Department of Forensic Science

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FORENSIC BIOLOGY PROCEDURES MANUAL

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

INTERPRETATION OF AMPFℓSTR™ YFILER™ CE DATA

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1 GENERAL INTERPRETATION WORKFLOW

The raw AmpFℓSTR™ Yfiler™ CE data obtained from the 3500*xl* is to be analyzed using the GeneMapper® ID-X (GMID-X) software prior to following these interpretation steps. Refer to FB PM, Analysis of CE Results using GeneMapper® ID-X, if necessary.

Information regarding known artifacts to be edited out, expected size standard patterns, ladder patterns and allele calls, etc., is detailed in Chapter 2, Interpretation of AmpFℓSTR™ Yfiler™ Data, of this manual.

For the purposes of this manual:

- M/m = Major/minor
- Probative Evidence = Evidence through testing that demonstrates the proposition that a biological fluid/material may or may not have been deposited by a specific "individual of interest" who is believed to be associated with the evidence in question.

1.1 Once data is analyzed using the GMID-X software and artifacts have been edited out such that the final profile is obtained, a determination will be made as to the value of each evidence profile as a whole.

1.1.1 Evidence profiles should be assessed for the number of loci with results, number of contributors, data above/below the STH at the DYS385a/b locus, amount of visible drop out (peaks observed below LOD but clearly discernable from noise), peak heights of called alleles, etc., when making this determination.

1.1.1.1 When a profile different from a known individual is sought, that person's known reference sample may also be evaluated as 1.2 describes and used to aid in this assessment.

1.1.1.2 Any other known reference samples must, to the extent possible, be evaluated after all associated evidence samples.

1.1.1.3 Sperm and non-sperm fractions from the same sample may be considered as one sample or independently.

1.1.2 Mixture profiles determined to be of value will be deconvoluted (refer to Chapter 3 of this manual, Mixture Deconvolution Procedures), if applicable.

1.2 Once all evidence profiles in a case have been assessed and deconvoluted (if applicable), the known reference samples will be assessed for value. If no known reference samples are available, proceed to 1.5.

1.2.1 No result at more than one locus for a known reference sample requires re-typing, re-amplification or re-extraction. If DYS385a/b has a homozygous allele at or below STH, all other loci must have a result for a reference sample to be used. Obtaining a complete profile is always preferable.

1.2.1.1 Exceptions may be made for alternate known samples, if necessary.

1.3 Evidence samples deemed of value for comparison will be compared to applicable known references.

1.3.1 The results of a comparison of a known profile to an evidence profile may result in one of the following conclusions:

- The individual is eliminated.
- The individual cannot be eliminated (nor can any of his patrilineally related male relatives).
- Insufficient information exists to draw a conclusion regarding the individual as a contributor.
- Because no statistical calculations will be conducted, no conclusions will be made. (This applies to mixture profiles for which a M/m deconvolution was not possible but is deemed to be of value for comparison).

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NOTES: These conclusions apply to non-assumed known references.

These conclusions may be in reference to a single source profile, a mixture profile in its entirety or a portion of a mixture such as a deconvoluted Major/minor or the portion of a mixture different from an assumed known reference.

1.4 Once conclusions are drawn, applicable statistics will be calculated.

1.4.1 All conclusions of not eliminated require a statistical calculation.

1.4.2 Qualitative (attribution) statements may be used in lieu of a statistical calculation for assumed knowns/knowns for evidence profiles in which a contributor different from that contributor is sought.

1.5 Y-STR profiles are not suitable for searching in CODIS. In some cases, a Y-STR profile may be entered into CODIS along with a sample's autosomal STR results.

1.6 All unaccounted for profiles (except those deemed to be of no value) will be searched against the Staff Index.

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1.6.1 Profiles accounted for by the non-elimination of or attribution to a known reference profile are not searched against the Staff Index.

1.6.2 This is a manual comparison to the Staff Index Spreadsheet.

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1.6.3 A potential match to a staff member will be vetted through the Program Manager (Technical Leader) or Assistant Technical Leader.

1.6.3.1 If the match is deemed adventitious, the evidence profile will be used.

1.6.3.2 If the match candidate cannot be eliminated as having possibly contributed to the profile, the evidence profile will be deemed to be of no value due to the quality control standard not being met.

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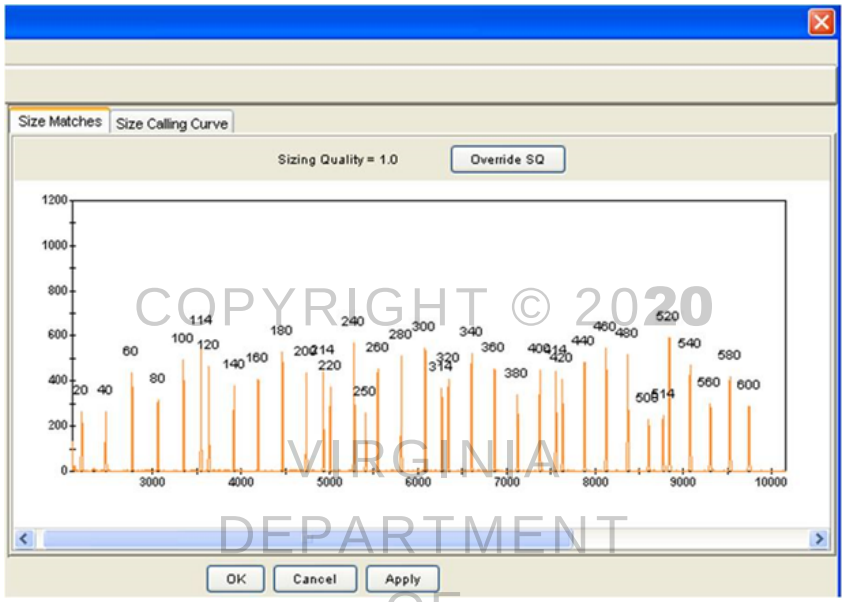
2 INTERPRETATION OF AMPFℓSTR™ YFILER™ CE DATA

