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1 Allele Calling Criteria

- 1.1 Results are interpreted by observing the occurrence of electropherogram peaks for the loci that are amplified simultaneously. The identification of a peak as an allele is determined through comparison to the allelic ladder. An allele is characterized by the labeling color of the locus-specific primers and the length of the amplified fragment. See the Fusion Appendix for a listing of each locus in the PowerPlex Fusion® multiplex. For each single-source sample, a locus can be either homozygous and show one allele, or heterozygous and show two alleles. In order to eliminate possible background and stutter peaks, only peaks that display intensity above the minimum analytical threshold (AT) based on validation data 50 Relative Fluorescent Units (RFU) are labeled as alleles.
- 1.2 Computer program processing steps for raw data:
 - 1.2.1 Recalculating fluorescence peaks using the instrument-specific spectral file in order to correct for the overlapping spectra of the fluorescent dyes.
 - 1.2.2 Calculating the fragment length for the detected peaks using the known in-lane standard fragments.
 - 1.2.3 Comparing and adjusting the allele categories to the sizing of the co-electrophoresed allelic ladder by calculating the offsets (the difference between the first allele in a category and the first allele in the allelic ladder at each locus).
 - 1.2.4 Labeling of all sized fragments that are above the AT and fall within the locus specific size range (see the Fusion Ladder, PE, and SS Appendix).
 - 1.2.5 Removing the labels from minor peaks (background and stutter) according to the filter functions detailed in the appendix of this manual.

2 Manual Removal of Labels from Non-Allelic Peaks

- 2.1 Additional **non-allelic peaks** may occur under the following instances (Clark 1988, Walsh et al. 1996, Clayton et al. 1998), which may be manually edited. Make sure not to remove any labels for potential DNA alleles. All edits must have a reference point on the editing sheet. Mixtures must be edited conservatively; labels may be removed from electrophoresis artifacts only.
- 2.2 Pull-up or Pull-down

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- 2.2.1 Pull-up or pull-down of peaks in one color may be due to very high peaks in another color. These are due to a spectral artifact that is caused by the inability of the software to compensate for the spectral overlap between the different colors if the peak height is too high.
- 2.2.2 The label in the other color will have a basepair size very close to the real allele in the other color. The peak that is considered an artifact or "pull up" will always be shorter than the original, true peak. It is possible for a particularly high peak in for example blue or green, to create pull up in red or orange.
- 2.2.3 Spectral artifacts could also be manifested as a raised baseline between two high peaks or an indentation of a large peak over another large peak. Labels placed on such artifacts can be removed and is known as "spectral over-subtraction".
- 2.2.4 Pull-down will appear as a valley in the electropherogram, in the color directly below a peak. For example, an allele at D3S1358 in blue could have a pull-down directly below in the green color at D16S539. This is due to oversaturation correction. The peak label may be present on either side of the valley and can be edited out.

2.3 Shoulder

2.3.1 Shoulder peaks are peaks approximately 1-4 bp smaller or larger than main alleles. Shoulder peaks can be recognized by their shape; they do not have the shape of an actual peak, rather they are continuous with the main peak.

2.4 Split peaks

- 2.4.1 Split peaks are due to the main peak being split into two peaks caused by the Taq polymerase activity that causes the addition of a single "A" to the terminus of the amplified product ("N+1" band). Since allele calling is based on N+1 bands, a complete extra "A" addition is desired. Split peaks due to incomplete non-nucleotide template A addition should not occur for samples with low amounts of DNA.
- 2.4.2 Split peaks can also be an electrophoresis artifact and attributed to an overblown allele. Additional labels can be edited out.
- 2.4.3 Split peaks may occur in overblown samples or amplicons due to matrix over-subtraction. For example, an overblown green peak may dip at the top where a pull up peak is present in blue and in red. The yellow peak will also display over-subtraction with a dip at the peak's crest. In this instance, the label on the left hand peak is usually edited.

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- Stutter -2, 3,4, 5 or 8 bp smaller or larger than the main allele for Powerplex Fusion
 - 2.5.1 The macro for each system has an automated stutter filter for each locus (see the Manual Appendix for PowerPlex® Fusion for stutter values)
 - 2.5.2 If a stutter peak is observed at the -8 bp position in a single source evidence sample or an exemplar sample, the label must be removed manually.
 - 2.5.3 Any other stutter peaks are not to be manually edited out for evidence samples; the stutter filter will be responsible for removing these peaks. Stutter peaks may be edited for exemplar samples and positive control samples.

2.6 Non specific artifacts

2.6.1 This category should be used if a labeled peak is caused by a not-otherwise categorized technical problem or caused by non-specific priming in a megaplex reaction. These artifacts are usually easily recognized due to their low peak height and their position outside of the allele range.

2.7 Elevated baseline

2.7.1 Elevated or noisy baseline may have labels. They may not resemble distinct peaks. Sometimes, an elevated baseline may occur adjacent to a shoulder peak.

2.8 Spikes

- 2.8.1 Generally, a spike is an electrophoresis artifact that is usually present in all colors.
- 2.8.2 Spikes might look like a single vertical line or a peak. They can easily be distinguished from DNA peaks by looking at the other fluorescent colors, including red or orange.
- 2.8.3 Spikes may be caused by power surges, crystals, or air bubbles traveling past the laser detector window during electrophoresis.

2.9 Dye Artifacts

- 2.9.1 Constant peaks caused by fluorescent dye that is not attached to the primers or is unincorporated dye-labeled primers. These "color blips" can occur in any color.
- 2.9.2 These artifacts may or may not appear in all samples, but are particularly apparent in samples with little or no DNA such as the negative controls.

2.10 Primer Front

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2.10.1 Common artifacts seen which result from primer front molecules. These low molecular weight peaks will appear as off-ladder artifacts at the very beginning of a dye color run.

