

Procedure for Analysis and Interpretation of STR DNA Profiles

1.0 Purpose-This procedure specifies the steps for performing analysis and interpretation of DNA samples amplified with Promega PowerPlex® Fusion 6C using the GeneMapper® ID-X (GMID-X) software and utilizing the STRmix™ probabilistic genotyping software and to provide guidance for the interpretation and reporting of these samples within Forensic Biology.

2.0 Scope- This procedure applies to casework analysts and trainees in the Forensic Biology Section who perform forensic casework or reviews on STR DNA samples.

3.0 Definitions – See Section Definition list.

4.0 Equipment and Materials

- Computer with GeneMapper® ID-X (version 1.6 or most current) software
- Microsoft Excel, Word
- Print2PDF software
- Computer with STRmix™ software (v2.7 or most current)
- CODIS software program (Analyst Workbench) located on CODIS computer(s)

5.0 Procedure

5.1 Introduction – The interpretation of results in casework is a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-set rule. The criteria are based on validation studies and literature references and set a minimum criteria developed by the laboratory for which analysts should adhere. These guidelines establish a framework to ensure that interpretation is made objectively and consistently between analysts, reporting conclusions that are supported by analytical data.

STRmix™ is a software program that applies a fully continuous probabilistic genotyping approach to DNA profile interpretation. It standardizes the analysis of profiles in the laboratory by using estimates of variance of results derived from both developmental and laboratory generated validation data. It is based on well-characterized biological models for interpretation of allelic and stutter peaks. STRmix can be used to analyze samples with or without reference profile samples. A key component of the software involves utilizing a well-described mathematical process called Markov Chain Monte Carlo (MCMC) to randomly assign weightings or probabilities in order to quantify the genotype combinations for a particular contributor. Implementation of STRmix does not invalidate previously reported results.


5.2 Documentation – Analysts shall document any assumptions used (e.g., use of assumed/conditioned profiles, number of contributors), determination of major profiles (to include listing the specific loci used/not used), the non-use of loci for interpretation and reason (e.g., imbalance, stochastic effects), and list loci which are not being used to perform statistical interpretation. Additionally, any other notes, remarks, and observations used to make an interpretation and/or conclusion regarding unknown samples shall be documented on the allele call tables or on data generated from the STRmix program.

5.3 Analyzing Samples Using GMID-X - Creating a Project

5.3.1 Open GMID-X and log in under the Forensic Scientist's name/log-in. Forensic Scientists shall log into and use the Casework Database Host.


5.3.2 To add samples to be analyzed to the project, locate the run folder containing the samples to be added to the project under the "Files" tab. Highlight the run folder (or select the specific samples to be analyzed) and select add to List. The file(s) then appears on the right-hand side. Select "Add."

5.3.3 In the Samples pane, the category Sample Type shall have the samples labeled as follows when amplified with Promega PowerPlex Fusion 6C®:


- Ladder: Allelic Ladder
- 2800M (Positive Amplification Control): Positive Control
- All Negative Controls (extraction and amplification): Negative Control
- All other samples shall be Samples
- Sample File names appear by well number (e.g., A12) followed by the sample name as entered by the Forensic Scientist prior to electrophoresis. Also, until the samples are analyzed, the Status icon to the left of the Sample File name has the unanalyzed icon () present next to it. Verify that the table setting (in top center of the main GMID-X window) is set to 31XX Data Analysis.

5.3.4 The remaining categories shall be filled out as follows:

- Specimen Category: no export
- Analysis Method: Fusion
- Panel: PowerPlex_Fusion_6C_Panels_IDX_v1.1
- Size Standard: WEN_ILS_500_CS
- Instrument Type: ABI3500, or similar (note: automatically filled in by the software and instrument)

5.3.5 Once all samples and categories are properly labeled and identified, select the Analyze icon () to analyze the data. When prompted, create a project name for the set of samples and select the "Casework Security Group" and select "OK."

5.3.5.1 The Analysis Summary and Genotypes tabs become available in the main GMID-X window once analysis is complete.

5.3.5.2 If any sample displays a solid red stop sign icon () on the right-hand side of the GMID-X window, assess the data as described in 5.4.

5.3.5.3 Forensic Scientists shall delete their projects from the software only after a copy of the project has been added to the associated case record and the case record has been published.

5.3.6 Analysis and Plot Settings

5.3.6.1 Access the Analysis Method Editor by selecting "Tools" from the menu in the main GMID-X window, and then select "Analysis Method Editor." Note that the user must have a single sample in the main GMID-X window selected in order to access the Editor.

5.3.6.2 Select the “Peak Detector” tab and ensure that these settings for Full Range analysis, Peak Detection (see analytical threshold), Smoothing (Light) and Baseline (51 points), and Size Calling Method (Local Southern) have been chosen when the samples have been amplified with PowerPlex® Fusion 6C. The analytical threshold was established through validation and performance check studies using the PowerPlex® Fusion 6C amplification kit. The analytical threshold is set per dye channel (Blue (FL-6C) – 75 RFU, Green (JOE-6C) – 75 RFU, Yellow (TMR-6C) – 80 RFU, Red (CXR-6C) – 100 RFU, Purple (TOM-6C) – 60 RFU, and Orange (WEN) – 35 RFU). Anything present below these thresholds is considered to be indistinguishable from background noise and shall not be considered for analysis. The “Allele” tab contents should also appear using the stutter as seen below and bin set of PowerPlex_Fusion_6C_Bins_IDX_v1.1.

5.3.6.3 Marker-specific stutter filter percentages for PowerPlex® Fusion 6C are listed below. All potential stutter peaks must be evaluated as GMID-X cannot set automatic stutter cutoffs for all stutter peaks noted in validation summaries. In addition to the N-4 stutter percentages listed below, N-8 and N+4 were evaluated in the validation studies. The maximum observed values were 1.1% for N-8 and 3.0% for N+4. Stutter filters in GMIDX are the validated values + 3 standard deviations. STR results shall not be considered inconclusive if stutter peaks are present in single source samples. In mixed samples with major/minor components, minor peaks in stutter position that are indistinguishable from stutter may be interpreted. Samples with overall peak height values within the stochastic range or close to overblown range should be evaluated carefully as increased stutter may be seen in these samples.

| Locus | N-4 Average Stutter % |
|----------|-----------------------|
| D3S1358 | 9 |
| D1S1656 | 8.5 |
| D2S441 | 4.8 |
| D10S1248 | 8 |
| D13S317 | 4.9 |
| Penta E | 2.7 |
| D16S539 | 6.4 |
| D18S51 | 8.1 |
| D2S1338 | 8.5 |
| CSF1PO | 5.5 |
| Penta D | 1.9 |
| THO1 | 2.2 |
| vWA | 6.9 |
| D21S11 | 8.2 |
| D7S820 | 4.7 |
| D5S818 | 6.1 |
| TPOX | 2.5 |
| D8S1179 | 7.3 |
| D12S391 | 9.1 |

| | |
|----------|-----|
| D19S433 | 7 |
| SE33 | 10 |
| D22S1045 | 7.2 |
| DYS391 | 6.7 |
| FGA | 7.1 |
| DYS576 | 8.5 |
| DYS570 | 8.9 |

5.3.6.4 In the main GMID-X window, ensure that the Traditional Allele Table is chosen; select “Tools” from the top menu; then select “Table Setting Editor.” Under the “Genotypes” tab, at the bottom of the window, ensure that the “Number of Alleles” under Allele Settings is set to at least 12.

5.3.6.5 Only the DNA Technical Leader or the GMID-X Administrators may change settings for the Analysis Method Editor or the Table Setting Editor.

5.3.7 Adding/Deleting samples

5.3.7.1 If a Forensic Scientist must add additional sample(s) to a current project, follow **5.3.2** through **5.3.5**. The newly added sample shall be analyzed (select the Analyze icon) before the data may be viewed.

5.3.7.1.1 If the sample is from a different injection (e.g., run folder), the associated Allelic ladder shall also be imported in order for the sample data to be sized properly.

5.3.7.2 If a Forensic Scientist must remove a sample(s) that has been added to the project (e.g., a sample from an unrelated case), highlight the sample to be removed and in the menu bar, select Edit and Delete from Project. Select “OK” when prompted.

5.3.7.2.1 Samples from the same case that are present in a project, but are not used (e.g., redundancy, sizing data issues or similar), shall not be deleted.

5.4 Analyzing/Preliminary Evaluation of Data from a GMID-X Project

5.4.1 Evaluate Sizing Data (i.e., WEN)

5.4.1.1 WEN sizing data for all samples must be evaluated to ensure all peaks are present and correctly sized (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500-base pairs). Samples and controls that show no sizing data but contain all correctly labeled sizing peaks (e.g. samples where noise causes an issue with the automatic labeling of peaks) may be used for comparison after the Override SQ function has been applied.

5.4.1.2 If any sample or reagent blank in the project indicates no sizing data (e.g., WEN failure), then that sample or reagent blank shall not be used for comparison. This information shall be noted on the Casework Table Worksheet.

5.4.1.2.1 If there is no sizing data for an allelic ladder, then no data associated with that ladder can be sized unless a ladder is imported from another run injected on the same instrument within 24 hours. Note: The source of this imported ladder shall be documented in the case file. When using an imported ladder, data must be evaluated for migration shift (e.g. numerous OL calls seen with imported ladder use).

5.4.1.2.2 If an amplification control has no sizing data, a duplicate control from the same amplification may be used.

5.4.1.2.3 Any sample/reagent blank that has no sizing data shall be reanalyzed (e.g. injected again on 3500, re-amplified, or re-extracted).

5.4.2 Assess Overall Quality of Data

5.4.2.1 Allelic Ladders and Controls

5.4.2.1.1 Once all samples have been analyzed, the Analysis Summary tab will appear. This tab shows an overview of all samples, ladders, and controls for the run.

5.4.2.1.2 Verify that all the alleles are sized and labeled correctly according to the expected calls for the PowerPlex® Fusion 6C Allelic ladder. All expected peaks must be present and above the appropriate analytical threshold.

5.4.2.1.3 All positive control(s) must have the expected allelic results at all loci. If these expected alleles are not in the correct position or are below the analytical threshold, then the control shall be re-injected. If re-injection is unsuccessful, then the controls and all associated samples shall be re-amplified and analyzed before that control may be used for analysis.

