DNA: Interpretation of DNA Typing Results

Introduction

The interpretation of results in casework is a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-set rule; however, it is important that the laboratory develops and adheres to minimum criteria for interpretation of analytical results. These criteria are based on validation studies, scientific literature references, casework experience, SWGDAM documents, the FBI's QAS document, and input from the forensic community at large.

Purpose

The purpose of these guidelines is to establish a general framework and outline minimum standards to ensure that:

- conclusions in casework reports are scientifically supported by the analytical data, including data obtained from appropriate standards and controls
- interpretations are made as objectively as possible, consistently from analyst to analyst, and within previously agreed limits

Terminology

See *DNA: Glossary*.

DNA Manager

DNA Manager is an *Excel* based workbook used to manage samples and document the steps of DNA analysis.

The workbook is located in a limited-access shared drive.

The embedded worksheets are not reviewed or approved by the Director as they are considered components of the examination documentation for the case file.

COSTaR

COSTaR is an Excel based workbook used to manage samples for possible upload to CODIS.

The workbook is located in a limited-access shared drive.

The embedded worksheets are not reviewed or approved by the Director as they are considered components of the examination documentation for the case file.

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Interpretation – Promega PowerPlex® Fusion 6C

The steps for the interpretation of STR data amplified with Fusion 6C are listed in the table below. The steps employ a fully continuous probabilistic genotyping system, STRmix™, which uses biological modelling, statistical theory, computer algorithms, and probability distribution to calculate likelihood ratios and/or infer genotypes for the DNA typing results of forensic samples.

Step	Action
1	Decide whether the profile is suitable for interpretation using STRmix™.
2	Assign the number of contributors.
3	Identify possible assumed contributors.
4	Compose the likelihood ratio (LR) propositions appropriate for the case circumstances, if needed.
5	If suitable, interpret the profile with STRmix™.
6	Review STRmix™ outputs.
7	Report conclusions and statistics. See DNA: Reporting DNA Typing Results.

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Profiles suitable for STRmix™ interpretation

STRmix™ should be used to interpret all qualifying profiles, where a qualifying profile is defined below.

- all probative samples, including single-source and 2, 3, and 4 person mixtures suitable for interpretation

STRmix™ should not be used to interpret profiles where:

- the number of contributors cannot be determined
- there are more than four contributors
- an inclusion does not provide probative value in the context of the case. For example:
  - an individual's own DNA on intimate swabs or clothing
  - alleles from a vaginal non-sperm fraction are consistent with carry-over from the sperm fraction and the known contributor
  - alleles from a steering wheel are consistent with one or more individuals who are known to drive the car

If it has been determined that a profile is unsuitable for interpretation, an explanation as to why the DNA evidence (or component of the DNA evidence) is uninterpretable should be documented.

If a person of interest (POI) is visually excluded as a contributor to a single-source profile, a likelihood ratio does not need to be calculated, but the profile should be interpreted with STRmix™ for potential future comparisons or for upload to CODIS. The POI is reported as an exclusion.

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Assigning the number of contributors to the DNA profile

The assignment of the number of contributors to a profile must be made by the analyst prior to starting a STRmix™ interpretation. The number of contributors chosen for the analysis should be the most likely number required to reasonably explain the observed profile. The following components of the mixture should be examined to estimate the number of contributors:

- the locus (or loci) that contain the greatest number of alleles
- the presence of peak height imbalance at more than one locus
- possible peaks that fall below the analytical threshold
- elevated stutter peaks

The presence of an assumed contributor(s) can help in assessing the number of contributors.

The presence of alleles at the YSTR markers (DYS391, DYS570, and DYS576) may provide additional information about the number of contributors.

After analysis of a STRmix™ output file, a deconvolution may not conform to qualitative expectation based on the assessment of STRmix™ diagnostics. If the assumed number of contributors is determined to be the cause, the deconvolution may be re-run under a different assumption for the contributor number. In this case, the course of action should be detailed in the case file and the electronic file of the first deconvolution should be included with the electronic data. The analyst should report the interpretation(s) that best conforms to scientific expectation.

At times, for profiles with low levels of DNA or high numbers of contributors, there may be so much allele dropout that it is difficult to estimate the number of contributors. If the number of contributors cannot be reliably estimated, the profile may be determined to be unsuitable for STRmix™ interpretation.

If a profile is determined to have five or more contributors, the profile is unsuitable for STRmix™ interpretation.