2.11 Other artifacts:

- 2.11.1 Low-level products can be seen in the n-2 and n+2 positions with some of the loci such as D1S1656, D13S317, D18S51, D21S11, D7S820, D5S818, D12S391, and D19S433. These low level products can be removed as an n-2 artifact.
- 2.11.2 See the <u>Manual Appendix for PowerPlex® Fusion</u> for a description of other common artifacts.

2.12 Saturated Peaks:

2.12.1 Samples which contain saturated peaks can be rerun at a dilution. Results from multiple injections of the same sample cannot be combined; therefore, a sample cannot be edited for saturated peaks at only some loci.

3 Detection of Rare Alleles

- 3.1 Off-ladder (OL) Alleles and Out of Bin (OB) Alleles
 - 3.1.1 In the GeneMarker® HID software, OL refers to Off Ladder alleles which are peaks outside of the marker range. OB refers to Out of Bin alleles which are peaks within the marker range but outside of a bin (frequently, microvariant alleles will be labeled as "OB").
 - 3.1.2 A peak labeled as an OL or OB allele may be a true allele not represented in the allelic ladder or may be a migration artifact.
 - 3.1.3 Examine the OL or OB allele closely in comparison to the ladder and other alleles present at that locus. If it is not at least one full basepair from a true allele, it is likely not a real off-ladder allele.
 - 3.1.4 If an OL allele does not appear to be a true off-ladder allele (ex., if it is 0.55 bp away from the closest allelic ladder allele call), this allele may be assigned the appropriate allele call based on its measurement in comparison to the allelic ladder and other alleles present at that locus.
 - 3.1.5 Compare the results of the run with other samples in the case and other amplifications of the sample for verification of the identity of the allele.
 - 3.1.6 If you are unsure of what the correct allele call is, re-amplify the sample for confirmation. Results from multiple injections of the same sample cannot be combined.

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- 3.1.7 If the allele appears to be a true OL or OB, assign the appropriate allele call based on its measurement in comparison to the allelic ladder. The peak label shows the length in base pairs and this value can be used to determine the proper allele nomenclature. For example, at locus D12S391, a peak with base pair size of 143.5, when compared to the allelic ladder and other peaks present at the locus, would be resolved as a 16.1 allele.
- 3.1.8 Off-ladder alleles which fall outside the range of the allelic ladder at that locus will be interpreted based on their measurement in comparison to the allelic ladder.

4 Interpretation of STR Data

4.1 Allele Nomenclature

- 4.1.1 After the assigning of allele names to the remaining labeled peaks, the software prepares a result table where all peaks that meet the above listed criteria are listed as alleles. The allele nomenclature follows the recommendations of the International Society for Forensic Haemogenetics (ISFH), (DNA recommendations, 1994) and reflects the number of core repeat units for the different alleles.
- 4.1.2 Subtypes displaying incomplete repeat units are labeled with the number of complete repeats and a period followed by the number of additional bases.
- 4.1.3 The Y chromosome allele nomenclature is also based on the number of core repeats and follows the nomenclature suggested in Evaluation of Y Chromosomal STRs (Kayser et al 1997) and used in the European Caucasian Y-STR Haplotype database (Roewer et al 2001).

4.2 Electropherograms

- 4.2.1 Capillary electrophoresis plot data containing case specific samples are a part of each case record. The electrophoresis plots are the basis for results interpretation.
- 4.2.2 The electrophoresis plot will display peak height information, unlabeled peaks, intensity differences that may indicate the presence of a mixture, and will show all peaks at each locus.
- 4.2.3 Reporting analysts will verify the edits made for their case samples while examining each page of the electrophoresis plot(s).

5 Interpretation of Electrophoretic Results

5.1 Refer to the <u>GeneMarker® HID</u> section of the manual to view the Fusion Allelic ladder, positive control and size standard.

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5.2	Electro	ophoresis Cont	rols	
	5.2.1	Allelic Lado	er	
		5.2.1.1	Evaluate the allelic ladder for expected results	s.
	5.2.2	Amplification	on Positive Control	
		5.2.2.1	Evaluate the positive control for the expected	type.
		5.2.2.2	If the positive control has been shown to give confirms the integrity of the electrophoresis ru	
		5.2.2.3	The amplification positive control may be run the corresponding samples and the amplification	
	5.2.3	Electrophore	esis run with failed positive control	
		5.2.3.1	Electrophoresis run containing one positive co	ontrol
		5.2.3.1.1	Indicate in LIMS that the positive cont	rol will be rerun
		5.2.3.1.2	Retest the positive control	
		5.2	3.1.2.1 If the positive control passes amplification set with the ret (The entire amplification set control, may be rerun togeth analyst.)	tested positive control. , including the positive
		5.2	3.1.2.2 If the positive control fails; to Complete a deviation against amp set in LIMS in order to failure report	t the output samples for the
		5.2.3.2	Electrophoresis run containing more than one	positive control
		5.2.3.2.1	Use another positive control to analyze	the run
		5.2.3.2.2	Indicate in LIMS that the positive cont	rol will be rerun

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passes

Retest the (failed) positive control

If the positive control passes; the amplification set

5.2.3.2.3

5.2.3.2.3.1

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5.2.3.2.3.2 If the positive control fails; the amplification set fails.