2.1 Examining Internal Lane Standard Results

2.1.1 The ILS Size Standard peaks should appear as follows; however, the peaks below 60 and above 400 may be left undefined and therefore may not be labeled as shown below:



2.1.2 To assess the ILS size standard for individual samples, highlight the sample(s) and select Tools→ Size Match Editor or click on the size match editor icon.

2.1.3 Check to see that all peaks between 60 and 400 are detected and the peaks are labeled correctly. If a sample or multiple samples are flagged as having a low or failing sizing quality (yellow triangle or red octagon, respectively), refer to the FB PM, Analysis of CE Results using GeneMapper® ID-X as appropriate.

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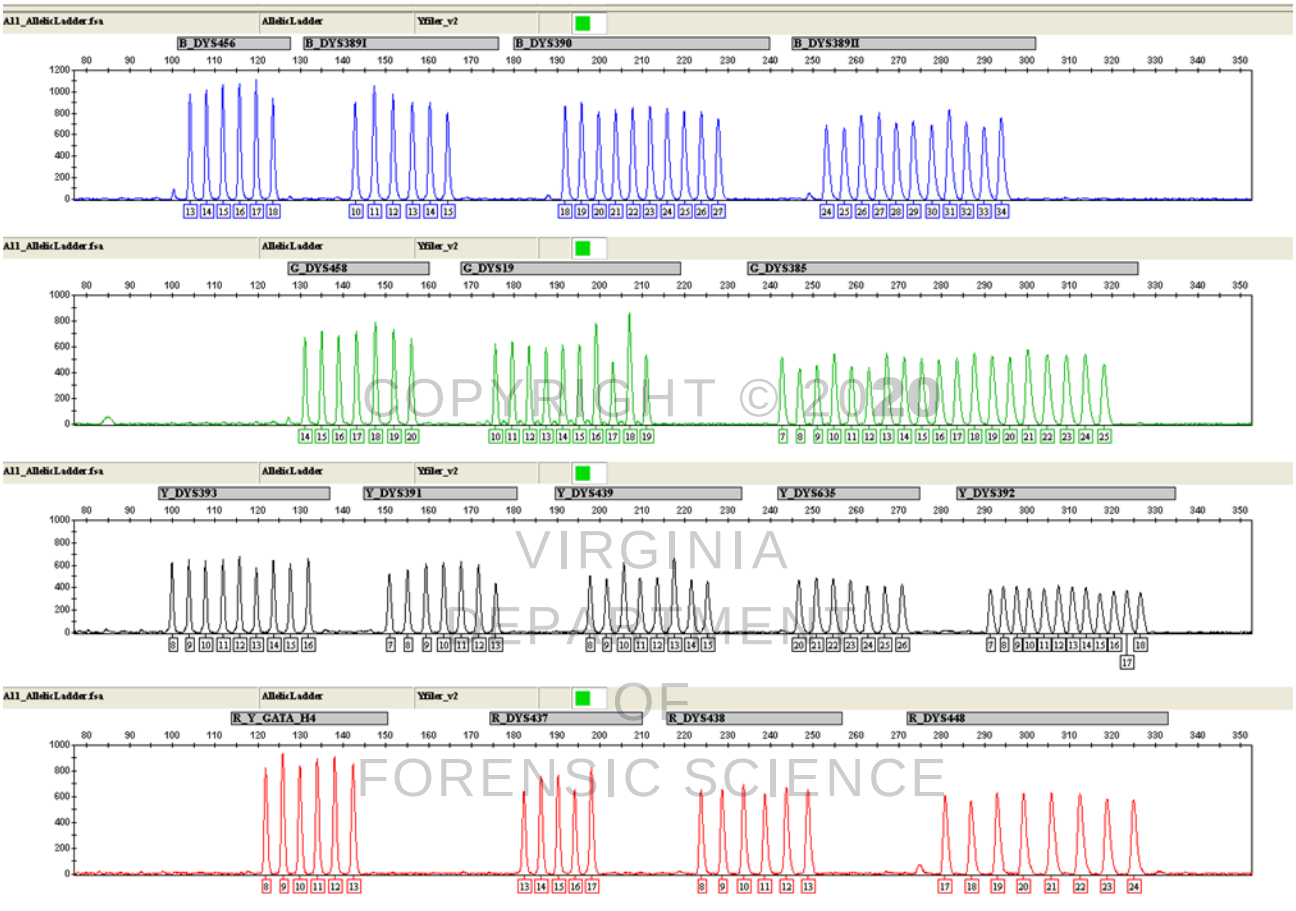
2.2 Examining Allelic Ladder Results

2.2.1 The AmpFℓSTR™ Yfiler™ Allelic Ladder should appear as follows:



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2.2.2 To display the plot for each ladder in a project, highlight the ladder(s) and select View→ Display Plots or click on the display plots icon.

