Department of Forensic Science

FORENSIC BIOLOGY PROCEDURES MANUAL

STRMIX™ SYSTEM

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1 INITIATION OF A STRMIX™ SYSTEM ANALYSIS REQUEST

Routinely, the STRmix™ System will be used for evidentiary profiles with up to three contributors generated with PowerPlex® Fusion (PPFusion) on which traditional statistical approaches either cannot be applied or discard too much information.

Mixtures that unequivocally include greater than three contributors will not be analyzed.

Mixtures generated with PowerPlex® 16 (PP16) will not be analyzed using the STRmix™ System.

Mixtures involving genetically related potential contributors (typically first- or second-order relatives – consultation with a TrueAllele® team member may be necessary) that do not qualify for a Major/minor or different deconvolution and statistic will not be analyzed using the STRmix™ System. These mixtures will be referred for TrueAllele® analysis.

Probabilistic genotyping analyses addressing the comparison of a contributor for whom insufficient information exists to draw a conclusion regarding elimination/non-elimination will not be conducted using the STRmix™ System. These mixtures will be referred for TrueAllele® analysis, as applicable.

In general, not all qualifying mixtures in a single case will be evaluated using the STRmix™ System. If multiple mixtures are developed in a single case, the most informative/probative mixture(s) for which a traditional statistical approach is not possible or discards too much information will be analyzed using the STRmix™ System, while the additional mixture(s) will be evaluated using traditional statistical methods, if possible.

Likelihood Ratios (LRs) for the Caucasian, African American, and Hispanic populations will routinely be reported. The original referring examiner will need to request that a statistic for the Asian population be reported in addition to the other three, when applicable.

A probabilistic genotyping referral request will be provided by the original referring examiner to the STRmix™ team or designee. STRmix™ qualified examiners will complete STRmix™ analysis on their own cases, as necessary.

The following will be forwarded to the STRmix™ examiner through upload to the Department server or other secure manner:

- A .pdf or paper copy of both the applicable RFLE(s) and the applicable technically reviewed/released Certificate(s) of Analysis including any Appendices
- The .hid file(s) of the evidence sample(s) and a .pdf or paper copy of each final edited electropherogram maintained in the original case file
- The .hid file(s) of the included reference sample(s), any assumed reference sample(s), and the profile(s) in landscape form
- The .hid file(s) of all associated allelic ladder(s) used in the analysis of both evidentiary and reference sample(s)
  - If multiple CE runs are involved, a folder for each run should be provided such that sample(s) and appropriate ladder(s) are easily paired
- A .pdf or paper copy of the electropherogram(s) for the positive control(s) associated with the evidence amplification(s)
- A .pdf or paper copy of the electropherogram(s) for the reagent blank(s) and negative control(s) associated with the evidence extraction(s) and amplification(s).
  - Scaled to 50 rfu for each channel not containing the ILS and preferably showing the bins

NOTE: A .pdf or paper copy of the known reference electropherogram(s) is optional.

2 INITIAL REVIEW AND PREPARATION OF DATA FOR STRMIX™ SYSTEM ANALYSIS

2.1 Evidence Profile Review and Preparation

2.1.1 Review the controls provided to determine if any loci will need to be excluded from the deconvolution or LR calculation(s).

2.1.2 Review the edited evidence sample electropherogram(s) provided by the original examiner for details such as the number of possible contributors (using allele counts, peak height ratio information and any assumed knowns) and other pertinent information such as the level of stochastic effects, potential differential degradation, and possible artifacts such as polymer spikes.

2.1.3 Create a project in GMID-X for the evidence profile(s) including any applicable ladder(s) or open and rename, if applicable, an existing project containing the sample(s) of interest.

2.1.4 Analyze the samples using the appropriate analysis method (e.g., "PPFusion_STRmix_evidence") which will not edit out stutter peaks.

2.1.5 Delete any non-stutter artifacts (e.g., Pull-up, Spikes, etc.) as well as any N-2 (repeats) stutter and any n-2 (bp) stutter. Do NOT delete N-1 or N+1 stutter peaks or any combined stutter peaks that fall in an N-1 or N+1 position.

NOTES: Any peaks, aside from stutter, that were edited out as artifact by the original referring examiner will be edited out for the STRmix™ file. Likewise, any peaks not in stutter position that were deemed real by the original referring examiner will be left in for the STRmix™ file. Exceptions to this must be approved by the Program Manager (Technical Leader) and/or the Assistant Technical Leader.

In rare instances, a peak in stutter position may be elevated above the stutter threshold set during validation by STRmix™ Model Maker due to the additive effect of pull-up at the same location. If this occurs, STRmix™ will model this stutter peak as a real allele. If this occurs or is suspected, consultation with the Program Manager (Technical Leader), the Assistant Technical Leader, and/or designee is appropriate.

2.1.6 Rename any OL peaks, as applicable.

NOTE: The STRmix™ software will not accept an import with any OL designations or microvariants that are named using the < or > convention. If a microvariant exists in the profile that must be named, per the FB PM, using the < or > convention, that locus must be edited out of the exported text file prior to import into the STRmix™ software.

2.1.7 Save the project.

2.1.8 Delete from the project any unnecessary samples (e.g., controls, ladders, samples not to be exported, etc.), but do NOT save the project again.

2.1.9 Click on the Genotypes tab and confirm that the appropriate table settings are chosen (e.g., "STRmix_evidence"). Using the appropriate table settings ensures that the parameters required by the STRmix™ software are included in the .txt file (i.e., Sample Name, Marker, Allele, Size and Height).

