STRmix™ Probabilistic Genotyping Software Operating Instructions

This procedure describes the use of STRmix™ V2.4 for the interpretation of PowerPlex® Fusion DNA profiles run on a 3130xL Genetic Analyzer within the NYC Department of Forensic Biology. Readers are also referred to the STRmix™ v.2.4 Users and Operation manuals for additional information.

For STRMix™ set-up instructions please refer to QC702 in the Quality Control Procedures Manual.

1 Preparing Data for a STRmix™ Analysis

1.1 Before performing your STRmix™ analysis, the following actions must be taken:

1.1.1 Verify that the sample is suitable for STRmix™ analysis (Refer to the STR Results Interpretation – PowerPlex Fusion & STRmix manual)

1.1.2 Determine the best described Number of Contributors to the sample (NOC). Refer to the STR Results Interpretation manual regarding the procedure for determining the number of contributors.

1.1.3 Evaluate your replicates. If there are drastic inconsistencies with the alleles present between replicates, only the amplification with the most information should be used or a third amplification may be warranted.

1.1.4 Create folders for the STRmix™ runs:

1.1.4.1 Navigate to the M:\STR_Data\STRmix Data Folder

1.1.4.2 Within the STRmix Data folder, create a new folder with the FB (or FBS) case number

e.g. FB16-01234 or FBS16-05678

1.1.4.3 Within the FB (or FBS) case file folder, create a folder for EACH evidence or suspect sample that will be run through STRmix™. Use the sample's OCME ID for the naming of the folder. Suffixes such as 'mcon' or 'reamp' should not be included.

e.g. FB16-01234_567_1_1.1_trig_GS
FB16-01234_890_1_1.1_shirt_BL
FB16-01234_123_1_1.1_VS_SF
FBS16-05678_999_1_1.1_(s)JS
FBS16-05678_888_1_1.1_cupJS

1.1.4.4 Note: If a suspect is being compared to multiple FB's, create a sub-folder within that suspect sample folder for each cross-referenced FB.

1.1.5 Confirm that the STR data is prepared correctly for STRmix™ analysis:

1.1.5.1 Evidence samples must only be amplified in PowerPlex Fusion® in order to undergo STRmix™ analysis.

1.1.5.2 Evidence samples must be edited to remove all artifacts, including pull ups, spikes, dye blobs, n-8 stutter and n-2 stutter, etc. before inputting into STRmix™. Standard forward and reverse stutters must not be removed before importing into STRmix™.

1.1.5.3 Sample data must be assembled into the appropriate format for STRmix™ input. The standard input for STRmix™ are .txt files. See "STRmix™ analysis for Evidence" and "Exporting Exemplar Table for STRmix™ input" sections within the GeneMarker manual.

1.1.5.4 Reference samples must be edited to remove all artifacts and all stutter. Incomplete or tri-allelic loci must not be imported into STRmix™ for a reference sample – remove all allele(s) for that locus within the text file. If a possible drop-in peak is present in a reference sample, remove this peak from the text file before STRmix™ import.

1.1.5.5 An attempt should be made to amplify reference samples in PowerPlex Fusion®. If unavailable, STRmix™ allows the user to calculate a likelihood ratio when the evidence and reference samples are analyzed in different autosomal typing kits. LRs will only be calculated for those loci in common between the two kits.

1.1.5.6 In the case of a reference sample not amplified in Fusion, the samples must input from .txt files and the locus order must match that of the evidence (PowerPlex Fusion® order). The reference sample data can be converted to the proper PowerPlex Fusion® order and appropriate .txt file format using the following macro: "Identifiler to Fusion Exemplar STRmix Input Creation".

1.1.5.7 If a DNA donor is being used from one sample to condition or compare to another, use the following macro: "Reference profile for STRmix Input Creation"

1.1.5.8 Non-numeric values such as OL or OB, < or > are not permitted within the STRmix™ input files. Unambiguous alleles including those that are rare should appear in the corresponding input file as their actual allelic size designation, for example D21: 30.1. If an actual allelic size designation cannot be determined, the data for this locus should be removed completely from the text file and the locus (or loci) should be ignored.

1.1.5.9 In order to modify a STRmix™ input text file:

1.1.5.9.1 Open the STRmix .txt file associated with the appropriate STR project (e.g. in Notepad)

1.1.5.9.2 Locate the sample and locus containing the non-numeric value within the .txt file and manually replace the value with the appropriate actual allelic size designation

1.1.5.9.3 Save .txt file replacing the original file

1.1.6 Evidence samples should be evaluated to determine if a locus needs to be ignored before STRmix deconvolution is performed. A comment should be added to the "Case Notes" field within the STRmix analysis to indicate why the locus was ignored for that run. For example, data must be ignored at loci that contain:

1.1.6.1 a tri-allelic pattern

1.1.6.2 an unresolved allelic or stutter peak that is visible above the AT

1.1.6.3 an OB/OL allele or stutter peak that cannot be assigned a correct allelic designation

1.1.6.4 a stutter or allelic peak for an allele belonging to locus A is being called in a neighboring locus B (ignore both loci)

1.1.6.5 where a conditioning sample does not have data at a locus that is present in the evidence sample

1.1.6.6 The technical leader should be consulted for any situation not covered above.

1.1.7 Loci should not be ignored for likelihood ratio calculations due to a partial comparison sample. Ensure that your reference sample text file has been updated appropriately as described in 1.1.5.4.

1.1.8 Prepare the scenarios to be run in STRmix™.

1.1.8.1 Are you performing a deconvolution on an evidence sample? Go to Section 2 Deconvolutions in STRmix.

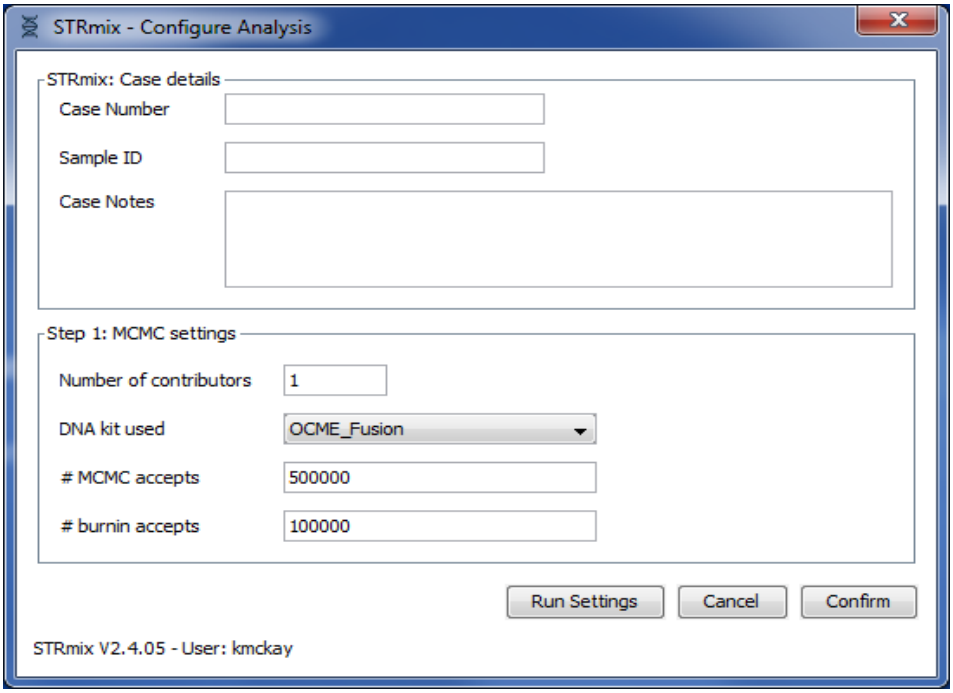
1.1.8.2 Are you comparing a reference sample against a previously deconvoluted evidence sample in order to generate an LR? Go to Section 3 Likelihood Ratio calculations with STRmix.

1.1.8.3 Are you performing a deconvolution of an evidence sample and comparing of a reference sample to generate the LR in a single step? Go to Section 4 Deconvolution and Likelihood Ratio Calculations (Combined) in STRmix.

2 Deconvolutions in STRmix™.

2.1 Launch the STRmix™ application. Open the STRmix™ software by locating STRmix™ in the task bar or by double clicking on the STRmix™ icon on the desktop.

2.2 **Select "Start Analysis" from the startup screen.** This will open the "STRmix – Configure Analysis" window.



2.3 Naming STRmix™ runs

2.3.1 STRmix™ output folder and file names are created by stringing together the values entered into the "Case Number" and "Sample ID" fields in the software followed by the date and time of the analysis run. The information in the file name is separated by dashes. Therefore, if other characters are entered, such as a comma, underscore, period, etc., the software will convert them into dashes.

2.3.2 The following naming convention should be used for deconvolutions:

Case Number = YY-XXXXX (leave out "FB")
Sample ID = remainder of the OCME ID* #p (NOC) cond elim initials (if applicable)
Case Notes = a comment should be added here if a locus is ignored, indicating the reason: e.g. "D2S441 was ignored due to an unresolved allelic peak"

*Suffixes such as 'mcon' or 'reamp' should not be included in the OCME ID.

e.g. deconvolution of 3-person mixture, FB16-01234_567_1_1.1_trig_GS, conditioned on John Doe

Case Number = 16-01234
Sample ID = 567_1_1.1_trig_GS 3p condJD

Examples	Resulting STRmix file name
1-person deconvolution	16-01234-567-1-1-1-trig-GS-1p
2-person deconvolution, no conditioning	16-01234-567-1-1-1-trig-GS-2p
2-person deconvolution, conditioning victim AB	16-01234-567-1-1-1-trig-GS-2p-condAB
3-person deconvolution, no conditioning	16-01234-567-1-1-1-trig-GS-3p
3-person deconvolution, conditioning elim CD	16-01234-567-1-1-1-trig-GS-3p-condCD
3-person deconvolution, conditioning elims CD and EF	16-01234-567-1-1-1-trig-GS-3p-condCD EF

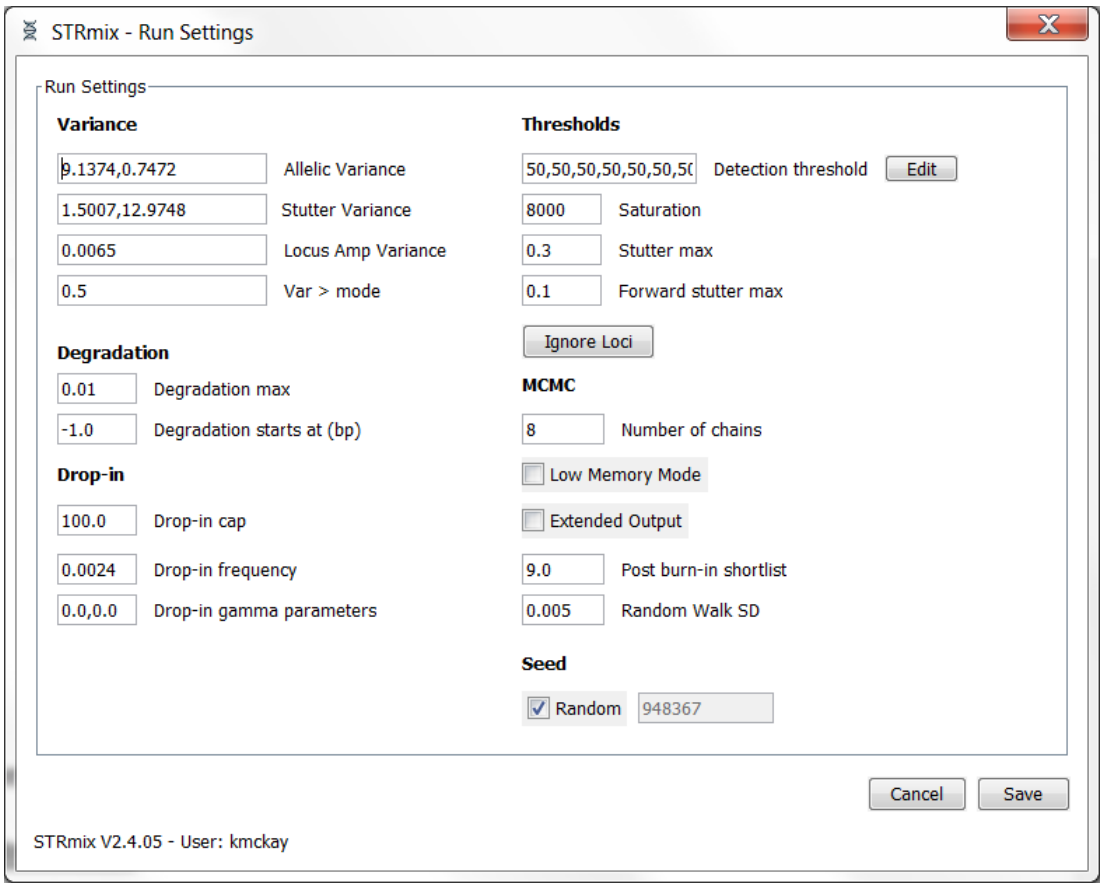
2.4 Set the number of contributors

2.5 Ensure that the following "Step 1: **MCMC settings"** are in place:

DNA kit used: OCME_Fusion
 # MCMC accepts: 500000
 # burnin accepts: 100000

2.5.1 NOTE: the # MCMC accepts and # burnin accepts must not be modified without documented approval from the technical leader (or his/her designee).