2.2.3 Verify that the allelic ladder is called correctly for each locus.

2.2.4 If a ladder has injected poorly, it can either be deleted from the project or designated as a sample and the project reanalyzed, as long as another ladder remains. If necessary, a new project can be created with an acceptable ladder.

2.3 Examining the Reagent Blank(s)

2.3.1 Each reagent blank will be checked to ensure that no called alleles are observed. A called allele is a peak which fits the criteria to be designated an allele or OL (off ladder) and is above the allele calling threshold (LOD) designated in the software and therefore is labeled. Known artifacts such as spikes or pull-up are not considered called alleles.

2.3.1.1 If a single peak above LOD is observed at a single locus, the associated sample results will be considered inconclusive at that locus.

2.3.1.1.1 Alternatively, if the reagent blank is re-loaded to assess if the peak was introduced during the CE preparation of the amplified product, and no peaks are observed, the full results of the associated samples may be used.

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2.3.1.1.2 Alternatively, if, upon re-amplification of the reagent blank and the associated samples, no peaks are observed, the full results of the re-amplification of the associated samples may be used.

2.3.1.1.3 If re-amplification of the reagent blank confirms the presence of DNA in the reagent blank itself (one or more peaks observed at a different locus/loci or multiple peaks at the same locus as in the original amp), the associated sample results from both the first and second amplification will be considered inconclusive. In accordance with 2.3.1.1, if a single peak is observed at the same locus as in the original amp, that locus may be considered inconclusive.

NOTE: Exceptions to 2.3.1.1.3 may be considered by the Program Manager (Technical Leader) or Assistant Technical Leader and, if granted, must be documented in the case file.

2.3.1.2 If a peak or peaks above LOD are observed at multiple loci, the results for the associated samples will be considered inconclusive at all loci.

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2.3.1.2.1 Alternatively, if the reagent blank is re-loaded to assess if the peaks were introduced during the CE preparation of the amplified product, and no peaks are observed, the full results of the associated samples may be used.

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2.3.1.2.2 Alternatively, if, upon re-amplification of the reagent blank and the associated samples, no peaks are observed, the full results of the re-amplification of the associated samples may be used.

2.3.1.2.3 If re-amplification of the reagent blank confirms the presence of DNA in the reagent blank itself (one or more peaks observed, regardless of which locus/loci), the associated sample results from both the first and second amplifications will be considered inconclusive.

2.3.2 Each reagent blank will also be assessed for low level peaks below the defined LOD but clearly discernable from noise.

2.3.2.1 If a single peak below the defined LOD but clearly discernable from noise is observed, the results of the associated samples may be used.

2.3.2.2 If multiple peaks (more than one) below the defined LOD but clearly discernable from noise are observed, the results for the associated samples will be considered inconclusive at all loci.

2.3.2.2.1 Alternatively, if the reagent blank is re-loaded to assess if the peaks were introduced during the CE preparation of the amplified product, and no peaks above LOD or below LOD but clearly discernable from noise are observed, the full results of the associated samples may be used.

2.3.2.2.2 Alternatively, if, upon re-amplification of the reagent blank and the associated samples, no peaks above LOD or below LOD but clearly discernable from noise are observed, the full results of the re-amplification of the associated samples may be used.

2.3.2.2.3 If re-amplification of the reagent blank confirms the presence of DNA in the reagent blank itself (one or more peaks observed, regardless of which locus/loci and regardless of the peak(s) being above LOD or below LOD but clearly discernable from noise), the associated sample results from both the first and second amplifications will be considered inconclusive.

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NOTE: Exceptions to 2.3.2.2.3 may be considered by the Program Manager (Technical Leader) or Assistant Technical Leader and, if granted, must be documented in the case file.

2.3.3 The raw data for each reagent blank will also be examined to ensure that the primer peaks are observed indicating that no pipetting error occurred and that the amplified product was indeed loaded into the plate for the Genetic Analyzer.

2.4 Examining the Negative Control (Negative Amplification Control)

2.4.1 Each negative control will be checked to ensure that no called alleles are observed. A called allele is a peak which fits the criteria to be designated an allele or OL (off ladder) and is above the allele calling threshold (LOD) designated in the software and therefore is labeled. Known artifacts such as spikes or pull-up are not considered called alleles.

2.4.1.1 If a single peak above LOD is observed at a single locus, the associated sample results will be considered inconclusive at that locus.

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2.4.1.1.1 Alternatively, if the negative control is re-loaded to assess if the peak was introduced during the CE preparation of the amplified product, and no peaks are observed, the full results of the associated samples may be used.

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2.4.1.1.2 Alternatively, the entire set of samples originally amplified with the negative control, including reagent blanks, may be re-amplified. The original amplification data will not be used and the subsequent amplification data will be used assuming no peaks are observed in the subsequent negative control.

2.4.1.2 If a peak or peaks are observed at multiple loci, the results for the associated samples will be considered inconclusive at all loci and all samples, including reagent blanks, will be re-amplified. The original amplification data will not be used and the subsequent amplification data will be used assuming no peaks are observed in the subsequent negative control.

2.4.2 Each negative control will also be assessed for low level peaks below the defined LOD but clearly discernable from noise.

2.4.2.1 If a single peak below the defined LOD but clearly discernable from noise is observed, the results for the associated samples may be used.

2.4.2.2 If multiple peaks (more than one) below the defined LOD but clearly discernable from noise are observed, the results for this amplification of the associated samples will be considered inconclusive and the samples, including reagent blanks, will be re-amplified. The subsequent amplification data will be used assuming no peaks are observed in the subsequent negative control.

