Interpretation of PowerPlex® Y23 data run on 3500xL

1 Guiding Principles and Scope

1.1 These guidelines for interpretation are applicable for samples amplified using PowerPlex® Y23 (PPY23) 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 Y-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® Y23 on 3500xLs

2.1 The PowerPlex® Y23 validation on the 3500xL Genetic Analyzers included experiments which determined the laboratory's Analytical Thresholds (AT), Stochastic Thresholds (ST), minimum and optimal amplification DNA input amounts, saturation point of the 3500xl instruments, and drop-in. These factors are fundamental for interpretation of PowerPlex® Y23 profiles run on the 3500xL Genetic Analyzers.

2.1.1 The optimal DNA input amount for amplification was determined to be **500pg** of male DNA for evidence and exemplars. The minimum DNA input amount is **100pg** of male DNA.

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

Fluorescein	(blue)	60 RFU
JOE	(green)	70 RFU
TMR-ET	(yellow)	90 RFU
CXR-ET	(red)	120 RFU

2.1.3 The within locus ST of **700** RFU was determined for the bi-allelic marker, DYS385a/b, at which an individual may have up to two alleles. The ST is the value above which it is reasonable to assume that allelic dropout of a sister allele has not occurred at this location within a single-source profile.

2.1.4 A between locus ST of 2500 RFU was determined for individual donors to samples. If an allele of an individual donor reaches 2500 RFU at any given locus besides DYS385a/b,

then there may be an expectation that the allelic peaks of the individual donor are detected at all loci, and that no drop-out of this donor has occurred.

2.1.4.1 To determine if a contributor meets the between locus ST, loci with distinct alleles for each individual contributor should be evaluated (eg. loci with 2 alleles in a two-person mixture, or three alleles in a three-person mixture).

2.1.5 An inversion threshold of 1200 RFU was determined for two-person mixtures. If the tallest peak(s) at a locus is above this threshold it is expected that this peak(s) belongs to the major contributor. Below this threshold, there is a possibility that the major contributor may not be the tallest peak(s).

2.1.6 Capillary electrophoresis has specific ranges of DNA amounts that are optimal for detection and analysis. For the 3500xl, peak heights approaching or above 30,000 RFU may be saturated. Samples with saturated peaks may still be analyzed if artifacts caused by the saturation can be clearly distinguished from the true contributor alleles, for example in single source or exemplar samples. Refer to the STR Analysis on 3500xL Genetic Analyzers manual for handling of samples with over-saturated peaks.

2.1.7 Drop-in is defined as the rare observation of non-reproducible, unexplained, low-level peak(s) in a DNA profile. Single drop-in peaks have been observed within negative controls in validation data. Drop-in may be considered for a single low-level (<300 RFU) peak within a control or a sample.

3 Y-STR General Guidelines

3.1 The Y-STR markers used in the PowerPlex® Y23 kit are typically single copy markers, with the exception of the DYS385a/b locus.

3.1.1 Rarely, a duplication or triplication event may occur where two alleles are present at a location other than DYS385a/b, or three alleles are observed at a single location, respectively. Refer to the Manual Appendix for PowerPlex® Y23 on 3500xL for locations where these events are known to occur.

3.2 A null (silent) allele is an allele which cannot be detected due to lack of amplification product, often caused by a mutation in the primer binding site, or deletion of the primer binding site or locus.

3.2.1 In a sample where a donor meets the between locus ST of 2500 RFU at one or more loci, another locus without a detected allele may be an indication of a null allele.

3.2.2 It may not be possible to distinguish null alleles from allelic dropout in mixtures and/or samples with a low amount of DNA, or those exhibiting degradation or inhibition.

3.3 For PPY23, various artifacts may be observed in samples that contain high amounts of female DNA in relation to male DNA. Refer to Manual Appendix for PowerPlex® Y23 on 3500xL for examples and base pair ranges where the artifacts have been observed.

