Interpretation of PowerPlex® Fusion data run on 3500xL

1 Guiding Principles and Scope

1.1 These guidelines for interpretation are applicable for samples amplified using PowerPlex® Fusion 5C run on the 3500xL Genetic Analyzers.

1.2 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, standard rules, and experience.

1.3 This manual may not cover all situations that arise, and not every situation can be covered by a pre-set rule. Equipped with these guidelines, analysts should rely on professional judgment and expertise as well as their supervisor for further guidance.

2 Validation Parameters for PowerPlex® Fusion on 3500xLs

2.1 The PowerPlex® Fusion validation on the 3500xL Genetic Analyzers, the associated STRmix™ validation, and supplemental studies included experiments which determined the laboratory's Analytical Thresholds (ATs), Stochastic Thresholds (STs), minimum and optimal amplification DNA input amounts, saturation point of the 3500xL instruments, and drop-in rate. These factors are fundamental for interpretation of PowerPlex® Fusion profiles run on 3500xLs.

2.1.1 The optimal DNA input amount for amplification is 375pg and the minimum DNA input amount is 37.5pg.

2.1.2 The AT was determined for each dye color as listed below in relative fluorescent units (rfu). The AT is the minimum rfu value at which peaks can be reliably distinguished from background noise.

Fluorescein	(blue)	85 rfu
JOE	(green)	120 rfu
TMR-ET	(yellow)	130 rfu
CXR-ET	(red)	160 rfu

2.1.3 The ST was determined for each dye color as listed below. The ST is the value above which it is reasonable to assume that allelic dropout of a heterozygous genotype has not occurred at an autosomal location within a single-source profile. It is considered by the OCME in the interpretation of single source profiles in order to assign genotypes to these profiles and can also be considered when determining the number of contributors for a mixture sample.

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Fluorescein	(blue)	900 rfu
JOE	(green)	1000 rfu
TMR-ET	(yellow)	900 rfu
CXR-ET	(red)	900 rfu

2.1.4 The saturation point was determined to be 30,000 rfu. Capillary electrophoresis has specific ranges of DNA amounts that are optimal for detection and analysis. See STR Analysis on 3500xL Genetic Analyzers for handling of samples with over-saturated peaks.

2.1.5 The drop-in rate (0.87%) and distribution parameters ($\alpha = 22.31$, $\beta = 2.65$) were determined using PowerPlex® Fusion data on 3500xLs and are applied during profile interpretation. Drop-in is defined as the observation of non-reproducible, unexplained, low-level peaks in a DNA profile. A drop in cap of 300 rfu is also applied during STRmix™ analysis.

3 Assessing the Number of Contributors (NOC) to Sample(s)

3.1 When assessing the best description of the number of contributors to a sample, **the sample should be evaluated as a whole**.

3.2 Running a **replicate amplification** can assist with determining the number of contributors. If there are replicate amplification(s), all qualifying replicates should be evaluated when determining the number of contributors.

3.2.1 If there are drastic inconsistencies between replicates, each replicate should be closely evaluated for use in interpretation. Generally, the amplification(s) with the most information should be used for STRmix™.

3.2.2 If a replicate will not be used for interpretation and/or STRmix™, it will be marked as "Not Suitable for Comparison."

3.2.3 In some cases, a third amplification can be performed.

3.3 An allele at the **DYS391 locus** may indicate the presence of at least one male contributor but should not be used for number of contributor determinations.

3.4 Follow the **process below by first looking at the most informative loci** to determine the number of contributors that best describes the sample. Generally, the most informative loci indicate the presence of the greatest number of contributors. This may be indicated by the characteristics described below.

3.4.1 The maximum allele count method is used for an initial estimate for the number of contributors.

3.4.1.1 Count the number of labeled alleles at each locus for sample data with stutter filters turned ON. Be sure to count and sum across replicates, if applicable.

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- For example, if the first replicate has 5 called alleles at FGA, and the second replicate has 6 called alleles (4 in common with the first replicate and 2 different), the allele count for this locus is 7.