2.4.3 The raw data for each negative control will also be examined to ensure no pipetting error occurred and that the amplified product was indeed loaded into the plate for the Genetic Analyzer.

2.5 Examining the Positive Control (Positive Amplification Control)

2.5.1 The positive amplification control DNA supplied with the AmpFℓSTR™ Yfiler™ kit is 007. The correct types are as follows:

Locus	Haplotype 007
DYS456	15
DYS389I	13
DYS390	24

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DYS389II	29
DYS458	17
DYS19	15
DYS385a/b	11,14
DYS393	13
DYS391	11
DYS439	12
DYS635	24
DYS392	13
Y GATA H4	13
DYS437	15
DYS438	12
DYS448	19

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2.5.2 If a positive control has injected poorly, it can be re-injected or re-prepared for the CE. The original sample injections may be used and interpreted as long as all of the correct types for the positive control are obtained upon re-injection/re-preparation.

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2.5.3 If incorrect types are obtained for any locus or if types are missing from any one locus, all samples, including all reagent blanks, originally associated with this positive control will be re-amplified.

2.5.3.1 For instances in which the Y-STR testing was consumptive and re-amplification is not possible, the positive control results may be evaluated by the Program Manager (Technical Leader) or Assistant Technical Leader for potential use on a case by case basis.

2.6 Examining Casework Samples

2.6.1 If it is determined that a sample contains elevated stutter peaks at a majority of the loci, there are off-scale peaks and other artifacts visible throughout the electropherogram due to injecting too much and/or amplifying too much sample DNA, the sample may be diluted and re-injected, re-injected using a reduced injection time and/or using less amplified DNA in the injection cocktail. Samples may also be re-amplified using a reduced amount of template DNA and then re-typed. If, however, the accurate profile can be determined from the original sample profile by both the analyst and the independent technical reviewer and the profile is single source, then the data may be used.

2.6.1.1 Off-scale data will not be used for mixture samples.

2.6.1.1.1 Exceptions may be made if a major profile is deconvoluted for use and the minor portion of the mixture is deemed to be of no value or if the deconvolution of a contributor different from the assumed known in a two person mixture is unaffected by possible elevated stutter and/or pull up due to an off-scale peak.

2.6.2 Each sample must be reviewed carefully and any artifacts, such as pull-up, stutter/elevated stutter, spikes, etc., identified and labeled appropriately. Refer to the FB PM, Analysis of CE Results using GeneMapper® ID-X as needed.

2.6.2.1 Incomplete +A nucleotide addition is indicated by a peak or apparent shoulder one base pair shorter than the true peak. The PCR process using AmpliTaq Gold® is optimized such that an additional adenosine nucleotide is added onto the extended fragment. Excessive input DNA makes the adenosine addition less efficient and thus PCR fragments are shorter by one nucleotide than the true amplicon size (-A).

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2.6.2.2 Pull-up refers to peaks that are not true alleles but result from poor color separation of the raw data or off-scale data in one or more channels. Repeated excessive pull-up indicates the need to perform a spectral calibration on the instrument.

2.6.2.3 Stutter peaks are most commonly 2, 4 and 8 nucleotides smaller or larger than the amplicon size (true peak). Careful consideration must be made when differentiating stutter peaks and a possible mixture. Stutter is typically more pronounced when excessive quantities of DNA are placed into the amplification cocktail. Not all of the loci contained within the AmpFℓSTR™ Yfiler™ System kit consist of four nucleotide repeats; the DYS392 locus consists of a three nucleotide repeat, the DYS438 locus consists of a five nucleotide repeat and the DYS448 locus consists of a six nucleotide repeat. Thus, stutter peaks are typically multiples of the locus repeat lengths; DYS392 will display stutter that is three nucleotides, DYS438, five nucleotides and DYS448, six nucleotides different in size. The expected stutter percentages listed below are based upon internal validation by the Department.

2.6.2.3.1 Peaks in the N-1 or N+1 position that fall below the percentages listed below must be edited out as stutter. The N-1 and N+1 stutter percentages are included in the GMID-X analysis method in use and are automatically applied.

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2.6.2.3.2 The N-2 stutter percentages are not automatically applied by the GMID-X software. Peaks in the N-2 position should be evaluated individually and may or may not be called stutter when below the percentage listed below.

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2.6.2.3.3 If a peak is observed between two larger peaks (1 repeat smaller than the larger peak and 1 repeat larger than the smaller peak), the maximum RFU value expected when combining the N-1 percentage for the larger peak and the N+1 percentage for the smaller peak must be calculated. The center peak, if below this RFU value, must be edited out and labeled as stutter.

Locus	N-2	N-1	N+1
DYS456	2.0	16	4.0
DYS3891	1.0	10	1.0
DYS390	2.0	14	3.0
DYS389II	4.0	23	4.0
DYS458	2.0	17	5.0
DYS19*	1.0	11	3.0
DYS385a/b	2.0	20	3.0
DYS393	3.0	13	3.0
DYS391	2.0	11	2.0
DYS439	1.0	11	5.0
DYS635	3.0	13	6.0
DYS392	4.0	20	11.0
Y_GATA_H4	2.0	11	3.0
DYS437	2.0	9	3.0
DYS438	2.0	6	2.0
DYS448	1.0	6	2.0

*DYS19 also exhibits both n-(2bp) ST and n+(2bp) ST. The following expected percentages are also automatically applied by the GMID-X software:

Locus	n-(2bp)	n+(2bp)
DYS19	13	4.0

2.6.2.4 Spikes are CE-related artifacts in which minor voltage changes or urea crystals passing by the laser can cause unexpected peaks. Spikes sometimes appear in one channel but often are

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easily identified by their presence in more than one channel at the same location. Spikes are typically characterized by their narrow width. Although GMID-X applies an algorithm to remove most spikes, some must be removed manually. Also, software identified spikes should be evaluated to be sure they are truly spikes.

