Department of Forensic Science

FORENSIC BIOLOGY PROCEDURES MANUAL

INTERPRETATION OF POWERPLEX® FUSION CE DATA

TABLE OF CONTENTS

1 GENERAL INTERPRETATION WORKFLOW

The raw PowerPlex® Fusion 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 PowerPlex® Fusion CE Data, of this manual.

For the purposes of this manual:

- URM = uRMP
- Traditional statistical calculations include any statistical calculation other than that generated by STRmix™ or TrueAllele® (URM, CPI, RM, traditional LR)
- M/m = Major/minor
- Probative Evidence = Evidence through testing that demonstrates the proposition that a biological fluid may or may not have been deposited by a specific "individual of interest" who is believed to be associated with the evidence in question.
- Breakout loci = loci at which a contribution from each contributor is definitely seen (3 or 4 alleles for 2 person mixtures, 5 or 6 alleles for 3 person mixtures)

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 contributors, amount of data above/below the STH, number of breakout loci based upon the assumed number of contributors for mixtures, amount of visible drop out (peaks observed below LOD but clearly discernable from noise) and with the applicable requirements listed in 2.10-2.16 below in mind in making this determination.

1.1.1.1 When determining the number of contributors to a mixture, more discriminating loci with fewer alleles OR less discriminating loci with full breakout OR total allele number in the mixture should be considered. There will be 3 person mixtures with only up to 4 alleles at any given locus and there will be 4 person mixture profiles with only up to 6 alleles at any given locus. Amount of total data along with peak height ratios and the information listed in 1.1.1 need all be considered.

1.1.1.2 Assumed known reference samples may be evaluated as 1.3 describes and used to aid in this assessment.

1.1.1.3 When a profile different from a known individual is sought, that person's known reference sample may also be evaluated as 1.3 describes and used to aid in this assessment.

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

1.1.1.5 Sperm and non-sperm fractions (fractions 2 and 1, respectively) 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.

1.1.2.1 Mixture profiles for which a CPI calculation will be conducted or that will be referred for probabilistic genotyping will not be manually deconvoluted; however, the assumption of the number of contributors and the intent to perform a CPI or refer for probabilistic genotyping will be documented on the electropherogram or landscape.

1.1.3 More than one deconvolution approach may be documented for possible future use, if desired (M/m and a URM, for example). However, it is preferable to avoid using both a traditional (i.e., major, minor, URM, CPI) statistic and probabilistic genotyping (i.e., STRmix™ or TrueAllele®) on the same mixture. If a mixture is developed for which it is clear a probabilistic genotyping referral will be made, the referral should be made to address all pending statistical comparisons/calculations, if possible.

EXAMPLES: A 2 person mixture for which one person of interest is not eliminated as the major and a second person of interest is not eliminated as the minor – may use Maj stats AND minor stats OR can choose to simply do a URM in regard to both people. The report wording should reflect what type of non-elimination is chosen. It is not acceptable, for example, to report a non-elimination as a Major and then only provide a URM stat.

A 3 person mixture for which one person is not eliminated as the major and a second person is not eliminated from the mixture as a whole, may use Maj stats in regard to first person and a URM_3 in regard to person 2.

1.2 Inter-comparisons of evidence profiles is not required and should only be conducted when doing so serves to further clarify the results for the reader of the Certificate of Analysis.

1.2.1 In certain instances, if multiple mixture profiles in a case are evaluated and appear to be similar, have a common contributor, or have a contributor in common with a single source profile also developed in the case, a single mixture profile may be taken through the remaining workflow. If the single source profile ***is of the most probative value***, all mixtures may be discontinued. The unselected mixtures, if this option is chosen, will remain available for further interpretation, if necessary or if requested.

1.2.1.1 This will only be done if the common contributor is what is of probative value to the case. If the remainder of a mixture/portion not attributable to this common contributor could provide investigative information to the case, the mixture workflow will not be stopped.

1.2.1.2 When choosing which mixture profile proceeds, the informative nature of the sample will be considered first and the complexity of the mixture profiles will be considered second. If equally informative samples are disparate in their complexity, the mixture allowing for the less complex interpretation will be chosen.

EXAMPLES: A vaginal/cervical (VC) sample profile is a mixture of 3 people. A perianal buttocks (PAB) sample is a mixture of 2 people. A sperm fraction (F2) from a pillow stain from the same case is single source. The single source sperm donor is included in both mixtures. The VC mixture, along with the pillow stain should continue through the process, as it is the most probative sample. The PAB sample interpretation may be discontinued at this time, but maintained for future use, if necessary.

Three trace DNA swabs from a home invasion yield mixtures. The three mixtures appear to have a common contributor who is not the homeowner. Any one of the three mixture profiles may proceed while the other two will remain available for further interpretation, if necessary or if requested. In this instance, the mixture which allows for the least complex/most informative interpretation should be chosen. If one mixture allows for the deconvolution of a Major contributor who is not the homeowner, but the other two mixtures require a URM, the one with the Major contributor should be chosen.

1.3 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 1.6.

1.3.1 An inconclusive result or no result at more than one locus for a known reference sample requires re-typing, re-amplification or re-extraction. Only one locus may have no result or have a homozygous allele below STH for a reference sample to be used. Obtaining a complete profile is always preferable.

EXCEPTIONS: Body Identification and cold cases may use a partial known profile when repeated attempts to obtain a full profile have been unsuccessful.

1.3.1.1 If the profile obtained for an alternate known sample is partial, a request for a traditional known sample will be made.

EXCEPTION: Body Identification cases may use a partial alternate known profile, if necessary.