3.4.1.2 Count the alleles at the locus with the most alleles and divide by 2. If the count is odd, add 1 before dividing by 2. This is your initial estimate of the number of contributors.

3.4.2 Next, evaluate the mixture ratio and peak height imbalance at the most informative loci (see Manual Appendix for PowerPlex® Fusion on 3500xL). Visually try to 'pair' the alleles and assign contributor(s). Be sure to consider allele sharing or 'stacking.'

3.4.2.1 If the peak imbalance between alleles is so great that the profile does not conform to your initial number of contributors estimate, this may indicate the presence of an additional contributor.

3.4.2.1.1 With higher order mixtures (3+ contributors), the mixture ratio across the profile can be an important indicator of number of contributors. If the mixture ratio does not hold, consider the presence of an additional contributor.

3.4.2.2 Since some loci are more polymorphic than others, consider the number of locations exhibiting the maximum allele count and the potential for allele sharing that occurs more commonly in higher order mixtures when determining NOC.

3.4.2.2.1 Note that based on allele count alone, as the NOC increases in a sample, it is less likely to see the maximum number of expected alleles at any one locus (where the maximum number of expected alleles is 4 at 2p, 6 at 3p, 8 at 4p). Alternatively, as the NOC decreases in a sample, it is more likely to see the maximum expected allele count.

3.4.3 Various characteristics of the sample should be taken into consideration including, but not limited to the following:

3.4.3.1 The amount of DNA amplified.

3.4.3.2 The presence of peaks below the analytical thresholds and global filter.

3.4.3.2.1 Peaks below the AT/global filter and not in positions of known artifacts may be useful to inform the presence of an additional low level or trace contributor(s). If peaks below AT/global filter are being used to infer the presence of an additional contributor(s), this should be supported by other characteristics elsewhere in the profile such as peaks falling just above AT/global filter and/or peak height imbalance, especially at the more informative loci.

3.4.3.3 The presence of peaks below the stochastic thresholds.

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3.4.3.3.1 If most or all peaks are below stochastic threshold, consider an increase of one contributor, especially when there is evidence of dropout throughout the profile and across replicate amplifications. Other characteristics such as multiple locations with the maximum allele count and/or peak(s) below the analytical threshold may also be present.

3.4.3.4 Indication(s) of degradation.

3.5 Apply the **general pattern of contributors** determined at the most informative loci (number and mixture ratio) to other loci in the mixture. If it holds, assign this number of contributors to the sample; otherwise, consider the addition of one contributor.

3.6 The presence of a **reasonably expected victim/elimination profile** within a mixture may also be used to assist with the estimation of number of contributors to a sample. The assumption of any contributor to a mixture must be supported by the data, regardless of case scenario.

3.6.1 If the reasonably expected elimination sample(s) are potential relative(s) and appear to have increased allele sharing with other contributors, consider an increase in the number of contributors.

3.7 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**. Tri-allelic patterns may be confirmed with a replicate amplification.

3.8 If no more than three additional trace peaks (e.g. a 3rd peak at a heterozygous locus, or a 2nd peak at a homozygote locus) are seen in a sample otherwise appearing to be a clear and robust single source sample, the sample may be considered single source if the trace peaks can be modeled as stutter (see Appendix for PowerPlex® Fusion Stutter) and/or drop-in (<300 RFU). If more than one of the trace peak(s) seen across all replicates is in a non-stutter position, the sample must be considered a mixture.

- NOTE: This should be applied ONLY AFTER the sample characteristics listed above are taken into account, especially the amount of DNA amplified and the presence of peaks below analytical threshold, as well as the context of the case (i.e. the presence of a reasonably expected victim/elimination profile).

3.9 Results from a STRmix™ analysis (deconvolution and/or LR calculation) may indicate that the initial estimation of number of contributors should be re-evaluated, and a subsequent run with **N+1 or N-**1 contributors may be warranted. Refer to the STRmix™ Probabilistic Genotyping Software Operating Instructions for more information.