2.1.10 Click on File > Export Table and save the .txt file for future import into the STRmix™ System.

NOTE: The profiles may be exported individually into multiple .txt files by removing all but one evidence profile from the project at a time OR they may be exported as one text file. If the option to export them as one file is chosen, the sample names field must differ for all samples.

2.2 Reference Profile Review and Preparation

2.2.1 Review the controls provided, if not reviewed during the preparation of the evidence profiles, and any other information provided by the referring examiner to determine if any loci will need to be excluded from the LR calculation(s).

2.2.2 Create a project in GMID-X for the reference profile(s) including any applicable ladder(s) or open and rename, if applicable, an existing project containing the sample(s) of interest.

2.2.3 Analyze the samples using the appropriate analysis method (e.g., "PPFusion_STRmix_reference") which will edit out stutter peaks.

2.2.4 Delete any artifacts (e.g., Stutter, Pull-up, Spikes, etc.).

2.2.5 Rename any OL peaks, as applicable.

NOTE: The STRmix™ software will not accept an import with any OL designations or microvariants that are named using the < or > convention. If a microvariant exists in the profile that must be named, per the FB PM, using the < or > convention, that locus must be edited out of the exported text file prior to import into the STRmix™ software.

2.2.6 Save the project.

2.2.7 Delete from the project any unnecessary samples (e.g., controls, ladders, samples not to be exported, etc.), but do NOT save the project again.

2.2.8 Click on the Genotypes tab and confirm that the appropriate table settings are chosen (e.g., "STRmix_reference"). Using the appropriate table settings ensures that the parameters required by the STRmix™ software are included in the .txt file (i.e., Sample Name, Marker, and Allele).

2.2.9 Click on File > Export Table and save the .txt file for future import into the STRmix™ System.

NOTE: The profiles may be exported individually into multiple .txt files by removing all but one reference profile from the project at a time OR they may be exported as one text file. If the option to export them as one file is chosen, the sample names field must differ for all samples.

3 ANALYSIS OF SAMPLES USING THE STRMIX™ SYSTEM

3.1 Deconvolution Using the Interpretation Module of the STRmix™ Software

3.1.1 Open the STRmix™ software, if not already open.

3.1.2 Click on Interpretation.

3.1.3 Case Number and Sample ID information are required while Case Notes are optional.

NOTE: The use of upper and lower case letters in the following listed conventions is interchangeable.

3.1.3.1 Case Number will, at a minimum, include the FS Lab #. It may also include the initials of the STRmix™ examiner and be formatted as follows: [FS Lab#].[STRmix™ Examiner Initials]

EXAMPLE: C21-1234.LCS

3.1.3.2 Sample ID will, at a minimum, include the evidence Item # and description AND the number of contributors assigned. It may also contain injection time and/or other comments, as desired, and be formatted as follows: [Item# and description].[# contributors assigned].[injection time].[additional comments, as desired]

EXAMPLE: 4knife.3c.24s
(Item 4 knife, 3 contributors assigned, 24s injection)

3.1.3.3 Case Notes may include other information helpful to the examiner such as, “attribution to V at 20 loci in original report.”

3.1.4 Under MCMC Settings, enter the total number of contributors assigned.

NOTE: If a mixture is referred for STRmix™ System analysis based on an assumption of a number of contributors by the original referring examiner, but the data suggest to the STRmix™ examiner the possibility that there is actually one fewer or one more than originally assumed, the STRmix™ examiner has the option to analyze the mixture under both assumptions. Therefore, although four contributor mixtures are not routinely analyzed, it is possible that a mixture referred as a three contributor mixture may also be run as a four contributor mixture.

The option to analyze a mixture under two different assumptions with regard to contributor number is not to be utilized as a routine course of action. This option is available for situations in which true ambiguity in the estimated number of contributors (two vs. three and/or three vs. four) exists.

3.1.5 Run Settings will not be modified.

3.1.6 Click Next and select the appropriate Profiling Kit for the sample being analyzed (i.e., Fusion_3500_24s for a sample that was injected for 24 seconds or Fusion_3500_12s for a sample that was injected for 12 seconds).

NOTE: The appropriate Profiling Kit must be selected prior to importing an evidence profile. If the wrong Profiling Kit is selected prior to import, the evidence profile must be deleted, the proper Kit selected, and then the evidence profile imported again.

3.1.7 Import the evidence profile by either dragging and dropping the .txt file into the “Evidence Profile Data” box or by using the green plus icon to navigate to the appropriate .txt file.

NOTE: Multiple evidence .txt files are not to be added to the "Evidence Profile Data" box for deconvolution. For instructions on setting up a batch style run of multiple subsequent deconvolutions, see the instructions below in 3.2.

3.1.8 If a locus must be excluded from the deconvolution (e.g., a locus is to be dropped due to a peak observed in the associated evidence RB), click on Kit Settings and check mark the "ignore" box for that locus. No other modifications to the Kit Settings will be made.

NOTE: D12S391 will generally be included in the deconvolution and not marked as "ignore" until conducting a LR From Previous calculation.

3.1.9 The use of an assumed known in a deconvolution will be limited to those known references for which a conclusion of not eliminated is reached when following the interpretation guidance found in 2.15 and 2.16 of the FB PM Interpretation of Fusion Data.

3.1.9.1 If an assumed known is to be considered as a part of the deconvolution, import the assumed known reference profile by either dragging and dropping the .txt file into the "Reference Profile Data" box or by using the green plus icon to navigate to the appropriate .txt file. Ensure that both the HP and HD boxes in the "Contributor To" area are marked.