5.4.2.1.4 Throughout developmental and internal validation studies sporadic instances of extraneous DNA were noted. These events are most likely due to the sensitivity of the kit and not resulting from sample handling. As a result, a single peak noted in a negative control that is seen below 300 rfus can be omitted during analysis as long as there are no other incidences of possible contamination or impact on interpretation of associated samples. If multiple peaks not attributable to artifacts are present above the analytical thresholds, the controls shall be reanalyzed (i.e., re-injected or re-amplified or re-extracted) unless multiple controls are present for the batch (e.g., casework direct processing), then an additional control from the batch may be processed for the check of the reagent and procedures. If re-analysis or alternate negative control use is not possible, then use of the controls and the associated samples for interpretation and reporting shall be done upon consultation with the DNA Technical Leader (TL). The TL shall consider the peak height and

number of peaks with respect to the profile. This consultation shall be documented.

5.4.2.2 Samples- Examine all samples to assess the quality and quantity of DNA present. Examine each sample to determine the number of contributors and to assist in artifact designation for samples that are to be interpreted using the STRmix software. Samples with resolution issues noted must be reinjected to resolve prior to import into STRmix.

5.4.2.2.1 Artifacts: If any peaks are to be removed from consideration as true alleles due to their presence as a known artifact (e.g., pull-up, dye-blob, stutter, spike), highlight the sizing box associated with the peak and right-click the mouse. The Forensic Scientist shall evaluate peaks before deleting to ensure they are artifacts and not peaks that are a part of a minor/low level profile. Select Delete Label(s) (multiple peaks can be deleted simultaneously by selecting them at the same time-when the warning dialog box appears, select OK). When prompted, type in artifact as the Reason(s) for Change. The Forensic Scientist should not relabel the peak if the GMID-X software automatically labels the peak (pink label).

5.4.2.2.2 Non-specific amplification peaks: Non-specific amplification is known to occasionally occur with STR kits and is documented in developmental validation and through on-going technical support from the manufacturer. The Forensic Scientist shall evaluate these peaks. Peaks may be designated as artifacts if they meet any of the following criteria: atypical peak morphology; OL designation without proper sizing (outside of +/- 0.5 bp bin); rfu value inconsistent with remainder of profile (e.g. 7000 rfu peak noted when remainder of profile is ~500 rfu); manufacturer documented non-specific amplification peak; and/or lack of stutter peak.

5.4.2.2.3 Off-ladder Alleles, Tri-alleles and Microvariants: If a sample(s) contains an off-ladder allele that is not an artifact or a microvariant is present in a sample and GMID-X has not automatically assigned it an allelic value, Forensic Scientists shall document the proper sizing/locus assignment of the off-ladder alleles, tri-alleles, and/or microvariants as follows:

5.4.2.2.3.1 In a single source sample, if the allele in question lies outside the marker range of a locus (e.g., between two loci, above or below the end of the color channel), the Forensic Scientist shall attempt to determine which locus the allele should be assigned based upon the presence or absence of heterozygosity at the loci in question.

5.4.2.2.3.2 If one locus exhibits homozygosity and the second heterozygosity, the off-ladder allele shall be assigned to the homozygous locus.


5.4.2.2.3.3 If both loci exhibit homozygosity or heterozygosity, the Forensic Scientist shall document either on the allele call table or the electropherograms that the allele was observed, but not assign the allele

to a particular locus, and mark the allele as inconclusive (INC). The called (non off-ladder) alleles at these loci shall be reported and uploaded into CODIS.

5.4.2.2.3.4 In some instances, an off-ladder allele from one locus may appear within an adjacent locus (i.e., appears to be a potential tri-allele next to a homozygous locus). If the Forensic Scientist believes such a situation has occurred, then both loci shall be marked as inconclusive.

5.4.2.2.3.4.1 A microvariant or an off-ladder shall be named/assigned per NDIS standards. For CODIS eligibility, refer to the Forensic Biology Section Procedure for CODIS.

5.4.2.2.3.4.2 In the main GMID-X window, select the sample(s) containing the microvariant/off-ladder allele and the allelic ladder. Display Plots. Zoom in to the locus containing the microvariant/off-ladder allele; the Sample Plot displays the allelic ladder in the top half of the Plot window and the sample is in the bottom half.

5.4.2.2.3.4.3 Highlight the microvariant/off-ladder allele in the sample, along with the alleles in the allelic ladder that lie the closest in size to the microvariant/off-ladder allele (to select peaks simultaneously, hold down the Ctrl key on the keyboard while selecting). Select the sizing table icon (). Select Ctrl + G to make the sizing table display the selected alleles.

5.4.2.2.3.4.4 Based upon the sizing information provided in the table, determine the appropriate allele call for the microvariant/off-ladder allele.

5.4.2.2.3.4.4.1 The correct allele call for the microvariant/off-ladder allele is dependent upon the base pair size when compared to the allelic ladder. For each base pair size difference, the naming convention is changed by .1 from the ladder allele (e.g., 17.1, 17.2, etc.) The size of the repeat motif (e.g. tri-, tetra-, or penta-) should be taken into account when naming. If the sizing difference is off by >0.5bp, then possible non-specific binding or sample shifting should be accounted for prior to naming. For samples that are either above or below the ladder alleles or between bins, then a

“>” or “<” sign will be added to the closest allele in the ladder for that locus.

5.4.2.2.3.4.4.2 Microvariants that vary by less than the consensus repeat unit for that locus must be designated as an integer of that variation (e.g., the 9.3 allele at TH01).

5.4.2.2.3.4.5 Rename the allele by right-clicking on it and selecting Rename Allele Label. Select Custom Allele Label and enter the call in the box. When prompted by the Reason(s) for Change box, type microvariant or off-ladder allele.

5.4.2.2.3.4.6 With the alleles highlighted still present in the sizing table, print to pdf adding to the electropherogram after the sample.

5.4.2.2.3.5 Verification of Off-Ladder Alleles, Tri-alleles, Microvariants:

5.4.2.2.3.5.1 Off-ladder alleles and microvariants do not require verification as long as they can be properly sized and there is no shifted alleles or injection issues.

5.4.2.2.3.5.2 Possible tri-alleles will be documented as such and the locus will be ignored in STRmix interpretation. There is no program that can account for tri-alleles in statistical calculations and STRmix cannot account within deconvolution. Re-amplification is required to verify the presence and size (if applicable) only if the locus is to be used for interpretation (e.g., confirm not elevated artifact).

5.4.2.2.3.5.3 When multiple occurrences of the same tri-allele are seen within the questioned samples of the same case, no verification is required.

5.4.2.2.3.5.4 Shifted alleles/loci shall not be used for comparison in any control, ladder or sample.

5.4.2.2.3.5.5 A known sample cannot be used to verify the presence of a tri-allele in a questioned sample.

5.4.2.2.3.6 Off-scale Samples: Off-scale samples should not routinely be used for comparison. Such samples shall be re-amplified with a lower concentration. If an off-scale sample is used for comparison, justification shall be included in the case notes.

5.4.3 Printing Egrams Electronically

5.4.3.1 The egrams for any well in which data is collected (e.g., blank, ladder, item, etc.) shall be printed and the egrams for each GMID-X project shall exist as an individual PDF file. Ensure the plot setting is set to “Traditional Genotype Plot” when printing to PDF. The egrams for the questioned items in a case shall be printed prior to the known items. The egrams for the negative controls and samples with no DNA peaks detected shall be printed scaled to approximately 100 rfus.

5.4.3.1.1 GMID-X projects are added to the FA case record object repository by exporting the projects: in GMID-X, select “Tools,” “GeneMapper® ID-X Manager,” select the applicable project and select “export.” The file is exported/saved as a .ser file.

5.4.3.2 Any individual sample/control/ladder with the case that is not being used (e.g., WEN failure, redundancy, further analysis) shall be added to the appropriate FA Case Record Object Repository. Such samples/controls/ladders shall appear in the Casework Table explaining why it was not used (e.g., WEN failure, redundancy, re-analysis).

5.4.3.2.1 Even if not used for comparison purposes, all instrument files (e.g. .hid) associated with such samples/controls/ladders shall be present in the run folder containing the electronic data in the FA case record object repository. If a sample is being reinjected with samples from different amplification setups, then printing those amplification controls is not required (only ladder and sample is required).

5.4.3.2.2 For any sample/control/ladder that is not used, corrections (labeling of artifacts) are not required.

5.4.3.3 If an entire run or project for a case is not used, it shall be added to the case record object repository. Corrections (e.g., labeling of artifacts) are not required in this instance. A Casework Table shall be generated for this instance and the reason for not using the entire run/project shall be noted on this page. An Allele Call Table is not required for such runs/projects.

5.4.3.4 The WEN sizing standard shall be printed along with the overall egram PDF file for each project. The WEN must be present and labeled correctly for all samples/controls/ladders, and all peaks (60 through 500) must be visible. The full width of all peaks must be visible; however, it is acceptable to zoom in on the heights of the peaks when printing.

5.4.4 View Raw Data

5.4.4.1 In order to verify the addition of amplification kit primers to all samples and controls which do not show amplification of at least one allele (which includes instances of WEN or injection failure), the raw data for these samples/controls shall be added to the case record object repository. Any sample/control that shows at least one allele is not required to be printed, as it may be inferred that the presence of an allele indicates primers were added to the

sample/control. Note: If the entire run is not used for analysis, then the negative raw data is not required to be printed.

5.4.4.1.1 In the main GMID-X window, highlight the sample/control and select Raw Data from the View option in the main GMID-X menu.

5.4.4.1.2 The subsequent window displays the raw data on the right-hand side and the sample and run information on the left-hand side. Expand the left-hand window so that the full run file name is clearly visible. The right-hand window shall show the maximum vertical and horizontal axis.

5.4.4.1.3 Raw data shall be saved/printed as a .pdf file and included in the case record object repository. The case number, sample identification, analyst's identifiers (initials or name) and data shall be present. Methods for transferring information from GMIDX to the case record are given below.

5.4.4.1.3.1 Using the "Alt" and "Print Screen" buttons on the computer, take a snapshot of the raw data. Open up a Word document and paste this snapshot into the document. Repeat this process for all samples/controls in each project that do not show amplification of at least one allele.

5.4.4.1.3.2 In the main GMIDX window, highlight the negative control/sample and select "Raw Data" from the "View" option in the main GMIDX window. Print to pdf the raw data for each negative control/sample, combining so that all the data is in one document.

5.5 Exporting and Creating Tables

5.5.1 Casework Table

5.5.1.1 Only samples and controls specific to the case shall be present in the Casework Table.

5.5.1.2 On the Casework Table, Forensic Scientists shall note any samples that are not to be used for comparison along with the reason or note what additional analysis is being performed. Notes used for the interpretation of a sample, including the number of contributors for questioned items, will be documented on this page or in the STRmix interpretation. Notation of uninterpretable profiles with reasoning will be noted on the casework table.