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Identifying possible assumed contributors

In some cases, profiles contain DNA from an individual who is reasonably expected to be a contributor to the sample. Whenever possible, the genetic profile of the assumed known contributor can be used to infer the genetic profile of the remaining contributor(s) in the sample. Examples of assumed contributor scenarios include:

- Intimate samples that are collected from an identified anatomical location (ex: body orifice, skin surface, fingernail scrapings) and/or a piece of clothing (ex: panties, bra, shirt).
- Samples in which an individual may be expected to contribute to a profile (ex: bedding, vehicle swabs, bloodstains from crime scenes in which a known individual was bleeding) as long as there is reasonable expectation of association.
- Samples with an additional known contributor, such as a consensual partner.
- Samples from which DNA is isolated by a differential extraction. In such situations, a contributor from one fraction (sperm or non-sperm) can be used to deduce information from its complementary fraction.
- For the purpose of obtaining a CODIS profile, the criteria for identifying an assumed contributor can be broader to include contributors that have already been identified in the case. For example, if one or more perpetrators have left clothing behind after a burglary, a contributor identified on a cutting from the sweatshirt can be used as an assumed contributor on a cutting from the cap to help STRmix™ deconvolute a complex mixture in order to develop one or more CODIS profiles from the cap. Before making this assumption, the proposed assumed contributor must give an LR $\geq$ 100 in the questioned evidence sample.

For intimate evidence samples, the analyst can make a visual comparison between the assumed contributor profile and the evidence profile to ensure the assumed contributor is present in the evidence profile. If it is unclear whether an intimate contributor is present in a profile, a STRmix™ deconvolution can be run, and an LR calculated for the potential intimate contributor can be assessed. If the LR is greater than the inconclusive range (LR $\geq$ 100), the profile can be re-run in STRmix™ with the intimate contributor assumed.

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Identifying possible assumed contributors (continued)

To identify assumed contributors from non-intimate evidence samples and samples with possible consensual partners, a STRmix™ deconvolution should be run before making the assumption, regardless of apparent contribution level. If the LR for the questioned contributor is greater than the inconclusive range (LR ≥ 100), the profile can be re-run in STRmix™ with the contributor assumed.

If the profile for the assumed contributor is partial (with any missing loci or single alleles under 450 rfu), an attempt should be made to obtain a full profile, if possible. If only a partial profile is available for assumption, only the loci with two alleles or one allele greater than 450 rfu should be used. The loci that do not have full information must be ignored during the deconvolution.

There may be times when the profile needed for the assumption is part of a mixture. In these instances, a STRmix™ deconvolution should be run on the mixture containing the proposed assumed contributor. Using the Contributor Summary document in the STRmix™ results folder for that sample, obtain the profile for the contributor where full genotypes are expected >98% of the time. Loci with an obligate allele (e.g. 10,0) will not be used. This profile can then be used as an assumed contributor, ignoring the loci that were not >98%. Because there is a level of risk in using the >98% genotypes, it is preferable to use a single source sample for assumption. If a mixture contributor is used for assumption, the run diagnostics should be closely examined.

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Setting up the Likelihood Ratio (LR) propositions appropriate for the case circumstances

The LR is represented by the following equation:

$$LR = \frac{\Pr(E \mid H_1)}{\Pr(E \mid H_2)}$$

The LR assesses the probability of the evidence given two alternate propositions or hypotheses, one that aligns with the prosecution (H_1) and one that aligns with the defense (H_2). H_1 is typically inclusionary of the person of interest (POI). H_2 is typically exclusionary of the person of interest (POI).

Depending on the case scenario, efforts are made to set propositions that will minimize the LR. This can be accomplished by minimizing the difference in the number of unknowns between H_1 and H_2.

H_1 should contain any assumed contributors, a POI (if applicable), and any unknown contributor(s) representing the total number of contributors in the sample. H_2 should contain any assumed contributors and the appropriate number of unknown contributors representing the total number of contributors in the sample. The total number of contributors in H_1 must equal the total number of contributors in H_2.

Examples:

- Two person mixture from intimate swab collected from C. One POI identified.
  - H_1: DNA originates from C and POI
  - H_2: DNA originates from C and unknown
- Three person mixture from firearm located at scene. One POI identified.
  - H_1: DNA originates from POI and two unknowns
  - H_2: DNA originates from three unknowns

- Three person mixture from firearm located at scene. Two POIs identified. Two LRs can be calculated.
  - LR_a:
    - H_{1a}: DNA originates from POI1 and two unknowns
    - $H_{2\,a}$: DNA originates from three unknowns
  - LR_b:
    - H_{1b}: DNA originates from POI2 and two unknowns
    - H_{2b}: DNA originates from three unknowns

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Setting up the Likelihood Ratio (LR) propositions appropriate for the case circumstances (continued)

This scenario represents an example where it is unavoidable to limit the difference in the number of unknowns under H_1 and H_2. Assuming that inclusionary LRs were obtained for POI1 and POI2, a third LR should be calculated.

- LR_c:
  - H_{1c}: DNA originates from POI1, POI2, and one unknown
  - H_{2c}: DNA originates from three unknowns

This third LR will typically be more than additive if POI1 and POI2 are present in the mixture together.

Multiple propositions may be required in certain instances. This information will be documented in the case file. If multiple runs are performed in STRmix™ for multiple propositions that are evaluated, maintain all analyses in the case file. The analyst will determine which proposition(s) to report based on available case information.