Other exceptions may be considered on a case by case basis by the Program Manager (Technical Leader) and/or Assistant Technical Leader.

1.3.1.2 If the profile obtained for an alternate known sample is a mixture and no additional information supports the decision to use the major/minor portion of the mixture (or no M/m can be deconvoluted), the alternate known will not be considered as a known sample, but rather as an additional evidentiary item, and a traditional known sample (or samples from parents/offspring) will be requested.

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

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

- The individual is eliminated.
- The individual cannot be eliminated.
- Insufficient information exists to draw a conclusion regarding the individual as a contributor (INC re: individual).
- Because no traditional statistical calculations can be conducted, no conclusions will be made. (This generally applies to samples on which a STRmix™ System or TrueAllele® analysis will not be conducted or when the inclusion is not probative and a CPI, RM, URM or LR using POPSTATS cannot be calculated).

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.

If a conclusion that an individual cannot be eliminated OR that insufficient information exists to draw a conclusion regarding an individual as a contributor is made with regard to a mixture profile that will be referred for TrueAllele® analysis, no conclusions will be reported by the primary examiner. The conclusion(s) and statistical analysis, if applicable, will be reported by the TrueAllele® examiner.

If a conclusion of INC re: an individual is made with regard to a sample/mixture, **AND** that individual is either eliminated or INC with regard to all other evidence samples in the case, no conclusions will be reported by the primary examiner for the most informative/probative sample for which the individual is INC, and that sample will be referred for TrueAllele® analysis.

1.5 Once conclusions are drawn, applicable statistics will be calculated or samples referred for probabilistic genotyping or for kinship statistics for conclusions and statistics.

1.5.1 All conclusions of not eliminated with regard to a known reference sample require a statistical calculation within the same Certificate of Analysis.

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

1.6 The evidence profiles will be evaluated for searching/entry into CODIS, if applicable. Refer to the FB PM, CODIS Operating Policies and Procedures Manual, if necessary.

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

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

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

1.6.1.1.2 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.

1.6.1.1.3 After technical review of the file is complete, the staff profile will be redacted and that redaction will be dated and initialed.

1.6.2 A profile deemed suitable for entry into CODIS need not match a deconvoluted profile exactly.

EXAMPLE: A predominant profile is observed throughout a mixture, but when the Major is deconvoluted, multiple loci are not included due to peak height ratio discrepancies or a failure to meet the 33% rule. The overall predominant profile may be searched/entered instead of the deconvoluted Major if agreed upon by both the examiner and CODIS reviewer. This may result in a full forensic unknown predominant profile or a partial profile if only the largest peak at a locus/multiple loci are used.

2 INTERPRETATION OF POWERPLEX® FUSION CE DATA

The amelogenin and DYS391 loci will be used only as an aid in indication of gender and do not apply to any locus/allele counts or other interpretation rules detailed in this manual.

In addition, the amelogenin and DYS391 loci will not be used for statistical purposes in any case.

The D12S391 locus, because of the complexity of its repeats, will sometimes exhibit a migration issue. Therefore, in comparing profiles, any allele call at the D12S391 locus may be up to 1 bp different between samples and still be considered to match.

EXAMPLE: Evidence profile types as 17,18.3. Known for comparison types as a 17,18.2. These samples match at the D12S391 locus.

The D12S391 locus will not be used for statistical purposes in any case.

EXCEPTION: For certain kinship statistical analyses, vWA may be dropped from a calculation in order to include D12S391, as addressed in Section 4.8 of this manual.

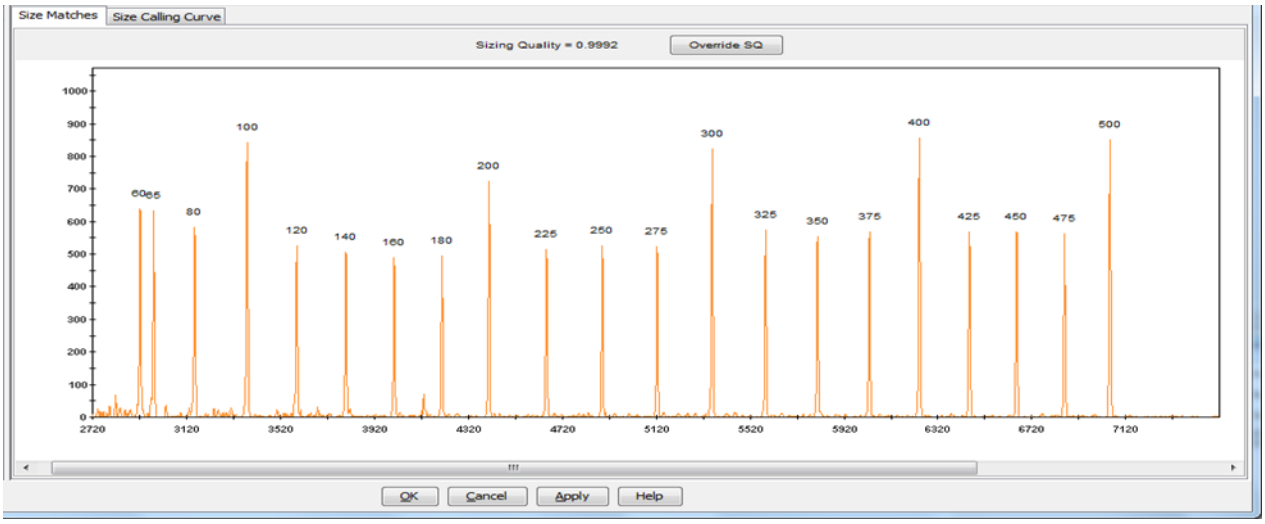
Where the procedures below indicate that statistics will be calculated at all loci with results, D12S391, DYS391 and amelogenin do not apply and are ignored (aside from the EXCEPTION stated above for kinship cases).