2.6 Select "run settings" to confirm run settings. The settings should be as follows for every STRmix™ analysis. Any changes that are made will appear in bold on the run report. If a locus needs to be ignored, follow the procedure below. Otherwise, press "Cancel" when done.



2.6.1 If a locus needs to be ignored for the deconvolution:

2.6.1.1 Under **Thresholds** settings click **Ignore Loci** button.

2.6.1.2 Select the locus within the **Include** window and click the **>** button to move the locus to the **Exclude** window.

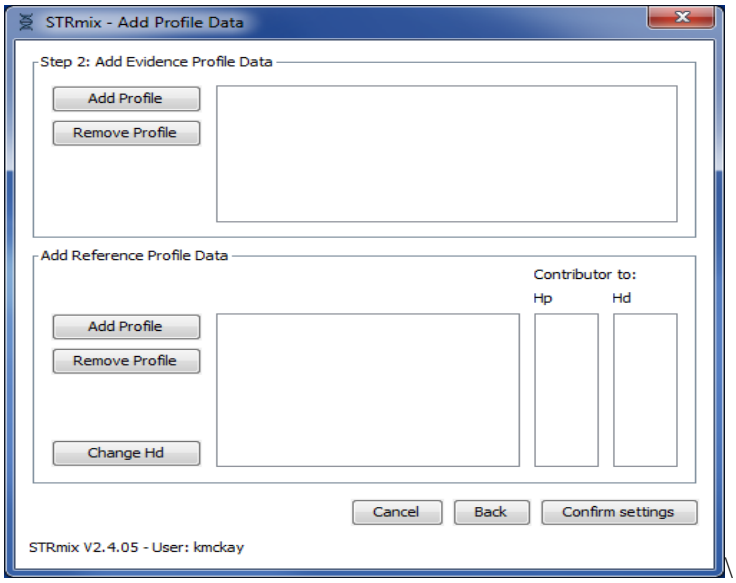
2.6.1.3 Click save when all appropriate loci have been added to the **Exclude** window.

2.6.1.4 Click save again in the **Run Settings** window.

2.7 Select "Confirm" to proceed to the "Add Profile Data" window or cancel to return to the Startup screen (canceling will not save any data up to this point). **Once "Confirm" is hit, a folder will be created in the STRmix results folder on the M drive. If incorrect nomenclature was used and you return to this screen to make changes, the empty folder on your STRmix results drive should be deleted.**

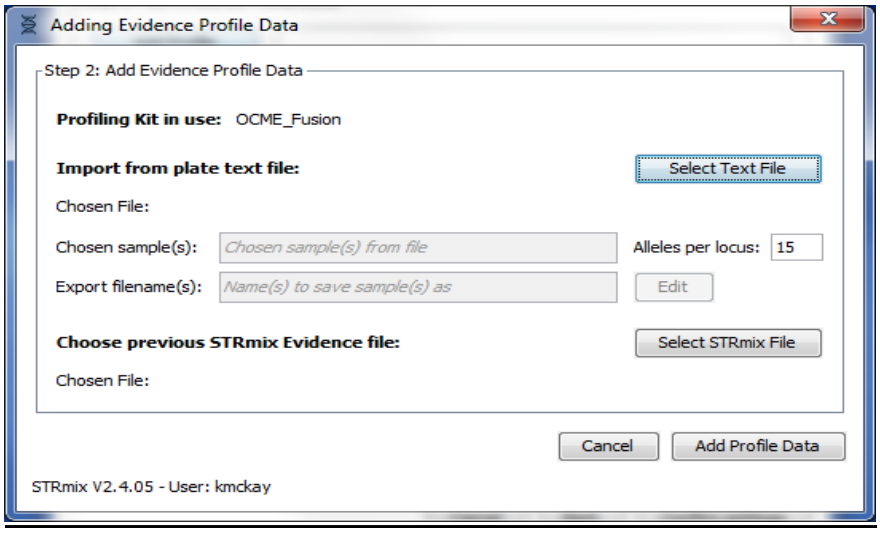
2.8 Add your Evidence Profile Data.

2.8.1 In the "STRmix – Add Profile Data" window, select "Add Profile". This will bring you to the "Adding Evidence Profile Data" window (see step 2.8.2 below) where you can select either a text file or STRmix file from which to run a STRmix™ analysis. Alternatively, you can navigate to your data folder and drag and drop the appropriate text file into the top box, and then proceed to step 2.8.4.

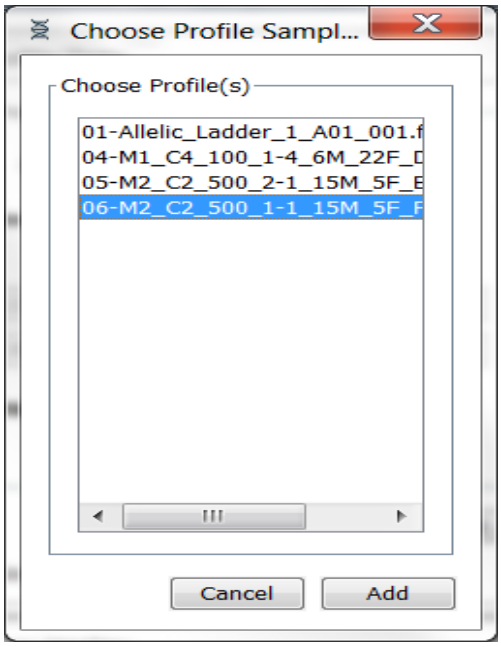


2.8.2 In the **"Adding Evidence Profile Data"** window (see below), ensure that **"Alleles per locus"** is set to 15.

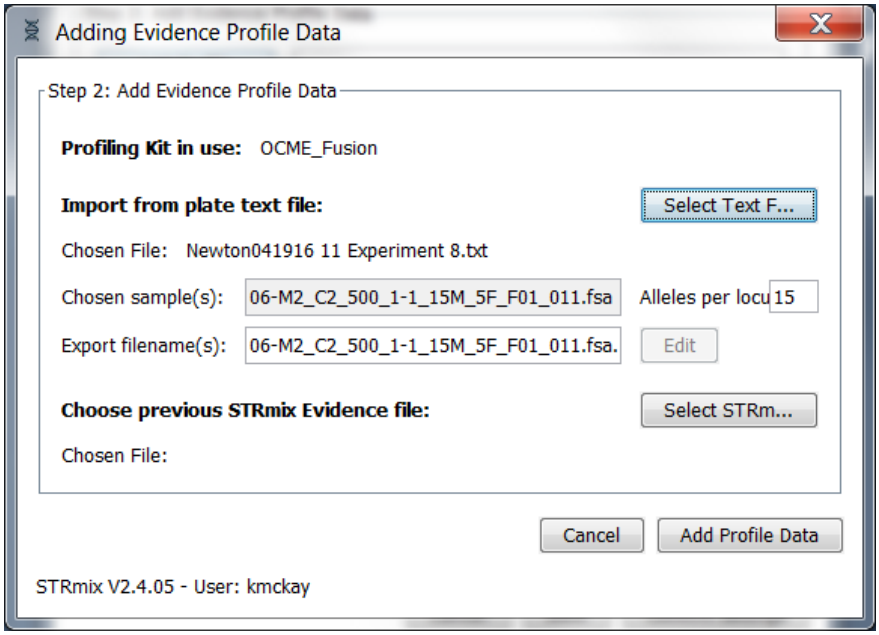
2.8.3 Choose "Select Text File" to navigate to and import your STRmix™ STR run data from the STR data folder.



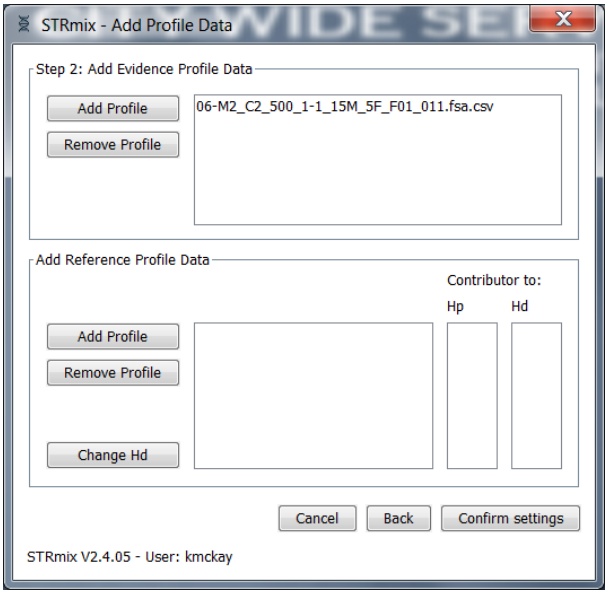
2.8.4 When you select a text file with multiple samples, it will look as follows:



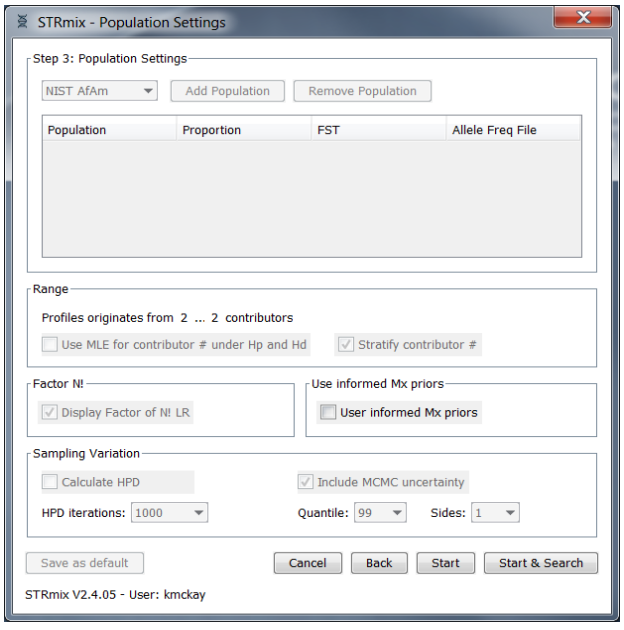
2.8.5 Select the appropriate sample by clicking on it, then select "Add" for the relevant text file. Your view will appear as follows:



2.8.6 Now select “Add Profile Data” and your text file name will appear in the “Add profile Data” screen as seen below. **Repeat steps** 2.8.1-2.8.6 **to add any replicates of the sample which were amplified.**



2.9 **Deconvolutions without a conditioned contributor**. Select "Confirm settings" and this will open up the "Population Settings" window. For a deconvolution without a conditioned contributor, population data is not needed, therefore the populations will appear grayed out in the screen below. Select "Start" to begin your analysis. Proceed to Step 2.10.6.1.



