Technical Leader about omitting the locus.

Procedure:

Examine the data from the known parent and child to determine the obligate alleles contributed by the unknown parent. Absent mutation, the known parent and child should share one allele at all loci. Mismatches (due to possible mutations) are allowed at 1 or 2 loci; if mismatches are present at 3 or more loci the alleged parent can be excluded as being the parent of the child.

a. For heterozygote loci in the child, the non-shared allele shall be attributed to the unknown parent.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 122 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

    b.   For heterozygote loci where the known parent could share either allele, both alleles could be attributed to the unknown parent.

    c.   For homozygote loci, the allele (above stochastic) shall be attributed to both parents

    d.   For single alleles below the stochastic threshold, if the obligate allele could be missing by drop-out then the loci will not be used in the comparison or calculations.

If the individual cannot be excluded as a biological relative, a parentage index or PI (paternity or maternity index) is calculated. The PI is a likelihood ratio based on two conditional probabilities: the probability of the child's genotype given the genotypes of the known and alleged parents, on the condition that the alleged parent is the other biological parent against the probability of the child's genotype given the known parent on the condition that the alleged parent is biologically unrelated to the child.

PI = $\dfrac{\text{P of genotypes observed given known and alleged are parents}}{\text{P of genotypes observed given the alleged is not the parent}}$

Parentage Trio – This mode uses a known parent along with the known biological child and a third person (not biologically related to the known parent) to assess the relative probability of the assumption that the third person is the biological parent of the child. This mode allows one or two mutations to exist between the alleged parent and child.

Reverse Parentage – Two known parents and an alleged child are tested in this mode to assess the relative probability of the assumption that the child is the biological child of the two known parents. PopStats in this mode does not allow mutations to exist between the alleged parent and child. If this is observed, confer with the DNA Technical Leader. The relationship of the parents is irrelevant to the statistics, per http://dna-view.com/glossary.htm#incest.

Single parentage – This uses a known and unknown sample to determine if they are a parent/offspring relationship pairing. This mode gives the relative probability of the relationship of the alleged parent and child. This mode allows one or two mutations to exist between the alleged parent and child.

Kinship – For kinship cases, two questioned samples will be entered into PopStats and a comparison performed. A visual comparison between genotypes/loci is not performed.

Trio/Reverse Parentage
1. Log onto CODIS computer and select Analyst Workbench.
2. Select "Popstats" on the menu bar on the bottom left side.
3. Manually enter SpecID (incident number/ME case number and sample name)
4. Select Parentage in the PopStats Calculations menu.
5. Ensure that the prior probability is equal to 0.5.
6. Select "Trio" for alleged parent scenario and "Reverse" for missing child scenario.
7. Manually enter all the alleles for each locus to be used in the calculations.
8. Any locus with a single allele observed at or below the stochastic threshold will be marked as inconclusive and not used for the calculations.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 123 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

9. Select Calculate. Use the default mutation rate and mean power of exclusion.
10. Print the parent/reverse parentage statistics report.
11. Population groups calculated will be African American, Caucasian, Hispanic and Asian
12. The Total Parentage Index for the Combined Population Group will be reported with appropriate verbal qualifier.

Kinship/Single Parentage

1. Log onto CODIS computer and select Analyst Workbench.
2. Select "Popstats" on the menu bar on the bottom left side.
3. Manually enter SpecID (incident number/ME case number and sample name)
4. Select Kinship in the PopStats Calculations menu.
5. Manually enter all the alleles for each locus to be used in the calculations. Kinship Parent-Offspring child profile is entered on left side and parent is entered on right to allow proper calculations for Single Parentage and Kinship.
6. Select the appropriate kinship relationships to test (or leave all selected)
7. Select Calculate. Use the default mutation rate and mean power of exclusion.
8. Print report for the Kinship statistics summary or single parentage.
9. Population groups calculated will be African American, Caucasian, Hispanic and Asian
10. The Total Parentage Index for the Combined Population Group will be reported with appropriate verbal qualifier.

Conclusions:

The three possible conclusions of the tested hypothesis are exclusion, inconclusive, or inclusion.

Mutational events must be accounted for in parentage/kinship investigations. According to the American Association of Blood Banks, the "two exclusion" rule is typically accepted with regards to exclusion in a parentage/kinship case. This means that if one to two loci do not match between the Reference and Evidence, the Evidence still cannot be excluded as being the true (Sibling/Parent/Offspring/other relation) due to the instance of naturally occurring mutational events.

For kinship analysis without an extensive pedigree, the results for relatedness will be qualified as either inclusion or inconclusive. This is based on inheritance patterns of siblings from their parents and the potential for full siblings to share 0 alleles at a locus. PopStats calculations cannot account for the possible linkage of D12S391 and vWA, the locus with the strongest statistical weight will be used in the calculations.

Exclusion – occur when obligate alleles at more than 2 loci are not consistent between a parent/child combinations.

Inclusions – occur when obligate alleles at 2 loci or less are not consistent between parent/child combinations. The analyst should bear in mind the strength of the inclusion based upon the PI result.

Inconclusive – follows laboratory current procedures in the SFPD Forensic Biology Section.

14.4.   General description of reports

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 124 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Use the wording and formatting in the applicable report templates to properly describe results. With the team workflow, the report author may be different than the person/people who performed the lab work. The report author is required to review the test record and document the review. All reports must follow the "Test Report" requirements outlined in the SFPD Quality Manual. In addition to elements required in the SFPD Quality Manual, Forensic Biology reports shall include a description of the technology (i.e. STR analysis), amplification system (i.e. name of PCR kit with loci described in SOP) and a quantitative/qualitative interpretive statement (i.e. weight of conclusion). For names that warrant anonymity, the first name and initial for the surname should be listed in the report; this includes victims, consensual partners and elimination samples.

The eligibility and/or suitability of the sample for CODIS upload with supporting documentation in maintained in the file. The report must indicate whether or not appropriate samples have been uploaded to CODIS. The report will reference the CODIS upload was performed in accordance with state and national regulations and that regular searches will be performed. The report will inform the client that notification will be issued if there is a hit in the database or if the profile(s) is/are removed from CODIS at any time in the future.

The lab may also issue supplemental and amended reports. Supplemental reports will be issued when additional evidence or references have been submitted for a case that has previously been processed. Each round of new testing after a report has been issued will be accompanied by an appropriate supplemental report. References will be compared to any interpretable profiles developed during testing of that case. Amended reports will be issued if an error has been detected after a report has been issued that affected any reported result. Changes in the amended reports will be clearly stated for the customer.

14.5.   Profile comparison

A comparison should be made between DNA profiles generated from evidence samples and those of the reference samples from the relevant case and a likelihood ratio will be calculated.

A reference statement needs to be included in reports if there is no comparison statements; this includes scenarios where all samples were terminated at quant, no STR results obtained, or results are inconclusive.

If there are multiple single source profiles originating from different contributors, each should be differentiated. i.e. Two unique single source male profiles are developed, "(unknown male #1)" and "unknown male #2) can be added to the reporting statement to help differentiate between the two male profiles.

Secondary/Alternate reference samples:
If an evidence item is indicated to be from a certain individual by the investigator, and yields a single source profile (determined by visual exam of electropherogram, not through STRmix), it can be used as a secondary reference accordingly.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 125 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

If the secondary reference sample yields a <u>mixture</u>, confer with the DNA Technical Leader to get approval to run STRmix. Genotype probabilities of 99% or greater can be used as a single source profile for comparison purposes. The profile can be manually entered into the reference text template used for QAdb hits.

<u>Evidence-to-evidence comparison:</u>

Upon request and approval by the DNA technical leader, comparisons can be made between single source evidence samples and other evidence samples within the case. In these cases the single source evidence sample will be treated as a reference sample when performing the comparison. A likelihood ratio will be calculated for these types of comparisons. Example: A single source profile from a cigarette butt is obtained. There are also evidence blood stains and touch DNA gun swabs in the case that yielded mixtures. The unknown profile from the cigarette butt will be compared to the mixtures and a LR will be calculated. These results will be included in the DNA report.

The intention of this use is for special investigation or by request, NOT as a mandatory application for every case because it would not be useful in every case. e.g. 10 different unknown blood samples from a scene and one mixture. It does not make sense to compare all 10 unknown samples (i.e. converting all to txt files, comparing to the one mixture and producing LRs for them all) without guidance from the investigator.

A likelihood ratio does not need to be calculated for a reference provided it meets any of the following:
- Samples where no STRmix deconvolution was done (i.e. inconclusive samples).
- Single source samples where a manual exclusion can be made (this also applies to samples where a STRmix deconvolution was done).
- The comparisons of evidentiary items to known samples will be relevant to the context of the case; a given evidentiary sample may/may not warrant comparison to a known elimination sample (i.e. two sexual assault kits with different victims submitted under a single incident number. In this case the victims do not need to be compared to evidence samples from the other SAK.)
- Genetic anomalies (e.g. tri-alleles, microvariants, etc.) may be observed in the reference samples and should prompt the analyst to review the original assessments.

14.6. Assumptions

All assumptions that are made as part of the interpretation must be stated within the report. This includes the assigned number of contributors and any assumed contributors. Assumed contributors can apply to any item of evidence where it can be reasonably assumed that a specific individual would be present.