NOTES: Known reference samples are not to be imported into the Interpretation module unless they are being considered as an assumed known for the deconvolution. LR calculations with regard to a known reference used for comparison will be conducted using the LR From Previous function.

Marking only HP, as opposed to both HP and HD, will result in a deconvolution that does not consider the reference as an assumed known and a LR result to be calculated for the comparison of the reference to the evidence.

3.1.10 Click Start to begin the deconvolution process.

3.1.11 Once the process is complete, click Finish to close the window and be brought back to the main menu.

3.2 Creating a Batch for Multiple Subsequent Deconvolutions

3.2.1 Open the STRmix™ Software, if not already open.

3.2.2 Click on Batch Mode.

3.2.3 Click on the Add to Batch arrow and select Add Interpretation. This allows all information associated with a single deconvolution in the batch to be entered.

3.2.4 Case Number and Sample ID information are required while Case Notes are optional.

NOTE: The use of upper and lower case letters in the following listed conventions is interchangeable.

3.2.4.1 Case Number will, at a minimum, include the FS Lab #. It may also include the initials of the STRmix™ examiner and be formatted as follows: [FS Lab#].[STRmix™ Examiner Initials]

EXAMPLE: C21-1234.lcs

3.2.4.2 Sample ID will, at a minimum, include the evidence Item # and description AND the number of contributors assigned. It may also contain injection time and/or other comments, as desired, and be formatted as follows: [Item# and description].[# contributors assigned].[injection time].[additional comments, as desired]

EXAMPLE: 4knife.3C.24s.+JC
(Item 4 knife, 3 contributors assigned, 24s injection, victim (JC) run as assumed known)

3.2.4.3 Case Notes may include other information helpful to the examiner such as, "attribution to V at 20 loci in original report."

3.2.5 Under MCMC Settings, enter the number of contributors assigned.

NOTE: If a mixture is referred for STRmix™ System analysis based on an assumption of a number of contributors by the original referring examiner, but the data suggest to the STRmix™ examiner the possibility that there is actually one fewer or one more than originally assumed, the STRmix™ examiner has the option to analyze the mixture under both assumptions. Therefore, although four contributor mixtures are not routinely analyzed, it is possible that a mixture referred as a three contributor mixture may also be run as a four contributor mixture.

The option to analyze a mixture under two different assumptions with regard to contributor number is not to be utilized as a routine course of action. This option is available for situations in which true ambiguity in the estimated number of contributors (two vs. three and/or three vs. four) exists.

3.2.6 Run Settings will not be modified.

3.2.7 Click Next and select the appropriate Profiling Kit for the sample being analyzed (i.e., Fusion_3500_24s for a sample that was injected for 24 seconds or Fusion_3500_12s for a sample that was injected for 12 seconds).

NOTE: The appropriate Profiling Kit must be selected prior to importing an evidence profile. If the wrong Profiling Kit is selected prior to import, the evidence profile must be deleted, the proper Kit selected, and then the evidence profile imported again.

3.2.8 Import the evidence profile by either dragging and dropping the .txt file into the "Evidence Profile Data" box or by using the green plus icon to navigate to the appropriate .txt file.

NOTE: Do not add multiple evidence .txt files to the "Evidence Profile Data" box for this deconvolution. Additional deconvolutions will be added at a later time using the queue function.

3.2.9 If a locus must be excluded from the deconvolution (e.g., a locus is to be dropped due to a peak observed in the associated evidence RB), click on Kit Settings and check mark the "ignore" box for that locus and click Apply. No other modifications to the Kit Settings will be made.

NOTE: D12S391 will generally be included in the deconvolution and not marked as "ignore" until conducting a LR From Previous calculation.

3.2.10 The use of an assumed known in a deconvolution will be limited to those known references for which a conclusion of not eliminated is reached when following the interpretation guidance found in 2.15 and 2.16 of the FB PM Interpretation of Fusion Data.

3.2.10.1 If an assumed known is to be considered as a part of the deconvolution, import the assumed known reference profile by either dragging and dropping the .txt file into the "Reference Profile Data" box or by using the green plus icon to navigate to the appropriate .txt file. Ensure that both the HP and HD boxes in the "Contributor To" area are marked.

NOTES: Known reference samples are not to be imported at this time unless they are being considered as an assumed known for the deconvolution. LR calculations with regard to a known reference used for comparison will be conducted using the LR From Previous function.

Marking only HP, as opposed to both HP and HD, will result in a deconvolution that does not consider the reference as an assumed known and a LR result to be calculated for the comparison of the reference to the evidence.

3.2.11 Click Queue, which will return the user to the Batch Mode screen, allowing for the process to be repeated for each deconvolution to be included in the batch.

3.2.12 Once all deconvolutions are included in the list within the Batch Mode screen, Click Start to begin processing the batched deconvolutions.

3.3 Evaluating the Results of the Deconvolution

3.3.1 Upon completion of the deconvolution, the Interpretation Report should open. If not, navigate to the appropriate Results Folder which contains the following for the sample/deconvolution in question: Extended Output, Inputs, Kits, Log, Reports, and Stutters folders, the config_input XML file, as well as other text and XML files. Click on the Reports folder to find the .pdf of the Interpretation Report.

NOTE: The appropriate folder can be found by clicking on STRmix™ Results under STRmix™ in the Windows Start menu OR by clicking on the folder icon at the top right corner of the software screen.