5.5.1.3 The Casework Table shall be saved as the first page of the egram file.

5.5.1.4 Create a Casework Table using Excel or through the GMIDX software.

5.5.1.4.1 Excel Version

5.5.1.4.1.1 In the main GMID-X window, select the "Traditional Allele Table" from the Table Setting Menu.

- 5.5.1.4.1.2 Select all samples and under “File” in the main GMID-X menu, select “Export Table.” Save the table to a location easily located by the Forensic Scientist (e.g., desktop or thumb drive). Ensure the file is exported/saved in the Tab-delimited Text (.txt) format.
- 5.5.1.4.1.3 Open Excel; open the .txt file saved in the previous step (note: the Forensic Scientist may have to change the file of type to All Files in order for the software to access the .txt file).
- 5.5.1.4.1.4 When the Text import Wizard opens, select “Finish.”
- 5.5.1.4.1.5 Adjust the columns to accommodate the full text for each line.
- 5.5.1.4.1.6 Select “Page Setup.” Under the Page tab, select landscape and fit to 1 page wide by 1 page tall; under the Header/Footer tab, select Custom Header – at a minimum, the Forensic Scientist shall enter the full case number, initials and the date; under the Sheet tab, select only the Gridlines box. Finish by selecting “OK.”

5.5.1.4.2 GMIDX Version

- 5.5.1.4.2.1 In the main GMIDX window, select “traditional allele table” from the setting menu.
- 5.5.1.4.2.2 Print to pdf the sample table, selecting landscape orientation with page size A3 transvers in the page setup tab before printing. Print the remainder of the egrams to pdf merging after the casework table.

5.5.2 Allele Call Table

- 5.5.2.1 In the main GMID-X window, select the “Traditional Allele Table” from the Table Setting menu. With all samples selected, select the “Genotypes” tab in the main GMID-X menu.
- 5.5.2.2 Under the “File” menu in the main GMID-X window, select “Export Table.” Save the table to a location easily located by the Forensic Scientist on his/her computer (e.g., desktop or thumb drive). Ensure the file is exported/saved in the Tab-delimited Text (.txt) format.
- 5.5.2.3 Open Excel; open the .txt file saved in the previous step (note: the Forensic Scientist may have to change the files of type to All Files in order for the software to access the .txt format).
- 5.5.2.4 When the Text Import Wizard opens, select “Finish.”
- 5.5.2.5 Open the Allele Call Table Excel file in the Forensic Biology Shared Folder. Copy and paste the allele calls generated from the .txt file (Select All) into the Results tab in the appropriate Allele Call Table Worksheet.

- 5.5.2.6 Return to the Allele Call Table tab (or the Mixture tabs if any samples have more than 2 alleles at any given locus) and verify that the copy and paste function worked properly.
- 5.5.2.7 If multiple injections are present in a case file due to re-extraction, re-amplification, or re-injection (non-serial in nature), the Forensic Scientist shall indicate from which run the sample came. Item descriptions shall also be typed into the designated areas in the worksheet. The Forensic Scientist shall ensure the full case number and the date are present.
- 5.5.2.8 It is permissible to combine results from different injections, dilutions and amplifications of the same sample when determining a final DNA profile. When separate extracts from different locations on a given evidentiary item are combined prior to amplification, the resultant DNA profile is NOT considered a composite profile. Unless there is a reasonable expectation of sample(s) originating from a common source (e.g., duplicate vaginal swabs or a bone), allelic data from separate extractions from different locations on a given evidentiary item should not be combined into a composite profile. Replicates cannot be used in STRmix deconvolution. Composite profiles are permissible for reference profiles or samples not subject to deconvolution in STRmix.
- 5.5.2.9 It is permissible for the Forensic Scientist to combine all Allele Call Table Worksheets into one Excel spreadsheet.
- 5.5.2.10 Allele call tables for known samples shall be generated and included as separate .pdf files in the appropriate Case Record Object Repository. If STRmix is used for all question samples in a case, then the STRmix reports may be used in lieu of an allele call table for the documentation of interpretation. An allele call table may be created for kinship cases that combines the question and known samples for comparison purposes.
- 5.5.2.11 Only samples specific to the case shall be present in the Allele Call Table (i.e., controls, unlabeled/unassigned allele call tables, and ladders shall not be printed).

5.5.3 CODIS Table - Refer to the Procedure for CODIS for CODIS Table export and Specimen upload.

5.6 GMID-X Use for Samples for STRmix™ Interpretation

- 5.6.1 Unknown/questioned samples will require a second analysis prior to export for STRmix interpretation. STRmix analysis may only be performed on samples amplified with Promega PowerPlex Fusion 6C. The additional analysis will use the same steps as the original analysis, with a change to the Analysis Method. The Analysis Method for samples for STRmix interpretation is “Fusion_no stutter”. STRmix will be used for single source samples and mixture samples up to four contributors. Reference samples are not to be analyzed a second time.
- 5.6.2 This project will be saved as a new GMID-X project, adding “_STRmix” to the original file name. The project will be exported and added to the case record object repository in the same manner as the original project.

5.6.3 After analysis of the selected samples, the electropherogram plot will be assessed for artifacts and the artifacts removed. The original plots can be used for comparison to assist the analyst. Stutter peaks must not be removed from these samples as STRmix requires them. During the validation and implementation of the STRmix software, various stutter positions were modeled. Back stutter, forward stutter, and double back stutter was modeled at all STR loci. Half back stutter was modeled at loci D1S1656, D18S51, D21S11, D19S433, and SE33. Stutter peaks identified at other positions along with off-ladder peaks should be removed since the retention could affect the results of the STRmix software.

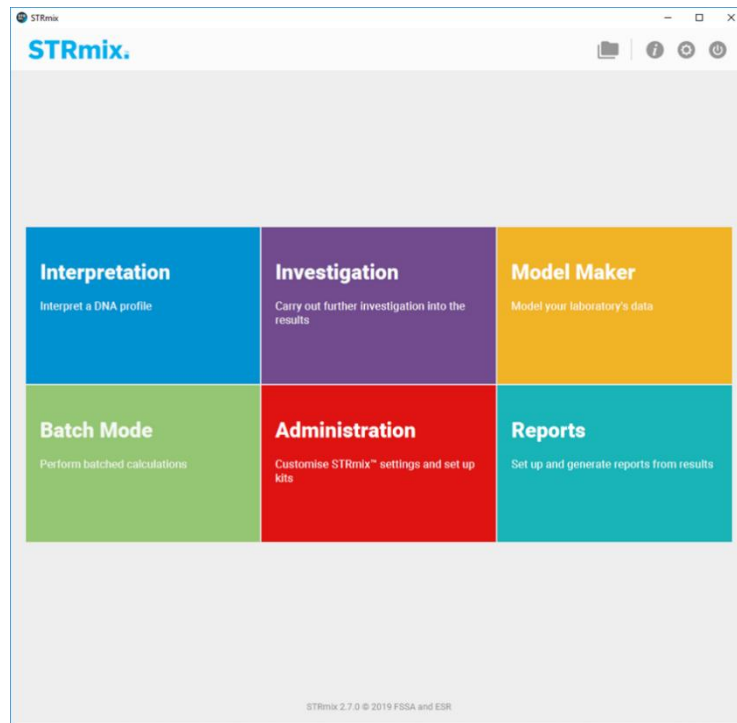
5.6.4 Samples with all stutter remaining will be exported for interpretation in STRmix.

5.6.4.1 Select the “Traditional Allele Table” in the table setting menu of the main GMID-X window. With all the samples selected, select the “genotypes” tab. Up to 25 alleles will be exported using this setting.

5.6.4.2 Under the “File” menu, select “Export Table.” Save the file to a location that can be accessed from the STRmix computers. Ensure this file is exported in the .txt file format.

5.7 Sample Interpretation Using STRmix Software

5.7.1 Open the STRmix program. Each of the functions with the software are accessible from the Startup screen.



5.7.2 The default settings were established during the implementation and validation of the software. They are saved within the program and are not to be changed without written permission from the Technical Leader. The default kit is NCDOJ_Fusion6C_3500 and the settings are listed in Appendix A.

5.7.3 Sample Analysis

5.7.3.1 A preliminary interpretation of samples to determine if the profile is suitable for interpretation and the number of contributors (NoC) present in the sample shall be performed. This preliminary evaluation will also determine if the profile can be conditioned upon using a known reference sample. Only interpretable samples deemed suitable will be analyzed in STRmix.

5.7.3.2 To start a deconvolution of a sample, select “Interpretation” in the main STRmix window.

5.7.3.3 Fill in the Case number, sample ID, any case notes (optional), and the number of contributors for the analysis. Click “Next.”

5.7.3.3.1 Evidence samples being interpreted as >4 contributors cannot be analyzed using the STRmix software.

5.7.3.3.2 Samples with excessive peak height cannot be analyzed using the STRmix software. Excessive peak heights will adversely affect the quality of the genotype data obtained from the analysis. Overblown samples should be re-amplified at a lower template amount prior to analysis with STRmix.

The screenshot shows the 'INTERPRETATION' window in the STRmix software. The window title is 'STRmix'. The main heading is '< INTERPRETATION'. Below the heading are four input fields: 'Case Number' (with placeholder 'Type your Case Number here'), 'Sample ID' (with placeholder 'Type your Sample ID here'), 'CODIS Specimen ID' (with placeholder 'Type your CODIS Specimen ID here'), and 'Case Notes' (with placeholder 'Type your Case Notes here'). Below these fields is the 'MCMC SETTINGS' section, which includes a 'Number of Contributors' field (with placeholder 'Type your value here') and a 'Contributor Range' checkbox. At the bottom of the window are three buttons: 'Run Settings' (purple), 'Cancel' (yellow), and 'Next' (blue). The footer of the window reads 'STRmix 2.7.0 © 2019 FSSA and ESR'.

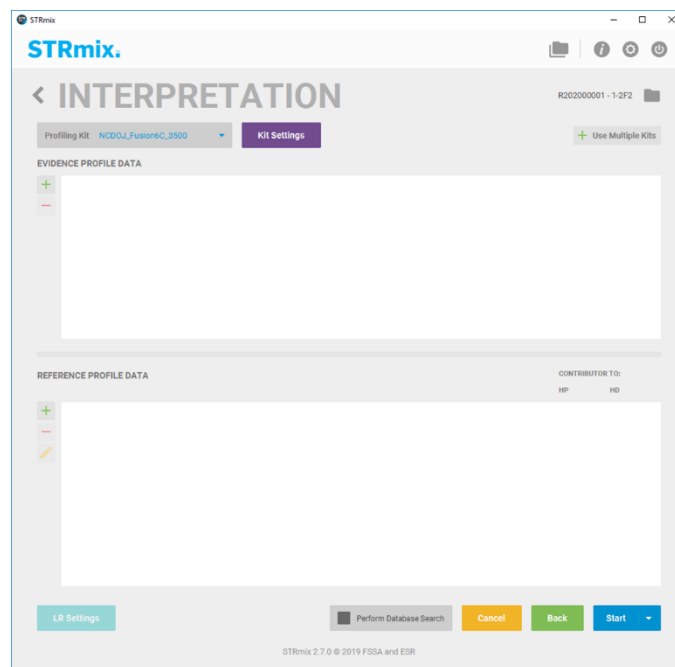
5.7.3.4 Kit settings are available by selecting the “Kit Settings” button in the next visible screen.