3.10 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 circumstances suggest that the possibility of additional

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contributors exists. Reporting analysts should use their professional judgment when assessing the number of contributors. See Section 4.3 if the number of contributors cannot be determined.

4 Samples for Comparison

4.1 Samples deemed as being best described as single source or two-, three-, or four-person mixtures will be used for comparison.

4.1.1 All DNA results that are interpreted under these guidelines are suitable for comparison in their entirety.

4.2 DNA samples that are best described as five or more person mixtures will not be used for comparison.

4.3 For samples best described as five or more person mixtures and in circumstances when the number of contributors cannot be determined, the analyst reports that no interpretations were made for the profile, and the profile is not used for comparison.

4.3.1 Fill out the Not Suitable for Comparison/Inconclusive Form.

4.4 Results from a STRmix™ analysis may also indicate that a sample is not suitable for comparison. See the STRmix™ Probabilistic Genotyping Software Operating Instructions for more information.

5 Determining the Profile of a Single Source Evidence Sample

5.1 If all called alleles are **equal to or above the ST** in a single source sample, the alleles may be assigned to the DNA profile with the following considerations:

5.1.1 Peak height imbalance is a feature of heterozygotes. (Refer to Manual Appendix for PowerPlex® Fusion on 3500xL 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.

5.1.1.1 If extreme peak imbalance is observed at several loci, consider performing a STRmix™ analysis to assist in the determination of allele assignments.

5.2 If a single source sample has **alleles below the ST**, the following considerations must be made:

5.2.1 If there is a single allele that is equal to or above the ST at a locus in at least one amp it can be assigned to the profile as a homozygote.

5.2.2 If there are two alleles at a locus with one or both below the ST, both alleles may be assigned as a heterozygote pair.

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5.2.3 If there is a single allele at a locus below the ST, the possibility of drop-out of a sister allele must be considered in the following manner:

5.2.3.1 In all samples, for the longest locus in each color: Penta E, Penta D, TPOX and D22S1045, the locus may be assigned as "A,Z". A homozygote call cannot be manually assigned to a single peak below the ST.

5.2.3.2 For samples that originate directly from an individual's body (body cavity swabs, swabbing from any skin surface, underwear within a SOECK, sample from fingernails or ligatures), where at least six loci can be fully assigned using 5.2.1 and 5.2.2, and all alleles present in the sample are the same as the reference sample's alleles, any locus with a single allele below ST may be interpreted as an "A,Z".

5.2.3.3 For all other samples, a STRmix™ analysis must be performed to determine the allelic assignment.

5.3 Samples that have 6 or more fully assigned loci are determined to be DNA profiles and are assigned a donor name. See General Guidelines for Building an Evidence DNA Donor Profile

5.3.1 If the sample profile is eligible for DNA databases, go to the Database Profile Creation Table.

5.4 Samples with less than 6 fully assigned loci, where no DNA profile is determined, are suitable for comparison only.

5.4.1 If the sample is from an intimate item (ex. body cavity swab or fingernails) and all the alleles labeled are the same as the alleles of the reference sample associated with the evidence sample, this may be reported as such.

6 Determining Profiles from Mixture Samples

6.1 Two, three, and four person mixtures are analyzed using STRmix™ for possible deconvolutions.

6.1.1 If a DNA profile deduced from a sample is eligible for DNA databases, go to the Database Profile Creation Table.

6.2 Deconvolutions that have 6 or more fully assigned loci for a contributor are determined to be DNA profiles and will be assigned a donor name. See General Guidelines for Building an Evidence DNA Donor Profile

6.2.1 Running a replicate amplification may increase the number of loci that are fully assigned within a deconvolution.

6.3 Deconvolutions with less than 6 fully assigned loci for any contributor are suitable for comparison only.

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6.4 For CPW cases, samples which the number of contributors can be estimated (Refer to SECTION 3,4, and 5), a STRmix™ deconvolution (including conditioning LR checks of elimination samples) is not required if a comparison sample is not available. In these situations, the sample should be deemed as a mixture suitable for comparison and reported out as such. If/when a comparison sample is received the necessary STRmix™ analysis/analyses can be done at that time. The STRmix™ deconvolution(s) will be placed in the evidence file, an additional evidence report with the new interpretations should be generated, and any LR calculations for the relevant comparison sample should be placed in the file associated with that comparison sample.