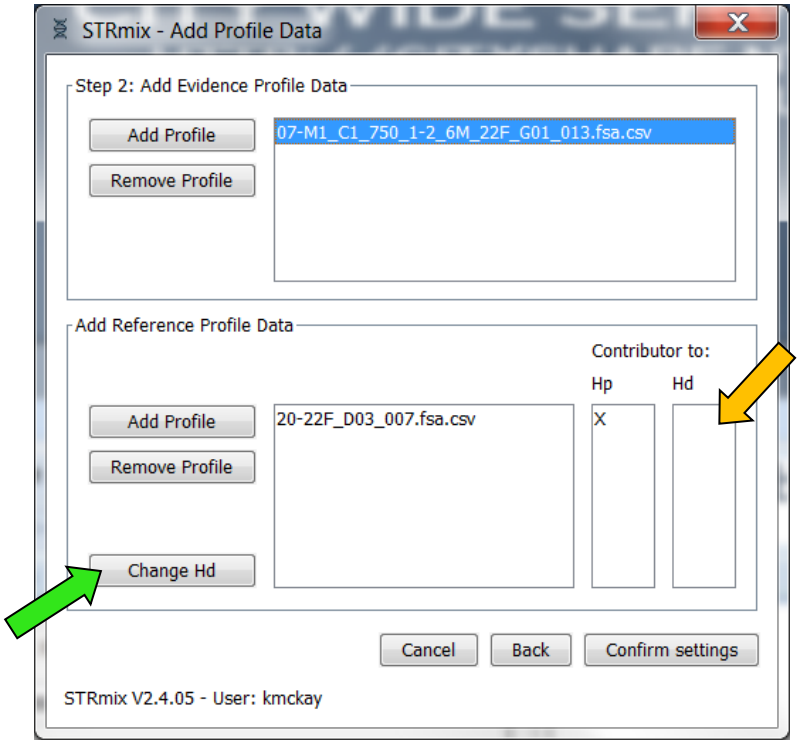
2.10 **Deconvolutions with a conditioned contributor:** For deconvolutions with a conditioned contributor, you must also add reference profile data. Refer to the STR Results Interpretation Manual for guidance on when a conditioned contributor may be applied. A deconvolution of the evidence sample without conditioning and a LR against a potential conditioned contributor may need to be run first.

2.10.1 Select “Add profile” under the “Add Reference Profile Data” section of the screen, or drag and drop the file in to the reference sample box and skip to step 2.10.3.

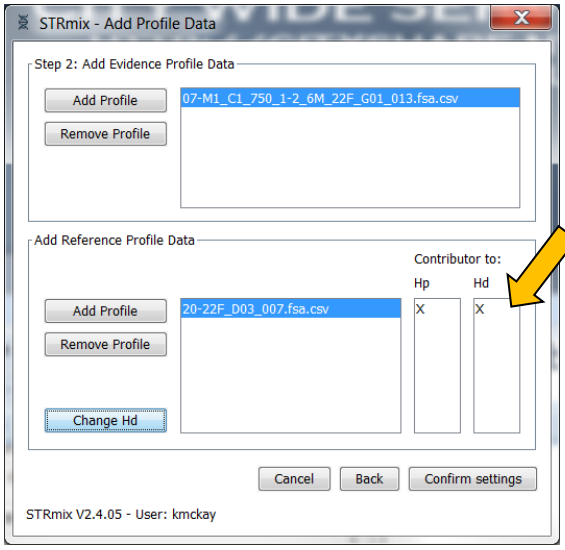
2.10.2 Click on "Select Text F..." to navigate to the case conditioned reference text file.

2.10.3 Once you open the reference text file, click on it and select "Add". This will bring you to the following "Adding Reference Profile Data" screen. Select "Add Profile Data" to complete the process of adding your conditioned contributor's profile to the analysis.

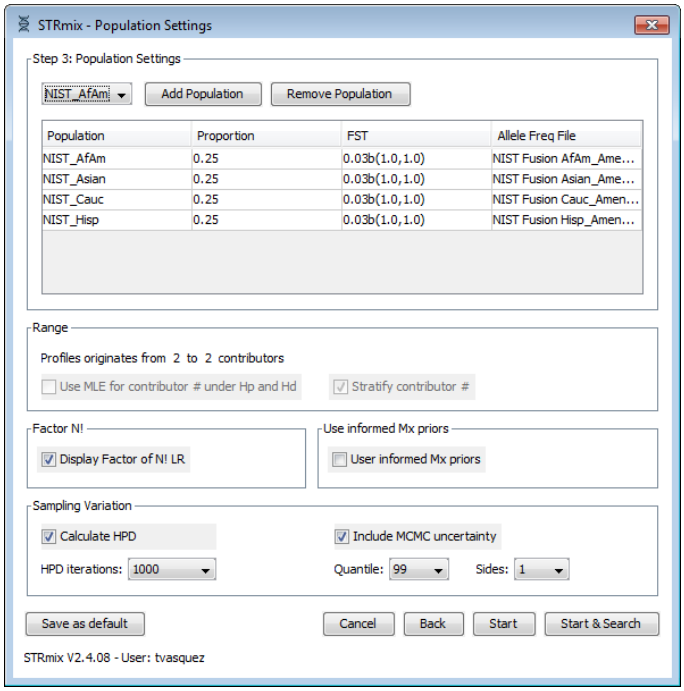
2.10.4 The conditioned sample will now appear in the "Add Reference Profile Data" section of the screen below.



2.10.5 Conditioned contributors are considered true donors in Hp and Hd. Therefore, you must assign the conditioned contributors as such by selecting “Change Hd”. This will allow the conditioned contributor to be chosen under H_p and H_d and will have an “X” marked in both columns.



2.10.6 Select “Confirm Settings”. This will open up the Populations Settings window. When a reference sample is conditioned, population data is needed in the calculation. Ensure that the following four populations are listed: NIST Caucasian, NIST Asian, NIST AfAm, and NIST Hisp and that the settings appear as below. Select "Start" to begin your analysis.



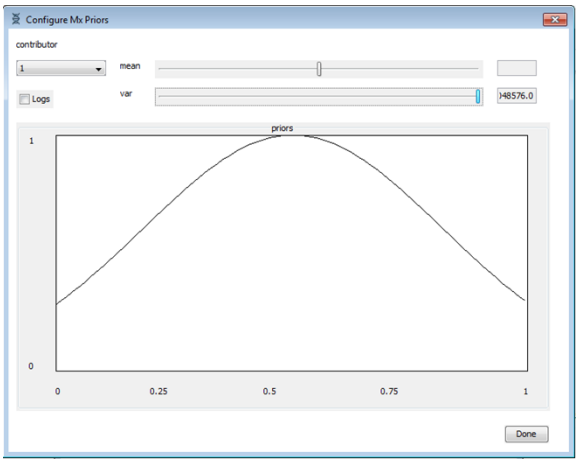
2.10.6.1 Applying User Informed Mixture Proportions (Mx) Priors: **THIS FUNCTION SHOULD NOT BE USED WITHOUT PRIOR APPROVAL BY THE TECHNICAL LEADER**

User Informed Mixture Proportions (Mx Priors) is a function within STRmix that allows users to set approximate mixture proportion percentages for each contributor. If a proposed genotype does not fit the proportion percentage set by the user within the set variance, a penalty will be applied to this iteration similarly to an overall poor fit to the observed profile.

2.10.6.1.1 This function is limited to use with two or three person mixtures with extreme mixture proportions (ex. 98%:2% or 97%:2%:1%) with at least some of the minor contributor(s) peaks being labeled.

2.10.6.1.2 Using the stutter filtered electropherograms, determine an approximate mixture proportion percentage for each contributor. This should be done by calculating mixture proportions percentages, based off labeled peak heights in the first few loci of each dye channel and averaging them together. If no minor peaks are labeled at a location, use the mixture percentage proportion of 100%:0% for that locus in a two-person mixture and 100%:0%:0% in a three-person mixture in the average. If five peaks are seen at a location for a two-person mixture, do not use that location in the average.

2.10.6.1.3 Once the Population Settings Window is open, check the box marked “User informed Mx priors”. This will open a new window like the one seen below:



2.10.6.1.4 Ensure contributor 1 is chosen in the drop down menu. Using the top slider bar marked “mean”, set the mean to the value as previously determined. Using the bottom slider bar marked “var”, set the variance to 1.22×10^{-4}

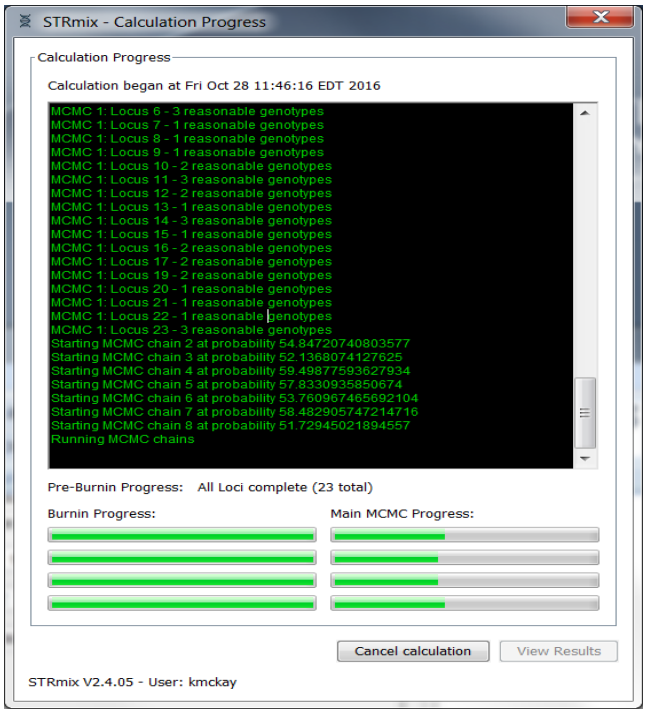
2.10.6.1.5 Using the drop down, change the contributor to number 2. Using the top slider bar marked “mean”, set the mean to the value as previously determined. Using the bottom slider bar marked “var”, set the variance to $1.22x10^{-4}$.

2.10.6.1.6 Repeat the same process for contributor 3 (if needed).

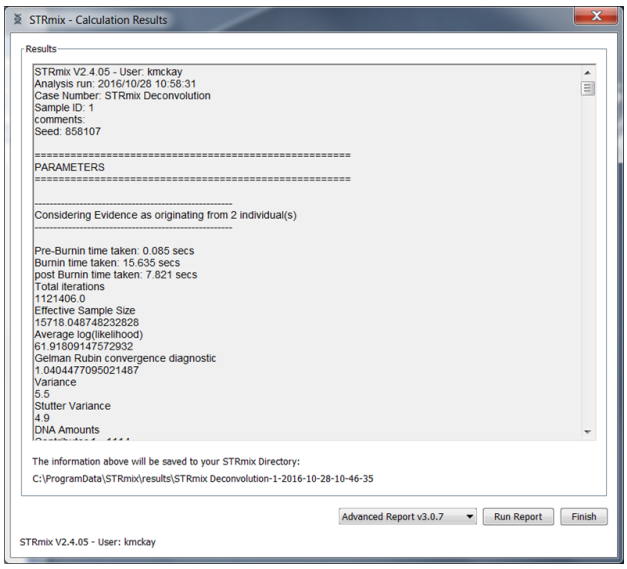
Even though the variances are the same for each of the contributors, you must move the slider bar off the original value and back to it in order to ensure the value is set correctly.

2.10.6.1.7 Select “Done”. This will return you to the Populations Settings window. Ensure the box marked “User informed Mx priors” is checked.

2.11 After selecting "Start", the "Calculation Progress" window will open showing the Burnin progress and Main MCMC Progress.

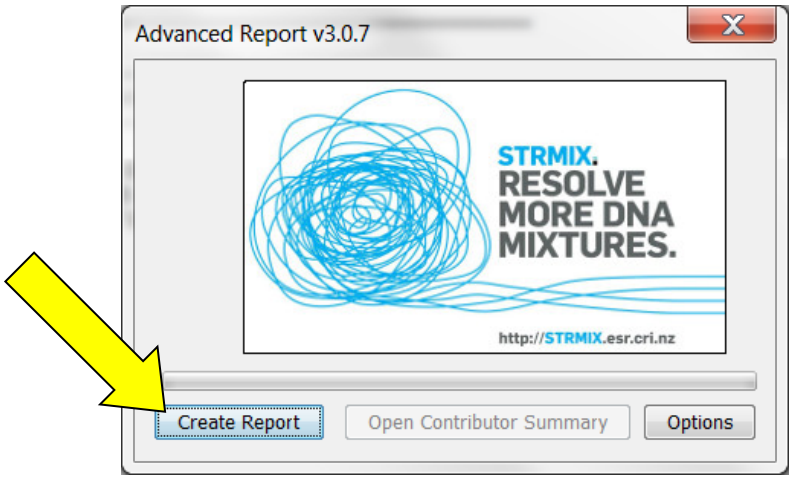


2.12 When the analysis is complete, the raw data report will open as follows:



2.13 Select "Run Report" from this screen to create the advanced report PDF which will be saved in the STRmix results folder for this analysis. Choose "Create Report" from the screen below

2.14 ***CAUTION: If you hit "Finish" a Run Report will NOT be generated, and the analysis will have to be re-done**.