3.3.2 Confirm that the evidence input file is correct.

NOTE: If a locus is ignored in Kit Settings for the deconvolution, the types at that locus will still appear in the input file chart.

3.3.3 In the Run Parameters section of the Interpretation Report:

- Confirm that the proper number of contributors is listed.
- Confirm that the proper Profiling Kit and Sample File are listed.
- If an assumed known was used, confirm that the proper file is listed as a known contributor under both HP and HD.

3.3.4 In the Summary of Contributors section of the Interpretation Report, confirm that the STRmix™ software derived mixture proportions make logical sense when considering the original electropherogram data.

Mixture proportions (considered a primary diagnostic) should be intuitive and in-line with the manual review of the data. If this is not the case, troubleshooting and a re-analysis are required.

3.3.5 In the Component Interpretation section of the Interpretation Report,

- Confirm that any loci to have been removed from the analysis were not included in the analysis.
- Confirm that the genotype weights displayed make logical sense when considering the original electropherogram data.

Genotype weights (considered a primary diagnostic) should be intuitive and in-line with the manual review of the data. If this is not the case, troubleshooting and a re-analysis are required.

3.3.6 In the Post Burn-In Summary section of the Interpretation Report:

- Confirm that the total number of iterations does not exceed 2.15 billion.
- Confirm that the effective sample size is ≥ 1000.
  - The effective sample size is the number of independent samples the MCMC has taken from the posterior distribution of all parameters.
- Confirm that the Gelman-Rubin convergence diagnostic (GR score) is ≤ 2.

- The GR score compares the MCMC within chain variation with the MCMC between chain variation and informs the user whether the MCMC analysis has likely converged.
- Ideally, the GR score should not exceed 1.2; however, it is possible for the value to be elevated, even for useable, high quality analyses.

- Confirm that the acceptance rate is equal to or greater than at least 1 in 1000 (1 in ≤1000).
  - The acceptance rate describes how often a new proposed set of parameters was accepted and is calculated by dividing the total number of accepts by the total number of post burn-in iterations.
- Confirm that the log(likelihood) is not negative or very low.
  - The log(likelihood) shows the average $\log_{10}$(likelihood) for the entire post burn-in MCMC and describes the fit to the observed electropherogram given the proposed profile and mass parameters or how well STRmix™ was able to describe the data. The higher the log(likelihood) value, the better the STRmix™ System has been able to describe the observed data.
  - Possible reasons for a low log(likelihood) are:
    - The profile is low level overall, so there is very little data making up the likelihood.
    - There are forced stochastic events in the STRmix™ run (e.g., large heterozygote peak imbalances or variation in mixture proportions across the profile). This can be caused by an incorrect estimate of the number of contributors.
    - Data was removed that was real, particularly stutter peaks, causing STRmix™ to describe the missing information as drop out.
    - Artifactual peaks have been left in and must now be accounted for by STRmix™ as drop-in.
- Confirm that the allele and stutter variances do not deviate significantly from the mode or approach the horizontal asymptote.
  - These variances are also visible in pictorial form in the Variance Charts portion of the Interpretation Report.
  - Significant deviations from the mode should elicit a review of the electropherogram data, but, alone, do not indicate the need for a re-analysis.
    - It is possible that, even with a large deviation, the analysis is still useable if the profile demonstrates, for example, a great deal of allele imbalance and drop-out and many stutter peaks are seen below the LOD and therefore missing from the imported .txt file.

3.3.6.1 A total iteration value of greater than 2.15 billion requires re-analysis.

3.3.6.2 A Gelman-Rubin convergence diagnostic (GR score) of greater than 2.0 requires both troubleshooting and a re-analysis.

3.3.6.2.1 If, after a re-analysis, the GR score is above 2.0, a third analysis will be conducted with additional iterations in the post burn-in phase.

3.3.6.2.1.1 To add additional iterations, follow 3.1.1 through 3.1.4 above.

3.3.6.2.1.2 Click on Run Settings.

3.3.6.2.1.3 Change the value under Post Burn-in Accepts (per chain) from 50,000 to 60,000 and click Apply.

3.3.6.2.1.4 Continue to follow 3.1.6 through 3.1.11.

3.3.6.2.2 If, after this third analysis, the total iterations exceed 2.15 billion or the GR score is above 2.0, consultation with the Program Manager (Technical Leader), Assistant Technical Leader, and/or designee is required.

3.3.6.3 Taken together, the remaining diagnostics (considered secondary diagnostics) may indicate the need for a re-analysis. However, any one of these alone will not indicate the need for re-analysis. Support for the deviation of one or more diagnostics from the expected values found during the manual review of the data (e.g., the profile is very low level, stochastic and understandably challenging) will generally negate the need for a re-analysis.

3.3.7 Once the deconvolution has been evaluated and deemed acceptable for use, the Investigation Module or Batch Mode module will be used to calculate likelihood ratios with regard to any applicable known reference profiles.

EXCEPTION: STRmix™ analysis will be discontinued and the mixture referred for TrueAllele® analysis when any one contributor for whom a LR will be calculated is a lower level contributor (25% or less in mixture weight).

In these instances, all potential comparisons will be evaluated with TrueAllele®, as opposed to STRmix™.

3.4 Calculating Likelihood Ratios Using the Investigation Module of the STRmix™ Software

If a mixture was analyzed successfully under two different contributor number assumptions, a LR will be calculated for each applicable known for both deconvolutions.