Notes are captured in the Evidence Sample table example:

Case No	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
NOC	2	3			
Minimum males	1	2			
STRmix analysis	Yes	Yes			
Assumed References	Yes	No			

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 126 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Assumed Reference 1	Yes				
Assumed Reference 2					

A "Notes" section is found at the bottom of the table. This should be used to document sample and testing specifics. For example, why a sample is inconclusive, explanation of number of contributors if it does not follow minimum allele rule, deviations from expected values for STRmix diagnostics or outputs, etc.

14.7. Reporting 99% HPD

The lowest (most common) 99% HPD LR value is normally reported. Testing done by STRmix has revealed that the 99% highest posterior density value calculated using STRmix software does not offer the desired 99% coverage. It has been found to be as low as 76% coverage. While the value being reported is still the most common (and conservative) value, it is not as common or conservative as the 99% HPD name implies.

14.8. Reporting point LR

If the difference in the LR total is at least one million times larger than the 99% HPD LR value in any of the population groups, then the LR total is reported. When an allele is required by STRmix for LR calculations that has a frequency of zero in one population but a non-zero frequency in the other populations, STRmix will continually sample very small frequencies for this zero allele, thereby calculating a very small value for the 99% 1-sided lower HPD interval for that one population. The statistically appropriate way to report the sample is through the lowest point LR total value.

The LR Assistant tool has been modified to accommodate this as follows:
1. It will flag when all populations do not favor the same proposition (see feature "All results favor the same proposition", with "TRUE" or "FALSE").
2. It will flag when the LR total is 1,000,000 times larger (1×10^6) than the 99% 1-sided lower HPD interval (see feature "Use LR Total" note).
3. If "Use LR Total" is displayed the tool may or may not have the lowest LR Total value highlighted in the tables as the teal highlight still indicates the lowest 99% 1-sided lower HPD interval. If the note is present, and lowest LR Total value is not highlighted, the analyst will use the lowest LR total value from the table.
4. If the lowest value for LR total is <1, the analyst must remember to report the number as 1/LR total.
5. The analyst will need to hand-write the LR total number and verbal qualifier on the LR Assistant print-out as this will not automatically be calculated like the other numbers. Report up to 3 significant digits.
6. The written laboratory report should state the LR Total was used.

Example:

Population	LR Total	99.0% 1-sided lower HPD interval	Result
Population: NIST1036_AfAm.csv	2185320.051	1.629236613	Favor Hp
Population: NIST1036_Asian.csv	81745.36009	0.059747313	Favor Hd
Population: NIST1036_Cauc.csv	10302.24258	0.013072492	Favor Hd
Population: NIST1036_Hisp.csv	18883.97071	0.021744932	Favor Hd

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 127 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Numbers to Words	Verbal Qualifier
1.63	Limited Support
16.7	Limited Support
76.5	Limited Support
46	Limited Support

All results favor the same proposition   **FALSE**

Use LR Total

The LR total of the AfAm population is >1 million larger than the HPD LR value. "**Use LR Total**" has appeared on the macro indicating this. The smallest LR total should then be selected for reporting purposes. In this case, that would be the Cauc database LR total of 10302.24258, which would be reported as 10.3 thousand favoring Hp.

Had the LR total been less than one, it would indicate an LR favoring Hd. Therefore the reported value would need to be converted to a whole number by taking the inverse of the LR total that is less than one. i.e. If lowest LR total is 0.01, the reported LR would 100 favoring Hd.

14.9. Support for inclusion (Hp) and exclusion (Hd)

An "inclusion" is where an individual/profile cannot be excluded as a potential contributor of DNA based on the comparison of two samples; typically this will be between a reference and a questioned sample. Support for inclusion can be defined as any comparison giving an LR > 1 favoring H_p. It is important to understand that there can be a large amount of variation in the LRs generated for inclusionary support.

Similarly, support for exclusion can be defined as any comparison giving an LR > 1 favoring H_d. This would equate to the inverse of the LR calculation for H_p. It is important to understand that there can be a large amount of variation in the LRs generated for exclusionary support. Full exclusions are defined in the "Exclusions" section.

14.10. Verbal qualifier

An LR will be accompanied by a verbal scale qualifier. Below is the verbal scale that will be used to express the degree of support for a specified proposition relative to an alternative proposition that will be included in the report wording.

LR for Hp Support and 1/LR for Hd Support	Verbal Qualifier
1	Uninformative
>1-<100	Limited Support
100-<10,000	Moderate Support
10,000-<1,000,000	Strong Support
≥1,000,000	Very Strong Support

14.11. Non-inclusions (exclusions and inconclusive)

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 128 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

Exclusions

An "exclusion" is where an individual is eliminated as a potential contributor of DNA to an evidence sample based on the comparison of reference and questioned DNA profiles given the interpretation under the given assumptions (including number of contributors). A contributor is normally excluded if they have an allele at any locus outside those alleles observed in the crime sample that cannot be accounted for by allelic dropout or drop-in. It is possible for single allele changes to be the result of a somatic mutation. This possibility should be considered in the event that a single allele difference is observed

An LR of 0 is considered an exclusion. Occasionally, where a LR Total value strongly supports Hd, you may observe a 99% 1-sided lower HPD interval value of 0 where per-locus LRs show non-zero values. STRmix does not continue calculating the HPD interval if the LR point estimate is smaller than 0.0000000000000001 (10^{-16}) and will just output 0 for each HPD.

Inconclusive

An inconclusive result relating to DNA interpretation is defined in one of several ways. Samples containing too little data present in the electropherogram to make a reasonable assumption regarding the number of contributors present in an evidence profile will be deemed inconclusive due to a limited amount of DNA present. Samples where there are indications of greater than four contributors to the DNA mixture present in an evidence profile will be deemed inconclusive due to the complexity of the mixture.

Other reporting conclusions can also fall under the inconclusive category. Samples where there are two or fewer additional autosomal alleles present in addition to an assumed contributor's DNA profile, no conclusions will be made regarding the additional alleles present. If all alleles detected in a DNA profile match an assumed contributor's profile but possible additional alleles are observed below analytical threshold, then a statement indicating all alleles detected are consistent with the assumed contributor. No assumptions regarding the number of contributors will be made in these instances.

14.12. CODIS

STRmix CODIS Report Rules for CODIS Sheet Maker Excel
The CODIS upload sheet has an excel macro that builds unknown profiles for CODIS entry using the STRmix deconvolution results. The upload sheet excel macro is based off of the following STRmix CODIS Report rules.

Rules:
1. If the top genotype is given a weight equal or greater to 99%, that genotype will be reported in the CODIS upload sheet
   a. Example results below: 15,16 genotype has >99% weight, therefore, 15,16 is listed in the CODIS upload sheet

Genotype Combinations	Genotype Weights
15,16	99.27
15,15	0.47
16,16	0.12
15,18	0.09

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 129 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

16,18	0.05

2. If the top genotype combination is <99%, then all alleles listed under the genotype combinations will be reported in the CODIS upload sheet (termed an unrestricted set)
    a. If there are more than 4 alleles in the unrestricted set, then only those individual alleles that are >0.99 in sum will be used
    b. If greater than 4 alleles are needed for a sum of >0.99, no entry will be listed on the CODIS upload sheet
    c. Examples results below: 15,16 genotype has <99% weight, therefore, 15,16,17,18 is listed in the CODIS upload sheet

Genotype Combinations	Genotype Weights
15,16	90.27
15,15	4.47
16,16	3.12
17,18	2.09
16,18	0.05

3. An allele will be labeled as an obligate (with "+"), if it is present in all the STRmix weighted genotype combinations.
    a. Example results below: 15,16 genotype has <99% weight and 15 allele is present in every genotype combination (Rules 2 and 3 in effect). 15+,16,18 will be listed on the CODIS upload sheet.
4. Q alleles – If there is a Q allele option with a genotype weight >1% (individually or in sum) no entry will be listed on the CODIS upload sheet

    Exception: If individual alleles are >0.99, then any individual allele >0.99 will be included for that locus

CODIS upload sheet
The CODIS upload sheet is created by the reporting analyst during interpretation and is reviewed during Technical, CODIS, and Administrative reviews. The upload sheet has case information, specimen details, the DNA profile, and information confirming the eligibility of the profile. The reporting analyst will create an upload sheet for all profiles that are potentially eligible and suitable for entry into CODIS.