2.15 The analysis run name will auto-populate based on your Case and Sample ID naming. Hit "Save". The advanced report PDF will now open. Print the report.

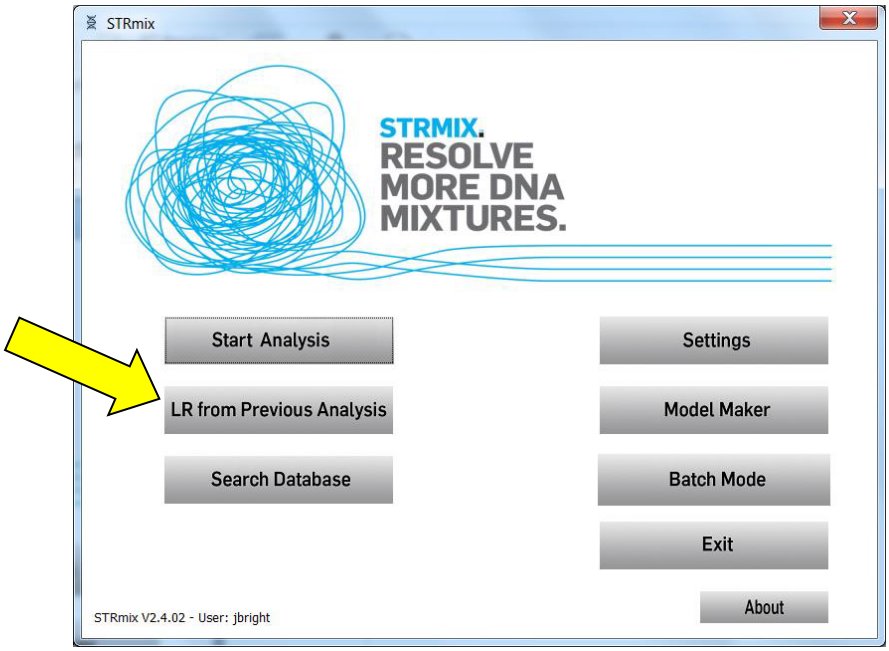
2.16 Close out of the Advanced Report window and then select "Finish" on the "STRmix - Calculation Results" window. This will return you to the STRmix start up screen.

2.17 Navigate to your STRmix Run Folder within the “STRmix Fileshare” folder (\\csc\ocme\OCME_STRmix_Fileshare). **COPY** your run folder into the previously created FB sample folder within the “STRmix Data” folder. Once you have copied the folder, **CONFIRM that all files for that run have transferred over correctly** to the “STRmix Data” folder. After confirmation, the copy of the STRmix Run folder located in the “STRmix Fileshare” folder should be deleted.

3 Likelihood Ratio calculations with STRmix™:

3.1 Note: Samples must undergo deconvolution prior to running an LR with a comparison sample. Refer to the STR Results Interpretation Manual for guidance on when a conditioned contributor may be applied. A deconvolution of the evidence sample without conditioning followed by an LR against a potential conditioned contributor may need to be run first.

3.2 **Launch the STRmix™ application.** Open the STRmix software by locating STRmix in the task bar or by double clicking on the STRmix icon on the desktop. Select "LR from Previous Analysis".



3.3 Navigate to the folder where the STRmix deconvolution on the M drive (STRmix data) for the relevant sample is saved. Double click on the "settings.ini" file for the sample to select it.

3.4 The STRmix - "Configure Analysis" window will open.

3.4.1 Rarely, a locus may be ignored at this step. For example, in the case of an unresolved peak or tri-allelic pattern that matches your reference sample, which was not recognized at the deconvolution stage:

3.4.1.1 Click **Run Settings** at the bottom of the window.

3.4.1.2 Under **Thresholds** settings click **Ignore Loci** button.

3.4.1.3 Select the locus within the **Include** window and click the **>** button to move the locus to the **Exclude** window.

3.4.1.4 Click save when all appropriate loci have been added to the **Exclude** window.

3.4.1.5 Click save again in the **Run Settings** window**.**

3.5 Naming STRmix™ Likelihood Ratio runs

3.5.1 The Case Number and Sample ID will auto-populate from the deconvolution settings file. **This should be updated to the appropriate naming convention for an LR run before proceeding.** The following naming convention should be used:

3.5.1.1 Evidence File:

Case Number = YY-XXXXX (leave out "FB")
Sample ID = remainder of the evidence sample OCME ID*_scenario for LR
Case notes = a comment should be added here if a locus is ignored, indicating the reason: e.g. "D2S441 was ignored due to an unresolved allelic peak"

e.g. LR of 2-person mixture, FB16-01234_567_1_1.1_shirt_BL, comparing to elim John Doe
Case Number = 16-01234
Sample ID = 567_1_1.1_shirt_BL JD1Uv2U

3.5.1.2 Suspect File:

Case Number = SYY-XXXXX (leave out "FB")
Sample ID = OCME ID* for evidence sample (leave out "FB")_scenario for LR
Case Notes = leave blank

3.5.1.3 *Suffixes such as 'mcon' or 'reamp' should not be included in the OCME ID

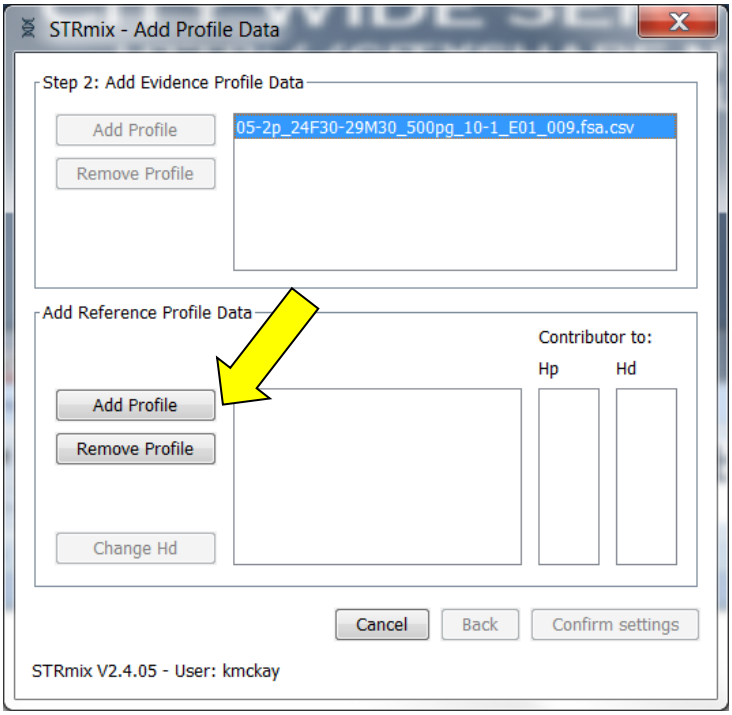
e.g. LR, FBS16-05678 suspect Tom Smith, comparing to 3-person mixture, FB16-01234_567_2_1.1_slide_GS
Case Number = S16-05678
Sample ID = 16-01234_567_2_1.1_slide_GS TS2Uv3U

3.5.1.4 Note – naming format for the LR scenarios should start with the comparison sample's initials, followed by any conditioned samples' initials, and then the number and "U" for unknowns, followed by a "v" to separate the numerator from the denominator hypotheses.

Examples	**Resulting STRmix file name**
Evidence File	
elimAB vs 1 unknown	16-01234-567-1-1-1-shirt-BL-ABv1U
elimAB+2 unknowns vs 3unknowns	16-01234-567-1-1-1-shirt-BL-AB2Uv3U
elimAB+cond elim CD vs cond elim CD+1 unknown	16-01234-567-1-1-1-shirt-BL-ABCDvCD1U
Suspect File	
suspTS vs 1 unknown	S16-05678-16-01234-567-2-1-1-slide-GS-TSv1U
suspTS+1 unknown vs 2 unknowns	S16-05678-16-01234-567-2-1-1-slide-GS-TS1Uv2U
suspTS+cond elim CD vs cond elim CD+1 unknown	S16-05678-16-01234-567-2-1-1-slide-GS-TSCDvCD1U
suspTS+cond elim AB+1 unknown vs cond elim AB+2 unknowns	S16-05678-16-01234-567-2-1-1-slide-GS-TSAB1UvAB2U

3.6 Select "Confirm". Once "Confirm" is hit, a folder will be created in the STRmix results folder on the M drive. If incorrect nomenclature was used and you return to this screen to make changes, the empty folder on your STRmix results drive should be deleted.

3.7 In the "Add Profile Data" window, this is where you will import comparison input files and set hypotheses. Select "Add Profile" and navigate to the .txt file for the comparison samples and select the file(s). Alternatively, you may drag and drop .txt files of your reference samples into the Reference Profile Data box. Click "Add Profile Data".

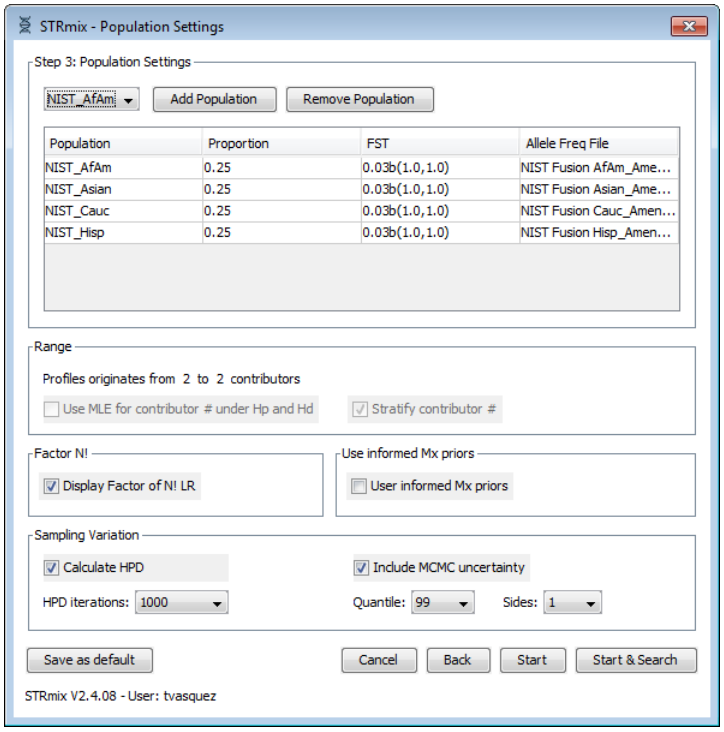


3.8 Each comparison sample (suspect, elim, or informative victim), that is added will be within the numerator of the LR (ie. assigned to H_p). If adding another comparison sample, repeat step 3.7 for that comparison sample.

3.9 If you are conditioning on a contributor, that conditioned sample should already be in the numerator (Hp) and denominator (Hd) from the deconvolution. Conditioning profiles may not be added at the LR step.

3.10 Select "Confirm settings".

3.11 The Population Settings window will open. Ensure that the following four populations are present in the list: NIST Caucasian, NIST Asian, NIST AfAm, NIST Hisp, as seen below. Ensure that the settings are as pictured in the screen shot below.



3.12 Select "Start" to calculate the LR

3.13 The Calculation Progress screen will open, and the software will progress through burnin and Main MCMC Progress.

3.14 The STRmix calculations Results window will then open. Choose "Run Report"

3.15 *****CAUTION: If you hit "Finish" a Run Report will NOT be generated, and the analysis will have to be re-done.**

3.16 The Advanced Report window will open. Select "Create Report".

3.17 The STRmix PDF report will open and will save in the relevant folder and then close the report.

3.18 Close out of the Advanced Report window and then select "Finish" on the "STRmix - Calculation Results" window. This will return you to the STRmix start up screen.