5.7.3.4.1 The general and stutter settings should not be adjusted, as they are the settings created during the implementation and validation process.

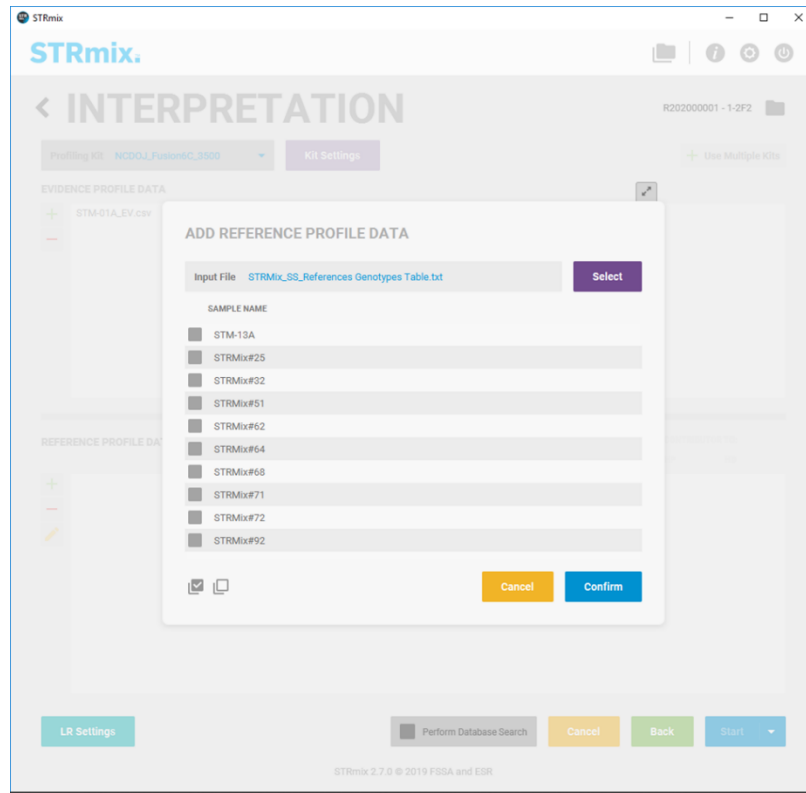
5.7.3.4.2 Loci settings may require adjustment depending on the input file. After review of the profile, if the analyst determines that loci need to be ignored during analysis, they would select those loci on this window.

5.7.3.4.2.1 STRmix cannot accommodate trisomy or somatic mutations. If a profile has a tri-allelic pattern, the locus at which it is seen will be ignored during analysis.

5.7.3.4.2.2 Non-numeric values are not permitted in a STRmix input file and will cause an error in analysis. Variant alleles must be assigned the appropriate numeric value prior to import. If a numeric value cannot be confidently assigned (e.g. >12), then the locus containing the variant allele must be ignored during analysis.



5.7.3.5 Add the evidence profile to be deconvoluted by selecting the “+” in the evidence profile data window. A new window will open. Drag the .txt file from the GMID-X analysis containing the samples into the “Input file” area. Select the sample for analysis and select “Confirm.”



- 5.7.3.6** To add a reference sample, repeat the process in the “Reference Profile Data” portion of the window.
- 5.7.3.6.1** References may be added during the deconvolution to samples interpreted as single source. This will allow the deconvolution and likelihood ratio to be generated simultaneously.
- 5.7.3.6.2** References may also be added during the deconvolution if the analysis is to be conditioned upon a profile. To condition, after adding the reference profile, select the boxes under both “HP” and “HD” to assign them as a contributor to both.
- 5.7.3.6.3** The full profile of a reference sample must be present for comparisons. STRmix will not perform comparisons at a locus where the reference profile does not contain any data (e.g., complete locus drop). STRmix considers a single allele entered into the input file as a homozygote (e.g. 10, 10 not 10, any) therefore any locus with partial information needs to be removed from the input file prior to entry.
- 5.7.3.7** After the evidence sample and any applicable reference sample(s) are added to the interpretation window, select “Start” to begin the analysis.

5.7.3.8 At the completion of the analysis, the report will automatically open. It can also be located in the STRmix results file.

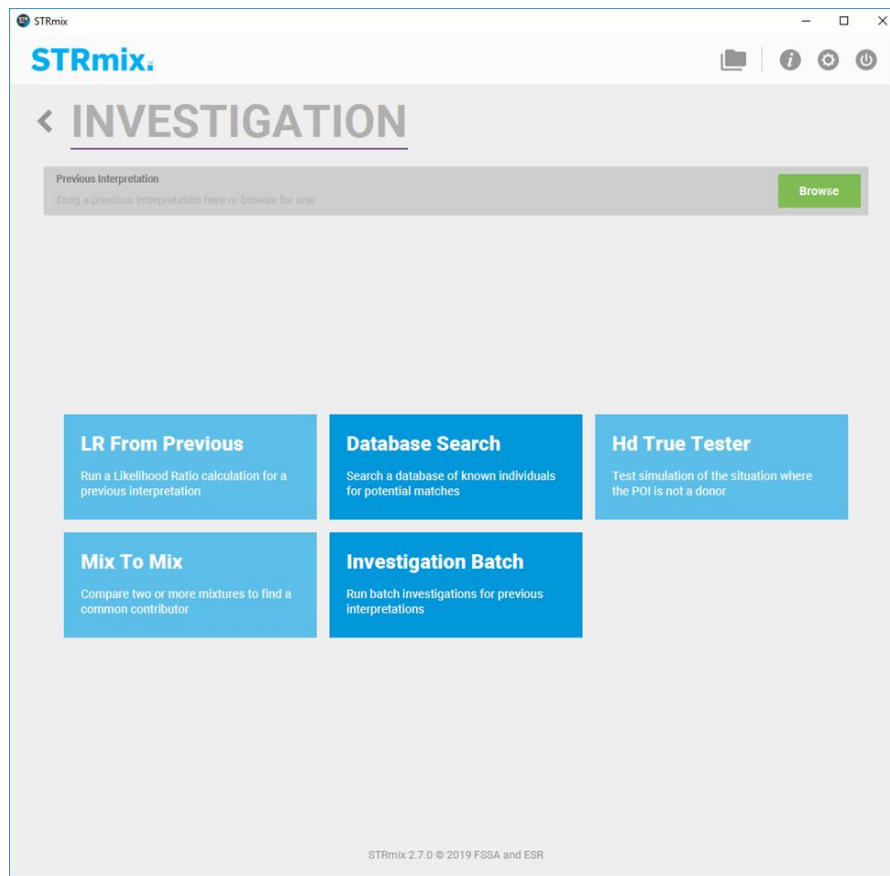
5.7.3.9 The Forensic Scientist will use the generated report for analysis and interpretation of the profile.

5.7.4 LR from Previous

5.7.4.1 After the deconvolution of the sample is completed, the Forensic Scientist shall review the reports and data to ensure that the results are intuitive.

5.7.4.2 A likelihood ratio shall be generated for reference samples submitted in the case that cannot be excluded as being a contributor to the sample. This will then be evaluated to determine the statistical support for the inclusion.

5.7.4.3 To start a LR from Previous analysis, open the Investigation window from the main menu.



5.7.4.4 Select the previous interpretation to be used, either by dragging the file from the results folder or by navigating to it using the “Browse” function.

5.7.4.5 Select LR from Previous. STRmix will use the previously generated genotype weightings to calculate an LR from a reference profile.

5.7.4.6 Add the reference profile that could not be excluded from the profile and start the LR function. Multiple reference samples can be selected to accommodate the LR propositions being considered.

5.7.4.6.1 The MCMC settings cannot be changed at this stage, the software utilizes the data that was previously generated. Evidence profiles cannot be edited under this function.

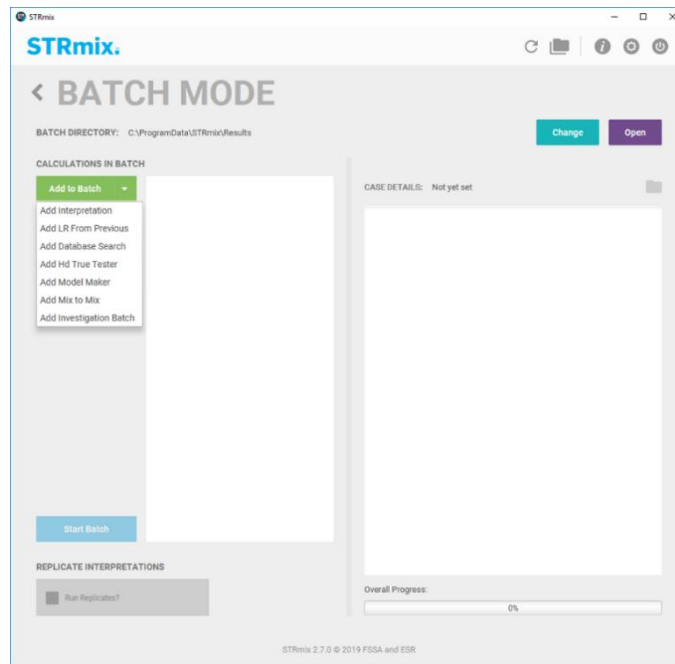
5.7.4.6.2 Samples will only be compared at loci that they have in common. If a locus is missing from either the evidence or the reference input, no comparison will be performed at that locus.

5.7.4.7 Upon completion the report will automatically open. It can also be located by navigating to the results file.

5.7.5 Batch Mode Processing

5.7.5.1 Batch mode processing allows a number of different analyses to be setup in turn and run sequentially without additional analyst interaction. Each analysis will be saved in individual folders.

5.7.5.2 From the main STRmix menu, select “Batch Mode”. This will open the Batch Mode window.



5.7.5.3 The analyst can then add each analysis to the calculations in batch window. For each selected analysis, the analyst will complete the setup in the same manner as performed for an individual analysis setup.

5.7.5.4 Once all analyses are added to the batch, select “Start Batch.” This will begin the analysis. They will be run in the order they were added to the batch.