7 Determining Profiles for Exemplars and Abandonment Samples

7.1 The alleles that are **equal to or above ST** can be assigned to the profile.

7.2 If there are two alleles at a locus with **one or both below the ST**, both alleles may be assigned as a heterozygote pair. If there is any ambiguity, then the locus should be deemed inconclusive. Refer to the Manual Appendix for PowerPlex® Fusion on 3500xL for further information.

7.3 If there is a single allele at a locus **below the ST**, the possibility of drop-out of a sister allele must be considered; therefore, the locus should be deemed inconclusive.

7.3.1 For samples process through the Fusion Direct procedure, the overall quality of the profile should be assessed to determine if there are increased stochastic effects (such as peaks below threshold, below a stutter or global filter, or peak height imbalance). Concordance with other samples in the case should also be evaluated. In these instances, a single peak above ST may be present even though a sister allele has dropped out. In these situations, and based on case information/results the locus should be deemed inconclusive, or the sample recut for extraction and quantitation.

7.4 Samples with < 6 fully assigned locations should be deemed inconclusive and will not be used for comparison.

7.5 Suspect samples that are eligible for search and/or upload to DNA databases with ≤ 10 assigned locations must have a match rarity estimate calculated before search and/or upload. Refer to the CODIS Profile Management manual for further details.

7.6 Exemplars or abandonment samples that appear to be mixtures, based on labelled alleles and characteristics described above, will not be used for interpretation.

7.7 If a third labelled 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 or a drop-in peak; the sample may still be used for interpretation.

7.8 Multiple results obtained from the same sample (ie. multiple cuttings, amplifications, or injections) may be combined in order to interpret the DNA profile of the individual.

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7.9 For Products of Conception (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.

8 Defining STRmix™

8.1 STRmix™ 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, (2) compare suspect/informative reference samples (comparison samples) to evidence samples and provide statistical weight in the form of a likelihood ratio (LR), and (3) calculate LRs for quality control purposes. See QC740 - QC Monitoring Program.

8.1.1 The deconvolution is performed using a Markov chain Monte Carlo process which creates possible genotype combination(s) based on the given sample data. Each combination is assigned a weight which reflects how well it explains the evidence profile.

8.1.2 LRs are calculated by comparing the probabilities of two hypotheses, H1 and H2 (H¬P and HD, in STRmix™). STRmix™ incorporates the assigned weights and sub-population models (Balding and Nichols, 1994, also known as NRC II recommendation 4.2) to calculate the LR.

8.2 STRmix™ requires an initial process to determine in-house parameters and Model Maker values that assist in the biological model used within STRmix™. The following parameters were determined by analysis of empirical data from the internal validation study:

- Analytical threshold
- Stutter ratios
- Drop-in parameters
- Saturation
- Allelic and stutter peak height variance
- Locus Specific Amplification Efficiencies (LSAE)

8.3 The default parameters specific to interpretation using STRmix™ with PowerPlex® Fusion data generated using 3500xL Genetic Analyzers at the NYC OCME Department of Forensic Biology can be found in the STRmix™ Probabilistic Genotyping Software Operating Instructions.

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

9 When to use STRmix™

9.1 STRmix™ is used for the interpretation of PowerPlex® Fusion DNA profiles as described below.

9.1.1 At the NYC OCME, evidence samples typed in any other PCR amplification kit cannot be interpreted in STRmix™.

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9.2 Exemplars may be characterized as conditioned or comparison samples. These may be typed in another PCR amplification kit and can be compared to PowerPlex® Fusion evidence profiles at the loci which they have in common. Consider re-typing reference samples in Fusion whenever possible (see Case Management).