3.19 Navigate to your STRmix Run Folder within the "STRmix Fileshare" folder (\\csc\ocme\OCME_STRmix_Fileshare). COPY your run folder into the previously created FB sample folder within the "STRmix Data" folder. Once you have copied the folder, **CONFIRM that all files for that run have transferred over correctly** to the "STRmix Data" folder. After confirmation, the copy of the STRmix Run folder located in the "STRmix Fileshare" folder should be deleted.

4 Deconvolution and Likelihood Ratio Calculations (Combined) in STRmix™.

4.1 This option may be used under the following scenarios:

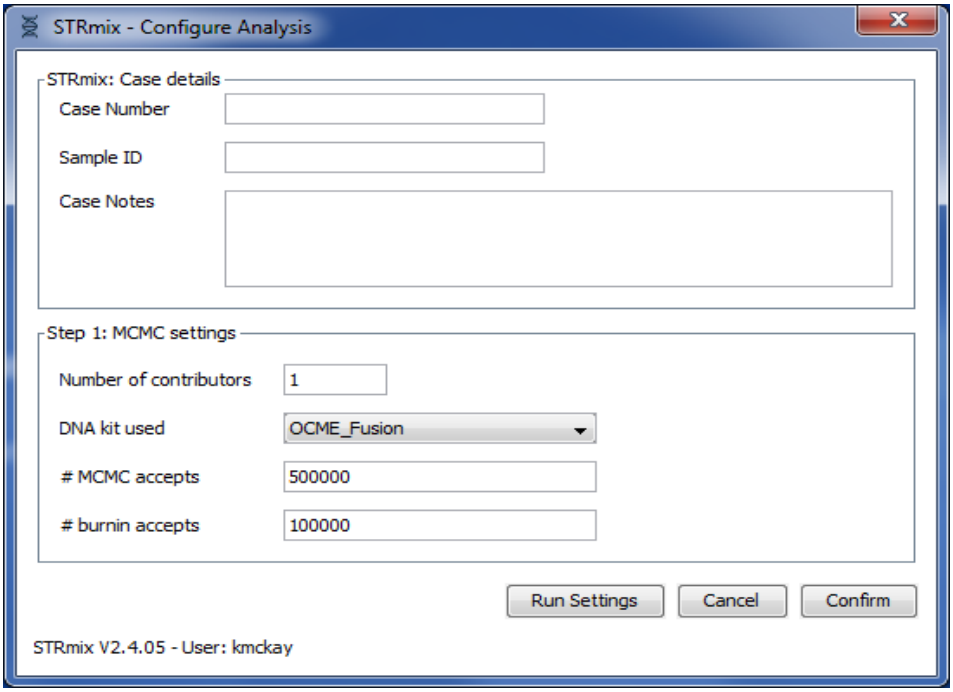
4.1.1 Single source evidence sample that did not require a STRmix deconvolution for determination of a profile, needing an LR to a matching comparison sample

4.1.2 Evidence mixture sample undergoing deconvolution and an LR check to determine if a reasonably expected reference sample can be used for further conditioning (ex. car owner on a swab from the steering wheel).

4.1.3 To assess a probative comparison sample's presence within a mixture where that reference sample data is contained within the same evidence file (ex. victim's blood on suspect's clothing.)

4.2 **Launch the STRmix™ application.** Open the STRmix™ software by locating STRmix™ in the task bar or by double clicking on the STRmix™ icon on the desktop.

4.3 **Select "Start Analysis" from the startup screen.** This will open the "STRmix – Configure Analysis" window.



4.4 Naming STRmix™ runs

4.4.1 STRmix™ output folder and file names are created by stringing together the values entered into the "Case Number" and "Sample ID" fields in the software followed by the date and time of the analysis run. The information in the file name is separated by dashes. Therefore, if other characters are entered, such as a comma, underscore, period, etc., the software will convert them into dashes.

4.4.2 The following naming convention should be used for deconvolution and LR combined runs:

4.4.2.1 Evidence Files

Case Number = YY-XXXXX (leave out "FB")
Sample ID = remainder of the evidence sample OCME ID* #NOC scenario for LR
Case notes = a comment should be added here if a locus is ignored, indicating the reason: e.g. "D2S441 was ignored due to an unresolved allelic peak"

e.g. decon and LR of 2-person mixture, FB16-01234_567_1_1.1_shirt_BL, comparing to elim John Doe
Case Number = 16-01234
Sample ID = 567_1_1.1_shirt_BL 2p JD1Uv2U

4.4.2.2 Suspect Files

Case Number = SYY-XXXXX (leave out "FB")
Sample ID = OCME ID* for evidence sample (leave out "FB") #NOC scenario for LR
Case Notes = a comment should be added here if a locus is ignored, indicating the reason: e.g. "D2S441 was ignored due to an unresolved allelic peak"

4.4.2.3 *Suffixes such as 'mcon' or 'reamp' should not be included in the OCME ID

e.g. LR, FBS16-05678 suspect Tom Smith, comparing to 3-person mixture, FB16-01234_567_2_1.1_slide_GS
Case Number = S16-05678
Sample ID = 16-01234_567_2_1.1_slide_GS 3p TS2Uv3U

4.4.2.4 Note – naming format for the LR scenarios should start with the comparison sample's initials, followed by any conditioned samples' initials, and then the number and "U" for unknowns, followed by a "v" to separate the numerator from the denominator hypotheses

Examples	**Resulting STRmix file name**
Evidence File	
Single source, elimAB LR	16-01234-567-1-1-1-shirt-BL-1p-ABv1U
2-person mixture, elimAB LR	16-01234-567-1-1-1-shirt-BL-2p-AB1Uv2U
3-person mixture, conditioned CD, elim AB LR	16-01234-567-1-1-1-shirt-BL-3p-ABCD1UvCD2U
Suspect File	
Single source, suspTS LR	S16-05678-16-01234-567-2-1-1-slide-GS-1p-TSv1U
2-person mixture, suspTS LR	S16-05678-16-01234-567-2-1-1-slide-GS-2p-TS1Uv2U
2-person mixture, conditioned CD, susp TS LR	S16-05678-16-01234-567-2-1-1-slide-GS-2p-TSCDvCD1U
3-person mixture, conditioned AB, suspTS LR	S16-05678-16-01234-567-2-1-1-slide-GS-3p-TSAB1UvAB2U

4.5 Set the number of contributors.

4.6 Ensure that the following "Step 1: **MCMC settings"** are in place:

DNA kit used: OCME_Fusion
MCMC accepts: 500000
burnin accepts: 100000

4.7 **NOTE: the # MCMC accepts and # burnin accepts must not be modified without documented approval from the technical leader (or his/her designee).**

4.8 Select "run settings" to confirm run settings. The settings should be as follows for every STRmix™ analysis. Any changes that are made will appear in bold on the run report. Press "Cancel" when done.

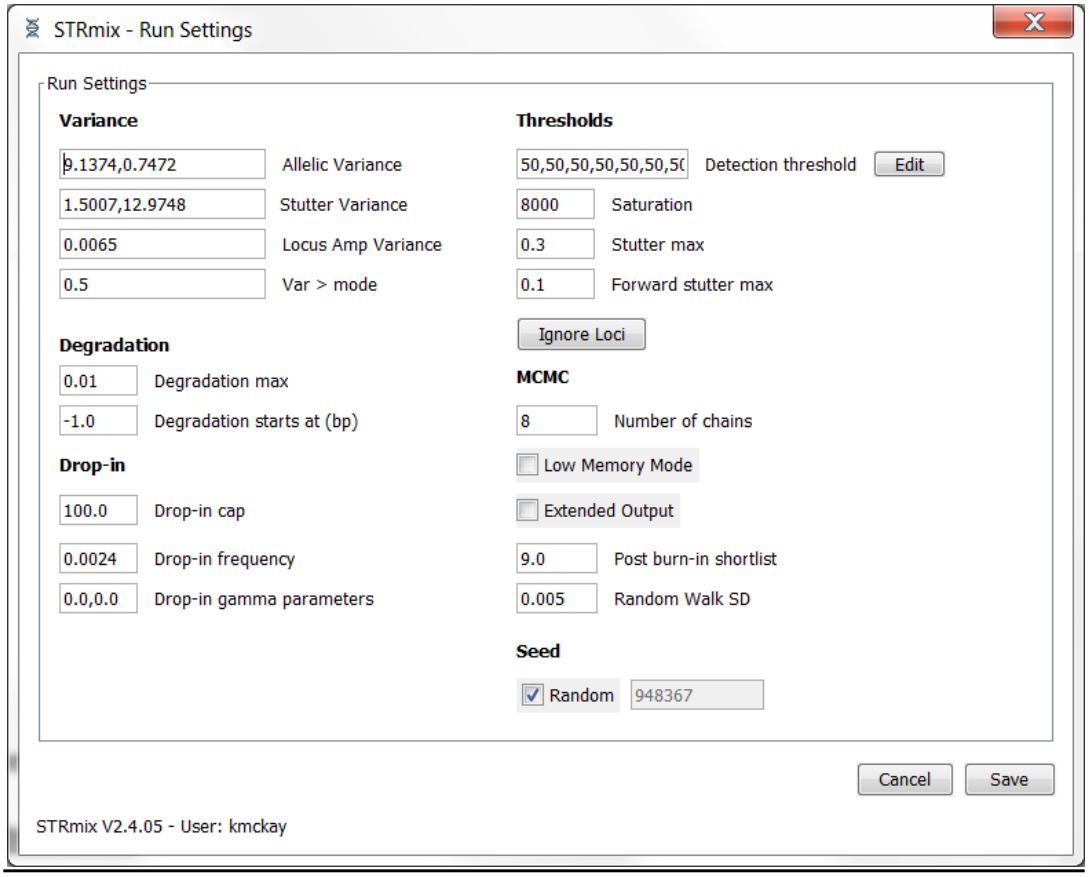
4.8.1 If a locus needs to be ignored for the deconvolution:

4.8.1.1 Under **Thresholds** settings click **Ignore Loci** button.

4.8.1.2 Select the locus within the **Include** window and click the **>** button to move the locus to the **Exclude** window.

4.8.1.3 Click save when all appropriate loci have been added to the **Exclude** window.

4.8.1.4 Click save again in the **Run Settings** window.

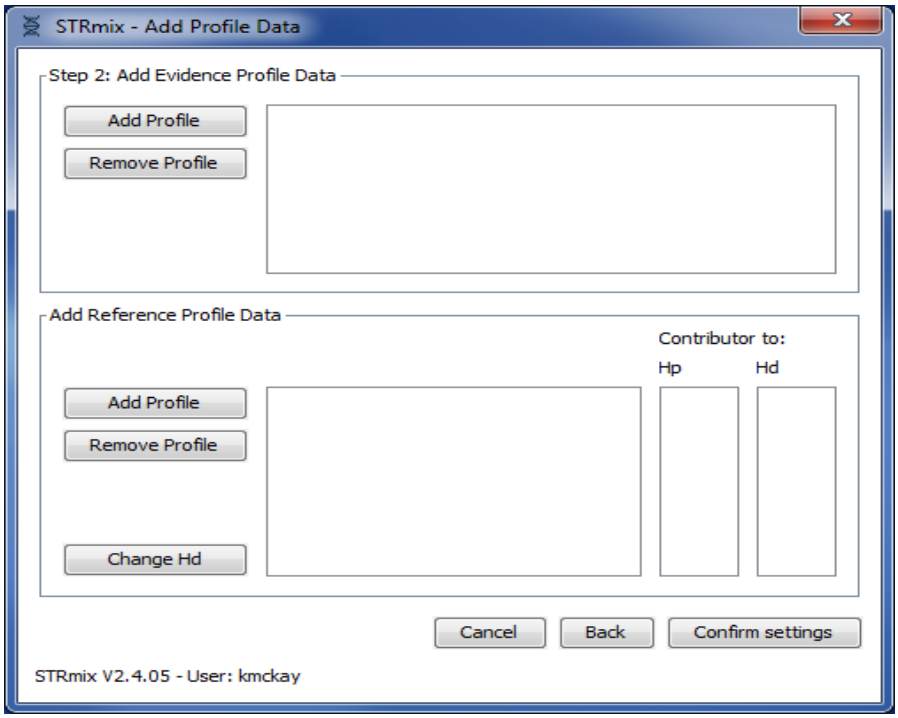


4.9 Select “Confirm” to proceed to the “Add Profile Data” window, or cancel to return to the Startup screen (canceling will not save any data up to this point). Once “Confirm” is hit, a folder will be created in the STRmix results folder on the M drive. If incorrect nomenclature was used and you

return to this screen to make changes, the empty folder on your STRmix results drive should be deleted.