2.7 Microvariant / Off Ladder Variant Interpretation and Nomenclature

2.7.1 If a peak is labeled as off ladder (OL) or is outside the ladder region and therefore not labeled by the GMID-X software or labeled "OMR" for out of marker range by the GMID-X software, review the data to determine that it is a true microvariant (MV) or off-ladder (OL) allele. True OL or MV peaks may be confirmed through re-injection or re-amplification, if necessary.

NOTE: Peaks outside of a locus labeled OMR by GMID-X and determined to be real OL peaks should be addressed as described in 2.7.3-2.7.6. Once those instructions have been followed, they can be 'assigned' to their proper locus in GMID-X by left-clicking the appropriate locus name to highlight it, left-clicking the peak to highlight it, right-clicking on the peak label→ Add Allele Label→[type in your allele call designation as described below].

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2.7.1.1 If multiple OL calls are made within one electropherogram, it may indicate an issue with the ladder(s) used for sizing. If this is the case, re-analysis by the software may be necessary.

2.7.1.2 The peak in question may be an artifact such as pull-up or a spike. If this is the case, edit the peak out and label it appropriately.

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2.7.2 If the peak is visually between two allelic ladder peaks of the same locus (a MV), assign an allele designation of the lower repeat value followed by the number of bases in the incomplete repeat.

EXAMPLE: An allele that migrates one base pair below the DYS456 14 allele will be designated as a DYS456 13.3. The "off ladder" value on the elecropherogram will be manually changed to reflect the allele designation.

2.7.2.1 To document that the proper allele call has been designated, the sample electropherogram and ladder electropherogram will be highlighted together and the plots displayed. Deselect all color channels except the one in which the locus in question exists and show two panes. Magnify the locus in question. The bins should be shown to better demonstrate where the MV falls. A printout of this documentation will be maintained in the case file.

2.7.3 If the peak is seen to the right of the largest ladder peak of the largest MW locus, assign the allele to the largest MW locus and assign an allele designation of >X, where X is the largest ladder peak in the largest MW locus.

NOTE: If a virtual bin exists in the GMID-X software that falls above the largest ladder peak, the allele designation will be >Z, where Z is the largest virtual bin peak designation in the largest MW locus.

2.7.4 If the peak is seen to the left of the smallest ladder peak of the smallest MW locus, assign the allele to the smallest MW locus and assign an allele designation of <X, where X is the smallest ladder peak in the smallest MW locus.

NOTE: If a virtual bin exists in the GMID-X software that falls below the smallest ladder peak, the allele designation will be <Z, where Z is the smallest virtual bin peak designation in the smallest MW locus.

2.7.5 If the peak is seen between two loci and either the locus to the right OR left of the peak contains no peak (for a single source sample), the allele will be considered to belong with the locus not containing a peak. The assignment of the allele designation will be based upon the nomenclature referenced below.

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2.7.5.1 If the allele is to the right of the largest ladder peak of the locus to which it has been assigned, it will be assigned the designation >X, where X is the largest ladder peak in the assigned locus.

NOTE: If a virtual bin exists in the GMID-X software that falls above the largest ladder peak in the assigned locus, the allele designation will be >Z, where Z is the largest virtual bin peak designation in the assigned locus.

2.7.5.2 If the allele is to the left of the smallest ladder peak of the locus to which it has been assigned, it will be assigned the designation <X, where X is the smallest ladder peak in the assigned locus.

NOTE: If a virtual bin exists in the GMID-X software that falls below the smallest ladder peak in the assigned locus, the allele designation will be <Z, where Z is the smallest virtual bin peak designation in the assigned locus.

2.7.6 If the peak is seen between two loci and neither of the surrounding loci have a peak (for a single source sample) OR the sample is a mixture:

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2.7.6.1 The base pair size for the allele in question will be compared to the base pair values for the largest allelic ladder peak (or virtual bin designation, as applicable) of the lower molecular weight locus and to the smallest allelic ladder peak (or virtual bin designation, as applicable) of the higher molecular weight locus.

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2.7.6.2 The physical location of the allele in question with respect to the surrounding loci will be evaluated.

2.7.6.3 An evaluation of the RFU values of the peak and loci in question may also be helpful.

2.7.6.4 The allele in question will be assigned to the locus with which it falls within an appropriate size distance (full repeat(s) away from the closest ladder peak/virtual bin designation). If it is within an appropriate size distance of both loci, it will be deemed inconclusive.

2.7.6.5 The allele will then be assigned the designation >X or <X as follows:

2.7.6.5.1 If the allele is to the right of the largest ladder peak of the locus to which it has been assigned, it will be assigned the designation >X, where X is the largest ladder peak in the assigned locus.

NOTE: If a virtual bin exists in the GMID-X software that falls above the largest ladder peak in the assigned locus, the allele designation will be >Z, where Z is the largest virtual bin peak designation in the assigned locus.