3.3.1 As the female DNA increases, the amount and intensity of artifactual peaks may increase. These artifacts may be observed in amplified extracts that have a male to female mixture ratio ≥ 1:2000.

3.3.2 Samples with a high female to male ratio displaying these artifacts may still be used for analysis and interpretation; however, caution should be taken when interpreting samples with multiple male contributors because excessive artifacts may make it difficult to distinguish true contributor alleles.

3.4 A replicate amplification may aid in the evaluation of allele count, peak height balance at the DYS385a/b locus, and mixture ratios.

3.4.1 If there are drastic inconsistencies between replicates, each replicate should be closely evaluated for use in interpretation.

3.4.1.1 If a replicate will not be used for interpretation, fill out the Not Suitable for Comparison/Inconclusive Form.

3.4.2 If there is a replicate amplification(s), all interpreted replicates should be evaluated together when determining the number of contributors and for the interpretation of DNA profiles.

3.5 Interpretations should be documented for Y-STR PPY23 single source or deconvoluted DNA profiles. See the Case Management manual for guidance on when a table is necessary for a casefile.

4 Assessing the number of male contributors (NOC) to sample(s)

4.1 When determining the number of male contributors to a sample, **the sample should be evaluated as a whole**.

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

4.2.1 The amount of DNA amplified and corresponding number of detected peaks.

4.2.2 The presence of peaks below the analytical thresholds.

4.2.3 Level(s) of potential degradation and inhibition.

4.3 Follow the **process below** to determine the number of male contributors that best describes the sample.

4.3.1 **Count alleles**: Count the number of labeled alleles at each locus. Use the most informative location(s) (greatest number of alleles) other than DYS385a/b to determine the initial assessment of the number of male contributors. The number of alleles observed at these location(s), excluding stutter and other explainable artifacts, corresponds to the initial estimate for the number of male contributors in the sample.

4.3.1.1 Be sure to count and sum across replicates, if applicable. For example, if the first run has an allele at DYS576 and the replicate has a different allele called, the total allele count for this locus is two.

4.3.1.2 If trace peak(s) are observed in stutter position in the sample, refer to the locus-specific stutter percentages for guidance in determining if peak(s) could be elevated stutter or an additional trace contributor. See Section 3 of Manual Appendix for PowerPlex® Y23 on 3500xL.

4.3.1.3 For a single low-level (<300 RFU) peak within a sample, the potential for drop-in may also be considered; however, the presence of additional peaks within the sample just above or below the analytical threshold, may indicate the presence of an additional contributor.

4.3.2 A sample can be considered single source if, excluding stutter, a single drop-in peak (<300 RFU), and other explainable artifacts, the sample does not demonstrate more than one labeled allele at each locus other than DYS385a/b, and no more than two labeled alleles at DYS385a/b.

4.3.2.1 If a sample appears single source but there are two or three alleles at a location(s), a duplication or triplication event can be considered. (See 3.1.1).

4.3.2.2 At the bi-allelic marker DYS385a/b, an individual may have up to two alleles present. Evaluate the peak height ratio at DYS385a/b by dividing the height of the shorter peak in a proposed pair by the height of the taller peak and expressing the result as a percentage.

4.3.2.2.1 Extreme imbalance at DYS385a/b may indicate the presence of an additional contributor.

4.3.2.2.2 If the shorter peak falls below 55% of the taller peak in a sample that appears single source, consider whether this may be due to primer binding site mutation, degradation, amount of template DNA, or extreme allele size differences. Under these circumstances, a sample may be considered single source even if greater imbalance is observed.

4.3.3 Samples that do not meet the single source criteria listed above should be considered mixed samples.

4.3.4 Using the allelic peaks at your most informative location(s), evaluate the **mixture ratios** of contributors in the sample. Be sure to consider allele sharing or ‘stacking’.