Counting drop out in regard to interpretation guidelines and requirements detailed in this manual will be conducted as described in the following examples:

- A locus is deconvoluted as 12x due to the presence of a 12 allele below the stochastic threshold. If the reference is a 12,12, there is no drop out. If the reference is an 11,12, this counts as partial drop out.
- A locus is deconvoluted as 10,10; 10,13; or 13,13. There is a 9 peak which was called stutter. If the reference is a 9,10, this counts as a partial drop out.
- A locus is deconvoluted as 10,12. If the reference is a 15,15, this counts as locus drop out.

2.1 Examining Internal Lane Standard Results

2.1.1 The WEN ILS Size Standard peaks should appear as follows:

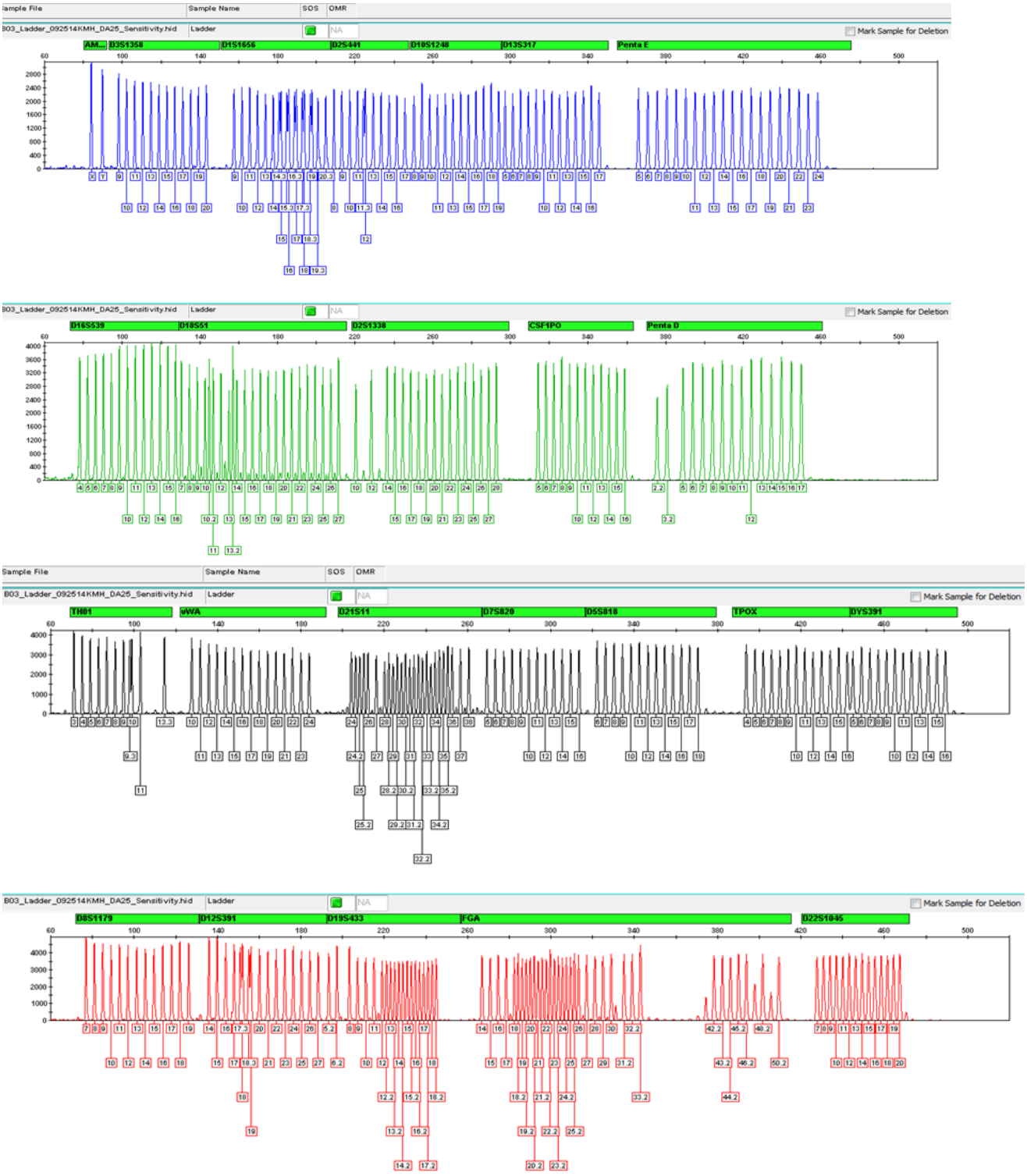


2.1.2 To assess the WEN ILS size standard for individual samples, highlight the sample(s) and select Tools→ Size Match Editor or click on the following icon:

2.1.3 Check to see that all peaks 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.

2.2 Examining Allelic Ladder Results

2.2.1 The PowerPlex® Fusion Allelic Ladder should appear as follows:



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 following 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 including, but not limited to, 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/fraction 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/fractions may be used.

2.3.1.1.2 Alternatively, if, upon re-amplification of the reagent blank and the associated samples/fractions, no peaks are observed, the full results of the re-amplification of the associated samples/fractions 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/fraction 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/fractions will be considered inconclusive at all loci.

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/fractions may be used.

2.3.1.2.2 Alternatively, if, upon re-amplification of the reagent blank and the associated samples/fractions, no peaks are observed, the full results of the re-amplification of the associated samples/fractions 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/fraction 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 Up to three peaks below the defined LOD but clearly discernable from noise and within a bin/bins may be observed and the results of the associated samples/fractions used.

