

DNA SOP-32 Using STRmix™ Software

32.1 Purpose:

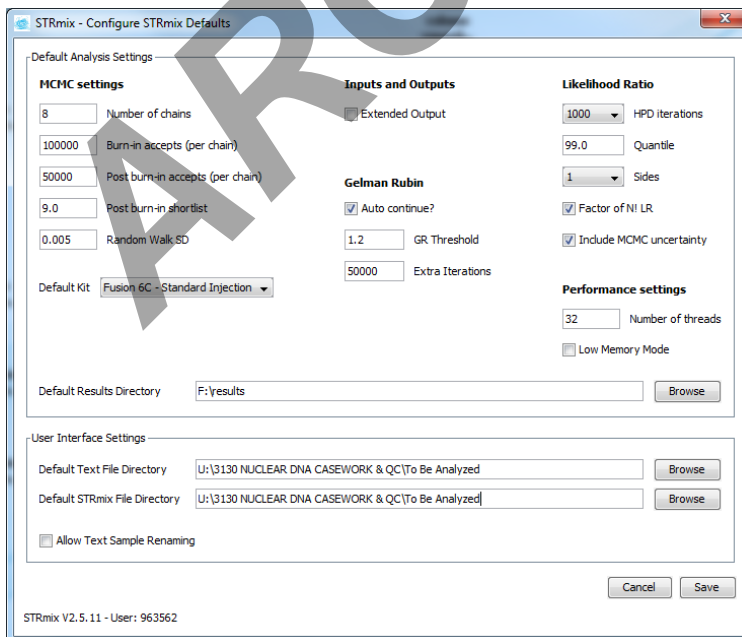
STRmix™ is used to deconvolute an evidentiary profile to obtain probabilistic weights of genotype combinations for all contributors to either a single source or mixture samples with, or without conditioning known profiles. Those weights are used to calculate likelihood ratios which will be reported out on all appropriate samples deconvoluted through STRmix™. The association may be, based on the likelihood ratio, included, cannot be eliminated, inconclusive, or an elimination. STRmix™ may also be used as a tool to determine possible CODIS entries, though this is not currently validated for casework. For further guidance on running STRmix™ software, please refer to the STRmix™ v 2.5 Operations Manual.

32.2 Responsibility:

DNA Unit personnel.

32.3 Set-up of STRmix™**32.3.1 STRmix™ Default Settings**

- 32.3.1.1 Default settings can be edited by clicking on “Settings” in the STRmix™ main menu, then clicking on “Configure STRmix™ Defaults”. See below for how your “Default Settings” should appear.

**32.3.1.2**

The number of MCMC chains, burn-in accepts, and post-burn-in accepts can be adjusted when scientifically valid (such as for a high Gelman-Rubin score or an expected genotype not being modeled) with TL approval.

*Approved by Director: Dr. Guy Vallaro*32.3.2 Kits

32.3.2.1 Kits can be edited by clicking on “Settings” in the STRmix™ main menu, then clicking on “Manage DNA profiling kits”.

32.3.2.2 Located in server desktop’s local disk (C:) ProgramData/STRmix™/Kits folder will be 3 different kit files for Fusion 6C: Maximum Injection, Standard Injection, and Low Injection. These correspond to the three allowed injections times on each 3130 instrument as described in SOP 30.5.2.4. Note that the utilization of the maximum injection is with TL approval.

32.3.2.3 Also present are two different kit files for Identifiler/Identifiler Plus: Standard Injection and Maximum Injection. The Standard Injection kit will be used for all injections at less than 15 seconds, and the Maximum Injection kit for all injections at 15 seconds or more.

32.3.2.4 These are the settings for the F6C – Standard Injection kit, with the loci listed, in order being AMEL, D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, SE33, D22S1045, DYS391, FGA, DYS576, DYS570:

32.3.2.5

Injection kit will have the same standard settings as the F6C – Standard Injection kit with the following exceptions:

Allelic Variance: 8.097, 1.622

Stutter Variance: 4.333, 3.092

Locus Amp Variance: 0.026

32.3.2.6 The F6C – Low Injection kit will have the same standard settings as the F6C – Standard Injection kit with the following exceptions:

Allelic Variance: 5.302, 0.882

Stutter Variance: 2.144, 4.105

Locus Amp Variance: 0.019

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32.3.2.6 These are the settings for the IDP – Standard Injection kit with the loci listed, in order being D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, AMEL, D5S818, FGA:

32.3.2.7 The IDP – Max Injection kit will have the same settings as the IDP – Standard Injection with the following exceptions:

Allelic Variance: 6.525, 1.161
Stutter Variance: 4.064, 2.638
Locus Amp Variance: 0.011

32.3.2.8 For all kits the detection threshold is set to 50 for all loci, the “Gender Locus?” checkbox is selected for AMEL only, and the “Ignore Locus?” box is selected for DYS391, DYS576, and DYS570 only.

32.3.2.8.1 Certain situations (such as tri-alleles, discrepant results at one locus as compared to the rest of the profile, or the possibility for stutter exceeding a stutter max threshold as described in 32.5.5.3) might warrant changing the “Ignore Locus?” setting for autosomal loci during a deconvolution, with TL approval.

32.3.2.8.2 Please see the Appendix for screen shots of the stutter text files.

*Approved by Director: Dr. Guy Vallaro*32.3.3 Populations/Alele Frequencies

32.3.3.1 Populations can be edited by clicking on “Settings” in the STRmix™ main menu, then clicking on “Manage Populations” (see figure to right)

32.3.3.2 Each population file could only be assigned to one kit in STRmix™ v2.4, therefore, for backward compatibility with previous deconvolutions each F6C population file is in triplicate in the ProgramData/STRmix/Populations folder, and named to reflect the kit that it is assigned to.

Population files for STRmix™ v2.5 are not kit-specific. Each file has an allele frequency file linked to it.