If the defense hypothesis suggests that a relative of the defendant is the true contributor to the DNA results, then an attempt should be made to obtain a reference sample from the relative. If a reference sample cannot be obtained, alternate LRs may be provided. STRmix™ automatically calculates LRs for siblings, parents/children, grandparents/grandchildren, aunts/uncles, and first cousins of the compared individual, and the appropriate LRs may be included in the report.

- For example, the sibling LR would be:
  - H_1: DNA originates from POI and one unknown
  - H_2: DNA originates from a sibling of POI and one unknown

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Special considerations for reference profiles

There may be times when the reference profile needed for a comparison is not a full profile. In this case, the loci that have partial information must be ignored for the STRmix™ analysis.

There may be times when the profile needed for a comparison is an evidence sample instead of a reference sample.

- If the profile is full and single-source, it can be analyzed with the stutter peaks removed and used directly in STRmix™.
- If the profile is partial and single-source, the loci that have partial information must be ignored for the STRmix™ analysis.
- If the profile is part of a mixture, a STRmix™ deconvolution should be run on the profile containing the target individual. Using the Contributor Summary document in the STRmix™ results folder for that sample, obtain the profile for that contributor where full genotypes are expected >98% of the time. Loci with an obligate allele (e.g. 10,0) will not be used. This profile can then be used for comparison, ignoring the loci that were not >98%. Because there is a level of risk in using the >98% genotypes, it is preferable to use a single source sample for comparison. If a mixture contributor is used for comparison, the diagnostics should be closely examined.

If reference samples from POIs are not available, deconvolute the sample in STRmix™ without setting up an LR. Assumed contributors can be included in the deconvolution, if appropriate. If reference samples become available at a later date, an LR can be calculated from this previous deconvolution.

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Importing data into STRmix™

STRmix™ requires input data describing an electropherogram (epg) in order to run, and this data must be formatted in a specific way for it to be entered. As STRmix™ models the height of both allelic and stutter peaks, +/- one repeat stutter must not be removed from evidence samples at epg analysis. Labels must be removed from all other analysis artifacts including pull-up, spikes, dye blobs, and additional stutter (aside from back and forward stutter). Non-numeric values such as OL or </> are not permitted within the STRmix™ input files. There is no function to accommodate somatic mutations or trisomy in STRmix™ calculations. If a profile has a triallelic STR pattern, STRmix™ can still use the input data if the locus is ignored.

Input file format

There are two types of .txt files that can be input into STRmix™:

- Evidence .txt files (which require sample name, marker, allelic designations, size (i.e. molecular weight) and height information).
  - As STRmix™ models back and forward stutter, the stutter filters will need to be turned off during analysis to capture potential stutter information within your input file.
- Reference .txt files (which require sample name, marker, allelic designations and size information).
  - As STRmix™ does not *interpret* the reference profile, stutter data should not be included in your reference input file.

Replicates

A replicate is defined as a repeated amplification of the same extract. The replicate can be amplified with different amounts of template DNA. Replicates may improve the sensitivity and specificity of the interpretation. Replicates get imported into STRmix™ together and result in one deconvolution. Multiple injections of the same sample cannot be treated as replicates.

Replicate profiles are not required to be included in the interpretation. For example, if a full profile was obtained from one amplification and no further information was obtained from previous or subsequent amplifications, the amplification with the most information should be chosen for STRmix™ analysis. A replicate PCR may help in determining the number of contributors. If the replicate amplifications of the sample indicate the presence of a different number of contributors, the highest likely number of contributors should be used and reported.