9.2.1 Conditioned samples: "Assumed contributors" are individuals who have a reasonable expectation to be present and you suspect may be included as a contributor to a mixture sample based on where the sample was taken from (i.e. victim's DNA profile from her vaginal swab, homeowner's DNA profile seen on an item recovered from the site of a residential burglary).

9.2.1.1 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.

9.2.1.2 If a profile is conditioned upon, even if you do not get an additional contributor profile, that analysis is preferred for future comparisons because it better informs the LR calculation.

9.2.2 Comparison sample: 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).

9.3 In general, multiple deconvolutions should not be run on the same set of data. Additional deconvolutions should only be run with additional data or with different settings (ie. conditioning, additional replicates, additional accepts).

9.4 For samples that are **single source**, a STRmix™ analysis may or may not need to be performed to determine the allelic assignment (refer to section 5). If the STRmix™ analysis was not performed at the time of profile determination, it will need to be performed if an LR calculation is needed for a comparison sample.

9.4.1 When a comparison sample is evaluated against a single source sample with a previously determined profile and an exclusion results, no LR calculation is needed.

9.4.2 If the comparison results in a positive association to a previously determined profile and a statistic is needed, an LR statistic must be calculated.

9.4.3 When a comparison sample is evaluated against a single source sample that has less than 6 fully assigned loci; an LR statistic must be calculated through STRmix™.

9.5 Samples best described **as two-, three-, or four-person mixtures** will have STRmix™ analyses performed for the purposes of **deconvolution**.

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9.5.1 If the deconvolution results in a profile (≥ 6 fully assigned loci for a contributor), the comparison sample can be directly compared to that profile. If an LR is needed, this must be calculated using STRmix™.

9.5.1.1 An LR may not be needed for all samples within one case. An LR for one sample along with a qualitative statement for the remaining samples may be reported when single source or deconvoluted profiles from the same item/location/area generate the same DNA donor and there is a positive association.

9.5.2 When a comparison sample is evaluated against an evidence mixture in which no profile was deconvoluted or the comparison sample is not positively associated to the deconvoluted profile, an LR statistic must be calculated through STRmix™.

9.5.2.1 For CPW cases, an LR may not be needed for all samples. If a sample from a firearm had a single source or deconvoluted profile (with a positive association) and a LR was calculated, no additional LR calculations to other non-deconvoluted mixtures on the same firearm are needed. Report as “no comparisons were made”.

9.5.3 For two-person mixtures in samples that originate from an individual’s body where the victim is conditioned (as per Section 9.9.2.1) and a donor profile is determined, consensual partner elimination profiles can be compared to the donor profile and do not require an LR calculation.

9.6 **Conditioning**: DNA profiles of victims/elimination samples/deconvoluted donors may be used as conditioned samples in a STRmix™ analysis to aid in the mixture deconvolution and/or better inform an LR calculation. If a potential conditioning sample does not have data at all Fusion loci, those loci will not be used in the deconvolution or subsequent LR calculations for the sample if that reference profile is used for conditioning. The number of loci that will not be used should be considered and evaluated before conditioning if this is the case.

9.7 If upon the initial deconvolution a full DNA profile is obtained, and the goal of the case has been met, no further conditioning is needed.

9.7.1 If the deconvoluted contributor profile is the same as a reasonably expected victim/elimination/donor, no LR calculation is needed and a second deconvolution conditioning upon that profile can be performed.

9.7.2 Consider if a comparison sample is available for that particular case and if a conditioned deconvolution of a reasonably expected victim or an elimination sample would be desirable for a more complete hypothesis statement.

9.8 If a probative association with a comparison sample is calculated for an evidence sample, that comparison sample cannot be subsequently conditioned upon for that same evidence sample.