2.3.2.2 If four or more peaks below the defined LOD but clearly discernable from noise and within bin(s) are observed, the results for the associated samples/fractions 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/fractions may be used.

2.3.2.2.2 Alternatively, if, upon re-amplification of the reagent blank and the associated samples/fractions, 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/fractions 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/fraction results from both the first and second amplifications will be considered inconclusive.

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 If the assessment of a sperm fraction (F2) reagent blank results in inconclusive results at all loci for the associated samples/fractions, but the non-sperm (F1) reagent blank is clean, the results for the non-sperm (F1) fraction(s) for the associated extraction set may be interpreted and reported. Alternatively (and in most cases preferably), the affected sample(s) may be re-extracted, if possible.

2.3.4 If the assessment of a non-sperm fraction (F1) reagent blank results in inconclusive results at all loci for the associated samples/fractions, the results of the entire extraction set (i.e., both sperm and non-sperm fractions (F1 and F2)) are deemed inconclusive at all loci, regardless of the results of the assessment of the sperm fraction (F2) reagent blank. The affected sample(s) will be re-extracted, if possible.

EXCEPTION: The results for the sperm fraction (F2) of an associated sample may be interpreted and reported if, and only if, **ALL** of the following conditions are met:

- Re-extraction of the associated sample is not possible (i.e., the sample was consumed).
- No peaks are observed above or below the LOD in the sperm fraction (F2) reagent blank.
- The sample is an intimate/ownership sample.
- The peak(s) observed in the non-sperm fraction (F1) reagent blank are attributable to the donor/owner of the sample.
- The sperm fraction (F2) results consist of a single source male profile different from the donor/owner of the sample.

NOTE: A single source profile deduced/deconvoluted from a mixture in the sperm fraction (F2) does NOT meet this condition.

2.3.5 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, including but not limited to, 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.

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.

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 Up to three peaks below the defined LOD but clearly discernable from noise may be observed and the results for the associated samples used.

2.4.2.1.1 If four or more peaks 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 only up to three peaks below the defined LOD but clearly discernable from noise 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 PowerPlex® Fusion kit is 2800M. The correct types are as follows:

Locus	Genotype 2800M
Amelogenin	X,Y
D3S1358	17,18
D1S1656	12,13
D2S441	10,14
D10S1248	13,15
D13S317	9,11
Penta E	7,14
D16S539	9,13
D18S51	16,18
D2S1338	22,25

CSF1PO	12,12
Penta D	12,13
TH01	6,9.3
vWA	16,19
D21S11	29,31.2
D7S820	8,11
D5S818	12,12
TPOX	11,11
DYS391	10
D8S1179	14,15
D12S391	18,23
D19S433	13,14
FGA	20,23
D22S1045	16,16

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.

2.5.3 If incorrect types or additional 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.

2.6 Examining Casework Samples

2.6.1 If it is determined that a sample contains elevated stutter peaks at a majority of the loci or 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 with Type I water or formamide 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 profile is single source and the accurate profile can be determined from the original sample profile by both the analyst and the independent technical reviewer, 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 at the discretion of the examiner 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 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).

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 observed 3 nucleotides smaller than the amplicon size (true peak) for the trinucleotide repeats, 4 nucleotides smaller for the tetranucleotide repeats and 5 nucleotides smaller for the pentanucleotide repeats. Stutter may also appear as multiples of the repeat unit (e.g., 8 nucleotides for tetranucleotide repeats) or may be larger than the amplicon size (+4 stutter or "up-stutter"). The expected stutter percentages listed below are based upon internal validation by the Department. The values marked with a * are maximum values observed due to fewer than 5 observations.

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. Therefore, the N-1 and N+1 stutter percentages are included in the GMID-X analysis method in use and are automatically applied.

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.

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
D3S1358	2	13	2
D1S1656	2	14	3
D2S441	2*	8	2
D10S1248	2	13	3
D13S317	1	10	4
Penta E	N/A	7	3
D16S539	3	11	2
D18S51	2	15	5
D2S1338	2	14	9
CSF1PO	1	10	4
Penta D	2*	4	4
TH01	2	5	5
vWA	2	14	5
D21S11	3	12	4
D7S820	2	12	5
D5S818	N/A	10	3
TPOX	2*	6	3
DYS391	2*	9	2
D8S1179	2	11	3
D12S391	3	18	5
D19S433	3	12	4
FGA	3	12	4
D22S1045	3	16	9

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 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.

2.6.2.5 Other artifact peaks can be observed at some of the PowerPlex® Fusion System loci:

- Low-level products can be seen in the n–2 and n+2 positions with some loci such as D1S1656, D13S317, D18S51, D21S11, D7S820, D5S818, D12S391 and D19S433.
- N–1 peaks are sometimes present at amelogenin and D2S441.
- N-3 peaks are sometimes present at D12S391.
- Amplification-independent artifacts may be observed in template and non-template samples in the fluorescein (FL) channel at 64–65, 69–71 and 88–90 bases and in the JOE channel at 74–76 bases. *
- Artifact peaks may be seen outside the locus panels in the FL channel at 70–74 bases, in the TMR-ET channel at 66–68 bases and in the CXR-ET channel at 58–65 bases. *
- Artifacts that may be seen within the locus panels include allele 5 (84 bases) in D16S539 and peaks at 71–73 and 75–77 bases in TH01, 214 bases in D18S51 and 247 bases in D2S1338. *

* These artifacts are typically below common minimum thresholds.