3.4.1 Open the STRmix™ Software, if not already open.

3.4.2 Click on Investigation.

3.4.3 Either drag and drop the entire STRmix™ interpretation results folder associated with the mixture deconvolution of interest into the Previous Interpretation box OR click Browse and navigate to the interpretation results folder associated with the mixture deconvolution of interest and double click on the "config_input" file within the folder to populate the Previous Interpretation box.

3.4.4 To compare and calculate a LR for a single reference profile, click on LR From Previous.

NOTE: Adding multiple reference samples in the LR From Previous module will calculate a combined LR, as opposed to subsequent individual LRs. Do not add multiple references for which individual LRs are needed using this module.

3.4.4.1 Case Number and Sample ID information automatically populates based upon the deconvolution file chosen, but may be edited, as applicable.

NOTE: LRPrev will automatically be appended to the previous Sample ID.

3.4.4.2 Click Next.

3.4.4.3 Import the known reference profile by either dragging and dropping the .txt file into the "Reference Profile Data" box or by using the green plus icon to navigate to the appropriate .txt file. The HP box will automatically be checked.

3.4.4.4 Click on Kit Settings and check mark the "ignore" box for D12S391 and click Apply.

3.4.4.4.1 Also check mark the "ignore" box for any other locus to be dropped from the calculation, as applicable (e.g., a peak is observed at a single locus in the RB associated with the reference sample, etc.) and click Apply.

NOTE: It is not necessary to "ignore' loci for which there are no results in the evidence mixture profile or loci that were previously ignored during the deconvolution step in the Interpretation module.

3.4.4.5 Click Start to begin the LR calculation process.

3.4.5 To compare multiple reference profiles and calculate multiple LRs with regard to the same mixture individually, repeat the above instructions for each known reference OR use the Batch Mode found in the main menu screen.

NOTE: Using LR Batch within the LR From Previous mode does not allow the option to ignore a locus in Kit Settings. Each LR must be set up individually as a LR From Previous, but these may be batched in the regular Batch Mode.

3.4.5.1 Open the STRmix™ Software, if not already open.

3.4.5.2 Click on Batch Mode.

3.4.5.3 Click on the Add to Batch arrow and select Add LR From Previous.

3.4.5.4 Either drag and drop the entire STRmix™ interpretation results folder associated with the mixture deconvolution of interest into the Previous Interpretation box OR click Browse and navigate to the interpretation results folder associated with the mixture deconvolution of interest and double click on the "config_input" file within the folder to populate the Previous Interpretation box. Browse and navigate to the interpretation folder associated with the mixture deconvolution of interest (e.g., C:\ProgramData\STRmix\Results…). Double click on the "config_input" file to populate the Choose Previous Interpretation for LR box and click Select.

3.4.5.5 Case Number and Sample ID information automatically populates based upon the deconvolution file chosen, but may be edited, as applicable.

NOTE: LRPrev will automatically be appended to the previous Sample ID.

3.4.5.6 Click Next.

3.4.5.7 Import one of the known reference profiles to be compared by either dragging and dropping the .txt file into the "Reference Profile Data" box or by using the green plus icon to navigate to the appropriate .txt file. The HP box will automatically be checked.

3.4.5.8 Click on Kit Settings and check mark the "ignore" box for D12S391 and click Apply.

3.4.5.8.1 Also check mark the "ignore" box for any other locus to be dropped from the calculation, as applicable (e.g., a peak is observed at a single locus in the RB associated with the reference sample, etc.) and click Apply.

NOTE: It is not necessary to "ignore' loci for which there are no results in the evidence mixture profile or loci that were previously ignored during the deconvolution step in the Interpretation module.

3.4.5.9 Click queue.

3.4.5.10 Repeat the process for each known reference sample to be compared.

3.4.5.11 Once all known reference LR From Previous analyses appear in the batch list, click Start.

3.5 Evaluating the Results of the Likelihood Ratio Calculation

3.5.1 Upon completion of the calculation, the LR From Previous Report should open. If not, navigate to the appropriate Results Folder which contains the following for the sample/deconvolution in question: AlleleFreq, Inputs, Log, Populations, and Reports folders, as well as other text and XML documents. Open the Reports folder to access the .pdf of the LR From Previous Report.

NOTE: The appropriate folder can be found by clicking on STRmix™ Results under STRmix™ in the Windows Start menu OR by clicking on the folder icon in the top right corner of the software.

3.5.2 Confirm that the imported reference profile(s) are correct.

NOTE: If a locus is ignored in Kit Settings for the LR From Previous, the types at that locus will still appear in the input file chart for the imported reference(s).

3.5.3 In the Run Parameters section of the LR From Previous Report:

- Confirm that the proper number of contributors is listed.
- Confirm that the proper Profiling Kit and Sample File are listed.
- Confirm that the proper file is listed as a known contributor under HP only.
- If an assumed known was used in the original deconvolution, confirm that the proper file is listed as a known contributor under both HP and HD.

3.5.4 In the Per Locus Likelihood Ratios section of the LR From Previous Report:

- Confirm that D12S391 and any other applicable locus were not included in the calculation.
- Confirm that the per locus likelihood ratios make logical sense and are consistent with a review of the electropherogram and contributor proportions.
    - An unexpected or unintuitive result may require troubleshooting and a re-analysis of the LR From Previous, or possibly of the original deconvolution.
- Review and examine the electropherogram for all loci with a per locus LR of zero or less than one.
    - A value of less than one should correlate logically with what is observed in the electropherogram for that locus. If not, troubleshooting and a re-analysis of the LR From Previous, or possibly the original deconvolution, may be required.
    - A value of zero may correlate logically with what is observed in the electropherogram for that locus (i.e., a true exclusion). If not, it will require possible troubleshooting and a re-analysis of the LR From Previous or possibly of the original deconvolution.