Complete a deviation against the output samples for the amp set in LIMS in order to generate an Electrophoresis Failure Report

5.2.3.3 Reruns / Re-injections

5.2.3.3.1 An injection set consisting of reruns or re-injections must have at least one positive control

Table 2 Interpretation of Electrophoresis Runs

Controls / Status	Resolution
Allelic Ladder – Pass	Run passes
Positive Control – Pass	
Allelic Ladder – Pass	Refer to Section 3
Positive Control – Fail	· ·
Allelic Ladder(s) – Fail	Run fails
Positive Control – Fail	Complete a deviation against the output
	samples for the amp set in LIMS in
	order to generate an Electrophoresis
	Failure Report

Table 3 Retesting Strategies for Positive Control

Table 5 Refesting Strategies for 1	FOSITIVE COULTOI	
Positive Control Result	Course of action	
No Data Available	Rerun	
- No size standard in lane		
No amplification product but size	Rerun	
standard correct		
Rerun with same result	Re-amplify amplification set	
Incorrect genotype	Reanalyze sample, if not able to resolve,	
-Could be caused by ill-defined size	rerun amplification product	
standard, other GeneMarker® HID		
problems or sample mix-up		
Rerun fails to give correct type	Re-amplify amplification set	
OL or OB alleles	Try re-analyzing with a different ladder	
- possibly GeneMarker® HID	in the run, if available	
problem	Rerun amplification product	

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5.3 Negative Controls

- 5.3.1 Negative controls injected under normal parameters:
 - 5.3.1.1 Evaluate the extraction negative, microcon negative and/or amplification negative control for expected results
 - 5.3.1.2 If peaks attributed to DNA are detected in an extraction negative, microcon negative and/or amplification negative control
 - 5.3.1.2.1 Retest the extraction negative control, microcon negative and/or amplification negative control
 - 5.3.1.2.2 Refer to Table 4, 5, or 6 for Retesting Strategies

Table 4 Retesting Strategies for Extraction Negative Control

Tuble Treetesting Strategies for Ex	action regative Control	
Extraction Negative Result	Course of action	
No data available	Rerun	
- No size standard in lane		
Misshaped size standard peaks	Control passes if no peaks are present	
Run artifacts such as color blips or	Edit	
spikes		
	Rerun only if the artifacts are so	
	abundant that amplified DNA might be	
	masked	
Alleles detected – Initial Run	Rerun	
Alleles detected – Rerun	Re-amplify control	
Alleles detected – Re-amplification	Extraction set fails	
	All samples must be re-extracted	

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Table 5 Retesting Strategies for Microcon Negative Control

Microcon Negative Result	Course of action
No data available	Rerun
- No size standard in lane	
Misshaped size standard peaks	Control passes if no peaks are present
Run artifacts such as color blips or spikes	Edit Rerun only if the artifacts are so abundant that amplified DNA might be masked
Alleles detected – Initial Run	Rerun
Alleles detected – Rerun	Re-amplify control
Alleles detected – Re-amplification	Microcon set fails; samples with data are deemed inconclusive

Table 6 Retesting Strategies for Amplification Negative Controls

Amplification Negative Result	Course of action
No data available	Rerun
- No size standard in lane	
Misshapen size standard peaks	Control passes if no peaks are present
Run artifacts such as color blips or	Edit
spikes	Rerun only if artifacts are so abundant
	that amplified DNA might be masked.
Peaks detected – Initial Run	Re-run
Peaks detected – Rerun	Amplification set fails
	Re-amplify amplification set

- 5.4 Discrepancies for overlapping loci in different multiplex systems
 - 5.4.1 The primer-binding site of an allele may contain a mutation.
 - 5.4.1.1 This mutation may make the annealing phase of amplification less efficient.
 - 5.4.1.2 Alternatively, if the mutation is near the 3' end, this may completely block extension (Clayton et al. 1998).
 - 5.4.2 Mutations may result in a pseudo-homozygote type.
 - 5.4.2.1 For a specific set of primers, this is reproducible.
 - 5.4.2.2 However, these mutations are extremely rare, estimated between 0.01 and 0.001 per locus (Clayton et al. 1998).

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- 5.4.3 If a pseudo-homozygote type for a locus was generated, evidence and exemplar samples amplified with the same primer sequence can be used for comparison.
 - 5.4.3.1 Fusion® does not have the same primer sequences as kits from different manufacturers, for example Identifiler and Minifiler.
 - 5.4.3.2 Therefore, the results from amplification with Fusion may not be concordant when compared with those of Identifiler.
- 5.4.4 If the same locus is amplified using a multiplex system with primer sequences that differ, it is possible to obtain a heterozygote type in one multiplex and the pseudo-homozygote in the second. The heterozygote type is the correct type and should be reported.

6 Guidelines for Interpretation of Results

- 6.1 The purpose of these guidelines is to provide a framework which can be applied to the interpretation of STR results in casework. The guidelines are based on validation studies, literature references, some standard rules and experience. However, not every situation can be covered by a pre-set rule. Equipped with these guidelines, analysts should rely on professional judgment and expertise.
- The Powerplex Fusion® validation and the STRmixTM validation included experiments which determined the laboratory's Analytical Threshold (AT), Stochastic Threshold (ST), minimum and optimal amplification DNA input amounts, saturation point of the 3130xl instruments, and drop-in rate. These factors are fundamental for interpretation of Powerplex Fusion® profiles.
 - 6.2.1 The optimal DNA input amount for amplification was determined to be **525pg** and the minimum DNA input amount is **37.5pg**.
 - 6.2.2 The AT was determined to be 50 relative fluorescent units (RFU).
 - 6.2.3 The ST was determined to be 300 RFU.
 - 6.2.4 The saturation was determined to be 8000 RFU.
 - 6.2.5 The drop-in rate of 0.0024 was determined using Powerplex Fusion® data in STRmixTM Model Maker to be applied during profile interpretation. A drop in cap of 100 RFU is also applied during STRmixTM analysis.
- 6.3 The ST, which is the value above which it is reasonable to assume that allelic dropout has not occurred within a single-source profile, will be considered by the OCME in the interpretation of single source profiles in order to assign alleles to these profiles, and can also be considered when determining a number of contributors for a mixture sample.

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- 6.4 (See the <u>STRmixTM- Probabilistic Genotyping</u> section of the manual, the document: "Estimation of STRmixTM parameters for OCME New York Laboratory" for further explanation of these factors and the PowerPlex Fusion validation).
- Refer to **Figure 1** below as a reference point for interpretation of DNA profiles. These flowcharts are general workflow processes. Other situations may arise that are not covered. Speak to a supervisor as needed.