32.3.3.2.1 The allele frequency file that the African American population is linked to is: AfAm_FBIextended.csv

32.3.3.2.2 The allele frequency file that the Caucasian population is linked to is: Cauc_FBIextended.csv

32.3.3.2.3 The allele frequency file that the Hispanic population is linked to is: SeHispanic_FBIextended.csv

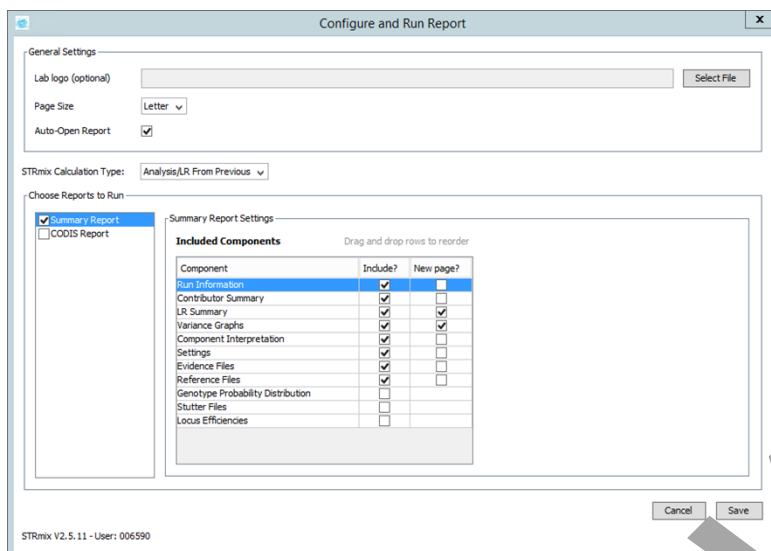
32.3.3.3 Allele frequencies are from the FBI’s allele frequencies in PopStats.

32.3.3.4 The allele frequencies listed in the CSV files do not have the posterior means equation applied to them.

32.3.3.5 When looking at the “Manage Populations” window for each population group, the Population Proportion and Default FST are the same. The bottom half of the screen does not currently apply to State of Connecticut SOPs.

32.4 **Initial Setup of Reports**

32.4.1 Setting Default Settings: From the Main Menu click “Settings”, then “Configure Report Defaults”.

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32.4.2 Highlight “Summary Report” (see below) from the list on the left and set the following options. (The order of items in the “Included Components” list is changed by dragging.)

32.4.3 If viewing additional components for a run is necessary, reports can be re-created retroactively with different options selected. To do this click “Tools” from the Main Menu then “Reports”, and supply the appropriate run folder by browsing or drag-and-drop. Select the desired

options, change the file name at the bottom of the window (as to not overwrite original report) and click “OK”. This will not change the default report settings. Please note that the Genotype Probability Distribution is not included in the default report, so if there is a need to look at individual locus/contributor weights, a new report will need to be created. A report including all components shall be created for discovery/FOIA purposes.

32.4.4 With the settings above, the Summary Report is generated automatically for every deconvolution and LR from Previous run.

32.5 Using STRmix™ to deconvolute a profile

After analysis of a DNA profile, a determination of the most reasonable number of contributors (refer to SOP 31) is chosen and documented on DNA QR-302. Analysts manually compare all knowns associated with a case to all questioned samples associated with that case. If a positive association or inconclusive conclusion (except in cases of insufficient data, complexity of a mixture, where STRmix cannot be run) is made to a questioned DNA profile that questioned sample shall be deconvoluted using STRmix™. Identical profiles compared to the same known, that will give identical conclusions, need only be run once through STRmix. This will be properly documented in the case jacket and DNA Report.

32.5.1 Launch STRmix™ software via remote desktop server: Whenever prompted, usernames, passwords, and settings can be stored.

32.5.1.1 Open the following website using your web browser: (Can be saved to favorites) <https://10.51.107.36/RDWeb/Pages/en-US/login.aspx>. This initially may come up as an unsecure website, but continue to the website when prompted by your browser. This link will bring up your remote application and desktop. If prompted to log in, Domain\username shall

be entered as: dps\employee#. Password shall be the same password used to log onto your DPS desktop.

32.5.1.2 Click on “explorer”. This will bring up the server desktop, or a prompt to save/open an application. Click “ok”, “continue” or “confirm” whenever prompted. Two drives will be accessed through this desktop:

32.5.1.2.1 The F-drive is the STRmix™ server. It is the results folder on this drive where your results will be automatically saved to. Located in F:\results are individual analyst folders. Any deconvolutions an analyst performs are to be saved temporarily in these individual analyst folders. If these are not automatically mapped to save in that location, the analyst shall move their results folders into their folder. Analysts further will create case specific folders in their results folders. This can be done by right-clicking in their folder → new → folder. The folder names will be DSS-YY-XXXXXX. Do not create any further sub-folders, as file name sizes are restricted. Upon issuance of a DNA report, this folder shall be moved to the F:\Results\Completed folder. A record of this move shall be recorded on QR-4, and confirmed by your technical reviewer. There shall be no sub-folders in “Completed” to assist with backing up files/archiving data.

32.5.1.2.2 The U-drive that you see on the server desktop is the U-drive that your GeneMarker/ GeneMapper text files have been previously exported and saved to. You will not be saving anything to the U-drive while using STRmix™. However, you must access those files from the server desktop, or you will not be allowed by STRmix™ to drag and drop those text files.

32.5.1.3 Click on “STRmix”. This will launch the STRmix™ software or a prompt to save/open an application. Click “ok”, “continue” or “confirm” whenever prompted. Only 12 analysts can concurrently run the STRmix™ software.