2.6.3 Indication of Gender Using the Amelogenin and DYS391 Loci

NOTE: The following criteria may also be used to indicate the gender of the donor of a deconvoluted single source profile if doing so is unambiguous (e.g., a clear major profile, a single source profile different from the owner of an intimate sample in a two-person mixture, etc.).

2.6.3.1 A single source sample exhibiting a peak only at ~89 bp (X allele) at amelogenin and no peak at DYS391 will generally be considered to have originated from a female.

2.6.3.2 A single source sample exhibiting a peak at both ~89 bp (X allele) and ~95 bp (Y allele) at amelogenin as well as a peak at DYS391 will generally be considered to have originated from a male.

2.6.3.3 A single source sample exhibiting only a peak at ~95 bp (Y allele) at amelogenin and a peak at DYS391 will generally be considered to have originated from a male.

2.6.3.4 A single source sample exhibiting a peak only at ~89 bp (X allele) at amelogenin and a peak at DYS391 will be considered inconclusive for gender.

2.6.3.5 A single source sample exhibiting only a peak at ~95 bp (Y allele) at amelogenin and no peak at DYS391 will be considered inconclusive for gender.

2.6.3.6 A single source sample exhibiting a peak at both ~89 bp (X allele) and ~95 bp (Y allele) at amelogenin and, based upon the quality and/or rfu values for the profile as a whole, there is a reasonable expectation that a missing DYS391 allele is due to drop out may generally be considered to have originated from a male.

2.6.3.6.1 If a robust profile exhibits both the X allele and the Y allele, but no DYS391 allele, the sample will generally be considered inconclusive for gender.

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, 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 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].

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.

2.7.2 If the peak is visually between two allelic ladder peaks of the same locus (a MV):

2.7.2.1 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 D16S539 14 allele will be designated as a D16S539 13.3. The "off ladder" value on the electropherogram will be manually changed to reflect the allele designation.

2.7.2.2 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:

2.7.3.1 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:

2.7.4.1 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 two peaks (for a single source sample), the allele will be considered to belong with the locus not containing two peaks. The assignment of the allele designation will be based upon the nomenclature referenced below.

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 two alleles (for a single source sample) OR the sample is a mixture:

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.

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 limit of detection and stochastic thresholds in use were derived from internal validation by the Department.

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 75 RFU, 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.

2.8.2 Stochastic Thresholds

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 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.

2.8.2.2 The stochastic threshold (STH) for a 24 second injection is 300 RFU. The STH for a 12 second injection is 210 RFU.

EXAMPLES: If a homozygous peak in a single source sample injected at 24 seconds is ***at or below*** 300 RFU, 2p will be used in any associated RM statistical calculation for that locus.

If a 12 allele observed in a mixture injected at 12 seconds is ***at or below*** 210 RFU, the "12, any" (12x) option will be used for any associated URM calculation.

2.9 Expected Minimum Peak Height Ratios for Heterozygous Loci

2.9.1 Expected minimum peak height ratios for heterozygous loci were derived from internal validation by the Department.

2.9.1.1 Heterozygous loci should generally meet a minimum peak height ratio of 60% for peaks from the same contributor.

2.10 General Interpretation of Single Source Samples

- 1 or 2 alleles are detected per locus (exceptions for tri-allelic patterns can be made).
- Heterozygous loci should generally meet the minimum peak height ratio of 60%.
  - For low level or partial profiles, heterozygous alleles do not need to be in peak height ratio to be used for comparison or in statistical calculations.
- DNA typing results are required at 6 or more loci before comparisons are conducted.

EXCEPTION: No minimum number of loci with typing results is required for a DNA profile developed from unidentified human remains or alternate knowns, believed to be single source, for the purposes of body identification.

EXCEPTION: Samples for which there is a reasonable expectation that a known (assumed) profile may be present must have the following minimum number of loci with typing results before comparisons are conducted and/or attribution is applied:

- Intimate sample (samples removed directly from body of a person) – no minimum
- Ownership item (e.g., cell phone stolen from victim – looking for profile different from victim) – 4 loci

        - Crime scene sample for which a profile different from a known individual is sought (e.g., victim is lying in own blood – looking for profile different from victim on a blood swab collected nearby) - 4 loci

- Possible allelic drop out can occur for data that is detected below the STH.
- Allelic/locus drop out is not counted at loci for which no results above LOD are obtained.
- For samples with typing results at 6-11 loci, a maximum of 3 loci can exhibit drop out for data below the STH when compared to a reference sample for a conclusion of not eliminated to be reached.
    - 4 or more loci with drop out will result in either an inconclusive or elimination conclusion with regard to a reference.
        - Exceptions to this will 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.
- For samples with typing results at 12-22 loci, a maximum of 5 loci can exhibit drop out for data below the STH when compared to a reference sample for a conclusion of not eliminated to be reached.
    - 6 or more loci with drop out will result in either an inconclusive or elimination conclusion with regard to a reference.
        - Exceptions to this will 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.
- Peaks below LOD but clearly discernable from noise will be considered during comparisons. When determining whether a conclusion of elimination or inconclusive will be reached, the following will be considered:
    - If peaks below LOD but clearly discernable from noise, are exculpatory, a conclusion of "eliminated" should be reached.
    - If peaks below LOD but clearly discernable from noise appear to be consistent with the known reference in question, a conclusion of "inconclusive" should be reached.
- Allelic drop out of a second allele at a locus for which the called allele is above the STH is less likely to occur.
    - For data above the STH, the DNA types from the evidence should match the DNA types from the reference for a conclusion of not eliminated to be reached.
- Statistics are calculated at all loci where DNA typing results are obtained.
    - Statistical calculations will incorporate 2p for homozygous alleles at or below the STH.