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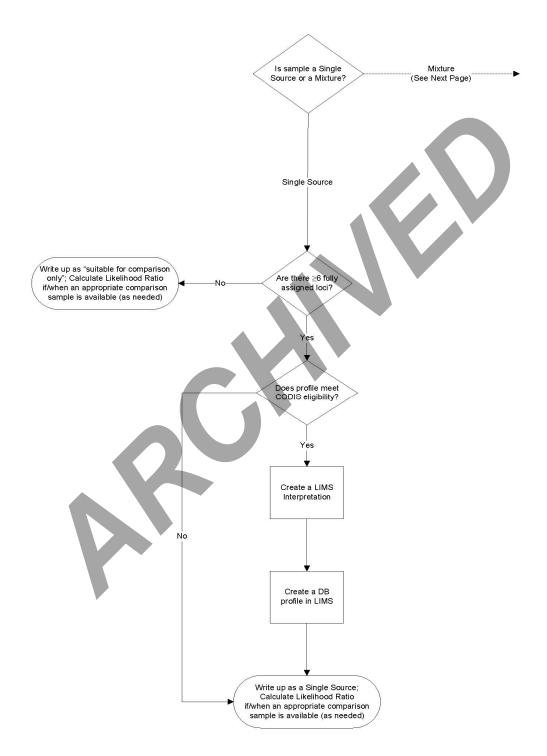


Figure 1a: Single Source general flowchart

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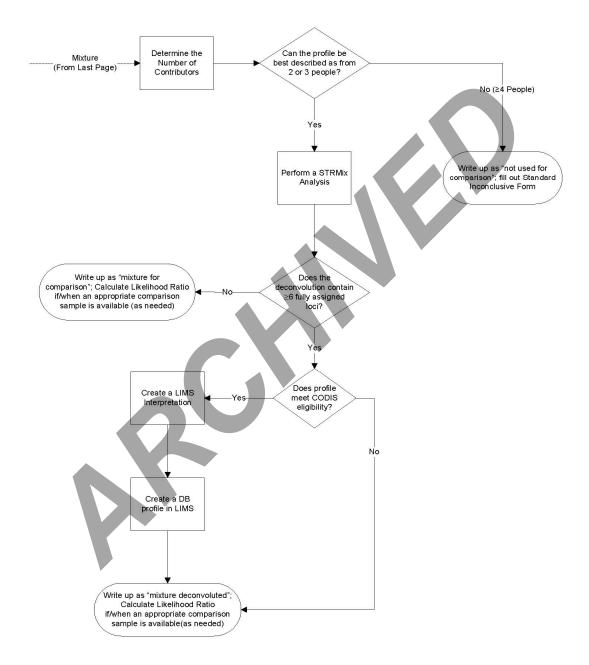


Figure 1b: Mixture general flowchart

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- 6.6 Assessing the **number of contributors** to the sample(s).
 - 6.6.1 When assessing the number of contributors to a mixture, the mixture as a whole should be evaluated. If there is a replicate amplification(s), all qualifying replicates should be evaluated when determining the number of contributors. If there are drastic inconsistencies with the alleles present between replicates, the amplification with the most information may be used for STRmix and the amplification with less information can be marked as Not Suitable for Comparison. In these situations, a third amplification may be warranted. Follow the process below in order to determine the number of contributors:
 - 6.6.1.1 **Count alleles**: Count the number of labeled alleles at each locus for sample data **with stutter filters turned ON.** If this number is an odd number, add 1. (# of alleles)/2 is the initial estimate of the number of contributors to the mixture.
 - 6.6.1.1.1 Be sure to count and sum across replicates as well, if applicable. For example if replicate one has 5 called alleles at FGA, and replicate two has 6 called alleles (4 in common with replicate one and 2 different), the allele count for this locus will be 7.
 - 6.6.1.1.2 The DYS391 locus should not be used for number of contributor determinations.
 - 6.6.1.2 Evaluate the mixture as a whole, assessing the amount of DNA amplified, level of degradation, presence of low level peaks below the stochastic threshold, noisy or clean baseline and general quality of the profile.
 - Evaluate peak height imbalances at the most informative locus (greatest number of alleles). Taking into account allele sharing or 'stacking,' visually try to 'pair' alleles and assign contributors. If there is too much imbalance between alleles this may mean the presence of an additional contributor above that indicated by allele count alone.
 - 6.6.1.3.1 If one or more contributors at this locus appear as either a trace contributor or a clear major contributor, check that this mixture ratio pattern is represented at other loci.
 - 6.6.1.4 Apply the general pattern of contributors (number and mixture ratio) to other loci in the mixture. If it holds, assign this number of contributors to the mixture; otherwise consider the addition or subtraction of one contributor.

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6.6.1.5 If trace peaks are seen in a sample otherwise appearing to be single source, (i.e. a small 3rd peak at a heterozygous locus, or a 2nd minor peak to a homozygote peak) consider the height and location of these peaks when determining if the sample is best described as single source or a two-person mixture. If the trace peak(s) fall into one of the following categories below, the sample may be considered single source. If more than one peak falls in a non-stutter position or more than three trace peaks are present overall, the sample must be considered a mixture for STRmixTM deconvolution. A drop-in peak <100 RFU that is in a nonstutter position A back stutter peak that is <30% of the parent peak 6.6.1.5.2 A forward stutter peak that is <10% of the parent peak 6.6.1.5.3 6.6.1.6 If a third allele is present at only one locus in a sample otherwise appearing to be single source, this may be an indication of a tri-allelic pattern. Peaks below the AT (50 RFU), but not in artifactual positions, may be 6.6.1.7 useful to inform the presence of low level or trace additional contributors. If sub AT peaks are being used to infer an extra contributor there should be small but above AT peaks elsewhere in the profile to support this. These peaks are unlikely to affect the interpretation of the other contributors. Results from a STRmixTM analysis may indicate that the initial 6.6.1.8 estimation of number of contributors may be incorrect, and a subsequent run with N+1 or N-1 contributors may be warranted. Refer to the STRmixTM Probabilistic Genotyping Software Operating Instructions for more information.