EXAMPLES: In the event that a true peak is not resolved (i.e., the peak is 1 bp larger or smaller than another true allele and appears as a shoulder, but is not called an allele by the GMID-X software and is therefore not considered in the deconvolution), either the LR From Previous should be repeated with the affected locus ignored OR the deconvolution should be repeated with the affected locus ignored. A new deconvolution will require a new LR From Previous.

In the event that the data supports a possible drop out allele, but a Q allele is not considered during the deconvolution, it may be appropriate to repeat the deconvolution.

NOTE: Consultation with the Program Manager (Technical Leader), Assistant Technical Leader, and/or designee in determining whether to repeat the deconvolution or to ignore a locus in the LR From Previous is recommended.

3.5.5 Once the LR result has been evaluated and deemed acceptable for use, the results may be reported.

4 REPORTING RESULTS

The LR values reported are the sub-source 99% 1-sided lower HPD interval values and will be truncated to two significant figures. They are listed, per population group, in the Summary of LR Tables of the LR From Previous Report and are labeled "Unrelated." The same value may also be found at the bottom of the Per Locus Likelihood Ratios section of the Report.

If a mixture was successfully analyzed under two different contributor number assumptions, the set of values to be reported for an individual are those for which the Unrelated value for the Stratified LR is lower (found in the Summary of LR Tables of the LR From Previous Report).

STRmix™ System Analysis Certificates of Analysis will follow the FB PM Report Writing with regard to the requirements listed in accordance with the FBI's Quality Assurance Standards for Forensic DNA Testing Laboratories and the Department Quality Manual.

4.1 Categories of Results to be Reported

The results will fall within one of the following categories:

- The match statistic favors the person of interest (LR > 1000)
- The match statistic favors a coincidental match (LR < 0.001)
- The meaning of the match statistic is uninformative (1000 ≥ LR ≥ 0.001)

4.2 General Format

4.2.1 The Certificate of Analysis will contain a METHODS section and RESULTS AND INTERPRETATIONS section.

4.2.1.1 The following statement will be included prior to the disposition statement:

Date(s) of testing: mm/dd/yyy – mm/dd/yyy. Supporting examination documentation is maintained in the case file. The above listed methods are those approved for use at the time of analysis. Current methods can be found in the Forensic Biology Procedures Manuals, which can be found at www.dfs.virginia.gov/documentation-publications/manuals/.

4.2.2 The METHODS section, placed prior to the RESULTS AND INTERPRETATIONS section, to be included will read:

4.2.2.1 Supplemental Certificates

STATISTICAL ANALYSIS METHODS:

- The DNA PowerPlex® Fusion profiles referenced in this report were previously developed and addressed in a Certificate of Analysis dated DATE.
- The STRmix™ System processed each evidence item in an independent computer analysis in which possible DNA contributor genotypes were inferred from the evidence profile(s).
  - The term "genotypes" used in this context refers to a probability distribution over allele pairs.
- The DNA statistics calculated herein used the 2017 revised population allele frequencies provided by the National Institute of Standards and Technology (NIST).
- The statistical calculations have been performed in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) 2017 Interpretation Guidelines and Departmental Procedures.
- The D12S391, DYS391, and Amelogenin loci are not used for statistical purposes.

4.2.2.2 Original Certificates with only STRmix™ Statistics

STATISTICAL ANALYSIS METHODS:

- The STRmix™ System processed each evidence item in an independent computer analysis in which possible DNA contributor genotypes were inferred from the evidence profile(s).
  - The term "genotypes" used in this context refers to a probability distribution over allele pairs.
- The DNA statistics calculated herein used the 2017 revised population allele frequencies provided by the National Institute of Standards and Technology (NIST).
- The statistical calculations have been performed in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) 2017 Interpretation Guidelines and Departmental Procedures.
- The D12S391, DYS391, and Amelogenin loci are not used for statistical purposes.

4.2.2.3 Original Certificates with Both Traditional and STRmix™ Statistics

STATISTICAL ANALYSIS METHODS:

- The DNA statistics calculated herein used the 2017 revised population allele frequencies provided by the National Institute of Standards and Technology (NIST).
- The statistical calculations have been performed in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) 2017 Interpretation Guidelines and Departmental Procedures.
- The D12S391, DYS391, and Amelogenin loci are not used for statistical purposes.
- For Item X/Items X and X, [the DNA PowerPlex Fusion® profile(s) was/were previously developed and addressed in a Certificate of Analysis dated XX/XX/XXXX]. The STRmix™ System processed [the evidence/each of these evidence items] in an independent computer analysis in which possible DNA contributor genotypes were inferred from the evidence profile(s).
  - The term "genotypes" used in this context refers to a probability distribution over allele pairs.

4.2.3 The RESULTS AND INTERPRETATIONS section will contain both narrative and statistical statements for each evidence profile.

NOTES: A conclusion statement with regard to each potential contributor will be included with the statistical statement.

If multiple known references are compared to a single evidence item, the narrative statement(s) may be combined.

Wording may be changed for grammatical reasons and/or to reflect the analysis conducted (e.g., two or three contributors, as opposed to three or four contributors, to reflect the use of an assumed known, to reflect whether the report is addressing a previously developed profile or one developed and reported along with the current STRmix™ results, etc.).