To create the CODIS Upload Sheet:
1) Open the CODIS sheet maker macro (S:\STRmix\CODIS sheet maker v3.0)
2) For evidence profiles, select the Click Me button
    a. Open the STRmix deconvolution folder for the specimen in question
    b. Within the Reports folder, select the applicable contributor XML file (e.g. "20-0636_5D_CODIS-C2" if contributor 2 is the contributor in consideration for CODIS)
        i. STRmix will only create an XML file if a contributor meets the minimum number of loci for SDIS
3) For reference profiles, or profiles that require manual entry, select "Reference Entry Only"
    a. Using the epg data, manually type in each genotype of the reference profile
4) Reporting analyst enters case specific information in top left and right

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 130 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

a. For reference: Incident no, Lab no, Item no, Reference Name, DOB, Juvenile (yes/no), Analyst
b. For evidence: Incident no, Item description, Analyst, Case Type, Specimen Category
    i. See below for Case Type and Specimen Category definitions.
    ii. Specimen category may change during CODIS review according to specimen MME results.
    iii. DISC sample Yes/No – to be completed during CODIS review

5) Case Type definitions: *Consider the profile situation to choose the appropriate case type*
   a. Profile Associated
       i. For profiles with reference comparison LR Hp > 1,000,000
       ii. For profiles that have a QA db hit to an associated evidence profile
   b. Unsolved – For unknown evidence profiles
   c. Known/Legal – For suspect reference profiles only

6) Specimen Category definitions: *Consider the profile situation to choose the appropriate specimen category*
   a. Forensic Unknown
       i. Complete (all loci) single source or fully deconvoluted profile, no more than 2 alleles at any locus
       ii. Weights for genotype combinations > 99%
       iii. Cross out all obligates present
   b. Forensic Partial
       i. Incomplete (missing loci) single source/fully deconvoluted profile OR
       ii. Complete single source/fully deconvoluted profile with possibility of dropout
       iii. No more than 2 alleles at any locus
   c. Forensic Mixture
       i. Incomplete or complete profiles with more than 2 alleles at any locus OR
       ii. Incomplete or complete profiles with weights for genotype combinations < 99%
   d. CA Forensic Mixture
       i. Same criteria as Forensic Mixture but only 7 core loci present
   e. CA Forensic Partial
       i. Same criteria as Forensic Partial, but only 7 core loci present
   f. LDIS designation
       i. 5 loci (do not need to be core loci, with ≥80% genotype weight

7) Reporting analyst selects checkboxes verifying eligibility and suitability of sample
8) Reporting analyst will document a secure ID and place in left side of casefile

Note: If a CODIS-eligible profile contains a microvariant not present in the population database STRmix will not be able to create the CODIS report used to generate a CODIS excel sheet used for upload. It is important to check that a CODIS report has been generated for all samples where a deconvolution has been performed. If no CODIS report has been generated, consult with a supervisor or technical leader.

Using electropherograms and STRmix interpretation, select the best samples to enter into CODIS. There is no need to generate a CODIS sheet for every sample. However, if the

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 131 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

analyst generates multiple CODIS sheets to assess the best for upload, save in the Casework Documentation folder so the technical reviewer can see that you assessed the profile.

Legal Profiles

The SFPD CODIS unit has a memorandum in place for use of the Legal specimen category. Reference profiles confirmed to be from active suspects in a case and confirmed to not be present in CODIS already will be entered as Legal samples and searched in CODIS. Existing legal samples stay in CODIS for a maximum of two years unless specific additional information is provided and applies.

Eligibility Criteria

All of the following criteria must be met to be deemed eligible for entry.

1) Checked proposition 69 box on the 235b form
2) Sample is from suspect in case
3) No associated forensic profiles being entered
4) Confirmation of eligibility by investigator

New CODIS Matches

New match information is typically available Mondays and Thursdays from SDIS and NDIS respectively. Matches from SDIS and NDIS are evaluated in the same way. Match information is received, documented, and then analyzed. Every forensic profile entered, regardless of match status, needs to have its information entered into the California Hit Outcome Project (CHOP) Every potential match determined by the SFPD must be evaluated by two qualified DNA analysts. If present, the reporting analyst and the technical reviewer should review the match. Cases should not be held up if either analyst is unavailable.

1) To complete CHOP entries, the analyst will do the following:
   a. Log in to CHOP
   b. Click on Caseload
   c. Search under Specimen ID with the police incident number in question
      i. Fill in the following fields:
         1. LDIS Administrator
         2. Lab Case #: enter with Detail following (e.g. 20-1234 SVU)
         3. Location Specimen Collected From
         4. Agency Name
         5. ORI # (automatically fills when Agency Name is selected)
         6. LEA Case #
         7. Date of Incident
         8. Crime Category
         9. Specific Crime
         10. Investigating Officer
         11. Office Telephone or Investigator Email
         12. Suspect name
            a. If sample is associated to a name through the QA database, enter name into CHOP.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 132 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

      b. For single source samples, add name and available information to LDIS Entered Subject Name fields.

      c. For mixture or partial samples, add name and available information (DOB or A#, see on QA database memo in left side of casefile) to the Comments section.

      d. Click Save.

2) The analyst will evaluate all matches within the case. They will indicate "match ok" or "no match", initial, and date the printout, and then pass the casefile to the technical reviewer

    a. Visual comparison can be used for assessment for high stringency matches

    b. If there are any matches involving SFPD STRmix mixtures or partials hitting to single source profiles that were not previously verified through the SFPD QA database, the analyst will run an LR from previous for each candidate match

      i. If a forensic profile has multiple matches to the same arrestee/offender profile, run the LR from previous using the profile with the most data (i.e. Globalfiler loci)

      ii. Open STRmix, select Investigation, and then LR from Previous

         1. If running multiple LR's: after opening STRmix, can select Batch Mode, and use the green arrow next to Add to Batch to select LR from Previous

      iii. Drag or browse and select the results data file for your sample into the Choose Previous Interpretation for LR window. Click select

         1. The XML file will be named "results" and is located within the STRmix deconvolution folder

      iv. Information is prefilled. Within the sample ID field, analyst can add additional information for sample being compared to so all future files will be labeled clearly. Click next

      v. Open S:\CODIS\CODIS dispos using STRmix\casework CODIS LR dispos and CODIS paperwork, and create a new folder for the case you are working on if a folder does not already exist

      vi. Open S:\CODIS\CODIS dispos using STRmix\LR Tool Dispo txt files and drag the file with the current date into the Reference Profile Data section in STRmix

         1. Can also click the green plus sign, click another green plus sign and browse for the txt file

      vii. Select the sample (reference or Forensic Unknown sample) that has matched to your case from the saved match details short report text file and click Confirm

      viii. Hit start

         1. If any error occurs here, check the text file to see if the offender number is listed multiple times. If so, consult the Monday CODIS team member to edit the document as follows before re-running

            a. Open the LR Tool Dispo txt file, select all, copy into a new excel workbook

            b. Filter within column A for the sample you need to use for LR from previous (a reference sample or single source evidence profile present multiple times within txt file)

            c. Edit file so the profile needed is only there once

            d. Save as txt file – add your case number to the file name and save in your case's folder within S:\CODIS\CODIS dispos using STRmix\casework CODIS LR dispos and CODIS paperwork

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 133 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

      ix.   Open the PDF and review the reference input file for the candidate match – make sure all alleles are present and correct, check weights with deconvolution for intuitiveness of LR

      x.   Make an LR worksheet and save in your case's folder within S:\CODIS\CODIS dispos using STRmix\casework CODIS LR dispos and CODIS paperwork

      xi.   Print for casefile

      xii.   Use LR results to determine match ok or no match

          1.   LRs > 1,000,000 for Hp for all populations = **match ok**

          2.   LRs < 1,000,000 for any population group = **no match**

          3.   For cases where one population group has an LR<1,000,000, discuss disposition with a CODIS team member

3) The second reviewer will evaluate any matches and indicate "match ok" or "no match," initial and date the printout, and then return the case folder to the CODIS terminal or dispositioning team member

    a.   For matches verified with LR from previous reviewer will look at:

      i.   Correct reference input data

      ii.   Genotype combinations from original deconvolution and/or epg

15. CODIS procedures

The Combined DNA Index System (CODIS) is a three tier DNA database maintained by the Federal Bureau of Investigation (FBI) and consisting of local (LDIS), state (SDIS) and national (NDIS) levels. The SFPD Crime Laboratory is a LDIS laboratory and uses the CODIS database for law enforcement investigative purposes.

Compliance with State and Federal Laws

The SFPD Crime Lab Forensic Biology Unit CODIS procedures are dictated by the current NDIS Operational Procedures. The Forensic Biology Unit also complies with current "Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories" published by the FBI Laboratory Director. In the event of a discrepancy between the QAS procedures and the NDIS Operational Procedures, the QAS procedures have precedence and supersede any of the provisions in the NDIS Operational Procedures. California state laws and guidelines are also followed by the Forensic Biology Unit.

DNA profiles stored in CODIS by the Forensic Biology Unit comply with applicable state and federal requirements, including the confidentiality, privacy and release of DNA records and databases as well as personally identifiable information. DNA records and personally identifiable information are only released to investigating officers, SFPD Inspectors, and/or SF Assistant District Attorneys, when the information is related to an active SFPD criminal investigation. The privacy and confidentiality of DNA records is assured by the CODIS Administrator.

User access to the CODIS computer network and computer terminals in the laboratory is restricted to the CODIS Administrator, DNA Technical Leader, qualified DNA analysts and DNA trainees who have been granted CODIS access by the FBI. In the event a casework analyst terminates employment with the SFPD, the CODIS Administrator notifies the FBI CODIS Unit and SDIS Custodian and requests removal of the user from CODIS. Any outstanding DNA profiles are re-assigned to the CODIS Administrator for upload to the system.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 134 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

All CODIS users must conduct mandatory annual training as provided by the FBI and coordinated by the CODIS Administrator. At a minimum, external audits are performed every other year to fulfill NDIS audit requirements.

15.1. Personnel

The CODIS Team consists of the CODIS Administrator, Alternate CODIS Administrator, and additional qualified DNA analysts as designated on the Laboratory's Organizational Chart. Members of the CODIS team will undergo the FBI QAS and FBI CODIS software training as outlined in the FBI QAS and NDIS Operational Procedures.