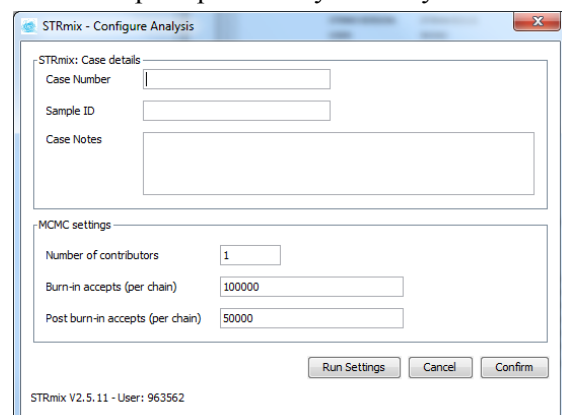
32.5.2 Click “Start Analysis”. The window to the right will appear.

32.5.3 Enter the following information into Configure Analysis” Window:

Case number: Lab ID# (ex. DSS-XX-XXXX)

Sample ID: Item #(s) (ex. 1G1 LR to 2)

Case Notes: Add any information that would be important to your technical reviewer. This can include, but not be limited to description of the item, knowns, conditioning information, reason (if applicable) deconvolution is being repeated, etc.



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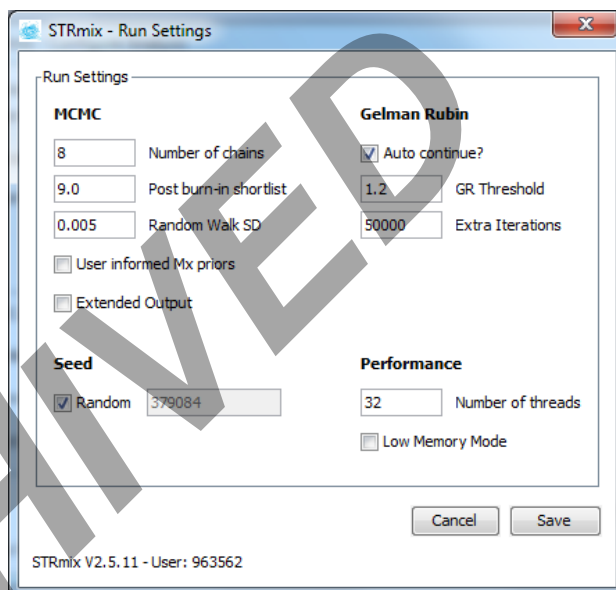
Number of contributors: This should have been pre-determined, and recorded on QR-302 during analysis.

of accepts (burn-in and post burn-in): default settings, need not be changed in routine casework. If warranted, and with TL approval, # of accepts can be changed here.

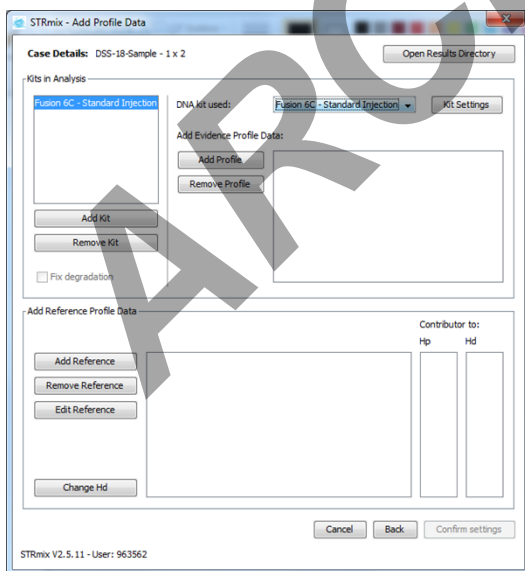
32.5.3.1 A click on “Run Settings” will bring up the window to the right, Nothing in here need be changed in routine casework.

32.5.3.2 After everything has been properly added to the “Configure Analysis” window, click “Confirm”. (Note: At any time you can return to earlier steps using the “Back” button.)

32.5.4 In the “Add Profile Data” window as shown below, select the kit appropriate to the amplification system and injection time for the evidentiary sample from the “DNA kit used” pulldown. The “Fusion 6C- Standard Injection” kit will be there



as a default. If this is not the kit needed, use the drop-down menu next to “DNA kit used:” to change kit.



32.5.4.1

results (the text file exported from GeneMarker/ GeneMapper) to be deconvoluted into “Add Evidence Profile Data”. Browse through the list of samples in that file, click on the one you are deconvoluting, and click “Add”. Alternatively, you can click “Add Profile”, then “Select Text File” and browse to the exported .txt file for the project. Select the appropriate evidentiary sample from the list, click “Add”, confirm that correct sample was

chosen, then click on “Add Profile Data”. The

evidentiary input file HAS n-1 and n+1 stutter peaks. (These stutter peaks HAVE NOT been filtered out).

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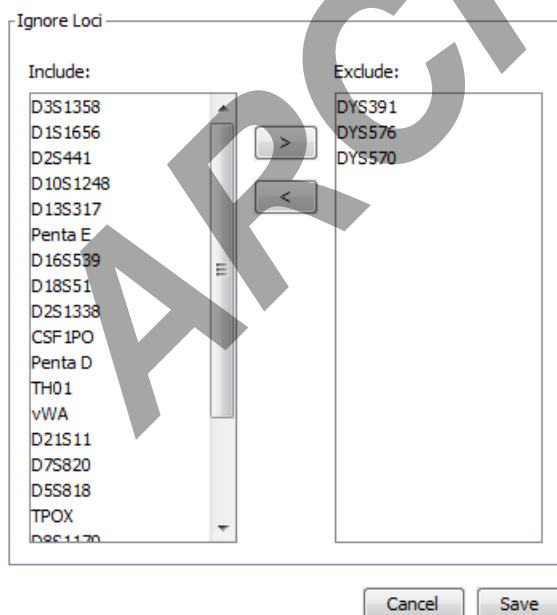
- 32.5.4.2 Add reference profiles to the “Add Reference Profile Data” box in the same way as above, with the exception that if you add a known with the “Add Reference” button that is a .csv file generated by QR-304, use the “Select STRmix File” button rather than “Select Text File”.