- 6.7 Samples which will not be used for comparison:
 - 6.7.1 DNA samples best described as four (or more) person mixtures will not be used for comparison. Fill out the "not suitable for comparison/inconclusive form".
 - 6.7.2 Sometimes the number of contributors may be unclear. This could be because the profile is complex and may contain putative indications of additional contributors, has a limited amount of data at only a few loci, or because case circumstance suggests that the possibility of further contributors exists. Reporting analysts should use their professional judgment when assessing the number of contributors, and may take into account peaks below the AT and high peaks in stutter positions if these appear genuine.

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- 6.7.2.1 In circumstances when the number of contributors cannot be determined, it will be reported that no interpretations were made for the profile and the profile was not used for comparison. Fill out the "not suitable for comparison/inconclusive form".
- 6.7.3 Results from a STRmixTM analysis may indicate that a sample is not suitable for comparison. See <u>the STRmixTM Probabilistic Genotyping Software Operating Instructions</u> for more information.
- 6.8 Determining the profile of a **Single Source** sample:
 - 6.8.1 If all called alleles are above the ST in a single source sample, the alleles may be assigned to the DNA profile with the following considerations:
 - 6.8.1.1 Peak height imbalance is a feature of heterozygotes. (Refer to the <u>Appendix for OCME Powerplex Fusion®</u> validation results.) For single source samples, heterozygote pairs may be assigned even if greater than average imbalance is observed. Consider the potential contribution of stutter if one labeled peak is in the stutter position of the other.
 - 6.8.1.1.1 If extreme peak imbalance is observed at several loci, consider performing a STRmixTM analysis to determine the allele assignments.
 - 6.8.2 If a single source sample has alleles below the ST, the following considerations will be made.
 - 6.8.2.1 The alleles that are above ST can be assigned to the profile.
 - 6.8.2.2 If there are two alleles at a locus with one or both below the ST, both alleles may be assigned as a heterozygote pair.
 - 6.8.2.3 If there is a single allele at a locus below the ST, the possibility of dropout of a sister allele must be considered.
 - 6.8.2.3.1 If this single allele is present at the following loci: Penta E, Penta D, TPOX and D22S1045 the locus may be assigned as "A,Z".
 - 6.8.2.3.2 If this single allele is present at any other locus, a STRmixTM analysis must be performed in order to determine the allelic assignment.
 - 6.8.3 Samples that have 6 or more fully assigned loci will be determined to be DNA profiles and will be assigned a donor name. See "General Guidelines for Building a Profile."

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- 6.8.4 Samples will less than 6 fully assigned loci will be considered as suitable for comparison only.
- 6.8.5 If sample is eligible for DNA databases, go to the "Database Profile Creation Table".
- 6.9 Interpretation of Two- and Three-person mixtures analyzed using STRmixTM:
 - 6.9.1 For samples with low amounts of DNA and/or limited data as indicated below, and for which the number of contributors can be estimated (Refer to SECTIONS 6.6 & 6.7), a STRmixTM deconvolution is not required upfront if a comparison sample is not available. In these situations, the sample should be deemed as a mixture for comparison and reported out as such. If/when a comparison sample is received; the STRmixTM analysis can be done at that time. The STRmixTM deconvolution should be placed in the evidence file, and any LR calculations for the relevant comparison sample should be placed in the file associated with that comparison sample.
 - 6.9.1.1 Mixed samples amplified with <75pg of DNA
 - 6.9.1.2 Mixed samples with less than 11 locations of data (at least 1 allele)
 - 6.9.2 Two- and three-person mixtures will be analyzed using STRmixTM for possible deconvolutions.
 - 6.9.3 Deconvolutions that have 6 or more fully assigned loci will be determined to be DNA profiles and will be assigned a donor name.
 - 6.9.4 Deconvolutions with less than 6 fully assigned loci for any component will be considered as suitable for comparison only.
 - 6.9.5 If sample is eligible for DNA databases, go to "Database Profile Creation Table".

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Table 7 - General Guidelines for Building a Profile.

Step	Question	Yes	No
1	Do you have 6 fully	Go to Step 2	Sample is suitable for
	assigned		comparison only, no
	(≥99%) loci?		DNA donor
			determination.
2	Are \geq 4 of the 6 assigned	Go to Step 4	Go to Step 3
	loci within the CODIS core 13?		
3	Does profile meet match	Report as DNA Donor	Sample is suitable for
	estimation threshold, if	X, eligible for LDIS	comparison only, no
	applicable?	entry only.	DNA donor
			determination.
4	Are there ≤4 alleles at	Eligible for NDIS	Eligible for SDIS
	≥8 CODIS core loci	upload.	Upload. Must meet
	suitable for entry?	Must meet match	match estimation
		estimation threshold, if	threshold, if applicable,
		applicable.	and have data at 6
			CODIS core loci.