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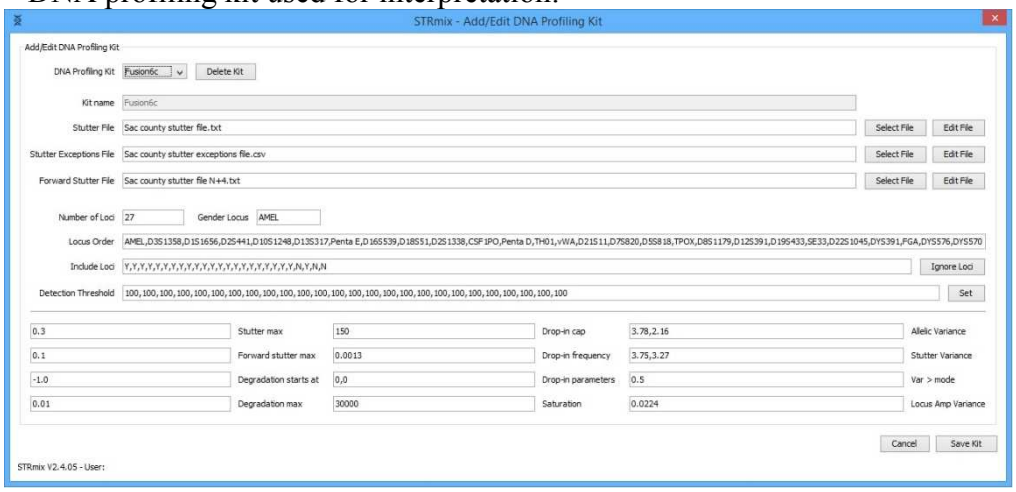
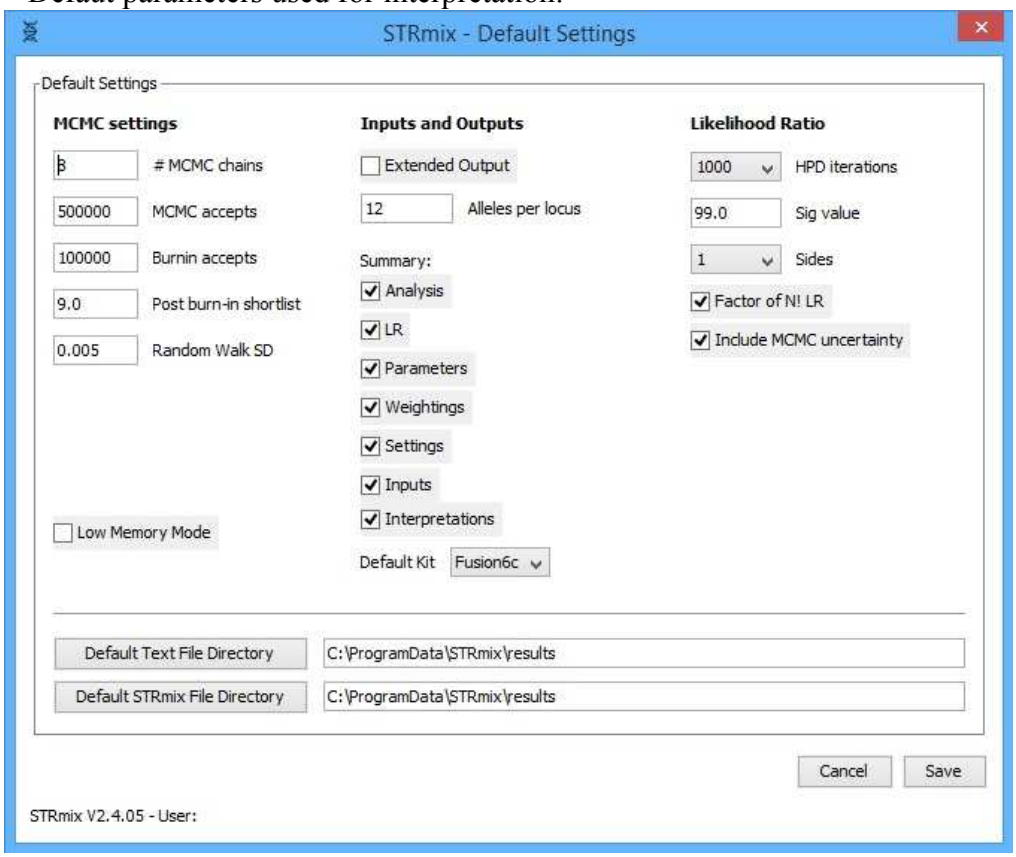
Run parameters in STRmix™

The following settings should be used for the interpretation of profiles.

- DNA profiling kit used for interpretation:



- Defaut parameters used for interpretation:



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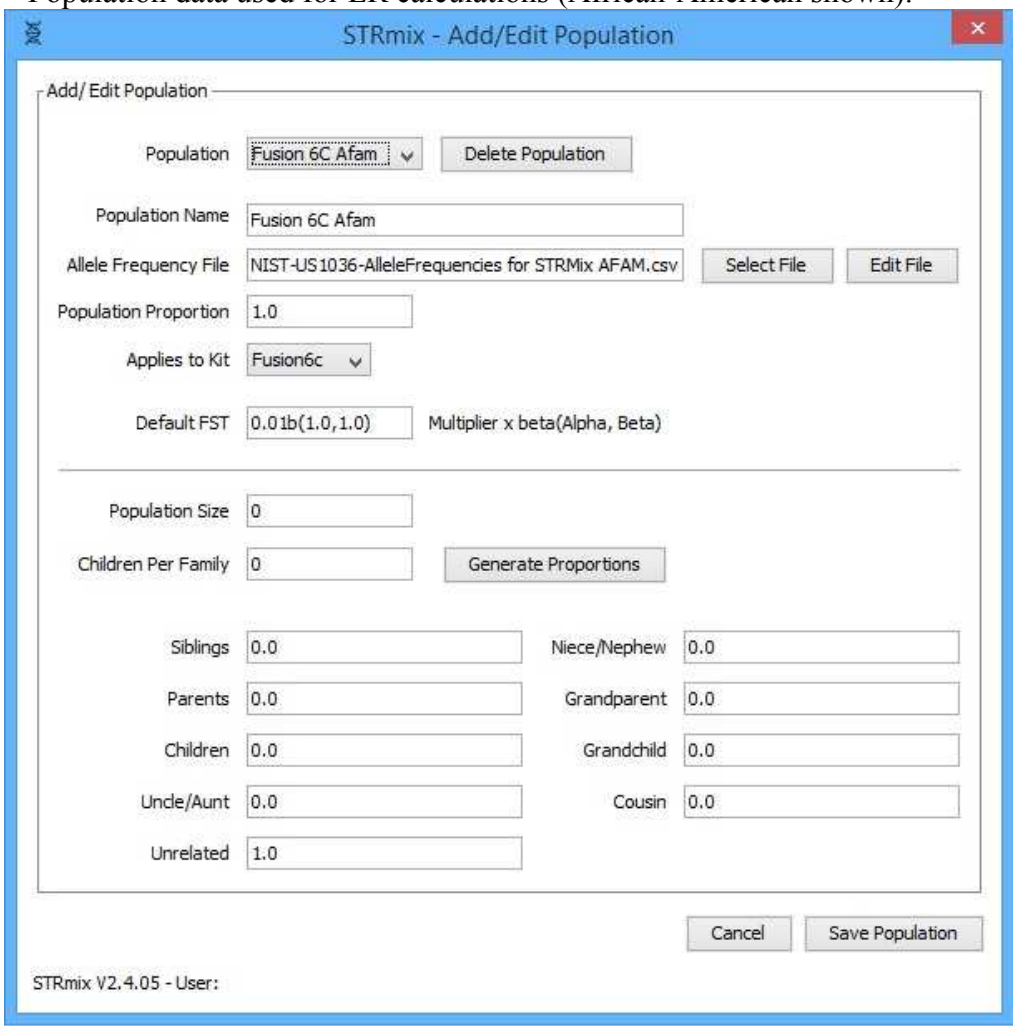
Run parameters in STRmix™ (continued)