The known input file does NOT have n-1 or n+1 stutter peaks. (All stutter peaks have been filtered out).

- 32.5.4.3 For any .csv input files that were generated for STRmix v2.4, STRmix v2.5 will prompt you for the kit used to type the sample being added. Providing any STRmix kit name that uses sample's amplification system will suffice for this; there is no need to look up the injection time used. This step is needed because these input files indicate marker only by reference numbers based on the order of loci in the kit, rather than containing locus names. The kit selected in step 32.5.4 will still be the kit used for the deconvolution.

- 32.5.4.4 If you are conditioning (a) known(s) to H_d , that profile must be added first. Click on the known in the “Add Reference Profile Data” section then click on “Change H_d ”. This will add an “x” to the “contributor to H_d ” column. Knowns only appearing in H_p must be listed after conditioned knowns.

- 32.5.5 Ignoring Loci (with TL approval)



- 32.5.5.1 Click the “Kit Settings” button toward the upper right of the “Add Profile Data” window. Click on “Ignore Loci”. The window to the left will appear.

- 32.5.5.2 Click on locus you wish to ignore, then click on “arrow right” button to move it to the “Exclude” column. Click “Save” will change loci for this run only. Default settings will not be changed.

- 32.5.5.3 As an additional note on kit settings, the stutter max thresholds are expected to be conservative for all loci in all kits, except for

D22S1045 in Fusion 6C, where typical forward stutter from larger alleles may exceed the threshold setting. If a sample has an allele at this locus with a peak in forward stutter position that is >10% of its height, it may be appropriate to leave the locus out of the STRmix analysis.

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32.5.6 Click “Confirm settings”. When just performing a deconvolution (i.e. not obtaining a likelihood ratio), whether or not a known is conditioned, there is nothing to do in the “Population Settings” window. Click on “Start” to deconvolute the profile.

32.5.6.1 When compared knowns are applied to any hypothesis, 3 populations must be added to “Population Settings”: African American, Caucasian, and Hispanic. This setting is completed the first time STRmix™ is run, and need not be completed on subsequent deconvolutions.

32.5.6.1.1 Select each population from the pull-down menu and click “Add population” to populate the

STRmix - Population Settings

Case Details: DSS-18-Sample - 1 x 2

Population Settings

Hispanic

Add Population Remove Population

Population	Proportion	FST	Allele Freq File
African American	0.3333333333333333	0.01b(1.0, 1.0)	AFAm_FBIextended.csv
Caucasian	0.3333333333333333	0.01b(1.0, 1.0)	Cauc_FBIextended.csv
Hispanic	0.3333333333333333	0.01b(1.0, 1.0)	SeHis_FBIextended.csv

Factor NI

☒ Factor of NI LR

Sampling Variation

☒ Calculate HPD ☒ Include MCMC uncertainty

HPD iterations: 1000 Quantile: 99 Sides: 1

Save as Default Populations Cancel Back Start Start & Search

STRmix V2.5.11 - User: 963562

window to the left after each population is selected. Click “Save as default” after all 3 are added so that this step does not have to be repeated with subsequent deconvolutions. The diagram to the left is how the population settings should look, when knowns are applied to any hypothesis, prior to hitting “Start”.

32.5.7 The report PDF file for the deconvolution opens automatically when the run completes. Print page 1 for a deconvolution-only run and pages 1-3 for a run with a likelihood ratio calculation for the case jacket.

32.5.8 The qualitative comparison categories for IDP likelihood ratios correspond to the ranges listed for F6C in SOP 31, with the exception that the maximum (rounded down) Inconclusive LR for IDP is 999.

32.6 Using STRmix™ to Create a Likelihood Ratio to a Reference Sample from previously deconvoluted sample

32.6.1 After launching STRmix™, click on “LR from Previous Analysis”, and navigate to the results file folder of the deconvolution that was previously completed in the F:\Results folder. Drag and

drop the outer run folder or the “config” .xml file within it into the Previous Analysis box (or click “Select File” and use the browser to populate it). Click OK.

- 32.6.2 In the “Configure Analysis” window, the case number shall remain the same. The sample ID may be changed to reflect the calculation currently being completed, (i.e. 1G1 LR 2 changes to 1G1 LR 3). This can be further described in the case notes. Since this is to a previous deconvolution, the MCMC settings, are unable to be changed. Click “Confirm”.
- 32.6.3 You will be unable to add or remove evidence profiles, or change kits in analysis. Add reference profiles to the bottom half of the “add profile data” window, as in 32.5.4.2-3. The known will only be added to H_p . Knowns cannot be added to or removed from H_d for conditioning purposes in “LR from previous analysis” settings, since conditioning a known affects the deconvolution, and not just the likelihood ratio from previously obtained weights.
- 32.6.3.1 If rework on this sample is being completed because a victim, consensual partner, or other known has been submitted that is to be conditioned to the profile, the deconvolution itself must be repeated with the conditioned known.
- 32.6.4 If this is your first time doing a LR in STRmix™, “Population Settings” will not have any populations. Add populations and confirm settings as in 32.5.6.1. Click “Start”.
- 32.6.5 Print pages 1-3 of each report for the case jacket.
- 32.7 Running samples in Batch Mode**
- 32.7.1 On the STRmix™ startup window, click “Batch Mode”.
- 32.7.2 Click “Add to Batch”, and add a sample as you would in 32.5. After a sample has been added to the batch, you will not be able to see your settings. Pay close attention to your settings. Entering something incorrectly could stop the batch from running.
- 32.7.3 If a sample needs to be run in low memory mode (a complex mixture, for example), please adjust in “Run Settings”.
- 32.7.4 If all samples have been added, but you do not wish to start the batch at this time, click “Exit Batch Mode”. The samples that have been added will be there when you return to “Batch Mode”.
- 32.7.5 If you wish to start the batch, click “Start Batch”.
- 32.7.6 If you wish to end the batch, click “Stop Batch”. The sample that is currently being deconvoluted will run to completion, and then the batch will stop. All samples that have yet to be deconvoluted will remain in the queue, until removed.