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9.8.1 Conditioning may be performed using a comparison sample that is qualitatively included and has data to support the inclusion in cases where it may help to resolve STRmix diagnostic issues. Consult the Technical Leader before proceeding.

9.9 The following two criteria must be met in order to use a reference sample for conditioning:

9.9.1 The victim/elimination sample/donor must be reasonably expected to be on the item based on case and sample information. Some examples of reasonable expectations are listed below.

9.9.1.1 Elimination homeowner on an item recovered from their house.

9.9.1.2 Person on any mixture on an item on which that person has already been demonstrated to be positively associated elsewhere on that same item.

- Victim in a mixture on a handle of a knife where that victim was single source or a deconvoluted major from the blade of the same knife.

9.9.1.3 Person on any mixture from an item where that person has already been demonstrated to be positively associated on a different item at the same location.

- Male Donor A in mixture on gear shift when Male Donor A was major or single source on steering wheel.
- Mixtures on sexual assault items/swabs/fractions where Male Donor A was already identified on one of the other items/swabs/fractions.

9.9.2 There must be data to support that their DNA profile is present within the mixture. An LR must be determined and be > 1,000 before conditioning. An LR is not required before conditioning for situations described below.

9.9.2.1 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.) - the majority of the individual's alleles must be present and labeled in the mixture.

9.9.2.2 In a mixture from their own clothing, all the alleles of an elimination or victim sample are present in a mixture.

9.9.2.3 Mixture where a previous deconvolution resulted in a profile matching the conditioning sample.

9.9.2.4 When a conditioning sample produced an LR > 1,000 when compared to the initial amplification, and a replicate amplification is subsequently performed on the evidence sample.

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9.10 If the **resulting STRmix™ LR > 1,000** (above the uninformative range, see Section 12 on LRs), the DNA profile should be used as a conditioned profile in a subsequent STRmix™ deconvolution. The original deconvolution should be noted on a STRmix™ "Not Interpreted/Reported" form in the case file.

9.10.1 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 separately for each against the initial STRmix™ deconvolution.

9.10.1.1 If more than one victim/elimination sample/donor generates an LR > 1,000 for a particular mixture, ensure that both reference samples are not aligning with the same contributor on the LR report. If this occurs and/or there is an indication of relatedness between the possible conditioned profiles, consult a supervisor or the Technical Leader before proceeding with any further conditioning. Refer to Section 12.8 for further guidance. Additional deconvolutions and/or LR scenarios may need to be considered.

9.11 If the **resulting STRmix™ LR ≤ 1,000** (within the uninformative range, see Section 12 on LRs), the victim/elimination sample/donor should not be conditioned upon. This STRmix™ analysis (LR) should be noted on a "Not Interpreted/Reported" form in the case file.

10 Determining Profiles using STRmix™

10.1 The following is a reference guide for determining a DNA donor profile. Not all DNA donor profiles are eligible for search and/or entry into DNA databanks. For further requirements relating to specific database eligibility of DNA profiles and procedures for searching local databanks, refer to the CODIS Profile Management manual.

10.2 The genotype weights are used to aid in the determination of DNA profiles, and the **Database Profile Creation Table** contains a guide for determining profiles.

10.3 When determining which alleles to include at a mixed locus, sum all weights from the COMPONENT INTERPRETATION section in which the allele is present for that contributor.

- For example, when determining the weight of a 14 allele, sum the weights for [8,14], [14,14], and [14,Q].
- When determining if Q is ⩾ 1.00%, sum all weights for the genotypes where a Q is present.