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Table 8 - Database Profile Creation Table

Sum of Genotype	Action	Interpretation	DB profile
Combinations*		_	assignment
Allele [A, B] ≥ 99%	Assign heterozygote	A, B	A, B
Allele A \geq 99%	Assign obligate to allele A	A, Z	A+, B,C,D
Alleles B-D $\geq 1\%$	Include additional alleles in DB profile (no		
	more than 4 total for DB profile)		
Allele A \geq 99%	Assign obligate to allele A	A, Z	A+
All other Alleles < 1%	Exclude additional alleles from DB profile		
Allele A ≥ 99%	Assign obligate to allele A	A, Z	A+
$Q \ge 1\%$ (even if other alleles	Exclude additional alleles from DB profile		
are also $\geq 1\%$)			
Allele [A,A] \geq 99%	Assign homozygote	A	A, A
No allele ≥ 99%	INC locus	INC	INC
Q or Q,Q \geq 1%			
Allele A ≥99%	INC locus	INC	A, B
Allele B ≥99%	Assign mixed DB profile		
Alleles A-D >1% & <99%	INC locus	INC	A, B, C, D
Q <1%	Alleles entered in as a mixed locus		
Alleles A-N > 1% (more than	INC locus, INC DB profile	INC	INC
4 total)			

^{*} The sum should be based on the individual genotype weightings from the STRmixTM analysis "Component Interpretation" section. The "Genotype >= 99%" in the STRmixTM analysis refers to individual alleles for each of the possible genotype combinations and not for the genotypes themselves. After performing the STRmixTM analysis, when evaluating the weightings in the "Component Interpretation" section, round accordingly to the nearest tenth before summing. For example, 78,652% would be rounded to 78,7%.

Note: The pre-rounded sum is used to support changing STRMix-called loci from A,B back to INC, in situations where the "A,B" genotype itself does not meet the 99% threshold, even though the individual alleles each do. The pre-rounded sum is NOT to be used to introduce a NEW allele call to the interpretation of a locus that STRMix has already called INC or A,Z.

6.9.6 The interpretation and DB profile assignment for the DYS391 locus must be entered as "INC."

6.10 Exemplars

Samples received as true and/or pseudo exemplars, whether they are from victims, suspects, eliminations, consensual partners, etc. will be interpreted as follows:

6.10.1 The alleles that are above ST can be assigned to the profile.

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	6.10.1.1	If there are two alleles at a locus with one or be alleles may be assigned as a heterozygote pair then the locus should be deemed inconclusive	. If there is any ambiguity,	
	6.10.1.2	If there is a single allele at a locus below the S out of a sister allele must be considered. There deemed inconclusive.		
	6.10.1.3	Samples with <6 fully assigned locations should and will not be used for comparison.	ald be deemed inconclusive	
6.10.2		amples that appear to be mixtures, based on the chaot be used for interpretation	aracteristics described	
	6.10.2.1	If a third allele is present at only one locus in appearing to be single source, this may be an apattern.		
	6.10.2.2	The presence of one trace peak may indicate a can still be interpreted as single source.	a drop-in and the sample	

6.11 Products of Conception (POC)

6.11.1 For POC cases, if an apparent mixture of the mother and POC is present, the profile of the mother can be utilized to determine the profile of the POC. Refer to Powerplex® Fusion Appendix for additional information.

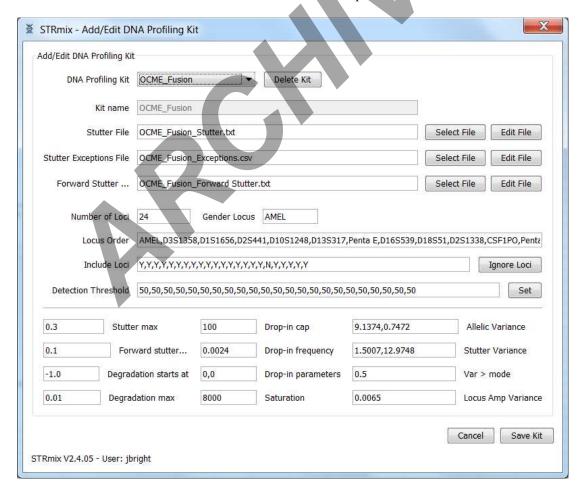
7 What is $STRmix^{TM}$?

- 7.1 **STRmix**TM is a fully continuous probabilistic genotyping forensic software which combines biological modeling with mathematical processes in order to (1) interpret and attempt to deconvolute DNA profiles in the presence or absence of conditioned samples, and (2) compare suspect/informative reference samples (comparison samples) to evidence samples and provide statistical weight in the form of a likelihood ratio (LR).
 - 7.1.1 The deconvolution is performed using a Markov chain Monte Carlo process which creates possible genotype combination(s). Each combination is assigned a weight which reflects how well it explains the evidence profile.
 - 7.1.2 LRs are calculated by comparing the probabilities of two hypotheses, H1 and H2 (H_p and H_d, in STRmixTM). STRmixTM incorporates the assigned weights and sub-population models (Balding and Nichols, 1994, also known as NRC II recommendation 4.2) to calculate the LR.

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.

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- 7.2 STRmixTM requires an initial process to determine in house "Model Maker" values that assist in the biological model used within STRmixTM. The following parameters were determined by analysis of empirical data from the internal validation study (see screen shot for actual values):
 - 7.2.1 Analytical threshold
 - 7.2.2 Stutter ratios
 - 7.2.3 Drop-in parameters
 - 7.2.4 Saturation
 - 7.2.5 Allelic and stutter peak height variance
 - 7.2.6 Locus Specific Amplification Efficiencies (LSAE)
- 7.3 Default Parameters for NYC PowerPlex Fusion® Interpretation



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7.4 These settings should typically **NOT** be altered by the users. If they are altered, they will show up as **bolded** on the STRmixTM printout settings.