- Population data used for LR calculations (African-American shown):



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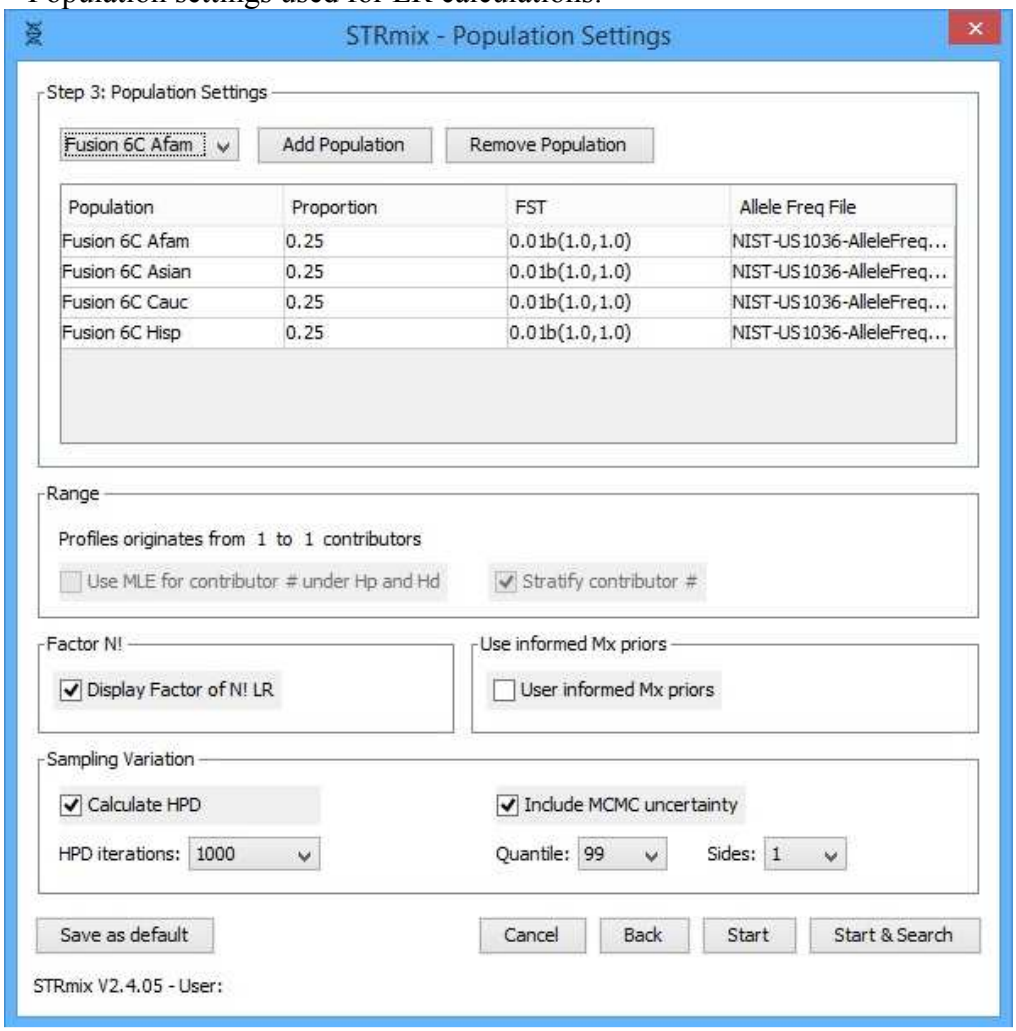
Run parameters in STRmix™ (continued)