15.2. Upload of DNA profiles

The workflow of samples for possible CODIS upload involves the following steps:

1) DNA profile is generated from an evidence sample or a suspect reference sample and the CODIS acceptability is reviewed by reporting analyst. All the following should be considered according to sample type present.

    a. For evidence samples:

        i. Is there documentation to support all the required eligibility criteria?

        ii. Does the profile meet the suitability requirements for NDIS? For SDIS? For LDIS? The STRmix CODIS reports will assist in the SDIS/NDIS suitability evaluation. The STRmix deconvolution reports will assist in the LDIS suitability evaluation.

    b. For suspect reference profiles:

        i. Is there documentation to support all the required eligibility criteria?

        ii. Are there any associated forensic profiles eligible for entry? If yes, do not enter the suspect reference profile into CODIS. The profile will be entered under the Forensic Specimen category.

        iii. Ask for a DNA verification. Email CAL DOJ (pc296.pc296@doj.ca.gov) or call (510-620-3300) and ask for a DNA verification to see if the suspect is already in SDIS. Provide the individual's name, their DOB, and their CII number during the inquiry.

            1. If DOJ confirms the samples is already at SIDS, then the sample will be entered into LDIS only.

            2. If DOJ confirms the sample is not at SDIS, then the sample will be entered into LDIS and SDIS.

2) If any profiles are deemed CODIS eligible and possibility suitable, the reporting analyst will make a CODIS upload sheet prior to submitting the case for technical review.

    a. Whenever possible the CODIS sheet maker macro will be used to create CODIS upload sheets, however, some evidence samples – particularly those that are only LDIS eligible, will require manual entry. For these samples, open the CODIS sheet maker, but select "Reference Entry Only" to manually enter the appropriate genotypes.

    b. For any CODIS eligible suspect references, open the suspect reference spreadsheet (S:\CODIS\Suspect Eligibility\Suspect Reference and Juvenile Samples)

        i. Enter the following information:

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 135 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

1. Incident #
2. Lab #
3. Item #
4. Name
5. Requested by and Detail
6. If suspect is a juvenile, enter "NA" for both fields
7. Upload date = Date of CODIS review
8. Exp date = 2 years from upload date
9. Investigator follow up date
10. If suspect is a juvenile, enter "Juvenile". Investigators cannot extend retention for juvenile samples
11. If suspect is NOT a juvenile, leave blank. Notice will be sent ~30 days before expungement
12. Expunged date (Leave blank)

   ii. Save and close

3) DNA profile is reviewed for acceptability by the technical reviewer during technical review of the associated case.

4) Profile suitability/eligibility, supporting documentation, correct assignment of DNA types, and correct specimen category are reviewed during CODIS review by an eligible CODIS reviewer.

   a. Forensic Mixture and Forensic Partial profiles to be submitted to CODIS are required to meet the Moderate Match Estimation (MME) threshold, as set by NDIS or SDIS, along with the minimum number of loci requirements. The CODIS team will review profile MME during the CODIS review.

5) The DNA profile to be submitted to CODIS is entered into the Specimen Manager panel of the CODIS software. (Duplicate keyboard strokes are used to ensure adequate verification of the data)

6) CODIS Administrator/Team performs final review of the profile upload request before "sending" the upload to SDIS.

DNA profiles transmitted to all levels of CODIS comply with applicable California state and federal eligibility requirements. Generally, all profiles that meet CODIS requirements will be uploaded. Exceptions include duplicate profiles from the same case.

15.3. Criteria for entry and searching CODIS
Eligibility criteria

Eligibility refers to the case scenario and item. For an evidence sample to meet CODIS eligibility criteria for entry and searching within LDIS, SDIS, or NDIS, it must have documentation of the following:

1) That a crime has been committed
2) That demonstrates the DNA sample was recovered directly from the crime scene and is attributed to the putative perpetrator
3) That the DNA sample was not taken directly from a suspect or suspect's possession
4) That elimination sample(s) have been requested, if applicable

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 136 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

For a suspect reference sample to meet CODIS eligibility criteria for entry and searching within LDIS, SDIS, or NDIS, it must have documentation of the following:
1) The individual is listed as a suspect in the case
2) The case has a SFPD 235b form with the proposition 69 box checked/initialed

Suitability criteria

Suitability refers to the rarity of the DNA profile. The following is a general guide for suitability for entry. The allele and MME requirements vary according to the size of the database. Samples that do not meet the criteria but are of interest to public safety may be suitable for a keyboard search at SDIS.

Evidence Samples

Database	Number of Loci	MME
NDIS	8 of 13 original core CODIS loci	1 in 10 million (1E+7)
SDIS	7 of 13 original core CODIS loci (D2S1338 and D19S433 included)	1 in 100,000 (1E+5)
LDIS	5 autosomal loci with 80% or greater genotype combinations per STRmix deconvolution report	None

Single source partial profiles with at least 8 original core loci and MRE >10E7 and MME <10E5 may be entered into the Forensic Targeted category, this will be assessed during the CODIS review.

Suspect Reference profiles

All reference profiles must be complete DNA profiles. Any exceptions must be approved by the CODIS Administrator.

15.4. **Review of DNA profile CODIS entry**

After technical review and prior to entry into the CODIS database, a CODIS review is performed by the CODIS Administrator, Alternate, or designee to verify:
1) Profile eligibility/suitability according to current CODIS requirements (e.g. MME, minimum # of loci, conditions of case scenario) and proper documentation is in case folder.
2) DNA types are consistent with data interpretation and report.
3) CODIS Search Request sheet is filled out correctly.

Completion of the CODIS review is indicated by the reviewer and dated on the case review sheet. Profiles are entered into the CODIS software during the CODIS review process. A Specimen Details Report will be printed and initialed for each entered profile and added to the casefile.

15.5. **CODIS upload**

Generally, the CODIS Administrator, Alternate, or designee sends uploads to CODIS at the end of the week. An Incremental Upload will be made. Full uploads will only be performed if recommended by SDIS or NDIS.

15.6. **CODIS Hit Outcome Project (CHOP)**

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 137 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

CHOP is managed by the California Department of Justice. It is a web-based database designed to follow the course of progress of CODIS hits and case-to-case matches. It also facilitates the dissemination of CODIS hit information to the applicable local CODIS laboratories, law enforcement agencies, and district attorney offices in the State of California. All CODIS forensic profiles generated by the laboratory are transferred to CHOP as forensic unknown records (FUR) after being uploaded to CODIS. CHOP data entry is performed by a CODIS tea member after the CODIS forensic profile has been uploaded to CODIS.

CHOP entry instructions:
1) Log onto CHOP.
2) From the homepage, click on "Investigative" and then select "CODIS Hit Outcome Project.
3) From the CHOP home screen, click on the "Caseload" tab.
4) In the "CODIS SpecID" column, enter in the CODIS specimen ID. Hit Enter to search.
5) Double-click on the FUR to open. Fill in the fields as complete as possible, including the investigator name and contact information. Required fields will be in red.
   a. Type in the "LEA Case #" with spaces (ex. 111 222 333).
   b. Select "San Francisco Police Dept" for agency name. ORI # will auto populate.
   c. Enter in date of incident.
   d. Select crime category entry.
   e. If the source of the FUR is known, enter in the subject's name in the "LDIS Entered Subject Name" section.
   f. Comments can be added as needed.
6) Click "Save." Close the record.

15.7. Verification and resolution of candidate matches

Candidate Matches will be dispositioned by the CODIS Administrator, Alternate, or designee in accordance with current NDIS Operational Procedures.

Generally, a review of Candidate Matches will be done by two (2) qualified DNA analysts; the CODIS Administrator, Alternate, or designee may also review the matches. The reviewers will initial and date the printed Match Detail Report. The casefile(s) associated with the candidate match may be reviewed to assist in the final disposition.

In case of a disagreement regarding the disposition of candidate matches, the DNA Technical Leader and CODIS Administrator will work to reconcile. In some instances, the lab's disposition of a candidate match is dependent on the disposition of the additional lab involved in the match (i.e., SFPD lab forensic unknown matched to lab B forensic partial). These types of matches do not require of a review by two DNA analysts.

Appropriate documentation is maintained in the casefile to support verification of database matches. STRmix related documentation of candidate matches determined by the analyst and reviewer to be "No Match" will be stored electronically in-house. For disposition of "No Match" or "Insufficient Data", comments are added at the CODIS terminal by the CODIS team and any related LDIS communication will be retained in the casefile.

15.8. Hit notifications

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 138 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

The laboratory report includes wording that communicates to the agency representative if a profile will be searched in the CODIS database.

The personally identifiable information relating to a Candidate Match shall not be released unless the laboratory confirms the match.

The Forensic Biology Unit notifies the appropriate agency and/or individual of 'cold hits' in accordance with SDIS/NDIS requirements. For a hit between an SFPD incident and a convicted offender/arrestee sample stored at SDIS, CHOP is utilized to provide the pertinent hit information to the agency representative(s).

For a hit between an SFPD incident and a convicted offender/arrestee stored in another state (e.g. NDIS level hits), Forensic Biology Unit follows the NDIS procedures in order to obtain the pertinent hit information from the other agency. Once the information is received by the Forensic Biology Unit through either a physical letter or electronic information exchange, the appropriate agency and/or individual is notified.