8 When will STRmixTM be used?

- 8.1 **STRmix**TM will be used for the interpretation of Powerplex Fusion® DNA profiles as follows:
 - 8.1.1 Conditioned samples: "assumed contributors". Individuals whom have a reasonable expectation to be on and you suspect may be included as a contributor to a mixture sample based on where the sample was taken from (ie. victim's DNA profile from her vaginal swab, homeowner's DNA profile seen on their jewelry box). A profile should be used as a conditioned sample (as needed) to improve the deconvolution of additional contributors to the mixture and/or to better inform the LR calculation. If the goal of the case has been satisfied with previous deconvolutions, conditioning may not be necessary. If a profile is conditioned upon, even if you do not get an additional contributor, that analysis is preferred because it better informs the LR calculation.
 - 8.1.2 <u>Comparison sample</u>: an individual whose association to an evidence sample is being described by a match statement and/or a likelihood ratio (i.e. suspect, informative victim, informative reference sample).
- 8.2 If a **single source sample has all alleles above the ST**, a STRmixTM analysis does not need to be performed.
 - 8.2.1 When a comparison sample is evaluated against a single source sample and an exclusion results, no STRmixTM LR calculation is needed.
 - 8.2.2 If the comparison results in a match and a statistic is needed, a STRmixTM analysis and an LR statistic must be calculated at that time.
- 8.3 If a **single source sample has alleles below the ST**, a STRmixTM analysis may need to be performed in order to determine the allelic assignment (refer to section 6.8.2).
 - 8.3.1 When a comparison sample is evaluated against a single source sample that has 6 or more fully assigned loci and an exclusion results, no STRmixTM LR calculation is needed. If the comparison results in a match and a statistic is needed, the LR must be calculated through STRmixTM.
 - When a comparison sample is evaluated against a single source sample that has less than 6 or more fully assigned loci an LR statistic must be calculated through STRmixTM.
- 8.4 Samples best described **as two- and three-person mixtures** will have STRmixTM analyses performed for the purposes of **deconvolution**.

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- 8.4.1 If the deconvolution results in a profile (≥6 fully assigned loci), the comparison sample can be compared to that profile to see if there is a match. If an LR is needed, this must be calculated using STRmixTM.
- 8.4.2 When a **comparison sample** is evaluated against an evidence **mixture** in which no profile was deconvoluted or the comparison sample does not match the deconvoluted profile, an LR statistic must be calculated through STRmixTM.
- 8.5 **Conditioned samples:** DNA profiles of victims/elimination samples/deconvoluted donors may be used as **conditioned samples** in a STRmixTM analysis to aid in the mixture deconvolution if they meet the following criteria:
 - 8.5.1 The victim/elimination sample/ donor has a reasonable expectation to be on the item
 - 8.5.2 Most or all of the alleles of the <u>victim</u> reference sample are readily apparent in a mixture for samples that originate directly from the individual's body: body cavity swabs, swabbing from any skin surface, underwear within a SOECK, samples from fingernails, or ligatures (zip ties, duct tape, rope, etc.).
 - 8.5.3 If all of the alleles of an <u>elimination</u> or <u>victim</u> sample are present in a mixture from their own clothing.
 - 8.5.4 If upon the initial deconvolution a full DNA profile is obtained:
 - 8.5.4.1 If the goal the case has been met, no further conditioning is needed.
 - 8.5.4.2 If the deconvoluted contributor matches a reasonably expected victim/elimination/donor, no LR calculation is needed and a second deconvolution conditioning upon that profile can be performed.
 - 8.5.5 A STRmixTM deconvolution on the evidence and LR calculation for the proposed conditioned profile must be performed <u>for all other situations</u>. Some examples are those types of situations are listed below.
 - 8.5.5.1 Elimination homeowner on any item from their house.
 - 8.5.5.2 Person on any mixture on an item on which that person has already been demonstrated to be present elsewhere on that same.item (Male Donor A on a mixture from cuffs scrapings of a shirt where Male Donor A was single-source or deconvoluted major from the collar scrapings on the same shirt)
 - 8.5.5.3 Person on any mixture from an item where that person has already been demonstrated to be present from a different item <u>at the same location.</u>

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8.5.5.3.1	Male Donor A in mixture on gear shift major or single source on steering whe	
8.5.5.3.2	Male Donor A and Male Donor B on to third and fourth cigarette butts are mix	•
8.5.5.3.3	Mixtures on sexual assault items/swabs Donor A was already identified on one items/swabs/fractions	

- 8.5.6 If multiple victim/elimination sample/ donors are available and they each have a reasonable expectation to be on an item, an LR should be calculated for each against the initial STRmixTM deconvolution.
- 8.5.7 If the resulting LR > 1,000 (above the uninformative range, see below), the DNA profile should be used as a conditioned profile in a subsequent STRmixTM deconvolution. The original deconvolution should be noted on a STRmixTM "Not Interpreted/Reported" form in the case file. If there is indication of relatedness between the possible conditioned profiles, consult a supervisor.
- 8.5.8 If the resulting STRmixTM LR \leq 1,000, this STRmixTM analysis should be noted on a "Not Interpreted/Reported" form in the case file and the sample should not be conditioned upon.
- 8.5.9 Refer to **Figure 2** below as a reference point for conditioning. These flowcharts are general workflow processes. Other situations may arise that are not covered. Speak to a supervisor as needed.

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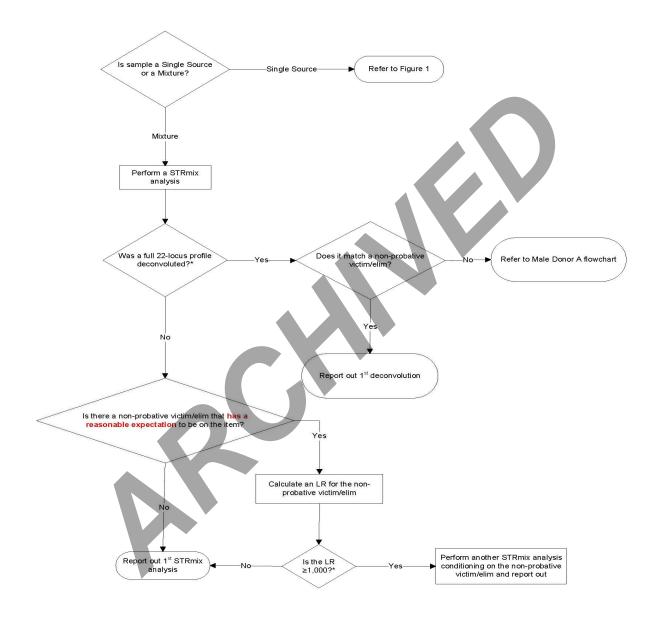