- Population settings used for LR calculations:



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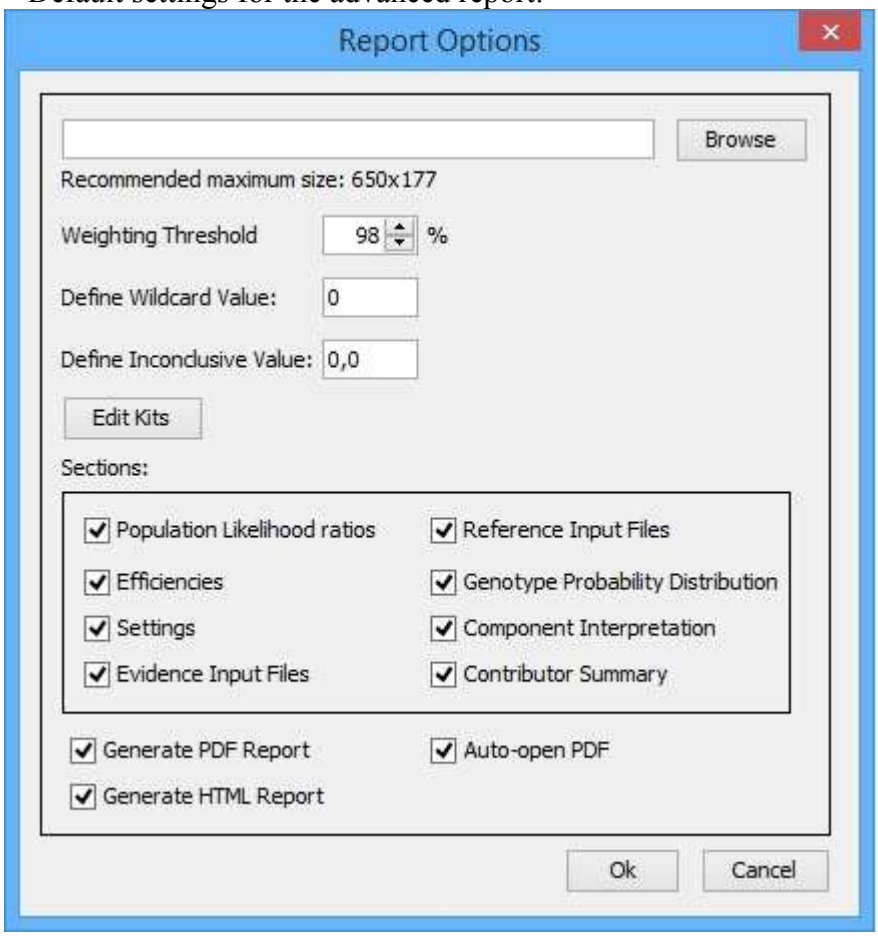
Run parameters in STRmix™ (continued)

- Default settings for the advanced report:



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Running STRmix™ - new deconvolution

This is the procedure for running a new deconvolution in STRmix™.

Step	Action
1	Select **Start Analysis**.
2	Enter the case number into the **Case Number** field.
3	The **Sample ID** should be the item number and should also include any run specific information, such as deconvolution, assumed contributors, and comparisons.
4	**Case Notes** may be used for other important information, such as loci ignored. Optionally, the item description can be entered here.
5	Enter the number of assumed contributors in the **Number of Contributors** box.
6	The **DNA kit used** should be Fusion 6C.
7	Click on **Run Settings** to ignore loci or change the number of MCMC and burn-in accepts. • The ignore loci function can be used to leave out loci during the deconvolution. This may be necessary if: – The evidence and/or reference profile has a tri-allele – The evidence and/or reference profile has an off-ladder allele that cannot be given an allele designation – The profile used for comparison has one or more loci with partial information (i.e. one allele typing below the stochastic threshold of 450 rfu). Loci with *no* alleles typing do not need to be ignored, but can be if desired. • The number of MCMC and burn-in accepts should be left at the default of 500,000 and 100,000, respectively. These values may be raised as needed during troubleshooting of STRmix™ diagnostics (see *Reviewing STRmix™ outputs*).
8	Select **Confirm** to proceed.
9	Add the appropriate evidence profile.
10	Reference profiles may be added for the purposes of comparing a POI or assuming a contributor. If assuming, ensure that the reference is included in both the H_1 and the H_2 columns. If a profile is not added to the Reference profile section, STRmix™ will deconvolute the profile, but an LR will not be calculated.