10.4 Database Profile Creation Table

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Amelogenin Determination	**Interp**	**DB Profile**
Consider Amelogenin peak heights and ratio, number of contributors, mixture proportions, data at DYS391, and quantitation data to determine allele assignment	X; X,Y; INC	X; X,Y; INC
Sum of Genotype Combinations	**Interp**	**DB profile**
Genotype [A,B] ≥ 99.00%	A, B	A, B
Genotype [A,A] ≥ 99.00%	A	A, A
Allele A ≥ 99.00% Allele B ≥ 99.00% Genotype [A,B] < 99.00% Alleles C-D ≥1.00% (if present) *Forensic Mixture***	INC	A, B, C, D
Allele A ≥ 99.00% Allele B ≥ 99.00% Genotype [A,B] < 99.00% *Forensic Partial***	INC	INC
Allele A ≥ 99.00% Q ≥ 1.00% (even if other alleles are ≥ 1.00%)	A, Z	A+
Allele A ≥ 99.00% All other Alleles < 1.00%	A, Z	A+
Allele A ≥ 99.00% Alleles B-D ≥ 1.00% *Forensic Mixture*	A, Z	A+, B, C, D
Allele A ≥ 99.00% Alleles B-N ≥ 1.00% (more than 4 total) *Forensic Mixture*	A, Z	A+
Allele A ≥ 99.00% Allele B ≥ 1.00% *Forensic Partial*	A, Z	A+
Alleles A-D ≥ 1.00% & < 99.00% Q < 1.00%	INC	A, B, C, D
Alleles A-N ≥1.00% (more than 4 total)	INC	INC
No allele ≥ 99.00% Q or Q,Q ≥ 1.00%	INC	INC

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10.5 ** The genotype listed in the COMPONENT ≥ 99% in the STRmix™ analysis may refer to individual alleles that sum to the 99.00% threshold and not the complete genotype (the weighting for the complete genotype is <99.00%). In this case, the interpretation for the locus is INC. If the profile is from a mixture, the database profile will include the two ≥ 99% alleles; if the profile is single source, the database profile will be deemed "INC." In these instances, the weight corresponding to the listed genotype is highlighted directly on the STRmix™ report.

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10.6 The sums of genotype weightings are NOT to be used to introduce a NEW allele call to the interpretation of a locus that STRmix™ has already assigned an INC or A,Z.

10.7 Interpretations based on the sums of the individual genotype weights should be evaluated by the analyst in comparison to the electropherogram(s). If, at a single locus, a genotype call that meets the COMPONENT ≥ 99% threshold does not conform to qualitative expectations based on analyst evaluation of the data, a more conservative call (ex. going from A,B to A,Z or A,Z to INC) may be considered if the listed genotype possibilities at the locus support the more conservative call. This should be done in consultation with a supervisor and/or manager.

10.7.1 In these instances, the ≥ 99% genotype must be highlighted on the STRmix™ report.

10.7.2 If multiple loci appear to have this issue, this may be a diagnostic indication that the sample NOC needs to be re-evaluated and STRmix™ analysis needs to be re-performed with N+1 or N-1 number of contributors, an additional rep, or additional accepts.

10.8 If there is ambiguity in the interpretation of Amelogenin, INC the location (rather than use X,Z) for both the interpretation and the DB profile, and the donor will be called a **DNA Donor**.

10.8.1 Consider Amelogenin peak heights and ratio, number of contributors, mixture proportions, data at DYS391, and quantitation data to determine allele assignment.

10.8.2 If multiple DNA donor profiles are determined from the same sample, use caution. You should not assign male or female designations to more than one deconvoluted donor from the same mixture.

10.9 The interpretation and DB profile assignment for the DYS391 locus must be entered as “INC.” This locus can be used to confirm the presence of male DNA in the sample but is not to be used for comparison.

10.10 If an allele meets the criteria to be entered into a DB profile but was filtered out as stutter in the stutter-filtered electropherogram, this allele must be entered into the LIMS Fusion Allele Table for that locus. Refer to the BEAST STR Analysis and Review Manual.

10.11 If an allele meets the criteria to be entered into a DB profile but was not present in the evidence sample (ex. in a deconvolution with a conditioned contributor) this allele is NOT to be used as part of the DB profile. The more conservative call (ex. going from A+,B,C to A+) should be used in the DB profile. This should be done in consultation with a supervisor and/or manager.