5.7.5.5 The results will be automatically saved in the results file.

5.7.6 STRmix Reports

5.7.6.1 There are three report types generated from STRmix: interpretation report, LR from previous report, and CODIS report. The default settings for these reports are saved within the software. Alterations to report settings will not be allowed without written permission from the Technical Leader.

5.7.6.1.1 If alterations to the default analysis settings are allowed, the settings will be added to the report.

5.7.6.2 Interpretation reports will be used in conjunction with the other data generated in the case to interpret samples and report on the results of the interpretations.

5.7.6.3 The analyst should review the weightings provided in the interpretation report along with the secondary diagnostics (e.g. Gelman-Rubin, allele and stutter variances, log (likelihood), LR) to ensure that the results are intuitive and do not warrant additional analysis.

5.7.6.4 LR from previous reports will be generated for all reference samples as directed in the section **LR from Previous**. These reports do not contain the results of the deconvolution, those are found in the Interpretation report.

5.7.6.5 Reports generated during analysis will be added to the case record object repository.

5.8 Sample Interpretation

5.8.1 Failure of any locus (loci) to amplify shall not preclude the Forensic Scientist from interpreting/reporting those loci that are present.

5.8.2 Reasoning for the determination of whether or not a locus is used for statistical interpretation shall be documented. Reasoning may be applied to the entire locus or to a portion of the locus (e.g., minor contributor(s)). Inconclusive loci (or portions) shall not be used for comparison purposes.

5.8.3 If no profile above analytical threshold is present, the scientist shall interpret that sample as no DNA profile obtained.

5.8.4 The presence of apparent degradation, inhibition, or significant stochastic effects in a profile shall be considered during the interpretation process and may influence the assessments made at each

locus. Additionally, the scientist may use additional criteria including quantitative values and/or the totality of the profile to determine if potential stochastic amplification has occurred. The stochastic threshold is set at 300 RFU. This threshold is used to assist the analyst in the preliminary interpretation of the quality of the profile and the number of contributors. STRmix is a probabilistic genotyping program and it does not use a stochastic threshold in the analysis of samples.

- 5.8.5** Data generated from quantitation results or electropherogram results may be used in the determination of the presence of a male contributor in a sample. Data obtained at Amelogenin and/or the YSTR loci (DYS391, DYS570, and DYS576) may be utilized for this determination even if the sample (or portion of sample) is interpreted as uninterpretable. This data may be used to clarify which samples would be recommended for YSTR processing following our current guidelines.
- 5.8.6** DYS391, DYS570, and DYS576 will be considered information only loci and can be used to assist in the determination of the number of contributors in the sample. These loci will not be used for inclusion or exclusion of a known standard or for statistical calculations. Lack of signal at these loci will not result in the profile being called "partial" and will not negate identity if all other loci meet the criteria. Results are required in the positive control to ensure appropriate amplification. These loci may be compared to any profiles generated with Y23 to ensure the concordance of samples.
- 5.8.7** Peak height ratio – During preliminary evaluation of samples balance at each locus will be assessed. Based upon validation studies, single source samples/single major contributor should exhibit heterozygote peak height ratios (PHR) greater than or equal to 60 % when both peaks are at/above the stochastic threshold. Low PHR may indicate a null allele, a primer binding site mutation, degradation, or the presence of inhibition. This evaluation will be used to assist the analyst in determining the number of contributors present in the sample. STRmix does not use peak height ratio thresholds during deconvolution steps as it is a probabilistic genotyping software.
- 5.8.8** Reinterpretation of Legacy Data – Comparison to single source (to include major profiles) is not considered reinterpretation. Additionally, it is not reinterpretation if documentation of previous interpretation includes possible contributor genotypes. Any assessment of allele calls, removing of alleles/loci for statistical calculations, or a change in assumptions is considered reinterpretation. Any reinterpretation shall require review by the Technical Leader and CODIS Administrator.
- 5.8.8.1** Requests to reanalyze samples amplified using Fusion 6C prior to implementation of the STRmix software shall be examined and performed per case and is not automatic. If the mixture was previously inconclusive (either in part or whole) and reevaluation of the sample using STRmix may render an interpretable profile, then reanalysis may proceed. This review will be documented in the case record.
- 5.8.8.2** STRmix software shall be used for samples previously amplified using Fusion 6C prior to the implementation of STRmix where statistical calculations are required as a result of additional comparisons (e.g. CODIS hits, additional reference standards submitted for comparison, statistics only requests, etc.). The likelihood ratio statistics generated shall be reported for these additional comparisons. No additional RMP calculations will be

performed on samples amplified with Fusion 6C without written documentation from the Technical Leader explaining the reasoning.

- 5.8.8.3** Samples amplified with kits previous to Fusion 6C shall be evaluated to determine if sufficient extract volume remains for amplification using Fusion 6C. If insufficient volume is present, then the sample will be re-extracted and amplified with Fusion 6C.
- 5.8.8.4** If statistical calculations are required on a profile amplified with a kit previous to Fusion 6C (such as a CODIS hit to a full profile) then the analyst will use the appropriate RMP statistic for the profile as listed on the allele call table from the case record. RMP calculations must be performed using either CODIS Popstats or ArmedXpert software, utilizing the 2017 NIST population database. The calculations performed are documented in the CODIS manual and in the ArmedXpert procedure. Statistics will be calculated using the Caucasian, African American, and Hispanic populations and the lowest value will be reported.
- 5.8.8.5** If reinterpretation of the legacy data is required, then the analyst shall utilize the Forensic Biology legacy case guidance document form to ensure the proper steps are performed in the documentation and reporting.
- 5.8.9** Reference standards that are submitted for comparison to outsource data that has been accepted by the Laboratory for upload shall be compared only to the profile(s) entered in CODIS. No other profiles/data from the case that was analyzed by an external laboratory (outsourced data) shall be reviewed. If a request is received to review additional profiles, the Forensic Biology Manager, Technical Leader, and CODIS Administrator shall make a determination of what items, if any, may be compared.
- 5.8.9.1** Data generated from an outsourcing lab and accepted by the NCSCL following an ownership review cannot be deconvoluted using the NCSCL STRmix software parameters. The outsourcing review procedures will be followed for these profiles and any subsequent comparisons and statistical analysis required.
- 5.8.10** Reference standards submitted for comparison to profiles generated within the Forensic Biology Section shall be compared to the profiles in the case (and listed cross-reference cases). No comparison is required if no DNA profile was generated from an item or if the profile (or component) was previously reported as uninterpretable.
- 5.8.11 Primary Evaluation of Samples** – Samples will be evaluated following initial GMID-X analysis to determine if the sample is interpretable and the number of contributors in a sample. The electropherogram plots are the basis for the results interpretation.
- 5.8.11.1** Samples will be evaluated for general overall quality, including an assessment of the amount of DNA amplified, degradation, and baseline.
- 5.8.11.2** Samples with peaks present near the saturation threshold can result in stutter peaks appearing larger than expected which can cause incorrect genotype assignments. Inaccurate peak heights can also affect the biological modeling of a sample. Samples with

high average peak heights will not be interpreted and will be amplified at a lower template amount. If there is insufficient extract to amplify, the interpretation of the sample will be discussed with the Technical Leader and documented.

5.8.11.3 Based on the results of the quality evaluation, profiles may be interpreted as uninterpretable. Profiles will be reported as uninterpretable if the profile does not have a minimum of 5 alleles over 3 loci.

5.8.11.4 Determination of Number of Contributors (NoC)

5.8.11.4.1 The minimum number of contributors will be based on the most reasonable interpretation of the data. The risk of false inclusions increases when additional contributors (more than the true NoC) are assumed to be present in the sample, while the risk of false exclusions increases when fewer contributors are assumed. Since the true number of contributors is never known in forensic samples, select the fewest number of contributors that explains the data seen. The assignment of NoC typically only affects the likelihood ratio and genotype assignments for the lowest level contributors.

5.8.11.4.2 Allele Count - The locus with the largest number of peaks greater than the analytical threshold will be used to base the NoC determination. The number of peaks divided by two (rounded up, if an odd number of peaks are detected.)

5.8.11.4.3 Peak height imbalances shall be reviewed and considered for the possibility of allele sharing or “stacking” which may indicate the presence of an additional contributor. Generally, homozygote alleles will appear with peak heights twice as large as heterozygotes.

5.8.11.4.4 The general contributor proportions across the profile should also be evaluated to check the pattern across multiple loci.

5.8.11.4.5 Excessive stutter amounts and position should be used to inform the number of contributors.

5.8.11.4.6 Data seen below threshold, not at artefactual positions, should also be evaluated to better inform the number of contributors in the sample. These peaks are unlikely to affect the interpretation of the other contributors. If sub-AT peaks are being used to infer an extra contributor there should be small but above AT peaks and/or peak height imbalance elsewhere in the profile to support this. This data seen below threshold must be at a level when compared to the alleles above threshold to have an impact on the interpretation of the genotypes of the contributors.

5.8.11.4.7 Results from a STRmix analysis (deconvolution and/or LR calculation) may indicate that the initial assessment of the number of contributors may need reevaluation. A subsequent run with N+1 or N-1 contributors may be warranted.

5.8.12 Not Suitable for Comparison Profiles

- 5.8.12.1** DNA samples that are being interpreted as a NoC greater than 4 will not be used for comparison. These samples will be interpreted as uninterpretable.
- 5.8.12.2** DNA samples which contain extremely limited data at only a few loci or contain data that is extremely low level (e.g. complex mixture with all peaks less than 100 rfu) will be interpreted as uninterpretable.

5.8.13 STRmix Interpretation Report

- 5.8.13.1** The interpretation report generated from STRmix will be reviewed and the results, along with the data from the electropherograms will be used by the analyst to generate their conclusions.
- 5.8.13.2** Review the weights and LR (if present), checking to ensure the results obtained are intuitive. The template amounts and mixture proportions should also be reviewed. If there are deconvolution results in a discrete contributor (Component $\geq 99\%$), this profile may be compared to other questioned samples in the case.
- 5.8.13.3** The template amount of a contributor as calculated by STRmix must be equal to or higher than the current lowest analytical threshold (60 rfu) in order for that component to be interpretable.
- 5.8.13.4** Review the secondary diagnostics, including the Gelman-Rubin (GR) result, variances, log(likelihood), and any evidence peak issues noted in the report. The analyst will use this review to ensure the STRmix analysis performed as expected and that no additional analysis is needed. Independently, the diagnostics may not indicate an issue; however, collectively they may be useful to gauge the quality of the results obtained from STRmix.
 - 5.8.13.4.1** Log (likelihood) – should be above 0. The larger the value, the better STRmix has been able to describe the observed data. A low value shows the software has not been able to describe the observed data.
 - 5.8.13.4.1.1** The profile may be low level and have little data to inform the value.
 - 5.8.13.4.1.2** The assigned contributors is incorrect, resulting in forced stochastic events.
 - 5.8.13.4.1.3** Input file is incorrect. Check for stutter peaks that were removed that should have been included in the input file and for artifact peaks left in the input file that should have been removed.
 - 5.8.13.4.2** Gelman-Rubin convergence diagnostic – should be less than 1.2. This value indicates how well the MCMC chains converged on the final deconvolution

profile. A higher value indicates that the chains may have not converged during the run. In conjunction with other diagnostics, running the analysis at greater iterations may improve this convergence. Performing the analysis with increased iterations will be done only after written documentation of the approval of the Technical Leader.