4.3.4.1 Caution should be taken when considering labeled peaks in stutter position.

4.3.4.2 Allele sharing may occur more commonly in higher order mixtures.

4.3.4.3 If the mixture ratio across the entire electropherogram does not conform to your initial number of male contributors estimate, this may indicate the presence of an additional male contributor.

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

4.3.5.1 "Assumed contributors" are individuals who have a reasonable expectation to be present in a mixture based on where the sample was taken from (e.g. consensual partner on a vaginal swab, victim on his own clothing).

4.4 Sometimes the number of male 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.

4.4.1 If a best estimate for the number of males cannot be determined, the sample may be deemed inconclusive.

4.5 Mixtures of DNA indicating the presence of four or more male contributors will not be interpreted.

5 Determining the profile of a single source male evidence sample

5.1 A single labeled allelic peak at each locus other than DYS385a/b may be assigned to the DNA profile.

5.2 If there are two alleles at any location other than DYS385a/b, a duplication event can be considered, and both alleles may be assigned to the contributor.

5.3 If there are three alleles at any location, a triplication event can be considered, and the three alleles may be assigned to the contributor.

5.4 Interpretation guidelines for DYS385a/b:

5.4.1 If two allelic peaks are present, both alleles may be assigned to the DNA profile.

5.4.2 The within locus ST for DYS385a/b is 700 RFU. A single allelic peak may be assigned to the profile if it is ≥700 RFU. If a single allelic peak is present below 700 RFU, this locus must be marked as inconclusive.

5.5 A minimum of 8 fully assigned loci is required to determine a Y-STR profile and assign as a male DNA donor.

5.5.1 If 8 loci cannot be assigned, the sample may be suitable for direct comparison.

6 Determining profiles from 2-male mixture samples

6.1 A mixture best described as originating from two males may be deconvoluted if the donors are distinguishable from each other using the guidelines described in this section.

6.1.1 Caution should be taken when deconvoluting samples with DNA amounts less than optimal as these may have more variable peak heights and mixture ratios.

6.1.2 Caution should be taken when deconvoluting samples that exhibit degradation and/or inhibition.

6.1.3 Assumed contributors, extreme mixture ratio, and/or peak heights of the minor contributor in the sample may aid in the interpretation.

6.1.4 Caution should be taken in deconvoluting the apparent minor contributor.

6.1.5 Mixed samples whose ratios approach 1:1 should not be deconvoluted unless there is an assumed contributor.

6.1.6 Mixtures that cannot be deconvoluted in whole or in part may be suitable for comparison.

6.1.6.1 At least one locus must have both contributors individually represented (with no evidence of drop out) in order for the mixture to be deemed suitable for comparison.

6.1.7 A deconvoluted major or minor profile must have 8 fully deconvoluted loci in order to determine a Y-STR profile and assign as a male DNA donor.

6.1.8 Caution should be taken applying any thresholds (between locus ST, within locus ST, or inversion) in samples that are amplified with low DNA amounts, or those that exhibit degradation, and/or inhibition.

6.2 A locus with one allelic peak present (excluding DYS385a/b)

6.2.1 If both major and minor contributors have peaks elsewhere in the sample that meet the between locus ST, it can be assumed that both donors have not dropped out and the allele may be assigned to both major and minor contributors.

6.2.2 If the major contributor has a peak elsewhere in the sample that meets the between locus ST but the minor contributor does not, there may be a possibility of drop-out of the minor donor and the allele can only be assigned to the major contributor.

6.2.3 If the major contributor does not meet the between locus ST at any other loci, the peak may be assigned to the major. However, in instances where the single peak is approaching the AT with other unlabeled peak(s) present within the sample just below AT, it should not be assigned to the major contributor.

6.3 A locus with two allelic peaks present (excluding DYS385a/b)

6.3.1 If at least one of the detected peaks is above the inversion threshold and the shorter peak is ≤50% of the taller peak, the taller peak may be assigned to the major contributor and the shorter peak may be assigned to the minor contributor.