2.7.6.5.2 If the allele is to the left of the smallest ladder peak of the locus to which it has been assigned, it will be assigned the designation <X, where X is the smallest ladder peak in the assigned locus.

NOTE: If a virtual bin exists in the GMID-X software that falls below the smallest ladder peak in the assigned locus, the allele designation will be <Z, where Z is the smallest virtual bin peak designation in the assigned locus.

2.8 Limit of Detection and Stochastic Thresholds

The limits of detection and stochastic threshold for DYS385a/b in use were derived from internal validation by the Department.

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2.8.1 Limit of Detection (LOD)

2.8.1.1 The LOD distinguishes peaks attributable to signal (amplified DNA) from those attributable to noise. If a peak does not reach the height of the LOD, it will not be labeled by the GMID-X software and will not be used as a called allele in the resulting profile.

2.8.1.2 The limit of detection (LOD) for each dye channel is shown below, with the exception of the orange (ILS) dye channel. The orange LOD default will be set to 50 RFU, but can be adjusted as needed to capture all ILS peaks in a sample.

Channel Color	LOD (RFU)
Blue	40
Green	50
Yellow	90
Red	120

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2.8.2 Stochastic Threshold

2.8.2.1 The Stochastic Threshold (STH) is a height (RFU value) above which one can expect that, in most instances, both peaks of a heterozygote at the multi-copy locus DYS385a/b will be observed. If the height of a homozygous peak is at or below this threshold, there is a possibility that a true sister peak has dropped out. The application of this value to casework analysis is designed to reduce the incidences of calling a false homozygote.

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2.8.2.2 The stochastic threshold (STH) for DYS385a/b is 150 RFU for 12 second injections and 315 RFU for 24 second injections.

2.9 General Interpretation of Single Source Samples

- 1 allele is detected per locus with the exception of DYS385a/b (exceptions for duplications can be made).
- DNA typing results are required at 4 or more loci before comparisons are conducted.

  EXCEPTION: Intimate samples (samples removed directly from body of a person) have no minimum number of loci with typing results for attribution to be applied.

- Possible allelic drop out can occur for data that is detected at or below the STH for DYS385a/b.
- Statistics are calculated at all loci where DNA typing results are obtained.
  - Because the Y-Chromosome STR Haplotype Reference Database (YHRD) requires [Allele, Allele] format at DYS385a/b and does not allow for the incorporation of a potentially missing allele in the search, results at this locus will be dropped from the statistical calculation if a homozygous allele at or below STH is observed.

2.10 General Interpretation of 2 Person Mixtures

- 1-2 alleles are detected per locus with the exception of DYS385a/b.
- A M/m deconvolution must be possible for a non-elimination to be reported and a statistic to be calculated.
  - Comparisons can be conducted to either the major or minor portion of a mixture.
    - For comparisons to a minor portion of a mixture, at least 4 distinct minor alleles must be present.
- Mixtures for which no M/m deconvolution can be conducted:
  - A conclusion of eliminated may be reached and reported.
  - A conclusion that insufficient information exists to draw a conclusion regarding a particular person may be reached and reported.
  - Because no statistics will be conducted, a conclusion of not eliminated will not be reported.

2.11 General Interpretation of >2 Person Mixtures

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- >2 alleles are detected at a locus with the exception of DYS385a/b.
- A M/m deconvolution must be possible for a non-elimination to be reported and a statistic to be calculated.
  - Non-eliminations can be reported only for the major portion of the mixture.
- Mixtures for which no M/m deconvolution can be conducted:
  - A conclusion of eliminated may be reached and reported.
  - A conclusion that insufficient information exists to draw a conclusion regarding a particular person may be reached and reported.
  - Because no statistics will be conducted, a conclusion of not eliminated will not be reported.

2.12 General Interpretation of Profiles from Intimate/Ownership Samples (Use of an Assumed Known)

Intimate samples will be defined as samples having come directly from or having been directly removed from the body of a person (i.e., vaginal swabs, fingernail scrapings, underpants removed by a clinician during the collection of a PERK, suspect clothing documented to have been removed by law enforcement, etc.).

Ownership Samples will be defined as samples for which a relatively certain assumption can be made that the owner/user's DNA profile will be detected (i.e., personal cell phone, etc.)

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Crime scene samples for which a profile different from a known individual is sought may be interpreted similarly to intimate/ownership samples, if applicable.

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- A general mixture approach may be used to interpret any intimate/ownership items and will, in most cases, be preferable.
- Alternatively, in rare instances, DNA types from an assumed known contributor may be subtracted or dosage considered when determining which types are different from that contributor at a locus (for 2 or 3 person mixtures).
- For 2 person mixtures (1 assumed known and 1 unknown contributor)
  - Results must be obtained for all loci in order to use an assumed known approach.
  - 4 or more loci with results different from the assumed known's are required for a non-elimination to be reported and statistics to be calculated.
- For 3 person mixtures
  - A general mixture approach will be used.

Exceptions to allow for the use of an assumed known approach may be considered on a case by case basis by the Program Manager (Technical Leader) or Assistant Technical Leader and, if granted, must be documented in the case file.

2.13 Interpretation of Criminal Paternity/Maternity Cases, Missing Person Cases, and Familial Search Comparisons

A single inconsistency may be observed without declaring the individual as eliminated as the source of genetic material. However, statistical analysis must be performed to incorporate the possibility that a mutation occurred. Mutations typically result in a full repeat difference, larger or smaller, for the allele. If more than one inconsistency is observed, then an inconclusive determination is made. Three inconsistencies in the Y-STR haplotype is an exclusion, even if the autosomal STR profile indicates an inclusion.