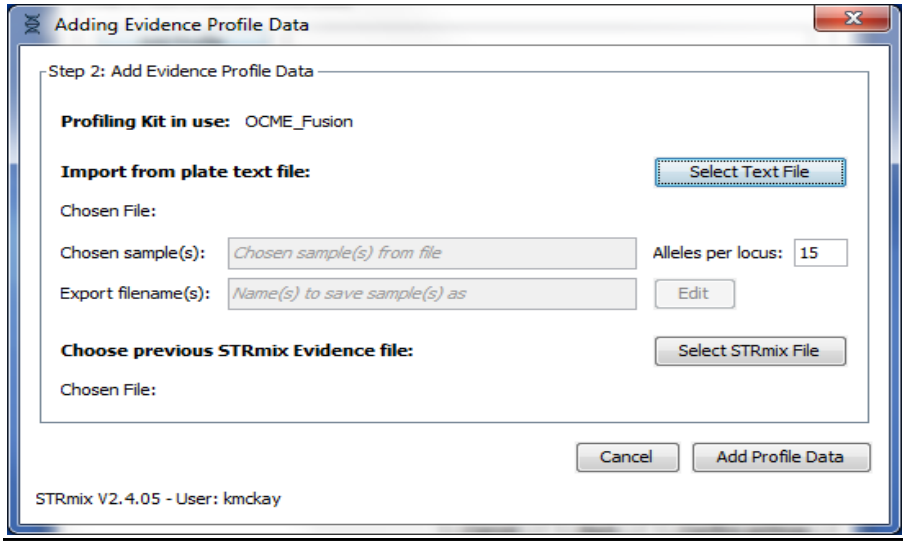
4.10 Add your Evidence Profile Data.

4.10.1 In the "STRmix – Add Profile Data" window, select "Add Profile". This will bring you to the "Adding Evidence Profile Data" window (see step 4.10.2 below) where you can select either a text file or STRmix file from which to run a STRmix analysis. Alternatively, you can navigate to your data folder and drag and drop the appropriate text file into the top box, and proceed to step 4.10.4.

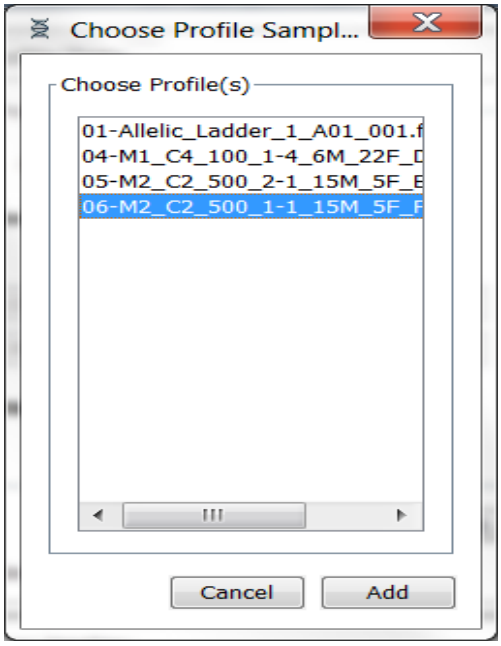


4.10.2 In the **"Adding Evidence Profile Data"** window (see below), ensure that **"Alleles per locus"** is set to 15.

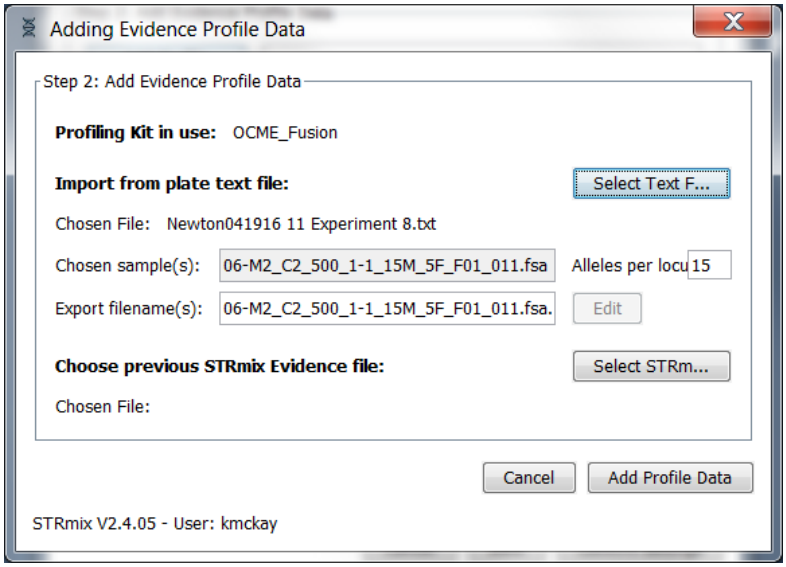
4.10.3 Choose "Select Text File" to navigate to and import your STRmix STR run data from the STR data folder.



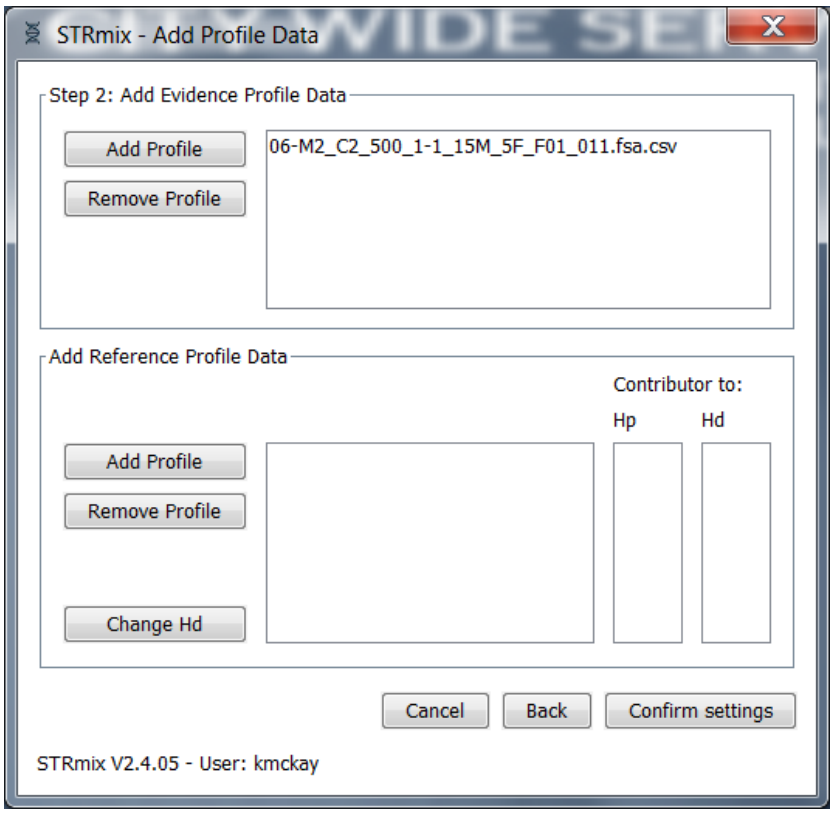
4.10.4 When you select a text file with multiple samples, it will look as follows:



4.10.5 Select the appropriate sample by clicking on it, then select "Add" for the relevant text file. Your view will appear as follows:



4.10.6 Now select "Add Profile Data" and your text file name will appear in the "Add profile Data" screen as seen below. **Repeat steps** 4.10.1 - 4.10.6 **to add any replicates of the sample which were amplified.**



4.11 **Deconvolutions without a conditioned contributor**. Proceed to Step 4.13.

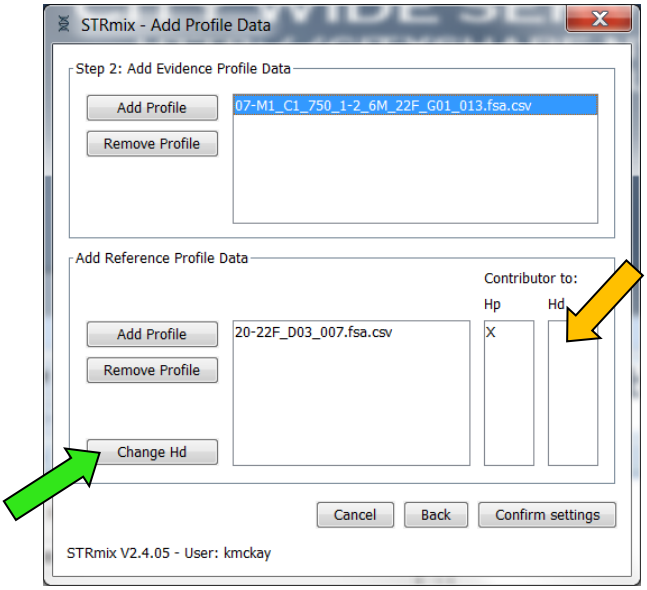
4.12 **Deconvolutions with a conditioned contributor**: For deconvolutions with a conditioned contributor, you must also add reference profile data. Refer to the STR Results Interpretation Manual for guidance on when a conditioned contributor may be applied. A deconvolution of the evidence sample without conditioning and a LR against a potential conditioned contributor may need to be run first.

4.12.1 Select “Add profile” under the “Add Reference Profile Data” section of the screen, or drag and drop the file in to the reference sample box and skip to step 4.12.3.

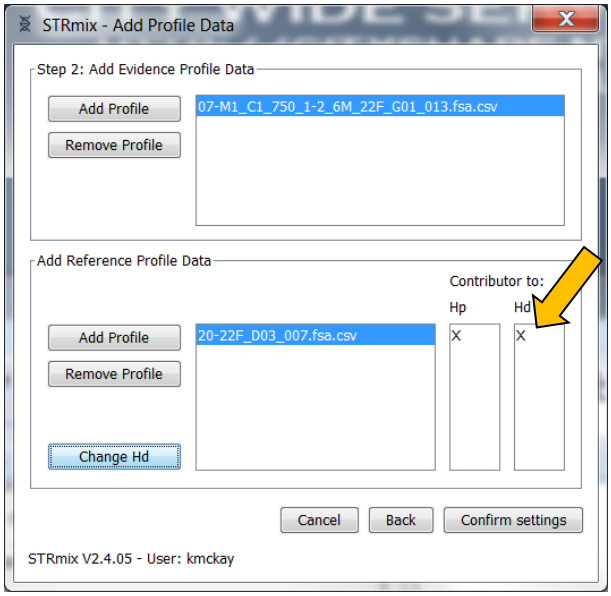
4.12.2 Click on "Select Text F..." to navigate to the case conditioned reference text file.

4.12.3 Once you open up the reference text file, click on it and select "Add". This will bring you to the following "Adding Reference Profile Data" screen. Select "Add Profile Data" to complete the process of adding your conditioned contributor's profile to the analysis.