4.2.3.1 Narrative Statements

Narrative Statements will address the following:

- The evidence sample being analyzed
- The number of unknown contributors tested
- The assumed known reference sample(s), if applicable

- The reference sample(s) for which comparisons were made

4.2.3.1.1 No Assumed Known Reference Used in the Deconvolution

Assuming the DNA profile data previously developed from the [EVIDENCE] is a mixture of three unknown contributors, the STRmix™ System objectively inferred genotypes solely from these data. The computer system then compared each inferred evidence contributor genotype to the provided reference genotype of [PERSON OF INTEREST], relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.

4.2.3.1.2 Assumed Known Reference Used in the Deconvolution

Assuming the DNA profile data previously developed from the [EVIDENCE] is a mixture of two unknown contributors and [ASSUMED KNOWN], the STRmix™ System objectively inferred genotypes solely from these data. The computer system then compared each inferred evidence contributor genotype to the provided reference genotype of [PERSON OF INTEREST], relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.

4.2.3.1.3 Newly Submitted Known Reference Sample, Previous STRmix™ Deconvolution

As previously reported in the Certificate of Analysis dated [DATE], the DNA profile data developed from the [EVIDENCE] is assumed to be a mixture of three unknown contributors. The STRmix™ System objectively inferred genotypes solely from these data. The computer then compared each inferred evidence contributor genotype to the newly provided reference genotype of [PERSON OF INTEREST], relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.

4.2.3.1.4 Two Deconvolutions Performed With Two Different Contributor Assignments

Assuming the DNA profile data previously developed from the [EVIDENCE] is a mixture of three or four unknown contributors, the STRmix™ System objectively inferred genotypes solely from these data. The computer system then compared each inferred evidence contributor genotype to the provided reference genotype of [PERSON OF INTEREST], relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.

4.2.3.2 Statistical Statements

4.2.3.2.1 The Match Statistics for All Population Groups Favor the Person of Interest (No Assumed Reference)

Based on these results, [PERSON OF INTEREST] cannot be eliminated as a contributor to this DNA mixture profile.

The DNA profile developed from the [EVIDENCE] at the PowerPlex® Fusion loci is approximately:

_____ times more likely to be observed if it originated from [PERSON OF INTEREST] and two unrelated unknown individuals in the Caucasian population than if it originated from three unrelated unknown individuals in the Caucasian population.

_____ times more likely to be observed if it originated from [PERSON OF INTEREST] and two unrelated unknown individuals in the African American population than if it originated from three unrelated unknown individuals in the African American population.

_____ times more likely to be observed if it originated from [PERSON OF INTEREST] and two unrelated unknown individuals in the Hispanic population than if it originated from three unrelated unknown individuals in the Hispanic population.

4.2.3.2.2 The Match Statistics for All Population Groups Favor the Person of Interest (Assumed Known Reference Used)

Based on these results, [PERSON OF INTEREST] cannot be eliminated as a contributor to this DNA mixture profile.

The DNA profile developed from the [EVIDENCE] at the PowerPlex® Fusion loci is approximately:

_____ times more likely to be observed if it originated from [ASSUMED KNOWN], [PERSON OF INTEREST], and one unrelated unknown individual in the Caucasian population than if it originated from [ASSUMED KNOWN] and two unrelated unknown individuals in the Caucasian population.

_____ times more likely to be observed if it originated from [ASSUMED KNOWN], [PERSON OF INTEREST], and one unrelated unknown individual in the African American population than if it originated from [ASSUMED KNOWN] and two unrelated unknown individuals in the African American population.

_____ times more likely to be observed if it originated from [ASSUMED KNOWN], [PERSON OF INTEREST], and one unrelated unknown individual in the Hispanic population than if it originated from [ASSUMED KNOWN] and two unrelated unknown individuals in the Hispanic population.

4.2.3.2.3 The Match Statistics for All Population Groups favor a Coincidental Match

Based on these results, [PERSON OF INTEREST] is eliminated as a contributor to this DNA mixture profile.

4.2.3.2.4 The Meaning of the Match Statistic for Any One or More of the Population Groups Is Uninformative

Based on these results, the DNA profile developed from the [EVIDENCE] at the PowerPlex® Fusion loci is approximately:

_____ times more likely/less likely/equally likely to be observed if it originated from [PERSON OF INTEREST] and two unrelated unknown individuals in the Caucasian population than/as if it originated from three unrelated unknown individuals in the Caucasian population.

_____ times more likely/less likely/equally likely to be observed if it originated from [PERSON OF INTEREST] and two unrelated unknown individuals in the

African American population than/as if it originated from three unrelated unknown individuals in the African American population.

_____ times more likely/less likely/equally likely to be observed if it originated from [PERSON OF INTEREST] and two unrelated unknown individuals in the Hispanic population than/as if it originated from three unrelated unknown individuals in the Hispanic population.

As a result of internal validation testing performed at the Virginia Department of Forensic Science, the foregoing match statistics are considered uninformative. Therefore, no conclusions can be made regarding [PERSON OF INTEREST] as a possible contributor to this DNA mixture profile.

NOTE: The verbiage 'equally likely' and 'as if' is used for a population group when the reported match statistic is equal to 1.

The option to use 'less likely' for a population group when the match statistic supports the proposition represented in the denominator of the LR is available. In this instance, the reciprocal value of the match statistic is reported.