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3 MAJOR/MINOR OR MAJOR DECONVOLUTION PROCEDURE

3.1 A clear predominant profile should be observed across the entire profile in order for a M/m or major deconvolution approach to be used.

3.2 A minor profile will not be deconvoluted for mixtures with more than 2 contributors. If the mixture, as a whole, is deemed suitable for comparison, it may be more appropriate to use a general mixture approach.

3.3 If the minor contributions are limited, using the major deconvolution and deeming the minor to be of no value may be more appropriate.

3.4 At least one allele must be above STH in order for M/m to be determined at the DYS385a/b locus.

3.5 For loci with both major and minor contributions, the peak height of the highest minor peak must be 33% or less of the peak height of the major peak in order for M/m to be determined.

3.6 If two or more loci with more than one allele do not meet the 33% rule in 3.5, no major may be deconvoluted.

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3.7 Examples of deconvoluted loci are below:

Parentheses may be used to document minor alleles on an electropherogram or the landscape at the examiner's discretion; however they will not appear in the Table of Typing Results when included with a Certificate of Analysis.

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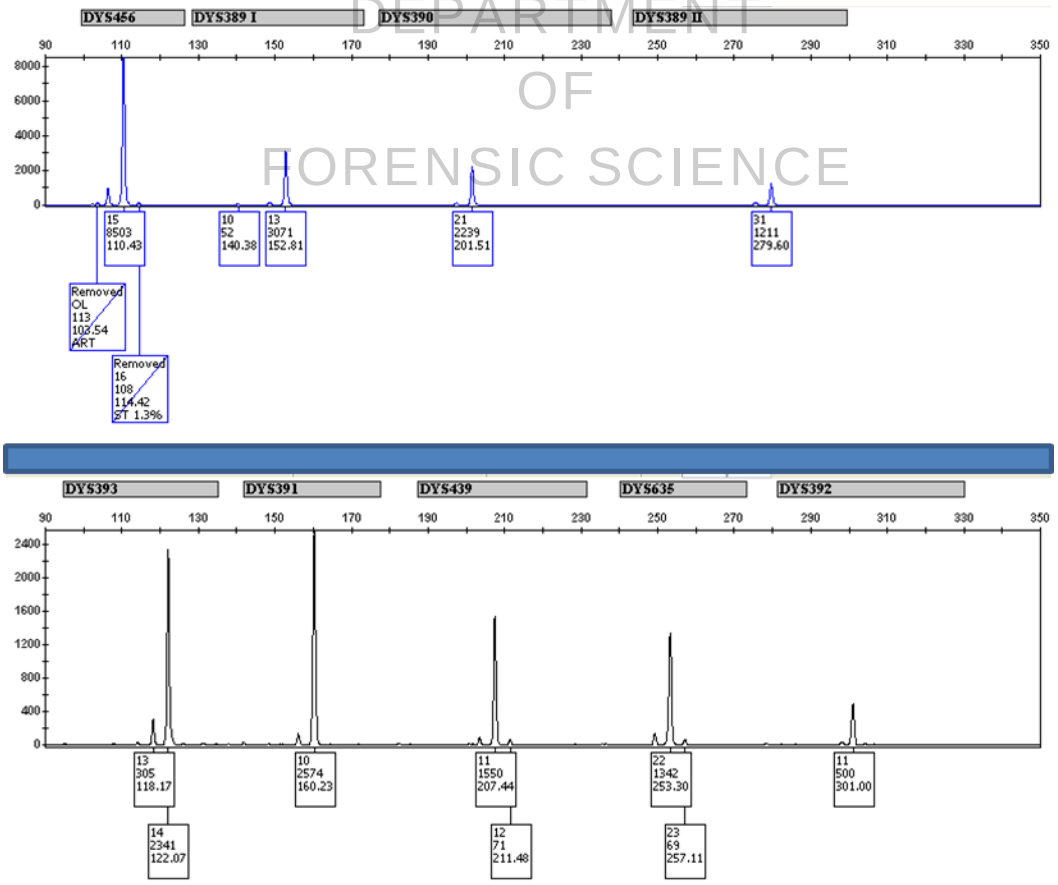

DYS456:	M: 15	/	no minor
DYS389I:	M: 13	/	m: 10
DYS390:	M: 21	/	no minor

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DYS389II:	M: 31	/	no minor
DYS393:	M: 14	/	m: 13
DYS391:	M: 10	/	no minor
DYS439:	M: 11	/	m: 12
DYS635:	M: 22	/	m: 23
DYS392:	M: 11	/	no minor

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4 STATISTICAL CALCULATIONS

Statistical calculations are required to be conducted and reported in the same Certificate of Analysis for any conclusion of not eliminated.

Qualitative (attribution) statements may be used in lieu of a statistical calculation for assumed knowns.

All loci analyzed by the AmpFℓSTR™ Yfiler™ kit are linked on the Y chromosome; therefore, the entire Y chromosome haplotype must be treated as a single locus. Given the male lineage pattern of inheritance, haplotype frequencies are estimated using the counting method.

The database used for estimating the haplotype frequency is located at: https://yhrd.org

For information regarding the database statistics and calculation methods used, see the Resources tab of the above referenced website.

The number of individuals in which the Y-STR haplotype would be expected to be observed will be truncated to 2 significant figures.