4.12.4 The conditioned sample will now appear in the "Add Reference Profile Data" section of the screen below.

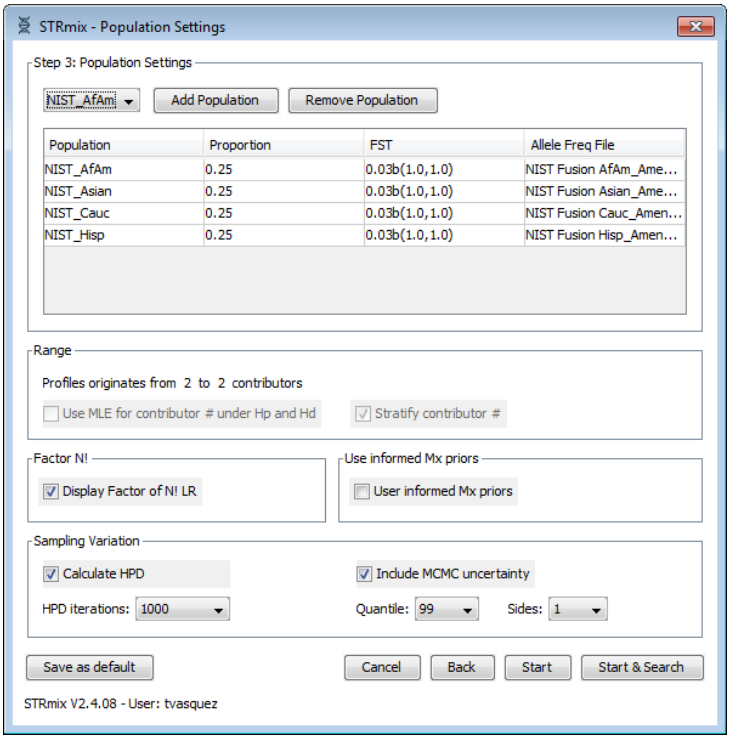


4.12.5 Conditioned contributors are considered true donors in Hp and Hd. Therefore, you must assign the conditioned contributors as such by selecting "Change Hd". This will allow the conditioned contributor to be chosen under H_p and H_d and will have an "X" marked in both columns.



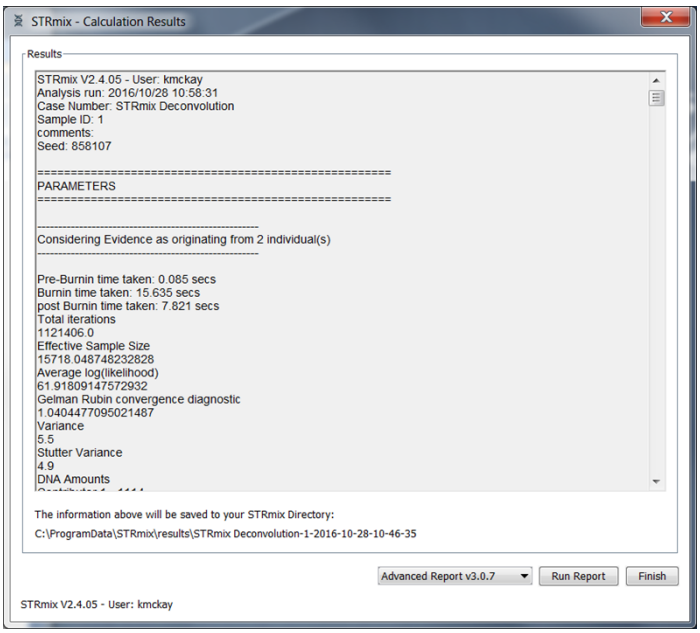
4.13 Add appropriate reference samples that need an LR calculated. Each sample that is added will be within the numerator of the LR (ie. assigned to H_p).

4.14 Select “Confirm Settings”. This will open up the Populations Settings window. Ensure that the following four populations are listed: NIST Caucasian, NIST Asian, NIST AfAm, and NIST Hisp and that the settings appear as below.



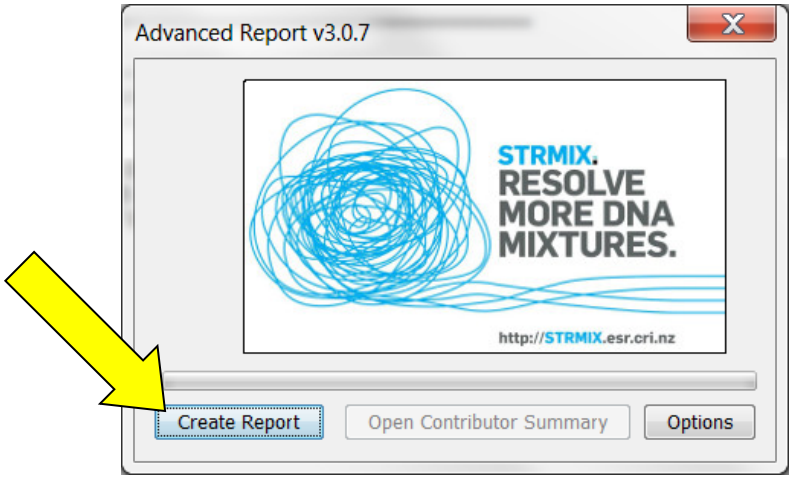
4.15 After selecting "Start", the "Calculation Progress" window will open showing the Burnin progress and Main MCMC Progress.

4.16 When the analysis is complete, the raw data report will open as follows:



4.17 Select "Run Report" from this screen to create the advanced report PDF which will be saved in the STRmix Results folder for this analysis. Choose "Create Report" from the screen below

4.18 * **CAUTION: If you hit "Finish" a Run Report will NOT be generated, and the analysis will have to be re-done.**



4.19 The analysis run name will auto-populate based on your Case and Sample ID naming. Hit "Save". The advanced report PDF will now open. Print the report.

4.20 Close out of the Advanced Report window and then select "Finish" on the "STRmix™ - Calculation Results" window. This will return you to the STRmix™ start up screen.

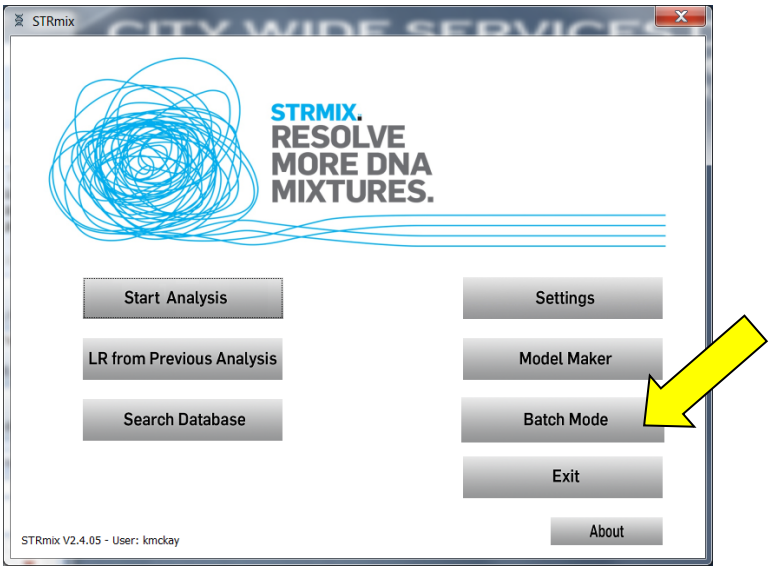
4.21 Navigate to your STRmix Run Folder within the "STRmix Fileshare" folder (\\csc\ocme\OCME_STRmix_Fileshare). **COPY** your run folder into the previously created FB sample folder within the "STRmix Data" folder. Once you have copied the folder, **CONFIRM that all files for that run have transferred over correctly** to the "STRmix Data" folder. After confirmation, the copy of the STRmix Run folder located in the "STRmix Fileshare" folder should be deleted.

5 How to Run STRmix™ using Batch Mode

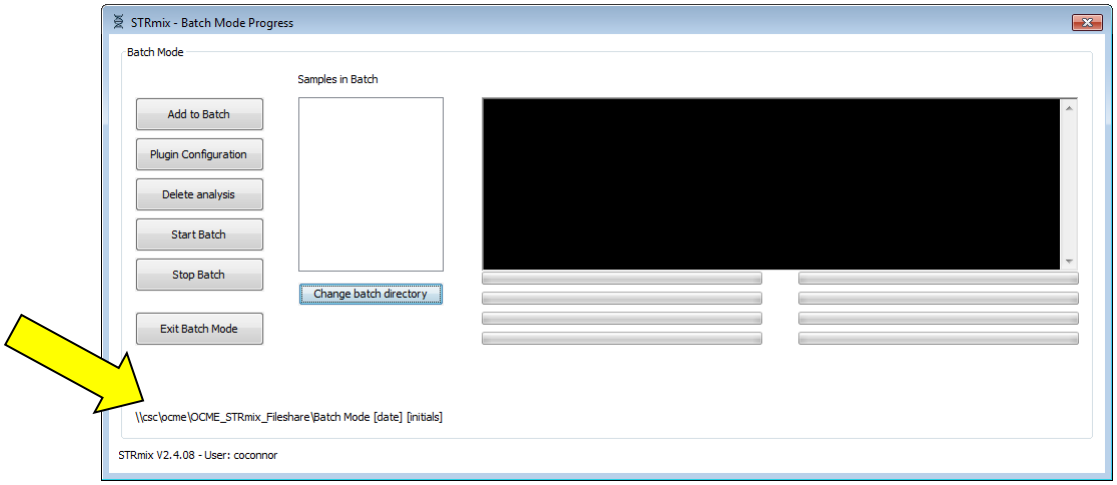
5.1 A number of STRmix™ deconvolutions can be set up and queued to run sequentially. Note, if STRmix™ cannot model the data by the chosen NOC, the batch mode will stop and **no data will be saved**.

5.2 Before setting up a batch, navigate to the "STRmix Fileshare" folder (\\csc\ocme\OCME_STRmix_Fileshare). Create a new folder within the "STRmix Fileshare" folder with the name of "Batch Mode [date] [your initials]."

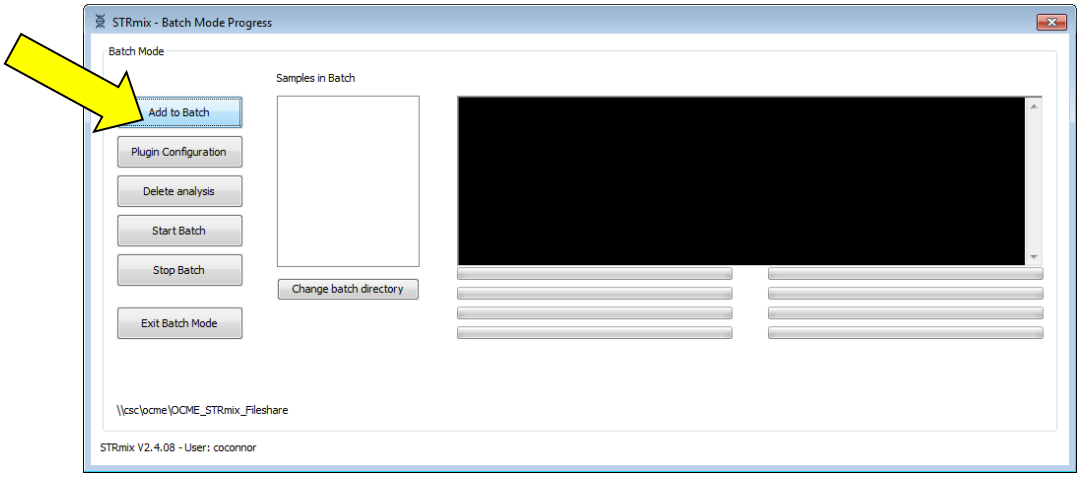
5.3 To set up a queued analysis for multiple samples, select Batch Mode from the STRmix™ main window.



5.4 Select "Change Batch Directory" and navigate to and select the created folder inside the "STRmix Fileshare." This new folder will now appear at the bottom left of the sample summary window as the directory.



5.5 Select “Add to Batch” from the Batch Mode window to open the Sample Summary window.



5.6 Complete the analysis set up for the first sample following Section 2 Deconvolutions in STRmixTM.

5.7 In the Population Settings window, select “Start” to return to the Batch Mode window.

5.8 In the Batch Mode Window, select “Add to Batch” to enter the next sample. Repeat steps 5.4 - 5.7 to add additional samples.

5.8.1 Note: to remove a sample from the batch mode, highlight the case/sample in the “Samples in Batch” section of the “Batch Mode” window then select “Delete analysis”.