2.11 General Interpretation of 2 Person Mixtures

- 1-4 alleles are detected per locus.
- Mixture profiles with results at fewer than 6 loci will not be interpreted.
    - Exceptions for eliminations 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.
- Allelic/locus drop out is not counted at loci for which no results above LOD are obtained.
- Major/minor can be determined for some 2 person mixtures. See Chapter 3, Mixture Deconvolution Procedures.
    - Deconvoluted major alleles shall be within peak height ratio.
    - Deconvoluted minor alleles should generally, but not always, be within peak height ratio.
    - Minor alleles are only deconvoluted at loci where major alleles are deconvoluted.
    - Minor alleles may be masked by major alleles and are therefore not deconvoluted in those instances.
    - Allelic/locus drop out for a minor contributor is not counted when minor alleles are masked by the major alleles.
    - Locus drop out for a minor contributor is counted when no minor contribution is observed.
    - 6 or more loci (excluding D12S391, DYS391 and amelogenin) are required for statistics to be calculated for major or minor contributors.
    - A known reference profile should match the predominant alleles across the mixture at loci where no M/m is determined in order for a conclusion of not eliminated as a major to be reached.
    - A known reference profile should match the apparent minor alleles across the mixture at loci where no M/m is determined in order for a conclusion of not eliminated as a minor to be reached.

- Unresolved 2 person mixtures (no Major/minor)
  - CPI will be calculated at all loci with results and will only be conducted if all alleles in the mixture are above the STH.
    - Exceptions 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.
  - If a CPI cannot be calculated, a URM will be calculated at loci where breakout has occurred (3 or 4 alleles at a locus) or the profile may be referred for probabilistic genotyping (STRmix™ analysis or TrueAllele® analysis).
    - URM may be calculated at a minimum of 1 locus.
    - Profiles will be referred for probabilistic genotyping (STRmix™ analysis or TrueAllele® analysis) when a URM discards too much information.
  - If neither a CPI nor a URM can be calculated (all alleles are not >STH and no breakout loci are available for the URM), the profile will be referred for probabilistic genotyping (STRmix™ analysis or TrueAllele® analysis).

NOTES: In general, not all qualifying mixtures in a single case will be evaluated using probabilistic genotyping. If multiple mixtures are developed in a single case, the most informative/probative mixture(s) for which a traditional statistical approach is not possible or discards too much information will be analyzed using probabilistic genotyping, while the additional mixture(s) will be evaluated using traditional statistical methods, if possible.

Probabilistic genotyping may not be performed for cases in which other equally probative samples are subjected to traditional statistics. Consultation with the STRmix™ or TrueAllele® team may be necessary. In these cases, a URM may be calculated on the mixture(s), if possible.

2.12 General Interpretation of 3 Person Mixtures

- 1-6 alleles are detected per locus.
- Mixture profiles with results at fewer than 6 loci will not be interpreted.
  - Exceptions for eliminations 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.
- Allelic/locus drop out is not counted at loci for which no results above LOD are obtained.
- Major can be determined for some 3 person mixtures. See Chapter 3, Mixture Deconvolution Procedures.
  - Major alleles are generally, but not always, within peak height ratio.
  - 6 or more loci (excluding D12S391 and DYS391) are required for statistics to be calculated for major contributors.
  - A known reference profile should match the predominant alleles at loci not designated as major for a conclusion of not eliminated as a major to be reached.
- Unresolved 3 person mixtures (no Major/minor)
  - CPI will be calculated at all loci with results and will only be calculated if all alleles in the mixture are above STH.
    - Exceptions 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.
  - If a CPI cannot be calculated, a URM will be calculated at loci where breakout has occurred (5 or 6 alleles at a locus) or the profile may be referred for probabilistic genotyping (STRmix™ analysis or TrueAllele® analysis).
    - URM may be calculated at a minimum of 1 locus.
    - Profiles will be referred for probabilistic genotyping (STRmix™ analysis or TrueAllele® analysis) when a URM discards too much information.
  - If neither a CPI nor a URM can be calculated (all alleles are not >STH and no breakout loci are available for the URM), the profile will be referred for probabilistic genotyping (STRmix™ analysis or TrueAllele® analysis).

NOTES: In general, not all qualifying mixtures in a single case will be evaluated using probabilistic genotyping. If multiple mixtures are developed in a single case, the most informative/probative mixture(s) for which a traditional statistical approach is not possible or discards too much

information will be analyzed using probabilistic genotyping, while the additional mixture(s) will be evaluated using traditional statistical methods, if possible.

Probabilistic genotyping may not be performed for cases in which other equally probative samples are subjected to traditional statistics. Consultation with the STRmix™ or TrueAllele® team may be necessary. In these cases, a URM may be calculated on the mixture(s), if possible.

2.13 General Interpretation of 4 Person Mixtures

- Mixtures with a 7th allele above LOD at any locus will not be interpreted.
  - Exceptions may be considered by the Program Manager (Technical Leader) or Assistant Technical Leader for a 4 person mixture for which a clear Major contributor can be deconvoluted.
- Mixtures with a 7th peak below LOD but clearly discernable from noise will generally not be interpreted.
  - Exceptions may be considered by the Program Manager (Technical Leader) or Assistant Technical Leader for a 4 person mixture for which a clear Major contributor can be deconvoluted.

Exceptions for eliminations 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.

2.14 General Interpretation of Profiles Using an Assumed Known Approach

An assumed known approach may only be used in the following situations:

- Intimate samples – donor of the sample and/or a known consensual partner may be assumed
- Ownership samples – owner/user may be assumed
- PTMB+ stains at a scene surrounding a decedent/homicide victim(s) – decedent/homicide victim(s) may be assumed and considered in determining what DNA profile(s) developed are different

Rare exceptions to use the assumed known approach in other situations may be considered on a case by case basis by the Program Manager (Technical Leader) and/or Assistant Technical Leader.