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DNA: Interpretation of DNA Typing Results, Continued

Running STRmix™ - new deconvolution (continued)

Step	Action
11	Select **Confirm settings**. NOTE: Default population settings should be used if calculating an LR (Fusion 6C Asian, Fusion 6C Afam, Fusion 6C Cauc, and Fusion 6C Hisp).
12	Select **Start & Search** to initiate sample deconvolution and a search of the employee database.
13	After each deconvolution, an advanced report should be run using the default settings.
14	The steps above may be achieved individually or in batch mode, which queues up the deconvolution of multiple profiles. See the *STRmix™ Operation Manual* for specific instructions on the use of batch mode.
15	Select **Exit** to close STRmix™.

Running STRmix™ - LR from previous analysis

If a profile has previously been deconvoluted, then the genotype probability distributions can be used to calculate an LR by comparing to additional references as they are obtained without the need to reanalyze the profile.

Step	Action
1	Select **LR from Previous Analysis**.
2	Navigate to the required results folder for the deconvolution of interest. Open the "settings.ini" file from the previous run.
3	Edit the sample ID and case notes with appropriate run information.
4	The ignore locus function can be accessed, if needed, by clicking on **Run Settings**.
5	Select **Confirm** to proceed.
6	Add reference profile(s) for comparison/LR calculation. NOTE: An assumed contributor cannot be edited at this stage. Changes to the assumed contributor(s) can be performed by starting a new analysis.
7	Select **Confirm settings**. Default population settings should be used.
8	Select **Start**.
9	When complete, an advanced report should be run using the default settings. Select **Exit** to close STRmix™.

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Reviewing STRmix™ outputs

Review the results of the database search to check for contamination from an individual in the employee database. The employee database consists of current and some former employees of the laboratory. The search will return a point estimate LR value using the Caucasian database for any database individual giving an LR greater than 1000. The LR proposition is as follows:

– H_1: DNA originates from database individual and N-1 unknowns
– H_2: DNA originates N unknowns

If an LR>1000 is obtained, the analyst should attempt to determine the reason for the association. A STRmix™ comparison of the target employee to the sample may be done, and if the associated LR is less than 100, the database "hit" can be disregarded, unless contamination is truly suspected. A STRmix™ comparison will return a lower LR than the database search because the database calculates LRs without the use of theta or HPD (highest posterior density), and it only returns values from one population group.

If the STRmix™ LR is greater than or equal to 100, and a means of contamination exists, then contamination should be investigated. Follow up with the Technical Leader if contamination is suspected.

In the Advanced Report of the STRmix™ deconvolution, there are numerous diagnostics that may indicate that an interpretation has not completed as expected.

The primary diagnostics include:
- genotype weights
- mixture proportions
- LRs

The secondary diagnostics include:
- total accepts
- effective sample size
- average log (likelihood)
- Gelman-Rubin value
- allele and stutter variances

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DNA: Interpretation of DNA Typing Results, Continued

Review of the primary diagnostics

- **Mixture proportions**: STRmix™ provides DNA amounts for each contributor in the profile (expressed in rfu units). These amounts are used to calculate mixture proportions. These mixture proportions should meet qualitative expectations when compared to the observed profile.

- **LRs**: If a review of the individual LRs reveals an inclusion or an exclusion of a contributor that is not intuitive, or if inclusionary LRs are obtained for every locus with one or two loci giving a low (even 0) LR, this could be due to:
  - extreme stochastic events at one or two loci, either large unexpected drop-in or large unexpected dropout
  - an error in the input file (e.g. pull-up peak left in the input file)
  - a partial genotype in a reference sample (and the locus has not been ignored)
  - an incorrect number of contributors assumed, especially one less than the true number

- **Genotype weights**: Where possible, the weights generated by STRmix™ should be assessed at each locus for their intuitiveness.

Review of the secondary diagnostics

No single run diagnostic alone is demonstrative of a problem with the STRmix™ deconvolution. When multiple diagnostics are affected (e.g., very low acceptance rate with very high Gelman-Rubin value), that may be indicative of a problem with the STRmix™ deconvolution and warrant further action. The secondary diagnostics are found in the Run Information section of the Advanced Report as shown in the example below.

RUN INFORMATION

Total iterations (Acceptance Rate)	568279.0 (1 in 1.42)	**Gelman-Rubin convergence diagnostic**	1.07
Inter replicate efficiency	PCR 1 - 100.00%	**Allele variance (mode=6.005)**	9.30
Effective sample size	1246.11	**Stutter variance (mode=8.992)**	9.90
Average (log) likelihood	44.84	**Seed value**	889149
Mx prior mean	n/a	**Mx prior variance**	n/a

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DNA: Interpretation of DNA Typing Results, Continued

Review of the secondary diagnostics (continued)

- **Total iterations (Acceptance Rate)**: The total iterations value is the number of post burn-in iterations run by the MCMC in order to reach the target of 500,000 accepts (100,000 burn-in plus 400,000 post burn-in accepts). The acceptance rate is 400,000 divided by the total iterations.
  - An acceptance rate of 1 in thousands or millions may indicate that the analysis needs to be re-run with additional accepts.