5.9 Select “Start Batch” to start the batch run.

5.10 After completion of analyses, select "Exit Batch Mode" to return to the STRmix™ main window.

5.11 Results folders from Batch Mode will be saved in the created folder inside of the "STRmix Fileshare" folder (\\csc\ocme\OCME_STRmix_Fileshare). **Move the results folders from the batch mode folder to the appropriate FB folders within the STRmix Data drive. Once confirming that all files for the batch mode runs have been transferred over correctly, DELETE the created batch mode folder from the fileshare.**

6 Evaluation of the STRmix™ Analysis

6.1 Verify the evidence and reference input sections of the STRmix™ printout against the associated electropherograms. Ensure that:

6.1.1 All appropriate edits were made, no artifact peaks were left labeled

6.1.2 No stutter (for evidence samples) or allelic peaks have been removed

6.1.3 Correct input file(s) have been selected

6.1.4 All suitable replicates have been utilized

6.1.4.1 Check the "Inter replicate efficiency" that is generated by STRmix™. If there are drastic inconsistencies between the two efficiencies that are consistent with the amount of data present in the replicates, the STRmix analysis may be marked as 'not reported' and a new analysis may be performed with the amplification with the most information. A third amplification may be warranted.

6.1.5 The correct settings file was imported into an LR from previous analysis, if applicable

6.2 The number of contributors that best describes the data has been chosen

6.3 The correct assumptions (conditioning) have been made, if applicable

6.4 The appropriate proposition has been selected (LR calculation), if applicable

6.5 Check the Parameters table against the settings listed below to ensure that the correct settings were used for the run (note that any edited values are bolded by the program).

6.6 For Interpretation (Deconvolutions)

Setting	**Value**	**Setting**	**Value**	**Setting**	**Value**
Allele Variance	9.1374,0.7472 mode=6.080	Drop-in Cap	100.0	HPD Iterations	0
Stutter Variance	1.5007,12.9748 mode=6.496	Drop-in Frequency	0.0024	HPD Significance Value	0.0
Minimum allowed variance from the mode	0.5	Drop-in Parameters	0.0,0.0	HPD Sides	0
Loci	23	RWSD	0.005	Alleles Per Locus	15
Locus Amplification Variance	0.0065	ESS Thinning	100000	Factor of N!	Yes
Maximum Stutter	0.3	MCMC Accepts	500000	MCMC Uncertainty	Yes
Forward Stutter Max	0.1	Maximum Degradation	0.01	Burn-in Accepts	100000
Excluded Loci	DYS391	Saturation	8000	Chains	8

6.7 For Likelihood Ratios (Comparison)

Setting	Value	Setting	Value	Setting	Value
Allele Variance	9.1374,0.7472 mode=6.080	Drop-in Cap	100.0	HPD Iterations	1000
Stutter Variance	1.5007,12.9748 mode=6.496	Drop-in Frequency	0.0024	HPD Significance Value	0.99
Minimum allowed variance from the mode	0.5	Drop-in Parameters	0.0,0.0	HPD Sides	1
Loci	23	RWSD	0.005	Alleles Per Locus	15
Locus Amplification Variance	0.0065	ESS Thinning	100000	Factor of N!	Yes
Maximum Stutter	0.3	MCMC Accepts	500000	MCMC Uncertainty	Yes
Forward Stutter Max	0.1	Maximum Degradation	0.01	Burn-in Accepts	100000
Excluded Loci	DYS391	Saturation	8000	Chains	8

6.8 The seed value is the starting number used within the random number generator. For deconvolutions and combined deconvolution/likelihood ratio calculations, the seed value is listed in the Run Information table on the first page of the report PDF. For likelihood ratio calculations, the seed value is listed in the Results text file within the run folder.

6.9 Verify that the following (**primary**) diagnostics conform to your qualitative expectations when compared to the electropherogram(s):

6.9.1 The **mixture proportions** and **template amounts** assigned to the contributor(s)

6.9.2 The **weights** assigned to the genotypes for each contributor listed in the Genotype Probability Distribution

6.9.3 The degradation values and Locus Efficiencies (LSAE)

6.10 Evaluate the following (secondary) diagnostics for the run information.

6.10.1 **Total number of iterations and acceptance rate.** A very low acceptance rate (e.g. 1 in thousands to millions) may, in combination with the other diagnostics, indicate that the analysis needs to be run for additional accepts. If the total iterations approaches or exceeds 2.15 billion (2.15×10^9), this may lead to incorrect genotype weightings being assigned.

6.10.2 **Effective sample size (ESS).** 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 a low acceptance rate. A low ESS value (tens or hundreds) means that there is potential for a large difference in weights if the analysis is run again.

6.10.3 **Average (log) likelihood.** 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. A low or negative value for the average $\log_{10}$ (likelihood) may indicate to users that the analysis requires additional scrutiny.

6.10.4 **Gelman-Rubin diagnostic.** If this value is above 1.2 then it is possible that the analysis has not converged.

6.10.5 **Allele variance and stutter variance.** These variances should be compared to the mode. If the numbers are significantly elevated, the analysis may require additional scrutiny.

6.11 For LR comparisons: The overall **category of support** (inclusion, uninformative, exclusion) should conform to your qualitative expectations in comparison to the data. Evaluate the **Per Locus Likelihood Ratio** table per locus and per sample, as well as the range of LR's between population subgroups; pay special attention to outliers and/or zero values.

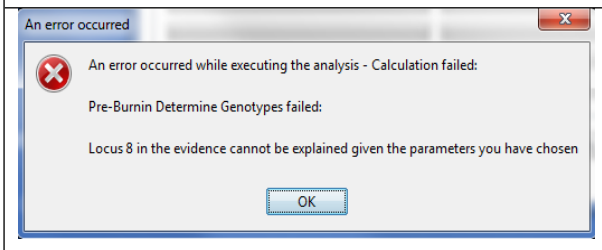
6.12 For LR comparisons resulting in support for an inclusion: check to ensure that the comparison sample falls in the appropriate "Contributor Order giving highest LR". If multiple comparison samples are positively associated with the same sample, results should be evaluated to ensure that they do not align with the same contributor.

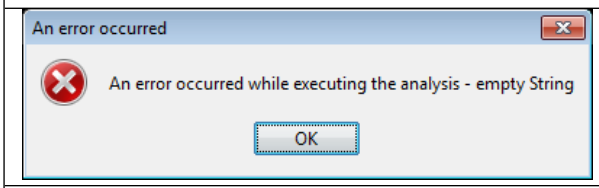
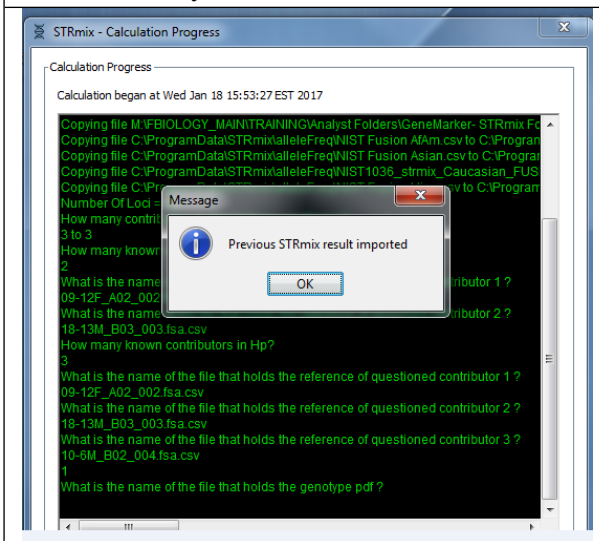
7 Troubleshooting Guide

The presence of a single sub-optimal diagnostic is not always an indication that rework is required. In some instances a sub-optimal diagnostic(s) may be due to the nature of the sample (ex. low amounts of input DNA and/or stochastic effects), and not due to an issue with the STRmix™ run. Refer to the Troubleshooting Guide below for further steps that may be taken to improve a sub-optimal diagnostic result.

The purpose of this guide is to address commonly seen scenarios which arise in casework. These guidelines are based on validation studies, literature references, and casework experience. However, not every situation can be covered by the Troubleshooting Guide. If a diagnostic issue arises that is not covered here, please discuss the issue with your supervisor, manager or the Technical Lead Team as needed.

Observation	**Action**
The mixture proportions or template amount do not reflect what is observed AND/OR the degradation does not reflect what is observed AND/OR the interpreted contributor genotypes do not conform to your qualitative expectations AND/OR the category of support for an LR comparison does not conform to your qualitative expectations.	Re-evaluate the number of contributors; consider another STRmix™ analysis with one higher or one lower number of contributors
	Consider amplifying a replicate if one has not already been done, with increased input amount when available and appropriate
	Inhibition has occurred—microcon to clean and reamplify sample
	Consider another STRmix™ analysis at greater number of accepts (typically, 1,000,000 burn-in accepts and 5,000,000 total accepts). **Note:** this requires approval by the Technical Leader (or his/her designee)
For a two or three person mixture with an extreme ratio: ex. 98:2 or 99:1; the mixture proportions and genotype weights do not conform to your qualitative expectations based on the electropherograms, and other trouble-shooting options (including additional iterations) have been exhausted.	Consider another STRmix analysis utilizing informed (Mx) priors. This requires approval by the Technical Leader (or his/her designee).
A low or negative average (log) likelihood	Reevaluate number of contributors; consider another STRmix™ analysis with one higher or one lower number of contributors
	Data has been removed that is allelic and/or stutter, and must be re-imported
	Artifact peaks have been left labeled and must be removed
Gelman-Rubin value is greater than 1.2	Consider another STRmix™ analysis at greater number of accepts (typically, 1,000,000 burn-in accepts and 5,000,000 total accepts). **Note:** this requires approval by the Technical Leader (or his/her designee)

Stutter and/or allele variance significantly elevated above mode (may be in conjunction with low average (log) likelihood)	Check to make sure no data has been omitted
	Reevaluate number of contributors; consider another STRmix™ analysis with one higher or one lower number of contributors
	Consider amplifying a replicate if one has not already been done, with increased input amount when available and appropriate
Large LR's (>1) obtained for each locus, except one where the LR = 0 and the POI reference is consistent with the evidentiary profile	Data entry problem—check input files
	Allele call not fully resolved at a given locus – ignore locus and perform analysis again. **Note:** discuss with supervisor as needed
	Inhibition has occurred—microcon to clean and reamplify sample
	Consider amplifying a replicate if one has not already been done, with increased input amount when available and appropriate
	Reevaluate number of contributors; consider another STRmix™ analysis with one higher or one lower number of contributors
	Consider another STRmix™ analysis at greater number of accepts (typically, 1,000,000 burn-in accepts and 5,000,000 total accepts). **Note:** this requires approval by the Technical Leader (or his/her designee)
	The number of contributors selected was too few and must be increased

	Consider amplifying a replicate if one has not already been done, with increased input amount when available and appropriate
The STRmix™ run does not progress at the MCMC burn in stage and the chains do not move. Error message also received that Degradation started at "0".	Check "alleles per locus" setting is set to 15.
	"Start & Search" was clicked instead of "Start". Analysis must set up and performed again.
The STRmix™ run does not progress past the MCMC burn in stage and the log likelihood are listed at Infinity and/or NaN	The number of contributors selected was too few and must be increased.
	One of the results files (GenotypePDF) from your deconvolution is corrupted or missing and you are trying to run an LR from previous analysis. Check your STRmix run folder for the associated deconvolution to check that all files are present. Additionally, check the input text files for your samples, ensure all values are present and aligned appropriately with their respective columns and are match the inputs on the STRmix report. If the error persists, the deconvolution may need to be re-run.
Multiple elimination and/or comparison samples that are positively associated with an evidence sample are aligning with the same contributor and/or there is an indication of relatedness.	Consult a supervisor and the Tech Lead Team to go over case specifics. Additional deconvolution and/or LR scenarios may need to be considered.
The 99% 1-sided HPD and Unified LR result(s) for one or more population subgroup(s) drops several orders of magnitude lower than the other population subgroups in comparison to the point estimate LR, and the lowest LR of the four	Consult your supervisor or manager and the Technical Lead Team. A stratified likelihood ratio using census data may be calculated and reported - this must be approved by the Technical Leader (or his/her designee).

population subgroups does not conform to your qualitative expectations of the comparison.	
The total iterations is approaching or exceeds 2.15 billion (2.15×10^9).	This could indicate that genotype weightings have been incorrectly assigned. Please contact the Technical Lead Team. An additional STRmix™ analysis may be required.

8 References:

8.1 STRmix™ v.2.4 Operation Manual

8.2 STRmix™ v. 2.4 Users Manual