32.7.7 To remove a sample from the queue, highlight the sample in the “Samples In Batch” window, then click “Delete Analysis”.

32.7.8 The “LR Batcher” feature allows sequential comparison of multiple previously deconvoluted questioned profiles to a known, or multiple knowns to a previously deconvoluted profile.

32.7.8.1 From the Main Menu click “Tools” then “LR Batcher.

32.7.8.2 Drag and drop the outer run folder or “config” file for completed analyses into the “Deconvolutions” window, and known input files into the “References” window (or add them by using the “Add analysis”/“Add reference” buttons to browse).

32.7.8.3 When all deconvolutions and all references have been added, click “Start”. As a note, all runs in the “Deconvolutions” window are compared to all knowns in the reference window.

32.7.8.4 Print pages 1-3 of each report for the case jacket.

32.8 **Review of Run Diagnostics:** In STRmix™, there are a number of diagnostics that may indicate that MCMC analysis did not perform as expected. These are found in the Post Burn-In Summary section of the Report.

32.8.1 For every deconvolution performed in STRmix, DNA-QR-303 must be completed to allow for an overall assessment of how the deconvolution was performed in STRmix. The ideal ranges for the quality parameters based on the internal validation are documented on this QR. No single diagnostic can determine whether a STRmix deconvolution was or was not successful. However, multiple values outside of their ideal ranges, or values falling far from their ideal ranges, are potential indicators of a problem with a deconvolution, and may warrant manual scrutiny of the genotypic weights assigned and possibly a re-run with TL approval.

In the event that any of the secondary diagnostic values are out of range, the analyst will document a brief justification in the “Comments” section of QR-303 for why the deconvolution is (or is not) valid.

32.8.2 Total iterations

The value indicates the total number of post burn-in iterations that the MCMC ran for during its analysis. This value, along with the number of accepts chosen for the analysis informs the analyst as to how often a new proposed set of parameters was accepted. This is referred to as the

acceptance rate. For example, the analysis that lead to the above results was carried out with 100,000 burn-in accepts and 500,000 total accepts. This leaves 400,000 post burn-in accepts spread across 800,000 total iterations giving an acceptance rate of 1 in 2.

A very low acceptance rate (e.g. 1 in hundreds or thousands) may, in combination with the other diagnostics, indicate that the analysis needs to be run for additional iterations. On its own, a low acceptance rate is not an indication that reanalysis is required.

As a note, if a deconvolution auto-continues past the standard number of post burn-in accepts (due to an above-threshold Gelman-Rubin diagnostic score), STRmix v2.5.11 factors the total number of post burn-in iterations but only the standard number of accepts into the acceptance rate on the STRmix Report. The macro in QR-303 uses the total post burn-in counts for both iterations and accepts when calculating acceptance rate. The value appearing on the worksheet is the appropriate one to compare to the threshold range.

32.8.3 Effective Sample Size

Effective sample size (ESS) is the number of independent genotypic/mass parameter combinations that the MCMC has evaluated. A low ESS in relation to the total number of iterations suggests that the MCMC has not moved very far with each step or has had a low acceptance rate. A low ESS (e.g. 10s or 100s) value means that there is potential for a large difference in weights if the analysis was run again. A low ESS on its own is not an indication that reanalysis is required. In general, an ESS of less than 1000 warrants additional scrutiny.

32.8.4 Average (\log_{10}) likelihood

This value is the average (\log_{10}) likelihood for the entire post burn-in MCMC. It is the log of the average likelihood (or probability) value created at each of the post burn-in MCMC iterations. The larger this value, the better STRmix™ has been able to describe the observed data. A negative value suggests that STRmix™ has not been able to describe the data very well given the information it has been provided. Some possible reasons for this value being low or negative are:

- a) The profile is simply very low level and there is very little data making up the likelihood.
- b) The number of contributors is wrong which can cause STRmix™ to consider incorrect genotypic combinations (e.g. large heterozygote peak imbalances or variation in mixture proportions across the profile).
- c) Data has been removed that was real, particularly stutter peaks, and must now be described in STRmix™ by dropout.
- d) Artifact peaks were not removed and must now be accounted for in STRmix™ by drop-in.

A low or negative value for the average (\log_{10}) likelihood may indicate that the analysis requires additional scrutiny.

Good quality (adequate template, high molecular weight) mixed DNA profiles often give higher average (\log_{10}) likelihood values than comparable single source profiles. So low average (\log_{10}) likelihood values alone are not necessarily an indicator of an issue especially if the profile is single source.

32.8.5 Gelman-Rubin convergence diagnostic

This diagnostic informs the analyst whether the MCMC analysis has likely converged. STRmix™ uses 8 chains to carry out the MCMC analysis and ideally each chain will be sampling in the same space after burn-in. If the chains spend their time in different spaces then it is likely that the analysis has not run for long enough. Whether or not the chains have spent time in the same space can be gauged by the within-chain and between-chain variances. This is known as the Gelman-Rubin convergence diagnostic (GR). If the chains fully converge, the GR is 1.

If the GR is above 1.2, then there exists the possibility that the analysis hasn't converged. If the GR value is above 1.2, the results of the analysis should be scrutinised. Running the analysis for a larger number of iterations may reduce the GR in these instances to below 1.2. With the default settings a run will continue automatically for an additional 50,000 accepts per chain if the GR score is above 1.2 after the standard accept count; this does not preclude analysts from increasing the iteration count further if appropriate.