- **Effective sample size**: Effective sample size (ESS) is the number of independent steps the MCMC has taken from the posterior distribution of all parameters.
  - A low ESS in relation to the total number of iterations suggests that the MCMC has not moved very far with each step or has had a low acceptance rate.
  - A low absolute value of ESS (e.g. 10s or 100s) will mean that there is potential for a large difference in weights if the analysis was run again. This will be taken into account during HPD interval generation in any LR calculations unless the genotype sets are completely resolved on a single combination, in which case there will be no effect of ESS on the HPD interval.
  - A low ESS on its own is not an indication that reanalysis is required.

- **Average (log) likelihood**: This value is the log of the average probability value created at each of the post burn-in MCMC iterations.
  - The larger this value, the better STRmix™ has been able to model the observed data.
  - A low or negative value may suggest that STRmix™ has not been able to model the data well, given the information provided. Reasons for this include:
    - The profile is very low level and there is little data making up the likelihood.
    - The incorrect number of contributors was entered and there are forced stochastic events in the STRmix™ run as a result (e.g. large heterozygote peak imbalances or variation in mixture proportions across the profile).
    - Data has been removed that was real, particularly stutter peaks, and must now be described by dropout.
    - Artifactual peaks have been left in the STRmix™ input and must be accounted for by drop-in.

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DNA: Interpretation of DNA Typing Results, Continued

Review of the secondary diagnostics (continued)

- **Gelman-Rubin (GR) convergence diagnostic**: This diagnostic is a measure of how well the eight STRmix ™ chains carrying out the MCMC have converged on a final profile deconvolution.
  - For a fully converged analysis, the GR value should be 1.
  - If the GR is above 1.2, the possibility exists that the analysis has not run long enough to fully converge, and the results of the analysis should be further examined. Running the analysis for a larger number of accepts will likely reduce the GR in these instances.
  - A GR value above 1.2 does not necessarily mean that a reanalysis should be done, especially for profiles with a high number of contributors where GR values above 1.2 are not uncommon.

- **Allele variance and Stutter variance**: These values are the average value for allele variance and stutter variance constants across the entire post burn-in MCMC analysis. These values can be used as an indication of the level of stochastic variation in peak heights present in the profile.
  - For the Fusion 6C kit, the allele variance mode is 6.005, and the stutter variance mode is 8.992.
  - If the allele or stutter variance constant values are significantly higher than the mode, this may indicate that the DNA profile is of poor quality or that an incorrect number of contributors was assumed.
  - Used in conjunction with the average log (likelihood), a large allele or stutter variance value can indicate poor PCR.
  - The deconvolution of low level samples may result in a low average log (likelihood) and average allele and stutter variance constants.
  - If some data has been inappropriately omitted, artifacts left in, or the profile was otherwise misinterpreted, the deconvolution should result in a low or negative average log (likelihood) and high allele and/or stutter variances relative to the mode.

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Troubleshooting

If the primary or secondary diagnostics indicate that further work should be done, then a number of options are available (listed below).

NOTE: Additional work requires a new STRmix ™ run.

- Re-amplify the sample.
- Re-inject or re-run the sample on the genetic analyzer.
- Edit the input file if an artifactual peak has been left in.
- Increase the number of MCMC accepts (requires documented DNA Technical Leader approval).
- Change the number of contributors.
- Introduce informed priors into the interpretation. See *Informed priors*.

If a sample is re-run using any of the previously listed options, the reasons will be documented in the case file. The analyses of all STRmix™ runs will be maintained in the case electronic data. Based on available case information, the analyst may choose to report all interpretations or the one that best meets scientific expectations.

Informed priors

Informed priors are a means by which an analyst can provide STRmix™ with a starting-point for contributor ratios. See the *STRmix™ Operation Manual* for specific instructions on the use of the informed prior functionality. This function may be especially useful if the evidentiary mixture is likely to be comprised of known first-order relatives.

The use of informed priors requires documented DNA Technical Leader approval. If informed priors are used, the Run Information section of the Advanced Report must be included in the case notes, indicating the mean and the variance for each contributor.

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Interpretation-Yfiler®

The following table outlines the steps involved in the interpretation of Yfiler® profiles.

Step	Action
1	Identify the profile as being from a single contributor or multiple contributors. For single-contributor profiles, identify DNA typing results at all loci, if possible. Proceed to Step 6.
2	Determine the assumed number of contributors.
3	Subtract alleles from an assumed known contributor, if applicable.
4	Evaluate the signal intensity to determine whether a major/minor contributor relationship exists.
5	Identify all loci in irresolvable mixtures that are suitable for comparison.
6	Compare to reference profiles.

Y-STR paternity

Due to the inheritance of a male haplotype from father to son, it is possible to perform paternity analysis in criminal paternity cases. This may be particularly useful in analyzing products of conception where it may not be possible to separate maternal DNA from the DNA of a male fetus.

In the instance that the alleged father's haplotype is very similar to the male child's haplotype, mutation rates for the locus or loci should be taken into consideration. For current Y-STR mutation rates, see the Y Chromosome Haplotype Reference Database website at www.yhrd.org.