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Only single source male profiles or a deduced single source male profile (i.e., the major/minor contributor to a mixed male profile where a clear major/minor contributor can be discerned or a profile different from an assumed known contributor) will be searched in the database and a haplotype statistic calculated.

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When performing a Y-STR Paternity Index, Missing Person calculation or Sibling Index on a partial DNA profile it is possible that an individual or an evidence sample may be identified that cannot be eliminated as a possible parent/offspring/sibling or have originated from the offspring/parent/sibling. However the individual's DNA profile or the evidence sample is missing an allele at a particular locus that is observed in the father/child/sibling's DNA profile. The allele may be missing as a result of allelic/locus drop out, mutation at the primer binding site resulting in a null allele or possibly the deletion of a portion of the Y-chromosome. To account for the possibility of allelic/locus drop out, the locus will not be used in the overall calculation.

4.1 Generating a Y Haplotype Frequency Estimate

4.1.1 Go to https://yhrd.org

4.1.2 Select Search the Database in the top ribbon menu.

4.1.3 Choose to import a file or to manually enter the haplotype/haplotypes to search.

4.1.3.1 If choosing to import a file, click on that option, navigate to the appropriate file and import it.

4.1.3.2 If choosing to manually enter the haplotype to be searched, click on that option.

4.1.3.2.1 Yfiler will be the default kit selected. Manually enter the appropriate alleles.

4.1.3.3 Verify that the DYS385a/b locus is entered in "allele, allele" format, which is required by the YHRD.

4.1.3.3.1 If a single 14 allele above the STH is observed, the entry will be 14, 14.

4.1.3.3.2 If a single allele below STH is observed, the locus will be dropped from the calculation.

4.1.4 Click Search.

4.1.5 Click "Add feature to this Report" and then "National Database (with Subpopulations, 2014 SWGDAM-compliant)".

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4.1.6 Close and remove the Worldwide portion of the report by clicking the "x" in the right hand side of the Worldwide ribbon.

4.1.7 To print the report, right click on the page and select Print.

4.2 Incorporation of the Y-STR Haplotype into the Autosomal Calculation for the Paternity Index (PI)

The Paternity Index is the ratio of the chance that the mother and a man of the Alleged Father's phenotype produced the child (passed the obligate allele; haplotype for Y-STRs) compared to the chance that the mother and a random man produced the child (passed the obligate allele; haplotype for Y-STRs).

4.2.1 A Paternity Index (PI) can be calculated using the Y-STR haplotype frequency as follows:

1/haplotype frequency (with the 95% upper bounds confidence interval)

For the YHRD search that provided the following result: 95% UCI*: 1 in 2,377, the PI for the Y-STR haplotype ($PI_{Y\text{-}STR}$) would be 2,300 (the 95% upper confidence interval value truncated to 2 significant figures).

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4.2.2 The PI for the Y-STR haplotype can be included in the overall Combined Paternity Index (CPI) as follows:

$$CPI = PI_1 \times PI_2 \times \ldots PI_n \times PI_{Y\text{-}STR}$$

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4.2.3 A mutation may be suspected when the Y-STR haplotype shows a one locus difference between alleged father and male child/fetus. This difference is typically one repeat larger or smaller than the alleged father's allele at that locus. If mutation is suspected, the following formula is applied to adjust the Paternity Index (PI) to account statistically for the potential mutation:

$$PI_{Y\text{-}STR} \approx m\bar{u}/2f$$

Where $\bar{u}$ is the average Y-STR mutation rate, m is the number of meioses that have occurred between the child's Y chromosome and that of the alleged paternal relative (e.g., father to son is one, grandfather to grandson is two) and f is the frequency of the child's haplotype.

4.2.3.1 The average mutation rate for the Y-STR haplotype, μ, is approximated at 0.0021.

4.3 Incorporating the Y-STR Haplotype into the Autosomal Calculation of Sibling Indices (SI)

The Y-STR haplotypes for both male individuals must be consistent, unless a mutation is suspected at one locus. If mutation is suspected, the ($SI_{Y\text{-}STR}$) must statistically incorporate the potential mutation as described in 4.2.3

4.3.1 The following formula will be used to calculate the SI for a Y-STR haplotype incorporating the :

$SI_{Y\text{-}STR} = PI_{Y\text{-}STR}$ = 1/haplotype frequency (with the 95% upper bounds confidence interval)

For a YHRD search that provided the following result: 95% UCI*: 1 in 2007, the $SI_{Y\text{-}STR}$ would be 2,000 (the 95% upper-confidence interval value truncated to 2 significant figures).

4.3.2 The Combined Sibling Index is calculated by multiplying together each independently derived Sibling Index as demonstrated below:

$$SI = SI_1 \times SI_2 \times \ldots SI_n \times SI_{Y\text{-}STR}$$

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4.4 Incorporating the Y-STR Haplotype into a Missing Person Calculation

A Missing Person Calculation may be performed when trying to determine if remains or other biological evidence could have come from the offspring of a known set of parents (both alleged mother's and father's genotypes are known). Since the Y-STR haplotype can only be assessed using male individuals, a comparison of both the alleged father's Y-STR haplotype and that of the evidence must be made. If the profiles are consistent, then a paternity index may be calculated using the Y-STR haplotype of the evidence following the procedure outlined in section 4.2. The $PI_{Y\text{-}STR}$ calculated can then be incorporated into the autosomal Missing Person likelihood ratio using the product rule as described in section 4.3 for the Sibling Index.

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APPENDIX A – REFERENCES

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