Figure 2a: Victim/elim conditioning flowchart

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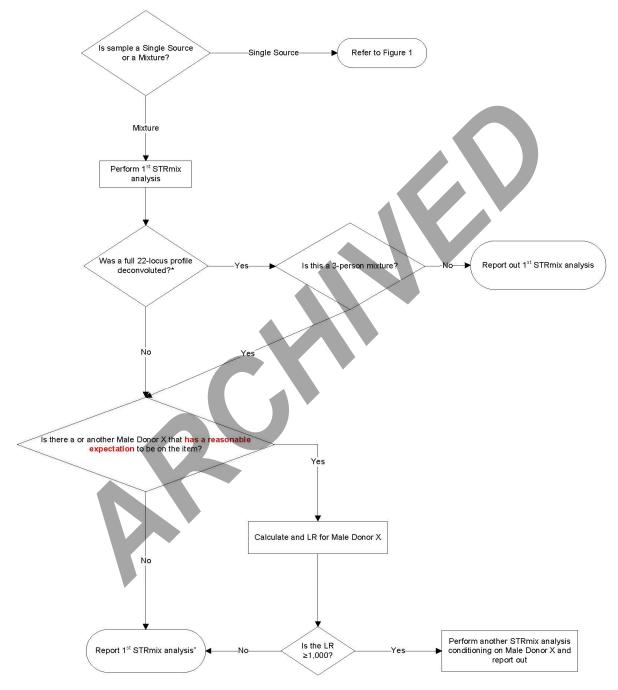


Figure 2b: Male Donor X conditioning flowchart

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- 8.6 Evidence samples typed in any other PCR amplification kit cannot be interpreted in STRmixTM. Conditioned or comparison samples typed in another PCR amplification kit can be compared to PowerPlex Fusion® evidence profiles at the loci which they have in common.
- 8.7 For testimony in relation to a convicted offender match, where a statistic is needed, an LR must be calculated through STRmixTM.
 - 8.7.1 Obtain the Convicted Offender profile through the CODIS software:

8.7.1.1	Go to the Specimen Manager window
8.7.1.2	Click the filter button at the top of the Specimen ID column
8.7.1.3	Click "custom"
8.7.1.4	Enter your specimen ID in the top line in the dialog box
8.7.1.5	Hit "search"
8.7.1.6	Once your specimen ID pops up, right click on the line and click "view matches"
8.7.1.7	Right-click and choose "print reports", choose "match details short report"

- 8.7.2 Add this to your case file, and use the convicted offender profile from this report in order to create a comparison sample profile for STRmixTM LR calculation.
- 8.7.3 Once the STRmixTM LR report is generated, this will need to undergo technical review and added to the case file prior to testimony.

9 What does the likelihood ratio calculation mean?

9.1 The likelihood ratio assesses the probability of the evidence (E) given two alternate propositions; one that aligns with the inclusion of a comparison sample, (H1, the equivalent of H_p) and one that aligns with the exclusion of that comparison sample, (H2, the equivalent of H_d). STRmixTM can calculate the following propositions:

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Table 9 – STRmixTM **Propositions**

Numerator (H1)	Denominator (H2)
Comparison	Unknown
Comparison + Unknown	2 Unknowns
Conditioned + Comparison	Conditioned + Unknown
*Comparison A + Comparison B	2 Unknowns
Comparison + 2 Unknowns	3 Unknowns
Conditioned + Comparison + Unknown	Conditioned +2 Unknowns
Conditioned X + Conditioned Y+ Comparison	Conditioned X + Conditioned Y +
	Unknown
*Comparison A + Comparison B + Unknown	3 Unknowns
*Comparison A + Comparison B + Comparison C	3 Unknowns

^{*}For these scenarios, since more than one exemplar is used as a comparison sample, a unified LR will not be calculated and the 99.0% 1-sided lower HPD LR will be reported. See section 10 for a discussion of the 99.0% 1-sided lower HPD and the unified LR. These situations should be rare and will usually only be calculated after a request is made.

10 Reporting STRmixTM Likelihood Ratio Results

- 10.1 When a likelihood ratio calculation is performed, the lowest **Unified LR** of all four racial groups (NIST Fusion AfAm, NIST Fusion Asian, NIST Fusion Caucasian, and NIST Fusion Hispanic) will be chosen and reported rounded down to three significant figures.
- 10.2 In the instance where a unified LR is not calculated (ie. when two samples are compared in H_p to unknowns in H_d), the lowest **99.0% 1-sided lower HPD** value will be chosen and reported rounded down to three significant figures.
- 10.3 If the chosen LR is between 0.001 and 1,000, the results will be considered uninformative. The results do not support an inclusion or an exclusion of an individual to a sample. This conclusion will be reported without numerical values.
- 10.4 If the lowest LR < 1.0 (and outside of the uninformative range), the reciprocal of the lowest LR is reported. The LR will be reported rounded down to three significant figures.
- 10.5 If the LR >1,000 in favor of H1 this supports that the individual is included as a contributor.
- 10.6 If the LR >1,000 in favor of H2 this supports that the individual is excluded as a contributor.
- 10.7 If the LR = 0 this indicates that an individual is excluded as a contributor to the sample. This conclusion will be reported without numerical values.

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10.8 Refer to the "STRmixTM Probabilistic Genotyping Software Operating Instructions" for operating instructions.

11 References:

- 11.1 Office of the Chief Medical Examiner- NYC PowerPlex® Fusion System Amplification Kit on the Applied Biosystems 3130xl Genetic Analyzer using GeneMarker® HID Analysis Software Validation Report, August 29, 2016.
- 11.2 Estimation of STRmixTM parameters for OCME New York Laboratory
- 11.3 Internal Validation of STRmixTM V2.4 for Fusion NYC OCME