5.8.13.4.3 Allele and stutter variances – these values indicate how well the data has been explained using the biological models. If the values have increased markedly from the respective modes, it may indicate that the data is not well explained. This issue may result from sub-optimal DNA profile quality or when the number of contributors assigned is incorrect.

5.8.13.5 Troubleshooting

5.8.13.5.1 If the results obtained do not appear intuitively correct, or the secondary diagnostics need further investigations, the analyst should examine the profiles, STRmix input, and reports to determine if additional analysis is required.

5.8.13.5.2 Examples would include:

5.8.13.5.2.1 Large LRs at all loci except for one where the LR = 0 but a visual examination shows the reference is consistent with the unknown sample.

5.8.13.5.2.2 The mixture proportions do not reflect the electropherograms data.

5.8.13.5.2.3 The degradation does not reflect observations.

5.8.13.5.2.4 The interpreted genotypes are not intuitively correct.

5.8.13.5.3 The analyst should investigate possible causes, to include:

5.8.13.5.3.1 The MCMC has not run for enough iterations based on the complexity of the profile.

5.8.13.5.3.2 The number of contributors needs to be reevaluated.

5.8.13.5.3.3 The PCR process was inhibited or otherwise affected.

5.8.13.5.4 The analyst should determine if additional analysis is required. This could include:

5.8.13.5.4.1 Verification that the input file is correct (no artifacts remain). If artifacts were still present, remove and rerun the analysis. The

analyst will use this updated analysis for comparisons and reporting.

5.8.13.5.4.2 Additional amplification (if sample remains).

5.8.13.5.4.3 Re-run the analysis at an increased number of iterations. This should not be done for samples with a GR reported as NaN.

5.8.13.5.5 If additional troubleshooting is needed, consult the Technical Leader.

5.8.14 Single Source Profiles

5.8.14.1 Generally, a sample is considered to have originated from a single individual if no more than two alleles are present at all loci for which typing results were obtained (although tri-allelic loci may occur).

5.8.14.2 The peak height ratios for all heterozygotes should meet or exceed the empirically determined PHR. It is noted that peak height imbalances may be seen as a result of a primer binding site mutation or also as peak heights are closer to the stochastic threshold.

5.8.14.3 Homozygote alleles will usually appear as peaks with peak heights approximately twice the height of heterozygote alleles.

5.8.14.4 Profiles being interpreted as single source that do not have reference profiles to compare against shall be analyzed with the STRmix software.

5.8.14.5 Single source profile(s) will be compared to the reference profile(s) submitted in the case (and any cross-referenced cases).

5.8.14.6 If a reference profile is determined to be included as the possible contributor to the single source profile, the sample will be analyzed with the assistance of STRmix to perform LR stats. The deconvolution and LR may be performed simultaneously.

5.8.14.7 If multiple single source profiles are analyzed that have the same profile with no concern for drop-out, then one item should be selected to generate the applicable statistics. Results statements may be combined in the report. For multiple single source profiles where drop-out is present, a single profile may be used for STRmix interpretation and statistics. The analyst should ensure that the results from sample selected do not overinflate the statistical result.

5.8.15 Mixture Profiles

5.8.15.1 General -There are three basic steps to the application of the mixture interpretation scheme: evaluate the overall quality of the profile, determine if the number of contributors can be inferred and decide if contributor genotypes at a particular locus can be restricted based on certain criteria.

Mixture interpretation will be performed utilizing the outputs from STRmix to assist the analysis in the interpretation of the profile.

5.8.15.2 All mixture profiles (2, 3, and/or 4 contributors) that are suitable for analysis will be interpreted with the assistance of the deconvolutions obtained from the STRmix report.

5.8.15.3 A reference profile may be used to condition the unknown profile during the STRmix analysis.

5.8.15.3.1 A conditioned sample (or “assumed contributor”) are individuals who have a reasonable expectation to be present on the sample (e.g., victim’s DNA profile on a vaginal swab, reference profile on an item of clothing identified as being owned/worn by that individual).

5.8.15.3.2 Conditioning improves the deconvolution of the additional contributors to the mixture and can better inform the LR calculation and should be used for applicable samples.

5.8.15.3.3 Loci that have drop out (full or partial) present in the reference sample should be ignored in the questioned item prior to conducting interpretation in STRmix.

5.8.15.3.4 Data should support that their DNA profile is present within the mixture. The quality of the data should be evaluated, i.e., the number of loci that will not be used should be evaluated before conditioning if there is missing data observed.

5.8.15.3.4.1 If, due to the low level of data seen, the analyst is unsure of whether conditioning on a reference standard will better inform the deconvolution, then a sequential approach should be taken for the interpretation of the mixture DNA profile.

5.8.15.3.4.1.1 The mixture will be interpreted using STRmix under the appropriate number of contributors without conditioning of the reference profile in question.

5.8.15.3.4.1.2 An LR from previous for the reference profile will be performed. If the LR previous yields an informative LR result, then a subsequent interpretation will be performed using the reference sample as a conditioning profile. This deconvolution report will be used for future comparisons and a note will be made of why the original is being omitted. If the LR result is

uninformative, then the original interpretation will be used for comparisons.

- 5.8.15.4** As average peak heights (template amount) decrease, so does the discriminatory power between contributors. This is also seen as the mixture complexity increases. Overlap of genotypes between contributors should be expected as the template decreases, or the mixture proportions grow closer. This overlap does not deem the contributors inconclusive.
- 5.8.15.5** If minor components are deconvoluted in a mixture and one contributor is below the interpretable threshold and one is above, but there is no discernable separation of the two components, the analyst shall evaluate the profile above threshold for overall quality. If the genotypes from two profiles are consistent with each other such that the comparison of a reference standard could not distinguish as to which contributor they best fit, then both may be reported as inconclusive components. The analyst may use LR from previous results to assist with the interpretation.
- 5.8.15.6 Template and Mixture Proportion** - As template amount decreases in a sample stochastic effects have a greater effect on the profile. This is also seen as the mixture proportion decreases. Imbalances and allelic dropout can affect the profile modeling.
- 5.8.15.6.1 Two Contributor Mixtures** - Each contributor with a template amount < 150 rfus must have a mixture proportion greater than 5% in order to be compared against any reference profile(s). If the template amount for a contributor is ≥ 150 rfus, the mixture proportion must be >1% in order to be compared against any reference profile(s).
- 5.8.15.6.2 Three Contributor Mixtures** – Each contributor with a template amount < 200 rfus must have a mixture proportion greater than 8% in order to be compared against any reference profile(s). If the template amount for a contributor is ≥ 200 rfus, the mixture proportion must be >3% in order to be compared against any reference profile(s).
- 5.8.15.6.3 Four Contributor Mixtures** – Each contributor with a template amount < 400 rfus must have a mixture proportion >15% in order to be compared against any reference profile(s). Due to the complexity of these profiles, if the template amount for a contributor is ≥ 400 rfus, the mixture proportion must be >10% in order to be compared to any reference profile(s).
- 5.8.15.6.4** Contributors that do not meet the above template and mixture proportion limits should be interpreted as inconclusive for comparison purposes. Interpretation that does not meet the above approach would require approval in writing by the Technical Leader.
- 5.8.15.7 Unknown Number of Contributors** – Every attempt should be made by the analyst to assign a number of contributors to the unknown profile. In rare occasions where the

number of contributors cannot be conclusively determined, a range +/- one contributor may be used in the STRmix analysis under the varNOC analysis settings. The set range cannot exceed four contributors. Consultation with the Technical Leader will be done prior to using the varNOC settings. This consultation will be documented in writing. The results obtained will be reviewed to include the primary and secondary diagnostic outputs and the analyst will determine which genotype results to compare profiles against. It is important to account for both template amount and the complexity of the profile when interpreting these profiles.

5.8.15.8 Mx Prior Function – The Mixture Priors function may be useful in cases where an extreme imbalance in contributor proportions is present which results in unintuitive genotypes following deconvolution in STRmix. Inconsistencies between the electropherogram results and the mixture proportions from the deconvolution may be noted. The Mx priors function allows the analyst to set the mixture ratio prior to deconvolution. The addition of this additional information may be useful to better inform the deconvolution process. Samples where an increased amount of allelic sharing may be better interpreted using this function. Prior to using this function the analyst and Technical Leader will decide based on electropherogram data and case information if this approach is warranted. This decision will be documented in writing and included in the case record.

5.9 Comparison of Profiles

- 5.9.1** When interpreting DNA typing results, the analyst must interpret the results from evidentiary items prior to comparison of any known samples, other than those of assumed contributors (e.g., victim standard on vaginal swabs). This includes whether each locus meets the analytical threshold, is single-source, mixed (distinguishable or indistinguishable), meets PHR expectations, and/or has potential allelic dropout.
- 5.9.2** Those loci which will be used in statistical calculations shall be determined prior to comparison to known reference samples. This will be determined from the STRmix reports.
- 5.9.3** Analysts will compare the LR results between reference samples to ensure that multiple reference samples within a case are not being placed as the same contributor.
- 5.9.4** There may be situations where identification of abnormalities in the questioned sample (e.g. a tri-allele or binding site mutation in mixture profiles) is not possible prior to the deconvolution and unmasking of the reference profile. If, after unmasking information contained within a reference profile, it appears that the original deconvolution may have been affected by the presence of these abnormalities, the number of contributors used in the interpretation will be re-evaluated.
- 5.9.4.1** If the number of contributors is still appropriate and a review of the results are intuitively correct given the mixture proportions, then the affected locus may be removed from the LR from Previous calculation. Documentation of why the locus was removed and that the genotypes at the removed locus does not result in an exclusion will be included in the case record.

5.9.4.2 If the number of contributors changes after this evaluation, the STRmix deconvolution will be re-run using the appropriate number of contributors and the affected locus will be omitted from the interpretation. Documentation will be included in the case record as to why the locus was omitted. The original deconvolution will be retained in the case record.

5.9.5 Comparison of Reference Samples to STRmix results – Analysts shall review the results from the STRmix analysis.

5.9.5.1 Manual exclusions may only be made to profiles where all interpretable components are resolved with only one genotype option at a minimum of three loci. Remaining loci should have resolution of a genotype >99% but may have additional options present.