11 Likelihood ratio scenarios

11.1 The likelihood ratio (LR) 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 HP) and one that aligns with the exclusion of that comparison sample, (H2, the equivalent of

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HD). STRmix™ can be used to calculate an LR for propositions with and without conditioned samples and/or multiple comparison samples.

11.1.1 If more than one exemplar is used as a comparison sample, a Unified LR will not be calculated, and the lowest value listed as the 99.0% 1-sided lower HPD LR is reported (see Section 12). These situations should be rare and will usually only be calculated after a request is made and there is a discussion with a supervisor and/or manager and the Tech Lead Team.

12 Reporting STRmix™ Likelihood Ratio Results

12.1 When a likelihood ratio (LR) calculation is performed, the lowest **Unified LR** of the four population groups (NIST Fusion AfAm, NIST Fusion Asian, NIST Fusion Caucasian, and NIST Fusion Hispanic) is chosen and reported **truncated to three significant figures.**

12.2 If the LR > 1,000 in favor of H1, this supports that the individual is included as a contributor.

12.3 If the LR > 1,000 in favor of H2, this supports that the individual is excluded as a contributor.

12.4 If the LR is ≤ 1,000 and ≥ 0.001, the results are uninformative. The results do not support an inclusion or an exclusion of an individual to a sample.

12.5 If the lowest LR is < 1.0, the reciprocal of the lowest LR is reported. The reciprocal LR is reported truncated to three significant figures.

12.6 The LR value is reported in both words (e.g. 598 million) and standard scientific notation (e.g. 5.98 x 108). For numbers less than 1 million, report the number (e.g. 15,500) as opposed to using the word "thousand." For reported LR values that fall into the uninformative range, the scientific notation does not need to be reported.

12.7 If the LR = 0 this indicates that an individual is excluded as a contributor to the sample. This conclusion is reported without numerical values.

12.8 If more than one comparison sample or possible conditioned sample(s) generates an LR > 1,000 for a particular mixture, ensure that both reference samples are not aligning with the same contributor. If this occurs and/or there is an indication of relatedness between the possible conditioned profiles or comparison samples, consult a supervisor or the Technical Leader before proceeding. Additional deconvolutions and/or LR scenarios may need to be considered.

12.8.1 Conditioning may help resolve issues with contributor alignment, however, take caution and evaluate the primary and secondary diagnostics (especially allele variance, Gelman-Rubin, and stutter variances) after conditioning. It may be necessary to condition on multiple individuals simultaneously (especially if relatives may be involved) to resolve the comparisons.

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12.8.2 Conditioning may be helpful to identify when an LR may be elevated due to the presence of multiple relatives within a sample

12.8.3 Combined LRs may be helpful to resolve alignment issues for robust contributors, however, a combined LR supporting inclusion of multiple contributors should not be used to specifically support the inclusion of a trace contributor.

12.9 Refer to the STRmix™ Probabilistic Genotyping Software Operating Instructions for operating instructions.

13 References

13.1 NYC OCME. PowerPlex® Fusion System Amplification Kit on the Applied Biosystems® 3500xL Genetic Analyzer with GeneMarker® HID 2.9.5 (July 2019)

13.2 NYC OCME Internal Validation of STRmix™v2.7 for Fusion 5C/3500xL (September 2021)

13.3 NYC OCME Internal Validation of STRmix™v2.7 for Fusion 5C/3500xL Data - STRmix™ Parameters (August 2021)

13.4 NYC OCME Stutter Study for GeneMarker® HID 3.0.0 and STRmix™ Version 2.7-PowerPlex® Fusion Data run on 3500xL Genetic Analyzers (September 2021)

13.5 NYC OCME Internal Validation of STRmix™V2.4 for Fusion (January 2017)

13.6 NYC OCME STRmix™V2.4.08 Performance Check. (July 2018)

13.7 NYC OCME: Decreasing the optimum input amount for the PowerPlex Fusion® kit: Evaluation of the Male to Female (M:F) Ratio at Various Amplification Amounts for Casework

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