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.).

- A general mixture approach without the use of an assumed known may always be employed and will sometimes be preferable even though the scenario may technically allow for the assumed known approach.
- Alternatively, DNA types from an assumed known contributor may be subtracted or dosage considered when determining which types are different from that contributor's at a locus (for 2 or 3 person mixtures).
- For 2 person mixtures (1 assumed known and 1 unknown contributor)
  - Alleles different from the assumed known should generally be within peak height ratio.
  - 6 or more loci with results different from the assumed known (excluding D12S391 and DYS391) are required for statistics to be calculated.
  - Allelic/locus drop out is not counted at loci for which no results above LOD are obtained.
  - Allelic/locus drop out is not counted at loci where a potential contributor's alleles are masked by the assumed known's.
  - Allelic/locus drop out is counted when the potential contributor's alleles are missing.
  - A traditional likelihood ratio calculation may be conducted using POPSTATS, if the sample is intimate, the entire profile different from the assumed known cannot be determined due to masking by the assumed known, and all of the alleles from the assumed known are above STH and no alleles from the potential contributor are missing.
- For 3 person mixtures
  - If only one assumed known, a general mixture approach will be used for non-intimate ownership samples.

        - Once the comparison to the assumed known using the general mixture approach has been conducted and if the conclusion reached, based upon the conclusion requirements detailed in 2.15 and 2.16, is "not eliminated" and non-probative, then that individual may be reported as an assumed known (using an assumption statement) and attribution may be used in lieu of a statistic for that non-elimination.
    - Intimate samples may be interpreted similarly to 2 person mixtures as detailed above using more than one assumed known (VC sample from victim (assumed known 1) and previous consensual partner (assumed known 2), for example).
    - Mixtures different from an assumed known developed from intimate samples for which only a single assumed known is submitted may be determined to be of no value for comparison, etc., as appropriate.
    - Non-intimate samples/ownership samples for which more than one assumed known is submitted (steering wheel with 2 drivers submitted, for example) will be interpreted using a general mixture approach.
        - Once the comparisons to the assumed knowns using the general mixture approach have been conducted and if a conclusion reached in regard to either or both of the assumed knowns, based upon the conclusion requirements detailed in 2.15 and 2.16, is "not eliminated" and non-probative, then that individual/those individuals may be reported as an assumed known(s) (using an assumption statement) and attribution may be used in lieu of a statistic for the applicable non-elimination(s).

2.15 Conclusion Requirements Specific to Comparisons of Known References to Mixtures with DNA Typing Results Above LOD at 6-11 Loci

- Up to 3 loci with ANY drop out (allelic or locus) may be tolerated for a conclusion of not eliminated to be reached.
- 4 or more loci with ANY drop out (allelic or locus) will result in either an inconclusive or elimination conclusion with regard to a reference.
    - Exceptions 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.
- Peaks below LOD but clearly discernable from noise will be considered during comparisons.
    - If peaks below LOD but clearly discernable from noise, are exculpatory, a conclusion of eliminated should be reached.
    - If peaks below LOD but clearly discernable from noise appear to be consistent with the known reference in question but 4 or more loci show drop out, a conclusion of inconclusive should be reached.

2.16 Conclusion Requirements Specific to Comparisons of Known References to Mixtures with DNA Typing Results Above LOD at 12-22 Loci

- Up to 5 loci with drop out may be tolerated for a conclusion of not eliminated.
    - Of these 5, a maximum of 2 can be full locus drop out to reach a conclusion of not eliminated.
- Peaks below LOD but clearly discernable from noise will be considered during comparisons.
    - If peaks below LOD but clearly discernable from noise, are exculpatory, a conclusion of eliminated should be reached.
    - If peaks below LOD but clearly discernable from noise appear to support a conclusion of not eliminated but the maximum allowable drop out options listed above are surpassed, a conclusion of inconclusive should be reached.

Exceptions to the requirements listed in 2.15 and 2.16 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.

2.17 Interpretation of Criminal Paternity/Maternity and Missing Person Cases

2.17.1 In general, the typing results for these cases will be referred to a member of the kinship statistics team for conclusions and statistics.

2.17.1.1 Eliminations for paternity/maternity will be reported by the original examiner. Non-eliminations will be referred.

2.17.2 An individual must be eliminated at three or more loci to account for the possibility of mutations before the individual is eliminated as a parent/offspring.

2.17.2.1 In criminal paternity/maternity and missing person cases, inconsistencies 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.

2.17.2.2 When a couple is evaluated as possible biological parents of a missing person, each possible parent's DNA profile will be evaluated separately to determine if the individual is included or eliminated as a biological parent. Subsequently the profiles from both individuals will be evaluated together to determine if as a couple they could have conceived the missing person.

3 MIXTURE DECONVOLUTION PROCEDURES

Maximum allowances for allelic/locus drop out with regard to comparison of known references apply across the entire mixture profile rather than for the deconvolution.

The deconvolution at D12S391 will be documented, if applicable, and used in the comparison of any known reference samples to a mixture. This locus will also apply to allele/locus drop out counts. However, this locus will not be used in statistical calculations.

Mixtures will be considered as a whole to determine the best deconvolution approach. Although a M/m deconvolution may be possible by strictly following the rules set forth, it may be more appropriate to use a URM or refer a mixture for probabilistic genotyping. Input from a supervisor, the Program Manager (Technical Leader) or Assistant Technical Leader may be sought.