EXAMPLE: Match statistics of 14, 1.0, and 0.57 for the Caucasian, African American, and Hispanic populations, respectively, may be reported in one of the following two ways (adjusting for the appropriate number of contributors):

Based on these results, the DNA profile developed from the EVIDENCE at the PowerPlex® Fusion loci is approximately:

14 times more likely to be observed if it originated from PERSON OF INTEREST and two unrelated unknown individuals in the Caucasian population than if it originated from three unrelated unknown individuals in the Caucasian population.

equally likely to be observed if it originated from PERSON OF INTEREST and two unrelated unknown individuals in the Caucasian population as if it originated from three unrelated unknown individuals in the African American population.

0.57 times more likely to be observed if it originated from PERSON OF INTEREST and two unrelated unknown individuals in the Caucasian population than if it originated from three unrelated unknown individuals in the Hispanic population.

As a result of internal validation testing performed at the Virginia Department of Forensic Science, the foregoing match statistics are considered uninformative. Therefore, no conclusions can be made regarding PERSON OF INTEREST as a possible contributor to this DNA mixture profile.

OR

Based on these results, the DNA profile developed from the EVIDENCE at the PowerPlex® Fusion loci is approximately:
14 times more likely to be observed if it originated from PERSON OF INTEREST and two unrelated unknown

individuals in the Caucasian population than if it originated from three unrelated unknown individuals in the Caucasian population.

equally likely to be observed if it originated from PERSON OF INTEREST and two unrelated unknown individuals in the Caucasian population as if it originated from three unrelated unknown individuals in the African American population.

1.7 times less likely to be observed if it originated from PERSON OF INTEREST and two unrelated unknown individuals in the Caucasian population than if it originated from three unrelated unknown individuals in the Hispanic population.

As a result of internal validation testing performed at the Virginia Department of Forensic Science, the foregoing match statistics are considered uninformative. Therefore, no conclusions can be made regarding PERSON OF INTEREST as a possible contributor to this DNA mixture profile.

4.2.3.3 Locus-Specific Comments

NOTE: Any applicable locus-specific comments will follow the statistical statement.

EXAMPLES:

The Penta E locus did not meet the quality control standard during the original analysis; therefore, this locus was not used in the statistical calculation.

The D13S317 locus was not used in the statistical calculation.

5 REQUIRED CASE FILE DOCUMENTATION

5.1 Paper Documentation

At a minimum, the following paper documentation will be maintained in the case file:

- Referral, if applicable
- Paper copy of both the applicable RFLE(s) and the applicable technically reviewed/released Certificate(s) of Analysis and Appendices provided by the original referring examiner, if applicable
- Copies of all evidence associated positive control, negative control, and reagent blank electropherograms provided by the original referring examiner
- Copies of all previously edited evidence mixture electropherograms provided by the original referring examiner
- STRmix™ System Analysis worksheet(s)
- Printed pages of the Interpretation Report(s) to include, at a minimum, the Details, Run Parameters, Summary of Contributors, Post Burn-In Summary, and Variance Charts sections of each report
- Printed pages of the LR From Previous Report(s) to include, at a minimum, the Details, Run Parameters, Summary of Contributors, Summary of LR, and Per Locus Likelihood Ratios sections of each report, if applicable

5.2 Electronic Data Retention

At a minimum, the following electronic files will be retained on disk (or other suitable media) and stored in the case file:

- All .txt files imported into the STRmix™ System for analysis
- STRmix™ Results folder(s) for deconvolutions
- STRmix™ Results folder(s) for LRs

APPENDIX A – REFERENCES

1. STRmix™ Operation Manual, v2.6.

2. STRmix™ Users Manual, v2.6.

3. A Guide to STRmix™ - March 2017 (Client Guide).

4. Bright, J., *et al.* (2013) Developing allelic and stutter peak height models for a continuous method of DNA Interpretation, *Forensic Science International: Genetics*, Volume 7, pages e296-e304.

5. Bright, J. *et al*. (2016) Developmental validation of STRmix™, expert software for the interpretation of forensic DNA profiles, *Forensic Science International: Genetics*, Volume 23, pages e226-e239.

6. Bright J.. *et al*. (2018) Internal validation of STRmix™ – A multi laboratory response to PCAST, *Forensic Science International: Genetics,* https://doi.org/10.1016/j.fsigen.2018.01.003.

7. Curran, J., *et.al.* (2002) Assessing uncertainty in DNA evidence caused by sampling effects, *Science and Justice,* Volume 42, No.1, pages 29-37.

8. Kalafut, T. *et al*. (2018) Implementation and validation of an improved allele specific stutter filtering method for electropherogram interpretation. *Genetics*, Volume 35, pages e50-e56.

9. Richey, M. (2010) The Evolution of Markov Chain Monte Carlo Methods. The Mathematical Association of America, Monthly 117, pages 383-386.

10. Taylor, D. *et al*. (2017) A fully continuous system of DNA profile evidence evaluation that can utilise STR profile data produced under different conditions within a single analysis, *Forensic Science International: Genetics,* Volume 31, pages e149-e154.

11. Taylor, D., *et al*. (2014) Considering relatives when assessing the evidential strength of mixed DNA profiles, *Forensic Science International: Genetics,* Volume 13, pages e259-e263.

12. Hansson, O., *et al*. (2017) Characterization of degradation and heterozygote balance by simulation of the forensic DNA analysis process, *International Journal of Legal Medicine,* Volume 131, pages e303-e317.

13. Tvedebrink, T., *et al*. (2012) Statistical model for degraded DNA samples and adjusted probabilities for allelic drop-out, *Forensic Science International: Genetics,* Volume 6, pages e97-e101.