5.9.5.2 LR from Previous will be calculated using STRmix to assist with the comparisons of all other interpretable components.

5.9.5.2.1 If the LR results in a value $>1.0e3$, report as included and report statistical results.

5.9.5.2.2 If the LR results in an uninformative value ($1.0e-3 - 1.0e3$), then no conclusion can be rendered to this reference due to the inconclusive LR result. If the reference is best fit as an inconclusive contributor (due to template amount or mixture proportion), then the reference will be excluded from the interpretable component(s) due to the quality of data obtained from the mixture. If the contributor best fit result differs for the different populations then the contributor that fits to the most population groups will be reported.

5.9.5.2.3 If the LR results are consistent with the exclusionary hypothesis ($<1.0e-3$), then the comparison shall be reported as an exclusion. The LR results (flipping the LR statement) will be reported only upon request.

5.10 Results

5.10.1 Inclusion – A known individual's profile is included in a single source or mixed sample if the genotype is present at all loci at which DNA typing results are deemed interpretable with no unexplained differences or if the individual cannot be excluded as a contributor to a mixture profile. The loss of an allele due to preferential amplification, stochastic effects, mutation, or other factors must be considered and does not necessarily indicate an exclusion.

5.10.2 Exclusion – A known individual's profile could not have contributed to or is not a part of the questioned profile from a single source or mixed sample if the genotype is missing at any loci at which the DNA typing results are deemed complete.

5.10.3 Inconclusive – There is not enough data to support and inclusion or exclusion. The inconclusive conclusion may be applied to the profile as a whole or to a minor contributor(s). No comparison shall be performed between an inconclusive profile/portion of profile and any reference standard. Such data is not suitable for inclusion or exclusion due to insufficient information.

- 5.10.4** Incidences of employee, vendor or batch case matches/associations shall be immediately conveyed to the DNA TL. Any incidences of the unintentional introduction of exogenous DNA into a control shall also be immediately conveyed to the DNA TL.
- 5.10.5** With unknown profiles in a case, comparisons between unidentified single source or 99% derived profiles to mixtures is not permitted (except for numbering of unknown profiles seen within a case).
- 5.10.6** DNA profiles for questioned items generated under prior technology (e.g., Identifiler, Quantifiler, etc.) may be compared to standards generated using new technology. The analysts will follow guidance provided in the procedure and in the Legacy guidance document. If questions arise, consultation with the DNA Technical Leader will be documented.

5.11 Statistical Calculations

- 5.11.1** Statistics shall be calculated on questioned items if comparison to a known item results in an inclusion. The exception to this requirement is for intimate samples where this association is made to the owner or a consensual partner.
- 5.11.2** Statistical calculations will be performed using the STRmix software which uses a fully continuous probabilistic genotyping likelihood ratio (LR) model. LR are calculated using the Balding and Nichols formula (NRC II recommendation 4.2)

$$\frac{2[\theta+(1-\theta)p_i][\theta+(1-\theta)p_j]}{(1+\theta)(1+2\theta)} \text{ for heterozygote loci}$$

$$\frac{[3\theta+(1-\theta)p_i][2\theta+(1-\theta)p_j]}{(1+\theta)(1+2\theta)} \text{ for homozygote loci}$$

- 5.11.3** The LR assesses the probability of the evidence (E) given two alternate propositions; one that aligns with the inclusion of the comparison sample (H1 or Hp) and one that aligns with the exclusion of that comparison samples (H2 or Hd).
- 5.11.4** The propositions chosen by the analyst will be the most reasonable based on the data from the profile and the case information provided.
- 5.11.5** The LR will be calculated using three groups (NIST Caucasian, African American, and Hispanic). The lowest value obtained from the 99% 1-sided lower HPD LR will be reported, truncated to three significant figures.
- 5.11.6** When determining which value should be used for reporting when the LR results are less than zero but range from within the inconclusive range to outside the inconclusive range (e.g., E-03 to E-04) then evaluation using the applicable hypothesis will be done. The applicable hypothesis would be the original H2/original H1. This calculation can be done by taking the STRmix output and performing the inverse (1/STRmix LR result). This result can then be used for results determination.
- 5.11.7** Amelogenin, DYS391, DYS570, and DYS576 frequencies are not used for calculations.

5.11.8 Failure of a locus to amplify in either the questioned or known sample does not preclude the generation of statistical results at loci where complete amplification occurred.

5.11.9 LR results between 1.00E-03 and 1.00E03 are uninformative. These results do not support an inclusion or exclusion of an individual to a sample. This conclusion will be reported without numerical values.

5.11.10 LR results >1000 in favor of H1 support that the individual is included as a contributor.

5.11.11 LR results >1000 in favor of H2 support that the individual is excluded as a contributor.

5.12 Deducing a Profile for CODIS Entry – Guidance for sample entry into CODIS is found within the Forensic Biology Procedures CODIS DNA Casework and Use of COSTaR.

6.0 Limitations - These guidelines are not meant to cover all situations and shall not be applied retroactively to analysis performed under previous versions of this procedure without the documented authorization of the DNA Technical Leader. Interpretation of low level samples should be approached with caution due to the limits in sensitivity of the STR typing testing procedure. Interpretation of low level results must also factor in the potential loss of data due to the inability to detect all genotypes present in a sample in an effort to ensure a reliable result is obtained. STRmix software can only be used on sample types that were included in the internal validation performed by each laboratory. Mixtures containing greater than 4 contributors were not explored during the validation studies and may not be interpreted with the use of this software. Samples amplified by another laboratory or utilizing a previous amplification kit (e.g. Identifiler Plus or previous) cannot be interpreted with the STRmix software.

7.0 Standards and Controls, Calibration, Maintenance – Controls are run in conjunction with each case to ensure the DNA analysis is performing as expected. The amplification and CE controls are evaluated during the GMID-X analysis. No additional controls are needed for STRmix analysis. Calibration of the software is not required, validations were performed prior to implementing into casework. Maintenance for STRmix is done as needed under the direction of the developer.

8.0 Sampling – N/A

9.0 Uncertainty of Measurement

9.1 Sampling uncertainty occurs during the STRmix analysis due to the finite number of possibilities associated with the population samples being used. An allowance for this uncertainty is implemented by adjusting the allele frequencies using a Bayesian posterior mean frequency (e.g. Highest posterior density (HPD)) to account for sampling uncertainty within a limited population data set.

9.2 The combined LR calculated within STRmix is referred to as a point estimate. The true answer is unknown; therefore a credible interval is applied around the point estimate. This interval is known as a one-sided 99% HPD credible interval. This is commonly applied in Bayesian statistical calculations and it gives a range of where the true allele proportions lie. The lower end of the HPD is reported from STRmix to be the most conservative reported value for the comparison of the reference to the unknown profile(s).

9.3 STRmix uses the Balding and Nichols model (NRC II recommendation 4.2) to account for uncertainty with alleles for a genotype that may be identical by descent. This model allows for a correction for any sub-population effects.

10.0 Safety - N/A

11.0 References

Applied Biosystems™. GeneMapper® ID Software Version 3.1 User's Guide. P/N 4338775 Rev. C.

Promega PowerPlex® Fusion 6C Amplification Kit User's Guide.

Forensic Biology Section Procedure for Use of the 3500XL Genetic Analyzer for Casework

Forensic Biology Section Procedure for CODIS-Casework

Forensic Biology Section Procedure for Use of COSTaR

Forensic Biology Section Procedure for Report Writing

NDIS STR Data Entry Software (accessible by registered CODIS users only)

STRmix v2.7 Operation Manual

National Research Council Committee on DNA Forensic Science (1996) An Update: The Evaluation of Forensic DNA Evidence. Washington, D.C., National Academy Press.

Butler, J.M. *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers*. 2nd ed. Burlington, MA: Elsevier Academic Press, 2005.

Federal Bureau of Investigation. "QUALITY ASSURANCE KNOWN SAMPLES FOR FORENSIC DNA TESTING LABORATORIES." *Forensic Science Communications*, October 2008, Volume 10, Number 4.

12.0 Records

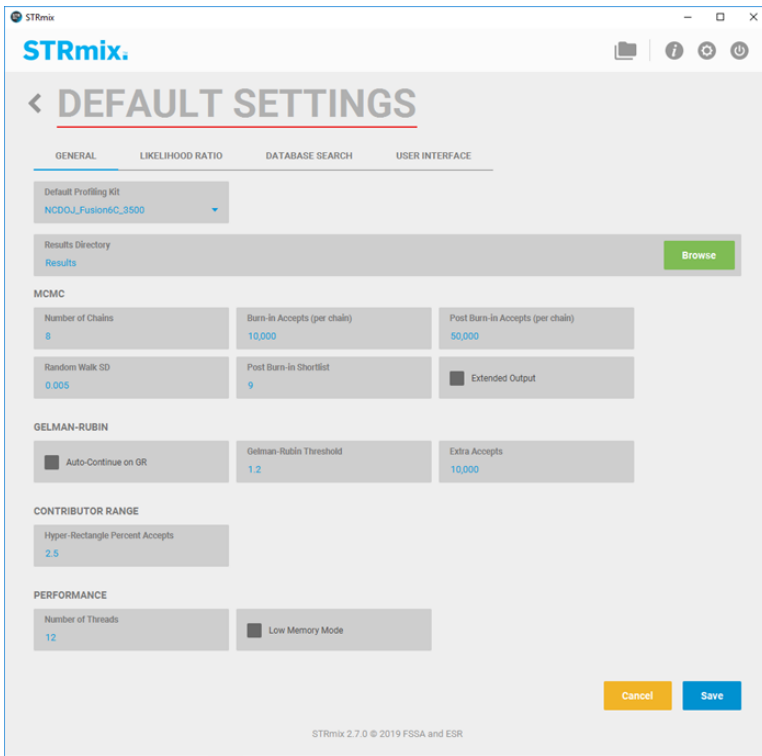
- PowerPlex® Fusion 6C Allelic Ladder Expected Results
- PowerPlex® Fusion 6C 2800M Expected Results