32.8.6 Allele Variance and Stutter Variance constants

Both of these values are the average value for variance and stutter variance constants across the entire post burn-in MCMC analysis. These values can be used as a guide as to the level of stochastic variation in peak heights that is present in the profile. A visual representation of how these values compare with the data seen in the laboratory's model maker data can be seen in "Variance Charts" section in the STRmix Report. The dot on the curve represents the value in the current run.

If variance is significantly above the mode value, additional scrutiny is warranted. It may indicate that the DNA profile is sub-optimal or that the number of contributors is incorrect. A higher than average Stutter Variance constant may be indicative of stutter peaks not being called in the STRmix GeneMarker project. A further look into the STRmix GeneMarker project may be necessary to see if all stutter peaks have been properly called. Pay close attention to those peaks adjacent to another peak with only a 1 base pair difference, since those are the more likely to be unresolved than those with greater than a 1 base pair difference.

Used in conjunction with the average (\log_{10})likelihood, a large allele variance or stutter variance may indicate that the PCR did not perform as expected.

If the sample is simply low level, this often results in a low average (\log_{10})likelihood and an average variance constant.

If some data has been omitted or mistakenly left in the STRmix™ input file, this often results in a low average (\log_{10})likelihood and high variances.

Glossary of Terms

Accept: An iteration that is accepted. Default settings in STRmix™ require 500,000 accepts in the MCMC process. The burn-in, the first 100,000 accepts, allows the MCMC chains to proceed into spaces where “good” genotypes are proposed. The Metropolis-Hastings algorithm determines if a new genotype combination will be accepted or rejected.

Allele Variance: A secondary diagnostic tool, that when compared to the kit’s mode, can determine how variable peak heights are in a particular sample, as well as to those samples most commonly seen in the laboratory’s model maker.

Average (log) likelihood: A secondary diagnostic tool that calculates the average of the common logarithm of all likelihoods obtained for the post burn-in MCMC process.

Effective Sample Size (ESS) – A secondary diagnostic tool that evaluates the total number of independent genotypic/mass parameter combinations that the MCMC has evaluated.

Gelman-Rubin convergence diagnostic: A secondary diagnostic tool that compares variances within and between MCMC chains, to determine if they have or haven’t converged.

Iteration: a proposed genotype combination.

Likelihood Ratio: A statistical test for comparing two competing hypotheses.

Markov chain Monte Carlo (MCMC): An algorithm based on standard mathematical principles that assigns a likelihood for each random genotype combination, in order to provide the best explanation for an observed data set.

Mass Parameters: 4 variable parameters used in the MCMC process to generate expected peak heights given a proposed genotype combination. These are: contributor specific **D**egradation rate, locus specific **A**mplification efficiencies, PCR **R**eplicate (not utilized by CT DSS SOPs), and contributor specific **T**emplate amount. A mnemonic phrase to easily remember these parameters is **DART**.

Model Maker: A tool within the software that uses laboratory specific empirical data to create kit specific STRmix™ parameters.

Probabilistic Genotyping: A tool that combines the use of biological modeling, statistical theory, computer algorithms, and probability distributions to calculate likelihood ratios for DNA typing results of forensic samples, providing statistical weighting to different genotype combinations

STRmix™: Forensic analysis software that utilizes a fully continuous (taking peak heights into account) approach to probabilistic genotyping to deconvolute DNA profiles into their most probable components,

then compare reference samples to that deconvolution, calculating a likelihood ratio to give that comparison a statistical weight.

Stutter Variance: A secondary diagnostic tool, that when compared to the kit's mode, can determine how variable stutter peak heights are in a particular sample, as well as to those samples most commonly seen in the laboratory's model maker.

Total Iterations: A secondary diagnostic tool that tells how many genotype combinations were proposed during the post burn-in MCMC process. Directly related to the acceptance rate, which divides the total number of post burn-in accepts (defaulted to 400,000) by the total number of iterations. Therefore, if the total iterations were 7 million, the acceptance rate would be 1 in 17.5.

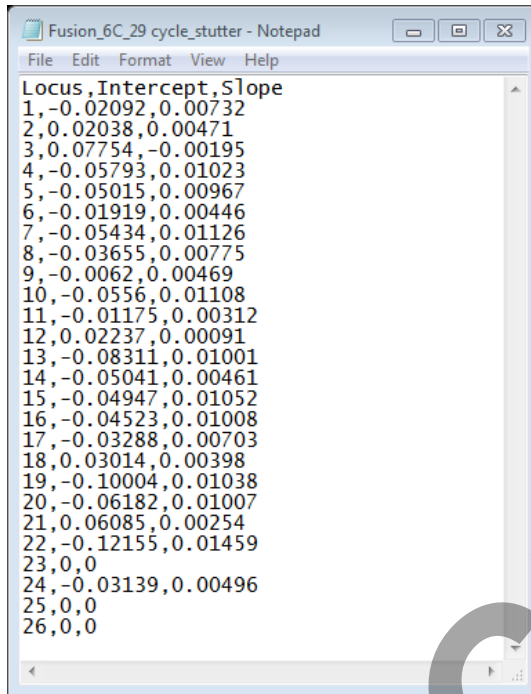
Weight: A probability given to a particular genotype combination that correlates to how well that combination explains the observed profile.

Note: In creating the above glossary, the following references were utilized: STRmix Training Workshop handouts, NYC OCME STRmix™ Glossary, the STRmix Support Website, the STRmix Operations Manual, and the STRmix user's manual.