6.3.2 If the detected peaks are below the inversion threshold, the taller peak may be assigned to the major contributor and the shorter peak may be assigned to the minor contributor if this shorter peak is <20% of the taller peak.

6.3.3 If the suspected allele(s) for the minor contributor is in stutter position to the major donor, the minor contributor may not be able to be assigned at this locus. Refer to the locus-specific stutter percentages for guidance in determining if the peak(s) could be elevated stutter or belong to the minor contributor. See Section 3 of Manual Appendix for PowerPlex® Y23 on 3500xL.

6.4 Interpretation guidelines for DYS385a/b:

6.4.1 Alleles may be assigned to the major and minor contributors if the following thresholds are met:

6.4.1.1 The peak height(s) of the major must be above the inversion threshold.

6.4.1.2 The peak heights between potential allele pairs are ≥75% of each other.

6.4.1.3 The peak heights of the potential shortest, non-major allele pairs are ≤50% to the potential major allele pairs

6.4.2 Caution should be taken if there is potential allele sharing, if one allele is in stutter position of the other, or in the instance where a contributor may only have a single copy allele (i.e., no allele duplication).

6.4.3 Apply caution when interpreting a minor contributor with labeled peaks below the within locus ST or samples that show a pattern of degradation. A single peak may be assigned to a donor profile if it is ≥700 RFU. If a single peak is present below 700 RFU, this locus must be marked as inconclusive.

7 Determining profiles from 3-male mixture samples

7.1 A mixture best described as originating from three males may be deconvoluted if the major is distinguishable from the other contributors using the guidelines described in this section.

7.1.1 Only deconvolution of the major donor should be attempted.

7.1.2 Caution should be taken when deconvoluting samples with DNA amounts less than optimal as these may have more variable peak heights and mixture ratios.

7.1.3 Caution should be taken when deconvoluting samples that exhibit degradation and/or inhibition.

7.1.4 Mixed samples whose ratios approach 1:1:1 or 1:1:trace should not be deconvoluted.

7.1.5 Mixtures that cannot be deconvoluted may be suitable for comparison.

7.1.5.1 At least one locus must have all contributors individually represented (with no evidence of drop out) in order for the mixture to be deemed suitable for comparison.

7.1.6 A deconvoluted major profile must have 8 fully deconvoluted loci in order to determine a Y-STR profile and assign as a male DNA donor.

7.2 If the sample is amplified at optimum amount and the major contributor is at least two times the sum of the two minor contributors (at loci with three allelic peaks) on average throughout the mixture, then the tallest peak(s) at every locus may be assigned to the major donor profile.

7.3 A locus with one allelic peak present (excluding DYS385a/b)

7.3.1 If the major contributor has a peak elsewhere in the sample above the between locus ST, the allele may be assigned to the major contributor.

7.3.2 If the major contributor does not meet the between locus ST, the allele may be assigned to the major contributor if the peak is not approaching the height of the minor contributor(s).

7.3.3 In instances where the single peak is approaching the AT with other unlabeled peak(s) present within the sample just below AT, it should not be assigned to the major contributor.

7.4 A locus with two allelic peaks present (excluding DYS385a/b)

7.4.1 The taller peak may be assigned to the major contributor if the shorter peak is ≤20% of the taller peak.

7.5 A locus with three allelic peaks present (excluding DYS385a/b)

7.5.1 The tallest peak may be assigned to the contributor if the second tallest peak is ≤25% of the tallest peak.

7.6 Interpretation guidelines for the DYS385a/b locus

7.6.1 Alleles may be assigned to the major contributor if the following thresholds are met:

7.6.1.1 The peak heights between the potential major allele pairs are ≥75% of each other.

7.6.1.2 The peak heights of the potential shortest, non-major allele pairs are ≤25% to the potential major allele pairs.

7.6.2 Caution should be taken if there is potential allele sharing, when one peak is in stutter position of another, or the instance where a contributor may only have a single copy allele (i.e., no allele duplication).