13.0 Attachments

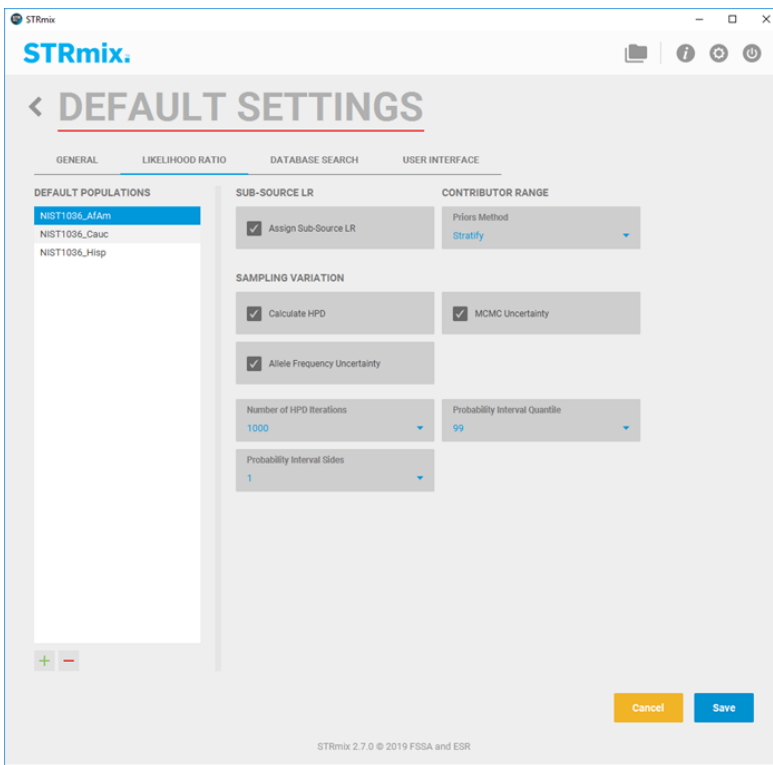
- STRMix Default Settings

| Revision History | | |
|-------------------------|----------------|---|
| Effective Date | Version Number | Reason |
| 01/21/2026 | 5 | 5.1, 5.3.6.2, 9.3 – correct typo, 5.4.2.1.4 – update requirement if multiple controls present, 5.4.2.2.3.4.6 – clarify printing, 5.4.2.2.3.6 – update language for consistency, 5.4.2.2.3.5.2 – update tri-allele language; 5.5.2.7 – remove (minor no longer marked), 5.5.2.9 – update replicate language, 5.5.2.11 – remove blank line, 5.8.9.1 – update outsourcing requirements, 5.8.14.4 – remove (statement in CODIS procedure), 5.8.14.7 – update language; 5.9.5.2.2 – added best fit clarification; 5.11.6 – added new section for inverse LR calculations |

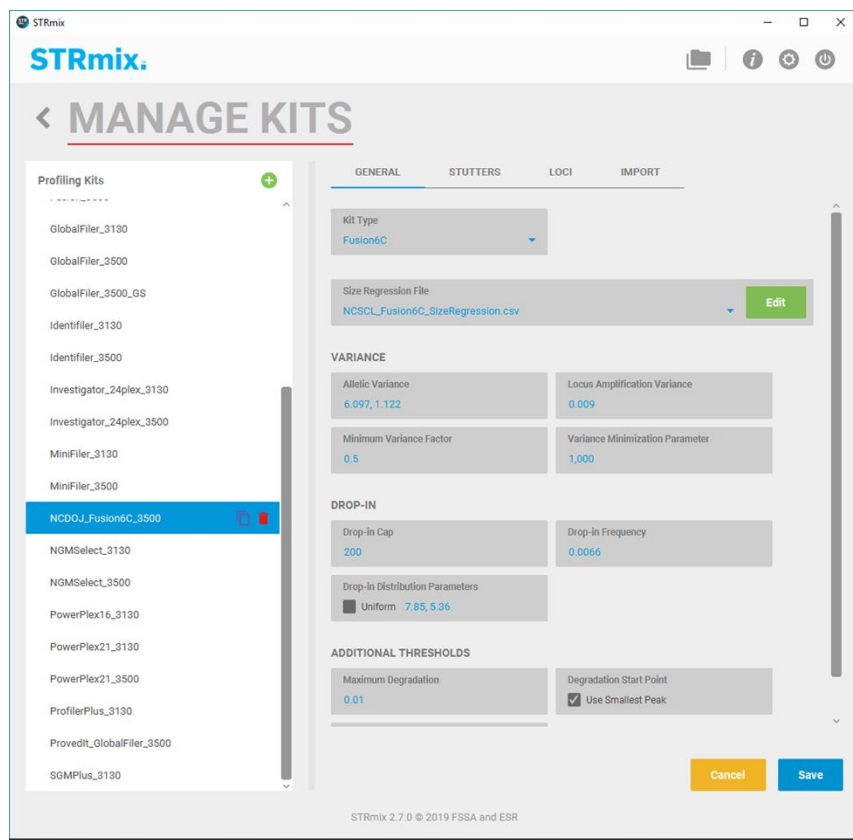
Appendix A: STRmix Default Settings

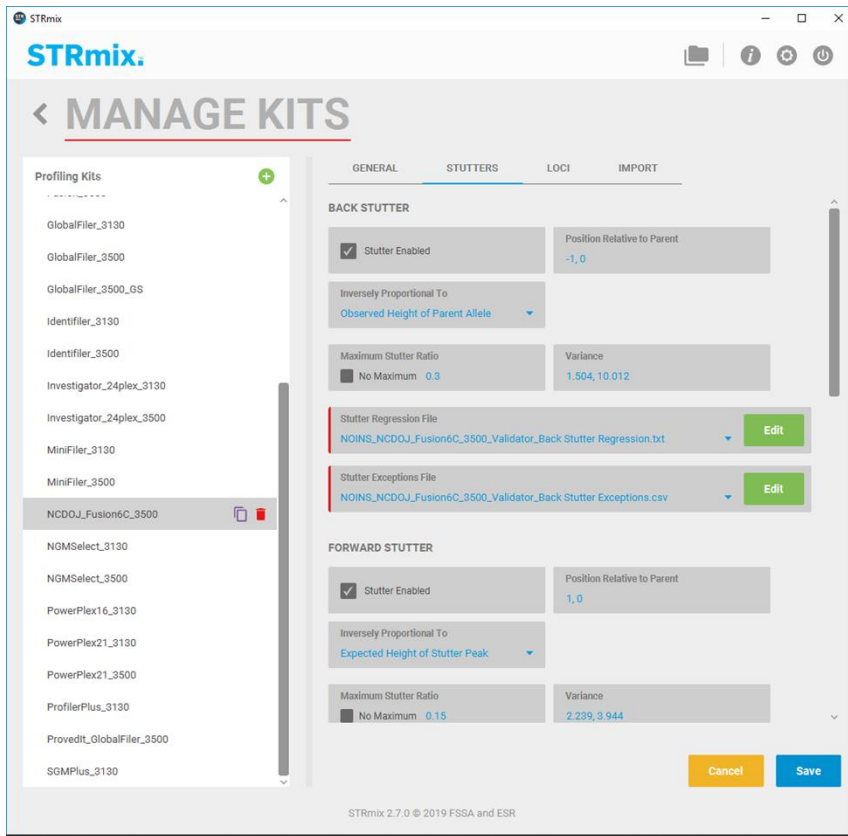


The screenshot shows the 'GENERAL' tab of the STRmix Default Settings. The interface includes a navigation bar with 'GENERAL', 'LIKELIHOOD RATIO', 'DATABASE SEARCH', and 'USER INTERFACE'. The 'GENERAL' tab is active. Settings include: Default Profiling Kit (NCDJ_Fusion6G_3500), Results Directory (with a 'Browse' button), MCMC parameters (Number of Chains: 8, Burn-In Accepts: 10,000, Post Burn-In Accepts: 50,000, Random Walk SD: 0.005, Post Burn-In Shortlist: 9, Extended Output: unchecked), GELMAN-RUBIN parameters (Auto-Continue on GR: unchecked, Gelman-Rubin Threshold: 1.2, Extra Accepts: 10,000), CONTRIBUTOR RANGE (Hyper-Rectangle Percent Accepts: 2.5), and PERFORMANCE (Number of Threads: 12, Low Memory Mode: unchecked). 'Cancel' and 'Save' buttons are at the bottom right. The footer reads 'STRmix 2.7.0 © 2019 FSSA and ESR'.



The screenshot shows the 'LIKELIHOOD RATIO' tab of the STRmix Default Settings. The interface includes a navigation bar with 'GENERAL', 'LIKELIHOOD RATIO', 'DATABASE SEARCH', and 'USER INTERFACE'. The 'LIKELIHOOD RATIO' tab is active. Settings include: DEFAULT POPULATIONS (list: NIST1036_AfAm, NIST1036_Cauc, NIST1036_Hisp), SUB-SOURCE LR (Assign Sub-Source LR: checked), CONTRIBUTOR RANGE (Priors Method: Stratfy), SAMPLING VARIATION (Calculate HPD: checked, Allele Frequency Uncertainty: checked, MCMC Uncertainty: checked), Number of HPD Iterations (1000), Probability Interval Quantile (99), and Probability Interval Sides (1). 'Cancel' and 'Save' buttons are at the bottom right. The footer reads 'STRmix 2.7.0 © 2019 FSSA and ESR'.





STRmix

< MANAGE KITS

Profiling Kits

- GlobalFiler_3130
- GlobalFiler_3500
- GlobalFiler_3500_GS
- Identifiler_3130
- Identifiler_3500
- Investigator_24plex_3130
- Investigator_24plex_3500
- MiniFiler_3130
- MiniFiler_3500
- NCDOJ_Fusion6C_3500**
- NGMSelect_3130
- NGMSelect_3500
- PowerPlex16_3130
- PowerPlex21_3130
- PowerPlex21_3500
- ProfilerPlus_3130
- Provedit_GlobalFiler_3500
- SGMPlus_3130

| LOCUS NAME | GENDER | REPEAT LENGTH | IGNORE? | DETECT ION THRES HOLD | BACK STUTTER | FORWARD STUTTER | DOUBLE BACK STUTTER | HALF BACK STUTTER |
|------------|--------|---------------|--------------------------|-----------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| | | | | | | | | |
| D3S... | | 4 | <input type="checkbox"/> | 75 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| D1S... | | 4 | <input type="checkbox"/> | 75 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |
| D2S... | | 4 | <input type="checkbox"/> | 75 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| D10... | | 4 | <input type="checkbox"/> | 75 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| D13... | | 4 | <input type="checkbox"/> | 75 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| Pen... | | 5 | <input type="checkbox"/> | 75 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| D16... | | 4 | <input type="checkbox"/> | 75 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| D18... | | 4 | <input type="checkbox"/> | 75 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |
| D2S... | | 4 | <input type="checkbox"/> | 75 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| CSF... | | 4 | <input type="checkbox"/> | 75 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| Pen... | | 5 | <input type="checkbox"/> | 75 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| TH01 | | 4 | <input type="checkbox"/> | 80 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| vWA | | 4 | <input type="checkbox"/> | 80 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| D21... | | 4 | <input type="checkbox"/> | 80 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |

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