Offender/arrestee hits may be subsequently confirmed by the Forensic Biology Unit through comparison of the subject's reference sample.

In the event of a case-to-case hit, notification of the possible link between the incidents is made to the appropriate agency contact(s) through CHOP or direct information exchange with an outside agency.

Hits generated within local CODIS database will be disseminated to investigators by CODIS team through either physical letter or electronic information exchange.

Electronic notification of hits to the appropriate agency and/or individual is acceptable.

15.9. Deletion of DNA profile from CODIS
In the event that the offender/arrestee hit is discovered to match an elimination sample (ex. consensual partner, victim, etc.) or additional investigative information is obtained that questions the CODIS eligibility of the profile, then at the discretion of the CODIS Administrator, Alternate, or designee, the profile may be subsequently removed from CODIS.

Reason for the deletion will be documented in the casefile and the agency will be notified.

15.10. Upload of Legally Obtained Suspect Known (LOSK) samples
The 2004 amendment to California's "DNA and Forensic Identification Data Base and Data Bank Act of 1998" (PC 295-297 and related) allows collection of DNA samples from suspects in criminal investigations by local law enforcement agencies for generation and upload of DNA profiles to CODIS by the local laboratory.

The SFPD Crime Lab has a memorandum in place for use of the Legal specimen category within CODIS. Eligible suspect reference profiles from active suspects in a case will be entered into CODIS and searched.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 139 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

SFPD investigators are responsible for (1) verifying the suspect's identity through administrative sources, print checks, etc. (2) determining that the suspect's profile has not been previously submitted to the California Department of Justice Bureau of Forensic Services Laboratory and (3) legally obtaining the sample. By checking the appropriate box on the 235b form submitted with suspect reference samples, SFPD investigators are indicating that the above criteria have been met. The Forensic Biology Unit maintains the appropriate documentation to support the suspect reference sample upload.

The disposition of candidate matches resulting from these profiles will be executed according to SDIS operational procedures.

Retention Policy
All suspect reference profiles entered into CODIS are tracked and expunged after a two-year retention period, per the legal requirement. As the two-year mark approaches, the investigator will be notified of the removal deadline.
- If the investigator informs the Forensic Biology Unit that the individual remains a suspect in an active investigation, the sample will remain in CODIS for another two years at which point it will again be due for expungement.
- If the investigator does not reply, or says the individual is no longer a suspect in an active investigation, the sample will be expunged from CODIS.
- Any suspect reference profiles that were juvenile individuals at the time of collection will be retained for two-years only; no extension of the retention period is permitted.

15.11. Notification of conviction
If an individual is convicted of a felony offense (e.g. requirement for convicted offender collection), and the Forensic Biology Unit receives notice of such a conviction, the Forensic Biology Unit will remove the suspect DNA profile from LDIS.

15.12. Software maintenance
Antivirus software updates and event view capture log backups are performed on a monthly basis by the CODIS Administrator, Alternate, or designee.

A backup tape of the CODIS database is sent offsite to the on a monthly basis. The CODIS Administrator, Alternate, or designee completes this process.

15.13. Disposition of mixture-to-mixture hits in CODIS
In the event that a STRmix CODIS mixture profile hits to another mixture or partial profile, the CODIS team will review the candidate match.
1) For mixtures that hit to other mixtures and/or low level partial profiles - "Insufficient Information" will most likely be the appropriate disposition if SFPD Crime Lab is the lab making the determination.
2) For mixtures that hit to partial profiles that have many heterozygote loci and no obligate loci, we can run an LR from previous (for example, a partial profile that has whole locus dropout at some locations).
3) For matches that are reviewed by another LDIS, SFPD Crime Lab will continue to disposition as usual, based on their dispo.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 140 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

15.14. Using LR from previous in STRmix to disposition CODIS candidate matches
CODIS TEAM MEMBER TO COMPLETE

1) In CODIS workbench, open Match Manager.
2) Select all new matches (highlighted in red), go to "File", "Print", select "Match details short report" and "Print Preview".
3) Click on the icon at the top left ("export" icon); name the file "Candidate matches MMDDYY".
4) Save as CSV file to the CODIS flash drive.
5) Save the CSV file (add "STRmix" to end of file name) to the S drive/CODIS/CODIS Dispos Using STRmix/ Export CSV Files.
6) Open the CODIS export tool located in S drive/ STRmix/ STRmix CODIS Export v1.0.
7) Select "Click ME" and navigate to appropriate Candidate matches MMDDYY export file.
8) Save as a text file in S drive/CODIS/CODIS Dispos Using STRmix/LR Tool Dispo txt files. Note: For any candidate match name with a dash / in the name – remove the dash.
9) Delete exported files from flash drive and return CODIS flash drive to CODIS area.
10) To verify matches using STRmix LR from previous, you will need to run an LR from previous for each candidate match. For duplicate offender/arrestee matches to the same forensic sample, run the LR from previous using the offender/arrestee profile with the most data (ie Globalfiler loci).
    a. Open STRmix, select Tools, then select LR batcher.
    b. Select the STRmix deconvolution data file for your sample (located within the lab number's Casework Documentation folder) and add to the Deconvolution side within the LR batcher tool.
    c. Open S drive/CODIS/CODIS Dispos Using STRmix/LR Tool Dispo txt files and find the file with the same date listed on the provided CODIS Match Details Report.
    d. Select the reference that has matched to your case from the saved match details short report text file and add to References side.
    e. Hit start.
    f. Open the PDF and review the reference input file for the candidate match – make sure all alleles are present and correct.
    g. Use the STRmix LRs tool to produce an LR worksheet. Save within the "CODIS LR Dispo" folder and print for casefile.
        i. LR's > 1,000,000 (very strong support for Hp) for all populations = match ok
        ii. LR's < 1,000,000 for any population group = no match.
        iii. For cases where one population group has an LR<1,000,000, discuss disposition with a CODIS team member.
    h. Save all electronic files in:
        i. S:\CODIS\CODIS dispos using STRmix\casework CODIS LR dispos
        ii. Create new folder if appropriate year does not exist.
11) Give to another CODIS team member for review.

16. Court testimony logs and discovery requests

16.1. Court testimony

A subpoena will be provided for any requests for court testimony. If testimony is requested by the defense, a memo must be submitted to your supervisor for approval. If testimony is requested by the prosecution, sign subpoena and return to requester. Once testimony is completed, submit a testimony log entry form to your supervisor and send an evaluation form to the attorney with whom you testified for. The testimony log entry form must also

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 141 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

be filled out if an analyst has gone to the courthouse for testimony, but was dismissed before taking the stand.

16.2. Providing discovery materials

Discovery requests may be submitted by either the prosecution or defense on a case. Requests will be fulfilled electronically via email or on a disc if requested. Refer to the Administrative Manual in PowerDMS for what is included in the Standard Technical Package for discovery requests. After discovery materials have been provided to requesting party, enter discovery request and fulfillment information into JusticeTrax as a communication log entry. If a control has an unexpected result (i.e. a reagent blank has peaks present), its electropherogram will also be included in the standard discovery package. A note in Justice Trax will be added after the discovery package has been sent.

Note: Data associated with category 1 QAdb hits shall be provided as part of discovery as applicable (i.e. STR data files, STRmix files, LRs, etc.).

17. Outsourcing procedures

The laboratory outsources, on an as-needed basis, DNA sample(s) to a vendor laboratory to generate DNA data that will be entered into CODIS. The laboratory maintains documentation of the vendor laboratory's compliance with the current FBI QAS and the accreditation requirements of federal law. Compliance with these standards/accreditation requirements are reviewed by the Technical Leader.

The Technical Leader shall approve the technical specifications of the outsourcing agreement with the vendor laboratory before it is awarded. If the SFPD is requested to take ownership and no outsourcing agreement exists between the law enforcement agency, vendor or SFPD, the SFPD Technical Leader shall document the following prior to acceptance of ownership of product(s) of forensic DNA analysis from the vendor laboratory:

1. Approval of the casework CODIS administrator and written permission from the NDIS custodian for any scenario that involves CODIS entry/searching
2. Approval of technical specifications of testing
3. Review documentation of or conduct an on-site visit of the vendor laboratory. This shall have been within 18 months of the conducted analysis and in accordance with current QAS.

The laboratory follows a procedure to verify the integrity of the DNA data received for the purposes of taking ownership of DNA from a vendor laboratory.   Prior to the search of DNA data in SDIS, an analyst, or CODIS Admin or technical reviewer at SFPD shall review the DNA data to verify specimen eligibility and the correct specimen category for entry into CODIS.

Prior to the upload of DNA data to SDIS or the reporting of search results, the "ownership review" of a vendor laboratory's DNA data shall be performed by an SFPD analyst or technical reviewer qualified in the technology, platform and typing test kit used to generate the data, or authorized to review a legacy technology, AND participates in the SFPD proficiency testing program.