7.6.3 Apply caution when interpreting a major contributor with labeled peaks below the within locus ST or samples that show a pattern of degradation. A single peak may be assigned to the profile if it is ≥700 RFU. If a single peak is present below 700 RFU, this locus must be marked as inconclusive.

8 Determining profiles for male exemplars

8.1 A Y-STR male DNA profile will only be developed for single source exemplar samples. If there is any indication of a mixture in an exemplar, the sample should be deemed inconclusive.

8.2 A single labeled allelic peak at each locus other than DYS385a/b may be assigned to the DNA profile.

8.2.1 A minimum of 8 fully assigned loci is required to designate a Y-STR profile.

8.3 If there are two alleles at any location other than DYS385a/b, a duplication event can be considered, and both alleles may be assigned to the profile.

8.4 If there are three alleles at any location, a triplication event can be considered, and the three alleles may be assigned to the profile.

8.5 Interpretation guidelines for the DYS385a/b locus:

8.5.1 If two peaks are present, both alleles may be assigned to the exemplar profile.

8.5.2 The stochastic threshold for the DYS385a/b locus is 700 RFU. A single peak may be assigned to the exemplar profile if it is ≥700 RFU. If a single peak is present below 700 RFU, the locus must be marked as inconclusive.

9 Samples for comparison

9.1 For each Y-STR based comparison, the following conclusions can be made:

9.1.1 Comparison to a single source profile, or to a deconvoluted profile from a mixed sample:

9.1.1.1 The comparison sample matches the profile

9.1.1.1.1 All, or most of the alleles seen in the comparison sample are also labeled in the single source profile or the deconvoluted profile. Any absent or unlabeled allele(s) may be explained (see 9.1.3).

9.1.1.2 The comparison sample does not match the profile

9.1.1.2.1 The donor of a comparison sample is not the source if one or more alleles seen in the DNA profile of the comparison sample are not seen in the deconvoluted profile or single source profile, and the absence cannot be explained (see 9.1.3).

9.1.1.2.2 Caution should be taken when evaluating DYS570 and DYS576, as these loci are known to have high mutation rates which could impact the comparison.

9.1.2 Comparison to a non-deconvoluted mixture – an entire mixture, or minor non-deconvoluted contributor(s)

9.1.2.1 The comparison sample can be included as a possible contributor.

9.1.2.1.1 All, or most of the alleles seen in the comparison sample are also labeled in the evidence sample. Any absent or unlabeled allele(s) can be explained (see 9.1.3).

9.1.2.2 The comparison sample is excluded as a contributor.

9.1.2.3 The donor of a comparison sample is excluded if one or more alleles seen in the DNA profile of the comparison sample are not seen in the evidence sample, and the absence cannot be explained (see 9.1.3).

9.1.2.4 No conclusions can be drawn.

9.1.2.4.1 The phrase no conclusions can be drawn is used if the criteria for “included” or “excluded” are not met. This should be documented in the case file using the Not Suitable for Comparison/Inconclusive Form.

9.1.3 Explanations for absent or unlabeled peaks may include the following:

9.1.3.1 Amount of DNA amplified

9.1.3.2 In a mixture, a trace contributor that is present at a similar height to stutter peaks may be affected and masked by stutter filters within a sample.

9.1.3.3 Degradation and inhibition

9.1.3.4 Null alleles

9.1.3.5 Locus amplification efficiency

10 Statistics using Y-Mixture Tool by Cal DOJ

10.1 A statistical calculation should be performed if a comparison results in a match or inclusion to a mixture. Refer to the protocol: Usage of the "Y-Mix Database Filter" manual for instructions on calculating a statistic.

10.2 A statistical calculation is not required if a comparison results in an exclusion, or no conclusions can be drawn.

11 References

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