Approved by Director: Dr. Guy Vallaro

Appendices

Appendix 1: F6C Stutter File



Locus	Intercept	Slope
1	-0.02092	0.00732
2	0.02038	0.00471
3	0.07754	-0.00195
4	-0.05793	0.01023
5	-0.05015	0.00967
6	-0.01919	0.00446
7	-0.05434	0.01126
8	-0.03655	0.00775
9	-0.0062	0.00469
10	-0.0556	0.01108
11	-0.01175	0.00312
12	0.02237	0.00091
13	-0.08311	0.01001
14	-0.05041	0.00461
15	-0.04947	0.01052
16	-0.04523	0.01008
17	-0.03288	0.00703
18	0.03014	0.00398
19	-0.10004	0.01038
20	-0.06182	0.01007
21	0.06085	0.00254
22	-0.12155	0.01459
23	0	0
24	-0.03139	0.00496
25	0	0
26	0	0

Appendix 2: F6C Forward Stutter File



Locus	Intercept	Slope
1	0.00855	0
2	0.00946	0
3	0.00833	0
4	0.00738	0
5	0.00827	0
6	0.00718	0
7	0.0092	0
8	0.00918	0
9	0.0081	0
10	0.01068	0
11	0.0074	0
12	0.00843	0
13	0.00874	0
14	0.01068	0
15	0.00812	0
16	0.00933	0
17	0.00805	0
18	0.01054	0
19	0.00877	0
20	0.00841	0
21	0.01125	0
22	-0.09332	0.0097
23	0	0
24	0.00828	0
25	0	0
26	0	0

Appendix 3: F6C Stutter Exceptions File:

What is shown in this appendix is a truncated version of the stutter exceptions file, listing only all of the stutter exceptions. The full version of this file lists all loci and all repeats in those loci.

DNA SOP-32 STRmix

Document ID: 4385

Revision: 5

Effective Date: 11/23/2018

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Page 17 of 21

Approved by Director: Dr. Guy Vallaro

Repeat	D3S1358	D1S1656	D2S441	D2S1338	CSF1PO	Penta D	TH01	D21S11	D8S1179	D19S433	SE33	FGA
6	0	0	0	0	0	0	0.02126	0	0	0	0	0
7	0	0	0	0	0.02889	0	0.03261	0	0	0	0	0
8	0	0	0	0	0.0446	0	0.03284	0	0	0	0	0
9	0	0	0	0	0.03888	0.02391	0.03904	0	0.05481	0	0	0
9.3	0	0	0	0	0	0	0.01725	0	0	0	0	0
10	0	0	0.04354	0	0.05278	0.02139	0	0	0.06995	0	0	0
11	0	0.06336	0.06581	0	0.06379	0.02226	0	0	0.08066	0.04463	0	0
11.3	0	0	0.0307	0	0	0	0	0	0	0	0	0
12	0	0.0734	0.07349	0	0.08068	0.023	0	0	0.08394	0.0616	0	0
13	0.06355	0.08337	0	0	0.09312	0.02947	0	0	0.07828	0.06884	0.07679	0
13.2	0	0	0	0	0	0	0	0	0	0.07345	0	0
13.3	0	0	0	0	0	0	0	0	0	0	0	0
14	0.07844	0.09366	0.04604	0	0	0.03312	0	0	0.08691	0.07851	0.08063	0
14.2	0	0	0	0	0	0	0	0	0	0.09034	0	0
15	0.09291	0.10519	0.05485	0	0	0.03318	0	0	0.0869	0.08811	0.08763	0
15.2	0	0	0	0	0	0	0	0	0	0.09411	0	0
15.3	0	0.06924	0	0	0	0	0	0	0	0	0	0
16	0.0933	0.11508	0	0.07082	0	0	0	0	0.09698	0.09051	0.10257	0
16.2	0	0	0	0	0	0	0	0	0	0.10265	0	0
16.3	0	0.07731	0	0	0	0	0	0	0	0	0	0
17	0.10154	0.12828	0	0.07211	0	0	0	0	0	0	0.10101	0
17.3	0	0.08733	0	0	0	0	0	0	0	0	0	0
18	0.11101	0	0	0.07885	0	0	0	0	0	0	0.11568	0
18.3	0	0.0956	0	0	0	0	0	0	0	0	0	0
19	0.1177	0	0	0.08786	0	0	0	0	0	0	0.12278	0.05522
19.3	0	0.11022	0	0	0	0	0	0	0	0	0	0

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DNA SOP-32 STRmix

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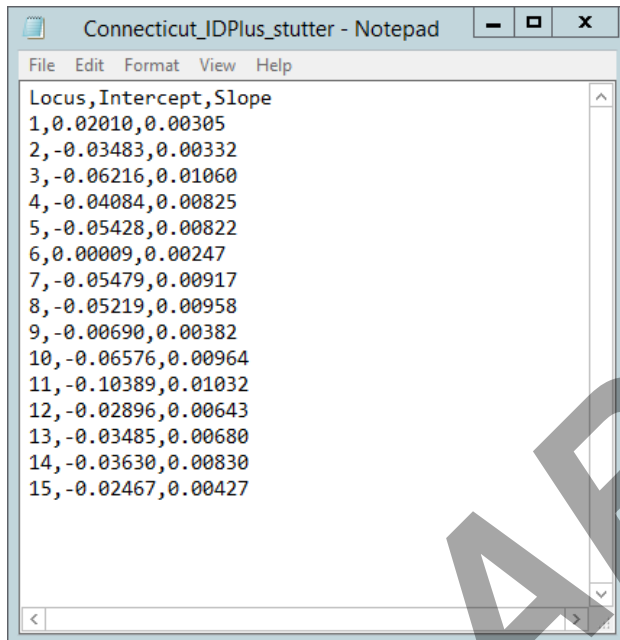
Page 18 of 21