17.1. Technical review of outsourced data/case ownership

The technical review, or "ownership review" of outsourced data includes the following elements:

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 142 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

1. A review of all DNA types that SFPD will take ownership of to verify that they are supported by the raw and/or analyzed data (electropherograms or images).
2. A review of all associated analytical controls, internal size standards and allelic ladders to verify that the expected results were obtained.
3. A review of the final report (if provided) to verify that the results/conclusions are supported by the data.
4. For samples to be entered into CODIS, verification of DNA types, eligibility and correct specimen category. Verification of eligibility must be performed by a current CODIS user. The reviewer must contact the vendor laboratory for clarification if questions arise about the interpretation and reporting of evidence items and maintain documentation/communications with the vendor laboratory in the casefile.

17.2. On-site visits

A documented initial on-site visit to assess the vendor laboratory's ability to perform analysis on outsourced casework is performed prior to the vendor laboratory's beginning of casework analysis.

The on-site visit shall be performed by the DNA Technical Leader, or a designated employee of the SFPD Forensic Biology Unit who is a qualified or previously qualified analyst in the technology, platform and typing test kit used to generate the DNA data. Alternatively, the DNA Technical Leader can evaluate and approve an on-site visit coordinated by a designated FBI employee. The review and approval of an on- site visit (performed by Technical Leader/designee/designated FBI employee) must be documented, and retained pursuant to the FBI Quality Assurance Standards.

If the outsourcing agreement extends beyond one year, an annual on-site visit is required. Each annual on-site visit shall occur every calendar year and shall be at least 6 months and no more than 18 months apart. The SFPD may accept an on-site visit conducted by another NDIS participating laboratory using the same technology, platform and typing test kit for the generation of DNA data, or coordinated by a designated FBI employee. The review and approval of an on- site visit (performed by Technical Leader/designee/designated FBI employee/another NDIS participating laboratory) must be documented and retained pursuant to the FBI Quality Assurance Standards.

18. Validation

The Forensic Biology Unit uses validated methods for DNA analysis. Validation procedures are based on current FBI QAS and SWGDAM Validation Guidelines.

Developmental validation studies, internal validation studies, modified procedure evaluations, and software testing, including the approval of the Technical Leader, are retained and available for review.

Validation procedures must be coordinated by and approved by the Technical Leader but authorized designees may participate in the work while maintaining effective communication among all parties involved.

18.1. Developmental validation

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 143 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

These studies precede the implementation of any new methods used for forensic DNA analysis.

Developmental validation will include as applicable: characterization of genetic markers, species specificity, sensitivity studies, stability studies, precision and accuracy studies, case-type samples, population studies, mixture studies and PCR-based studies. PCR-based studies include reaction conditions, assessment of differential and preferential amplification, effects of multiplexing, assessment of appropriate controls and product detection studies.

All developmental validation studies will be documented. Peer-reviewed publication of the underlying scientific principle(s) of a method is required.

18.2. Internal validation
These studies are conducted of all manual and robotic methods with the appropriate sample number and type to demonstrate reliability and potential limitations of the method.

Internal validation will include as applicable: known and non-probative evidence samples or mock evidence samples, precision and accuracy studies, sensitivity and stochastic studies, mixture studies and contamination assessment studies.

Internal validation will define quality assurance parameters and interpretation guidelines, including as applicable guidelines for mixture interpretation and the application of appropriate statistical calculations. Mixture interpretation validation studies will include samples with a range of the number of contributors, template amounts, and mixture ratios expected to be interpreted in casework.

Internal validation studies are conducted prior to implementing a change in platform instrument model or typing test kit.

All internal validation studies will be coordinated and designed by the Technical Leader, and designated individuals. Studies will be will be documented and summarized. Studies will be reviewed and approved by the Technical Leader prior to implementing a procedure for forensic applications.

Newly validated DNA methods (from amplification through characterization), typing test kit or platform instrument model will be checked against an appropriate certified reference material (e.g. NIST) or traceable to certified material prior to implementation of the method for forensic analysis.

18.3. Material modifications
Modified procedures will have their performance evaluated by comparison to the original procedure using similar DNA samples and the evaluation documented. The evaluation will be reviewed and approved by the Technical Leader prior to implementation into casework

18.4. Performance checks

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 144 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

In the Forensic Biology Unit, performance checks are quality assurance measures to assess the functionality of laboratory instruments and equipment that affect the accuracy and/or validity of forensic sample analysis and must be conducted before use in casework.

They are required after service calls, repairs and/or scheduled maintenance of any critical equipment in the DNA laboratory (excluding in-house calibration unless necessary) or for software upgrades that do not impact interpretation or the analytical process. Performance checks are also required for each new critical instrument of the same make or model already in use within the Forensic Biology Unit, or if the physical location or environment of the instrument changes.

18.5. Software evaluation

New software or new modules of existing software and modifications to software are evaluated to assess the suitability of the software for its intended use in the laboratory, and to determine the necessity of validation studies or software testing. This evaluation will include the determination of which studies will/ will not be conducted and will be documented.

Software validation and testing will be documented, and will be reviewed and approved by the Technical Leader prior to implementation.

18.5.1 Developmental validation of software

New software or new modules of existing software that are used as a component of instrumentation, for the analysis and/or interpretation of DNA data or for statistical calculations will be subject to developmental validation prior to implementation in DNA analysis.

Except where legally protected, the underlying scientific principle(s) utilized by software with an impact on the analytical process, interpretation or statistical calculations shall be publicly available for review or published in a peer reviewed scientific journal.

Developmental software validation studies for new software or new modules of existing software:

a. **Used as a component of instrumentation**: will include at a minimum functional testing and reliability testing.
b. **For the analysis and/or interpretation of DNA data**: will include at a minimum functional testing, reliability testing, and as applicable accuracy, precision, sensitivity and specificity studies.
c. **For statistical calculations:** will include at a minimum functional testing, reliability testing, and as applicable accuracy and precision studies.

18.5.2 Internal validation of software

New software or new modules of existing software that are used as a component of instrumentation, for the analysis and/or interpretation of DNA data or for statistical calculations will be subject to internal validation specific to the laboratory's intended use prior to implementation in DNA analysis.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 145 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Internal software validation studies for new software or new modules of existing software:

a. **Used as a component of instrumentation**: will include functional testing and reliability testing.
b. **For the analysis and/or interpretation of DNA data**: will include functional testing, reliability testing, and, as applicable precision and accuracy studies, sensitivity and specificity studies.
c. **For statistical calculations:** will include at a minimum functional testing, reliability testing, and as applicable precision and accuracy studies.
d. **Software that does not impact the analytical process, interpretation or statistical calculations:** will include at a minimum a functional test.

18.5.3 Software testing

Modifications to software are evaluated to determine if the modifications result in major or minor revisions to the software.

A major revision to software used:

a. **As a component of instrumentation**: will require validation prior to implementation. Software validation studies will include functional testing, reliability testing and regression testing.
b. **For the analysis and/or interpretation of DNA data**: will require validation prior to implementation. Software validation studies will include functional testing, reliability testing, regression testing and, as applicable, precision and accuracy studies, sensitivity and specificity studies.
c. **For statistical calculations:** will require validation prior to implementation. Software validation studies will include functional testing, reliability testing, regression testing and, as applicable, precision and accuracy studies.

19. Approved abbreviations

-A = minus A peak
ALS = alternate light source
AP = acid phosphatase
ART = artifact
AT = analytical threshold
BPB = brown paper bag
CBB = cardboard box
CIID = case, item, initials, date
cm = centimeter
ctrl = control
D/diff = differential
DNU = data not used (includes "not amplified" at TECAN stage)
DTT = dithiothreitol
EC = epithelial cell fraction
Env = envelope
epg = electropherogram
ES = evidence storage

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 146 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

ET = evidence tape
Exp = expiration
GS or g/s = glue sealed
Immed = immediate
Inc = inconclusive
int/ext = interior/exterior
KM = Kastle-Meyer test
LIP = labeled in part
ME = manila envelope
mm = millimeter
MPE = manila property envelope
Mx= mixture proportion
N+#/N-# = forward/back stutter type
NA or N/A = not applicable
NC = nucleated cells/fraction
NCC = no color change
ND = non-differential
NE = not examined
Neg/-/∅/ = negative
NR = no results
NS = non-sperm, not sampled
NT = not tested
OL = off-ladder allele
OP = original packaging
o/n = overnight
PBT = peaks below threshold
PCD = property control division
PHR = peak height ratio
PD = pull down
pg = page
PHR = peak height ratio
Pkg = package
Pos/+ (circled or not) = positive
ProK = proteinase K
PT = presumptive test
PU = pull-up
R/ref = reference
R/B = red brown
RB = reagent blank
RBL = raised baseline
RBS = red-brown stain
RES = remaining evidence swabs
RFU = relative fluorescent unit
RSID = rapid stain ID test (saliva)
RT = room temperature
Rxn = reaction
SAK = sexual assault kit
SAEK = sexual assault evidence kit

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 147 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

SP = sperm cells/fraction
SPK = spike
ST = stochastic threshold
TE = tan envelope
Temp = temporary
TS or t/s = tape sealed
WC = water control
w/d = wet/dry
Wk = weak
w/ and w/o = with and without
WP = work product
WPE = work product envelope
UD = undetermined
< / > = less than/greater than
Δ = change
~ = approximately
[ ] = allele detected at or below stochastic threshold

20. STRmix glossary

STRmix: a fully continuous probabilistic genotyping forensic software which combines biological modeling with mathematical processes in order to (1) interpret and attempt to deconvolute DNA profiles in the presence or absence of conditioned samples, and (2) compare suspect/informative reference samples (comparison samples) to evidence samples and provide statistical weight in the form of a likelihood ratio (LR).

- The deconvolution is performed using a Markov Chain Monte Carlo process which creates possible genotype combination(s). Each combination is assigned a weight which reflects how well it explains the evidence profile.

- LRs are calculated by comparing the probabilities of two hypotheses, H1 and H2 (Hp and Hd, in STRmixTM). STRmixTM incorporates the assigned weights and sub-population models (Balding and Nichols, 1994, also known as NRC II recommendation 4.2) to calculate the LR.

Mass parameters: variable parameters used to generate expected peak heights during a deconvolution, collectively referred to as DART.
DART: **D**egradation rate for each contributor to the DNA profile. **A**mplification efficiencies for each locus within the profile. **R**eplicate amplification strength for each PCR replicate. **T**emplate for each contributor (measured in relative fluorescence units (RFU).

Model Maker: estimation of the STRmixTM parameters for an STR amplification kit using empirical data. The parameters within Model Maker must be determined before casework samples can be analyzed in STRmixTM.

Weighting or Weight: A probability that reflects how well a particular genotype combination explains the evidence profile. For example, if a proposed combination of genotypes is unlikely to lead to the observed evidence profile then that combination will be given a low weighting (close to zero).

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 148 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

MCMC: Markov Chain Monte Carlo is a mathematical method that uses a random re-sampling process in order to give a best explanation for an observed set of data

- **Markov chain**: A process that steps from one position to another. In STRmix, the positions define certain combinations of genotypes and parameters that are being tried as explanations for the profile. At each iteration, the algorithm may either step or stay put. The chain is the path of steps and stay puts.

- **Markov property**: the property of the MCMC analysis where the values in each step are independent of the values in the previous step.

- **Monte Carlo**: a random re-sampling method used to address complex problems. It is used to systematically search the entire range of possibilities that are being considered to ensure that all likely combinations are considered. In STRmix, this range of possibilities consists of series of proposed genotypes for each given locus.

Iteration: a proposed genotype combination that is either accepted or rejected during the MCMC process. These proposed genotype combinations are not the final result.

Accepts or Moves: an iteration that is accepted and 'moved' towards the next step in the MCMC process, e.g. 50,000 accepts

- **Burn**-in: During the MCMC process, the first 10,000 accepts are discarded. This is done to allow the chains time to reach a desired or 'good space'.

Metropolis-Hastings algorithm: The Metropolis-Hastings algorithm is an equation used to determine whether or not to accept or reject a new profile combination.

Summary of Contributors
DNA Amounts (RFU): The best estimate of the "amount" (proportion) of DNA, expressed in RFU, determined by the mean of all post burn-in accepts of the calculated template (t) for each contributor.

Mixture Proportions (%): approximate percentage of each contributor to the sample.

Degradation: estimated for each contributor, the decreasing trend of peak heights with increasing molecular weight – a higher number indicates a steeper slope of degradation.

- Degradation starting at (rfu/bp) - the lowest RFU peak at the smallest molecular weight locus in the sample.

Run Information
Total Iterations: total number of proposed genotype combinations made during the MCMC process. The value displayed in this section indicates the total number of post burn-in iterations that the MCMC ran for during its analysis.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 149 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Acceptance rate: total number of accepts during the MCMC process, divided by total iterations. The acceptance rate informs the user as to how often a new proposed set of parameters was accepted. A very low acceptance rate (e.g. 1 in thousands to millions) may, in combination with the other diagnostics, indicate that the analysis needs to be run for additional iterations. On its own (and without any other indication of sub-optimal results) a low acceptance rate is not an indication that rework is required.

Inter-replicate efficiency: The comparison of the variation in sampling between replicates. This value is reported as a percentage.

Effective Sample Size (ESS): the number of independent samples that have been taken from the posterior distribution of the MCMC likelihood.

A low ESS in relation to the total number of iterations suggests that 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) will mean that there is potential for a large difference in weights if the analysis was run again. This will be taken into account during HPD interval generation in any LR calculations (unless the genotype sets are completely resolved on a single combination, in which case there will be no effect of ESS on the HPD interval). Again, a low ESS on its own is not an indication that rework is required.

99%1-sided lower HPD: the combined LR calculated by STRmix is referred to as a point estimate. Because the true answer is not known, a confidence interval is then applied around the point estimate known as a 1-sided 99% highest HPD credible interval. This interval accounts for the uncertainty associated with the point estimate LR, and gives a range (i.e. with 99% confidence) of where the true allele probabilities actually lie. The lower end of the HPD interval is reported from STRmix to be the most conservative to the person of interest.

Average (log) likelihood: this value is the average $\log_{10}$(likelihood) for the entire post burn-in MCMC. It is the 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 suggests that STRmix™ has not been able to describe the data very well given the information it has been provided. Reasons why this value may be low or negative are:

- The profile is simply very low level and there is very little data making up the likelihood
- The number of contributors is wrong 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)
- Data has been removed that was real, particularly stutter peaks, and must now be described in STRmix™ by dropout
- Artifactual peaks have been left labeled and must now be accounted for in STRmix™ by drop-in.
- A low or negative value for the average $\log_{10}$(likelihood) indicates to users that the analysis requires additional scrutiny.
- Note, good quality mixed DNA profiles are more likely to give higher average $\log_{10}$(likelihood) values than good quality single source profiles. So, low average $\log_{10}$(likelihood) values alone are not necessarily an indicator of an issue especially if the profile is single source.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 150 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Gelman-Rubin convergence diagnostic: this value informs the user whether the MCMC chains have converged. This is calculated by comparing the within-chain and between-chain variances of the MCMC chains.

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 that the analysis has not run for 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 GR will be 1.

If the GR is above 1.2 then there exists the possibility that the analysis hasn't converged. If the GR value is above 1.2 the results of the analysis should be closely scrutinized.

Allele Variance (c^2): Parameter that describes how variable allele peak heights are within the run

Stutter Variance (k^2): Parameter that describes how variable stutter peak heights are within the run

Both the allele and stutter variance values are the average value for variance and stutter variance constants across the entire post burn-in MCMC analysis. These values are plotted separately on the derived prior distributions. 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 that the DNA profile is sub-optimal or that the number of contributors is incorrect. Used in conjunction with low average $\log_{10}$(likelihood), a large variance or stutter variance constant can indicate poor PCR.

If the sample is simply low level this should result in a low average $\log_{10}$(likelihood) and an average variance constant.

If some data has been omitted, left on or misinterpreted this should result in a low average $\log_{10}$(likelihood) and high variances.

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Page 151 of 168
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Page 154 of 168
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Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 159 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

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Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 160 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

Appendix A. <u>Decision trees for assessing contamination and controls</u>

NTC (negative quantitation control)

Negative amplification control

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 161 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Positive amplification control

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS
Page 162 of 168
Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Reagent blank

A.   Initial assessment of reagent blank

```
┌──────────────────────────┐      Yes      ┌──────────────────────────────┐
│ Are any alleles called   │ ────────────► │ Contamination detected.      │
│ above analytical         │               │ Continue to part B.          │
│ threshold?               │               │ Complete steps below.        │
└──────────────────────────┘               └──────────────────────────────┘
            │                                            │
            │ No                                         ▼
            ▼                          ┌────────────────────────────────────┐
┌──────────────────────────┐          │ Visually check other samples on    │
│ Are ≥5 possible autosomal│   Yes    │ plate for possible source of       │
│ alleles ≥20 RFUs present?│ ───────┐ │ contamination                      │
└──────────────────────────┘        │ └────────────────────────────────────┘
            │                        │ ┌────────────────────────────────────┐
            │ No                     │ │ Search QA database to possible QC  │
            ▼                        │ │ source of contamination.           │
┌──────────────────────────┐        │ └────────────────────────────────────┘
│    No contamination      │        │ ┌────────────────────────────────────┐
└──────────────────────────┘        │ │ Notify DNA supervisor or TL to     │
                                     │ │ start action report.               │
                                     │ └────────────────────────────────────┘
                                     ▼
                       ┌──────────────────────────────┐
                       │ Contamination detected.      │
                       │ Continue to part B.          │
                       │ Complete steps below.        │
                       └──────────────────────────────┘
                                     │
                                     ▼
                       ┌────────────────────────────────────┐
                       │ Visually check other samples on    │
                       │ plate for possible source of       │
                       │ contamination                      │
                       └────────────────────────────────────┘
                       ┌────────────────────────────────────┐
                       │ Search QA database to possible QC  │
                       │ source of contamination.           │
                       └────────────────────────────────────┘
                       ┌────────────────────────────────────┐
                       │ Notify DNA supervisor or TL to     │
                       │ start action report.               │
                       └────────────────────────────────────┘
```