20	0	0	0	0.09115	0	0	0	0	0	0	0.12455	0.0641
21	0	0	0	0.0908	0	0	0	0	0	0	0.1356	0.0703
21.2	0	0	0	0	0	0	0	0	0	0	0.08847	0
22	0	0	0	0.08697	0	0	0	0	0	0	0.13586	0.08002
Repeat	D3S1358	D1S1656	D2S441	D2S1338	CSF1PO	Penta D	TH01	D21S11	D8S1179	D19S433	SE33	FGA
22.2	0	0	0	0	0	0	0	0	0	0	0.09782	0
23	0	0	0	0.09679	0	0	0	0	0	0	0	0.08644
23.2	0	0	0	0	0	0	0	0	0	0	0.10263	0
24	0	0	0	0.10597	0	0	0	0	0	0	0	0.09284
24.2	0	0	0	0	0	0	0	0	0	0	0.1108	0
25	0	0	0	0.12193	0	0	0	0	0	0	0	0.09859
25.2	0	0	0	0	0	0	0	0	0	0	0.10887	0
26	0	0	0	0.11848	0	0	0	0	0	0	0	0.10413
26.2	0	0	0	0	0	0	0	0	0	0	0.12206	0
27	0	0	0	0	0	0	0	0.06409	0	0	0	0.09188
27.2	0	0	0	0	0	0	0	0	0	0	0.12681	0
28	0	0	0	0	0	0	0	0.077798	0	0	0	0
28.2	0	0	0	0	0	0	0	0	0	0	0.13212	0
29	0	0	0	0	0	0	0	0.083498	0	0	0	0
29.2	0	0	0	0	0	0	0	0	0	0	0.13372	0
30	0	0	0	0	0	0	0	0.094359	0	0	0	0
30.2	0	0	0	0	0	0	0	0.082745	0	0	0.14036	0
31	0	0	0	0	0	0	0	0.10219	0	0	0	0
31.2	0	0	0	0	0	0	0	0.086892	0	0	0.15647	0
32	0	0	0	0	0	0	0	0.110109	0	0	0	0
32.2	0	0	0	0	0	0	0	0.096907	0	0	0.15267	0
33.2	0	0	0	0	0	0	0	0.10599	0	0	0	0

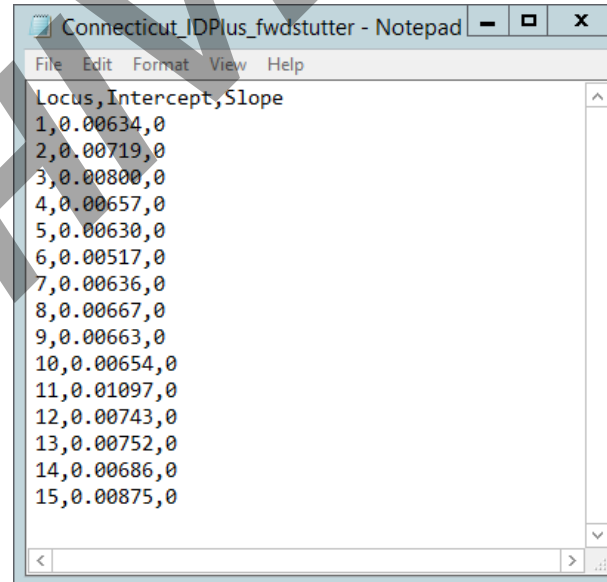
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35 0 0 0 0 0 0 0 0.088913 0 0 0 0

Appendix 4: ID/IDP Stutter File

```
File Edit Format View Help
Locus,Intercept,Slope
1,0.02010,0.00305
2,-0.03483,0.00332
3,-0.06216,0.01060
4,-0.04084,0.00825
5,-0.05428,0.00822
6,0.00009,0.00247
7,-0.05479,0.00917
8,-0.05219,0.00958
9,-0.00690,0.00382
10,-0.06576,0.00964
11,-0.10389,0.01032
12,-0.02896,0.00643
13,-0.03485,0.00680
14,-0.03630,0.00830
15,-0.02467,0.00427
```

Appendix 5: ID/IDP Forward Stutter File

```
File Edit Format View Help
Locus,Intercept,Slope
1,0.00634,0
2,0.00719,0
3,0.00800,0
4,0.00657,0
5,0.00630,0
6,0.00517,0
7,0.00636,0
8,0.00667,0
9,0.00663,0
10,0.00654,0
11,0.01097,0
12,0.00743,0
13,0.00752,0
14,0.00686,0
15,0.00875,0
```

Appendix 6: ID/IDP Stutter Exceptions File

What is shown in this appendix is a truncated version of the stutter exceptions file, listing only all of the

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stutter exceptions. The full version of this file lists all loci and all repeats in those loci.

Allele	D21S11
27	0.047908
28	0.054311
29	0.062966
30	0.070931
30.2	0.052559
31	0.077371
31.2	0.062083
32	0.08521
32.2	0.071276
33.2	0.080434
35	0.045518

Allele	TH01
4	0.00245
5	0.00759
6	0.01273
7	0.01787
8	0.02301
8.3	0.00759
9	0.02815
9.3	0.01273
10	0.03329
10.3	0.01273
11	0.03843
12	0.04357
13.3	0.02301

Allele	D2S1338
16	0.049954
17	0.057781
18	0.065838
19	0.06834
20	0.07168
21	0.074861
22	0.070758
23	0.074588
24	0.085853
25	0.091494
26	0.09872

Allele	D19S433
6.2	0.00402
8	0.01308
9	0.01308
10	0.0312
11	0.04026
11.1	0.0312
11.2	0.04932
12	0.04932
12.1	0.00402
12.2	0.05838
13	0.05838
13.2	0.06744
14	0.06744
14.2	0.0765
15	0.0765
15.2	0.08556
16	0.08556
16.2	0.09462
17	0.09462
17.2	0.09915
18	0.10368
18.2	0.11274
19.2	0.1218

Allele	FGA
18.2	0.050704
19	0.046956
20	0.052723
21	0.06027
22	0.074733
23	0.077721
24	0.082077
25	0.09072
26	0.090288
27	0.079836

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