B.   Determine reprocessing (refer to Section 3.8 for Reprocessing Guide)

```
               No     ┌──────────────────────────┐     Yes
        ┌──────────── │ Is there a quantification│ ───────────┐
        │             │ value for any of targets?│            │
        │             └──────────────────────────┘            │
        ▼                                                      ▼
┌──────────────────────────┐                    ┌──────────────────────────┐
│ Do contamination peaks   │                    │ Begin at Step 3 in       │
│ match those from another │                    │ reprocessing guide.      │
│ sample injected in same  │                    └──────────────────────────┘
│ capillary?               │
└──────────────────────────┘
      │             │
   Yes│             │No
      ▼             ▼
┌──────────────┐ ┌──────────────┐
│ Begin at     │ │ Begin at     │
│ Step 1 in    │ │ Step 2 in    │
│ reprocessing │ │ reprocessing │
│ guide.       │ │ guide.       │
└──────────────┘ └──────────────┘
```
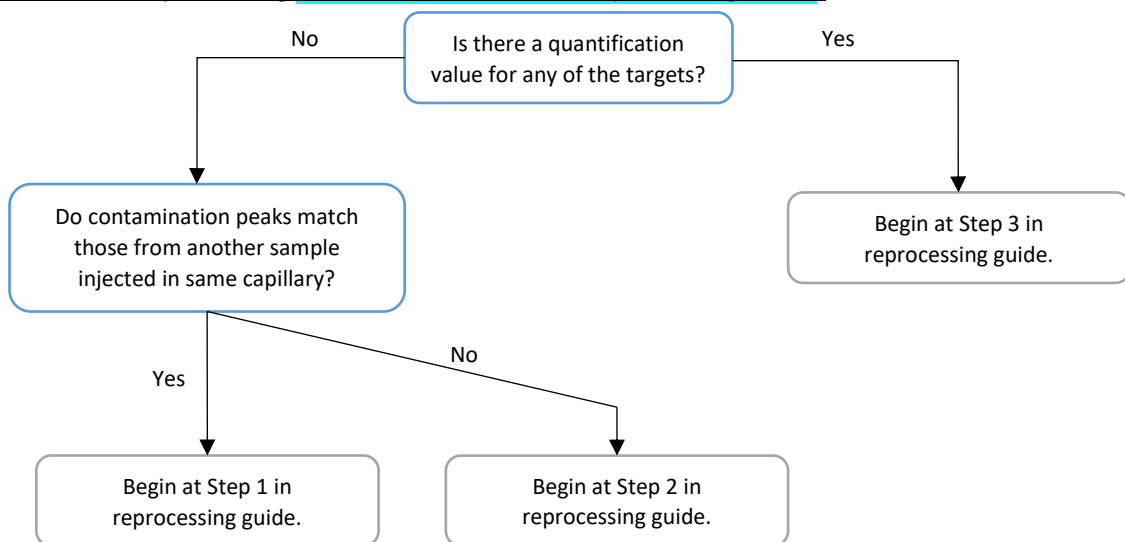
*After reprocessing is completed, proceed to section C.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS   Page 163 of 168
Approved by: EJS

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

C.   Proceeding after reprocessing lab work has been completed

```
Was contamination sourced      ──Yes──▶   Source profile will be used for comparisons in all
to a QC profile at any point?              associated samples. LRs will be reported in case report.
        │
        No
        │
        ▼
Was contamination             ──Yes──▶   Is interpretation affected?
confirmed after reprocessing?            Contamination cannot be accounted
        │                                for? Discuss with supervisor or TL.
        No                                    │              │
        │                                    No             Yes
        ▼                                    ▼              ▼
Note on CE sheet peaks were   | Statement regarding        | Receive permission to consume and re-extract
resolved following reprocessing. | contamination is added to report. | associated samples OR samples are reported as
Cases can be reported per usual. | Case can be reported as usual.    | unsuitable for comparisons with note.
```

Assessing contamination in reference samples

```
Are both loads of the         ──Yes──▶   No contamination
reference single source?
        │
        No
        │
        ▼
Is one load of the reference  ──Yes──▶   Does non-single source reference
single source?                           profile have ≥5 foreign alleles?
        │                                    │              │
        No                                  Yes             No
        │                                    ▼              ▼
        ▼                            Contamination detected. | Contamination detected. Add
Contamination detected.           Begin at Step 3 in        | note to CE sheet. Use single
Begin at Step 3 in                reprocessing guide.        | source load for reporting.
reprocessing guide.
```

Assessing contamination in evidence samples

Possible contamination is most likely to be detected from a QA database search hit to a personnel profile. If this occurs notify a supervisor or Technical Leader so that an action report can be started.

1. If contaminant profile is be sourced to an individual who has recently had access to the lab, proceed to Step 3 of reprocessing guide.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 164 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

2. If contaminant profile is sourced to non-lab personnel or visitor (i.e. CSI member), no reprocessing is necessary.

3. Determine whether contaminant profile can be conditioned on and report accordingly.

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 165 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

Appendix B. Profile Comparison Tool Guide

Profile Comparison Tool Guide

The sample comparison function within the profile comparison tool shall be utilized in GMID-X to assess clear contamination. This is only a tool and shall not be used as an all-encompassing quality control assessment. The profile comparison tool has sample concordance, lab reference comparison, and Control/QC comparison functions which are optional for use.

The tool ignores samples with OLs present. Therefore, before running the tool, all OLs must be resolved (i.e. deleted or re-labeled). This will be done as part of the data verification steps after CE data is generated. OLs should be either artifacts or OLs, both of which can be resolved prior to data interpretation during reporting. Comparison reports shall also be reviewed by the second verification analyst. If contamination is suspected notify a supervisor or Technical Leader before distributing data for reporting.

To find all OLs in project:

1) Click on Genotypes tab.
2) Scroll across and find the "bin" column.
3) Hold shift key and click on bin column header. Depending on how you have your table display set up. *Sometimes you have to click more than once.*
4) This sorts our samples based on the bin symbol (we want all samples with a yellow triangle sorted to the top).
5) Once it is sorted use your mouse to highlight those yellow triangles in the bin column.
6) Then press the "Display Plots" button in your toolbar and it will bring up all samples in the display with an OL.

Tips for using tool:

- Matches between different cases should be carefully examined.
- Matches to negative controls or reagent blanks should be carefully examined.
- Samples with little data (few alleles called) can yield many matches. Likely no determinations can be made based on these samples. Exceptions are matches with negative or reagent blank samples.
- Samples with higher order mixtures can yield many matches. Matches between higher order mixture samples will likely be uninformative based on the complexity of comparison and amount of allele sharing possible.
- Matches within cases may be expected.
- While some of these scenarios can yield adventitious matches not associated with a quality issue, all match results should be looked at.

Sample Concordance (optional)
Function:

Forensic Biology Unit Operating Procedure
Author: PLS/EJS
Approved by: EJS

Page 166 of 168

Published: 03/18/2022
Effective date: 03/18/2022
Replaces published version: 12/20/2021

The software performs a sample concordance check to group samples with identical profiles and minimize the number of comparisons performed on the other tabs of the Profile Comparison tool. For 100% concordance all markers and all alleles must match.

Use:

This can be used for references to help confirm duplicates are identical matches. Note: If one reference has stutter at a marker and other sample does not, this would not be 100% concordance. This type of match would be seen in the sample comparison tab.

In assessing the sets of grouped concordant samples, analysts should look to see whether there is 100% concordance between samples with different case numbers. If this occurs, it should be investigated to determine whether a sample-switch or contamination incident has occurred.

Sample Comparison (required)

Function:

The software compares samples in the project against all other samples in the project and returns matches based on % match threshold. % match threshold can be set from 50 to 100. It is recommended to use % match of 100 to begin. This can be decreased incrementally to help identify less obvious contamination. Higher order mixtures (NOC = 3 or more) and low level samples can yield many matches as the threshold is decreased.

Use:

This tool would help identify sample to sample contamination and contamination in negative controls from other samples on the batch.

The % match is calculated using the following formula:

$$\frac{\text{\# reference profile alleles found in comparison profile}}{\text{Total \# reference profile alleles}}$$

Example:
   a. Sample 1 is a single-source sample
   b. Sample 2 is a mixed-source sample
   c. Sample 1 contains 10 alleles (all of which are found in Sample 2)
   d. Sample 2 contains 20 alleles
   e. When Sample 1 is used as the reference profile, the % Match = 100%
   f. When Sample 2 is used as the reference profile, the % Match = 50%

In this example, the comparison in which Sample 1 is used as the reference profile is reported. Samples 1 and 2 would show as a match if a 100% match threshold is set.

Single – Single

Check to see if profiles from different case numbers are matching. This may be an indication that a contamination event or a sample switch could have occurred.

Single – Mixture

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 167 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021

Look to see if profiles from different case numbers are matching. All 100% matches between different cases should be further investigated. Any 100% inclusion of a single source sample to a mixture from a different case should be examined to determine if an analytical error has occurred.

Mixture – Mixture
Look to see if profiles from different cases are matching. Any 100% match between mixtures from two different cases should be examined carefully to determine if an analytical error has occurred.

Lab Reference Comparison (optional)
Function:
The software determines whether lab reference sample profiles stored in the Profile Manager are potential contributors to sample profiles in the project.

Use:
This tool will help identify contamination from quality control samples.

Control/QC Comparison (optional)
Function:
The software performs a blind QC check by comparing the observed profiles for the custom control and QC samples present in a project to the custom control profiles stored in the Profile Manager (QC samples use the Custom Control profile type).

Use:
This tool can confirm the positive control yielded the correct genotype.

Forensic Biology Unit Operating Procedure   Published: 03/18/2022
Author: PLS/EJS   Page 168 of 168   Effective date: 03/18/2022
Approved by: EJS   Replaces published version: 12/20/2021