Examples of deconvolutions provided are not meant to be all-inclusive. The training and experience of each examiner is meant to be relied upon when following these procedures.

The assumed number of contributors will be documented for each deconvolution in such a way that a reviewer is clear as to what the assumption is.

Documentation of the deconvolution must be interpretable by other examiners.

- A 2 contributor URM is conducted on a locus with alleles 12, 13, 17, 19.
  - Examples of possible documentation for this locus are: listing all possible combinations of the four alleles (12,13; 12,17; 12,19; 13,17; 13,19; 17,19) or URM or URM_2, etc.
- A 2 contributor URM is conducted on a locus with alleles 12, 13, 17 – all above STH.
  - Examples of possible documentation for this locus are: listing all possible combinations (12; 12,13; 12,17; 13; 13,17; 17) or URM + H or URM_2 + H or URM + homozygotes, URM, URM_2 etc.
- Commas/spaces between allele calls or between the allele call and an x may be used at the examiner's discretion.

3.1 Major/minor or Major Deconvolutions

3.1.1 A clear predominant profile should be observed across the majority of the entire profile in order for a M/m or major deconvolution approach to be used. It is not recommended for mixture profiles for which stochastic effects or stacking is likely the cause for a peak or peaks at individual loci to appear to be predominant.

3.1.2 A minor profile will not be deconvoluted for 3 person mixtures. If the mixture, as a whole, is deemed suitable for comparison, it may be more appropriate to use a URM approach or refer the mixture for probabilistic genotyping. If the minor contributions are limited, using the major deconvolution and deeming the minor to be of no value may be more appropriate.

3.1.3 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 shortest major peak in order for M/m to be determined.

3.1.4 In order to designate M/m at a locus, at least one allele must be above STH.

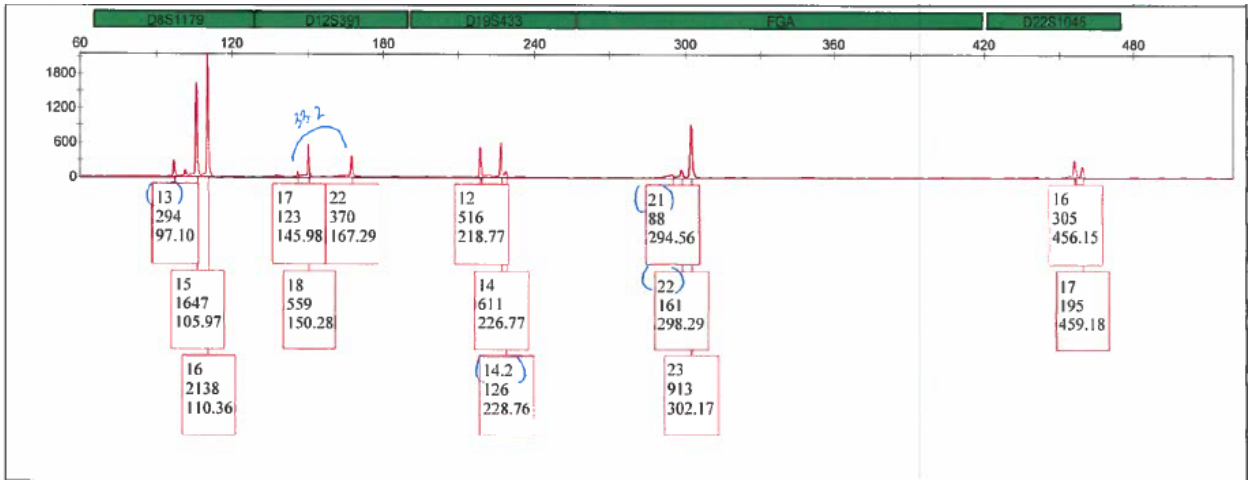
3.1.5 For 2 person mixtures, the major alleles for a locus must be within expected peak height ratio (60%) in order for M/m to be determined at that locus.

3.1.6 For 3 person mixtures, the major alleles for a locus should generally, but do not have to be within expected peak height ratio in order for the major to be determined.

3.1.7 In order to apply a M/m or major deconvolution to a mixture as a whole, 50% or more of the breakout loci (3 or 4 alleles for a 2 person mixture; 5 or 6 alleles for a 3 person mixture) must meet the requirements in 3.1.3 – 3.1.5 for designation of a M/m. If this requirement is met, the M/(m) deconvolution will then be applied across the profile as a whole.

3.1.8 Single minor alleles at a locus that are at or below STH will be designated with an x (allele, x) to account for a possible missing sister allele.

3.1.9 Examples of deconvoluted loci are below:



The STH for this sample is 300 RFU.

This is a 2 person mixture for which a M/m was deconvoluted.

Parentheses may be used to document minor alleles on an electropherogram or the landscape at the examiner's discretion.

Note that in some instances the peak height ratio calculation is not shown. If the peak height ratio for a minor allele to a major allele or for the two major alleles can be seen to certainly meet the associated requirement, it does not need to be documented.

D8S1179:	M: 15,16	/	m: 13x
D12S391:	no major	/	no minor
D19S433:	M: 12,14	/	m: 14.2x
FGA:	M: 23,23	/	21,22
D22S1045:	M: 16,17	/	no minor

3.2 Unrestricted Random Match Deconvolutions (URM)

3.2.1 URM deconvolutions will only be conducted at break out loci (loci with 3 or 4 alleles for a 2 person mixture; loci with 5 or 6 alleles for a 3 person mixture).

3.2.1.1 The assumption of number of contributors must be designated. An option is to designate the deconvolution as URM_2 or URM_3.

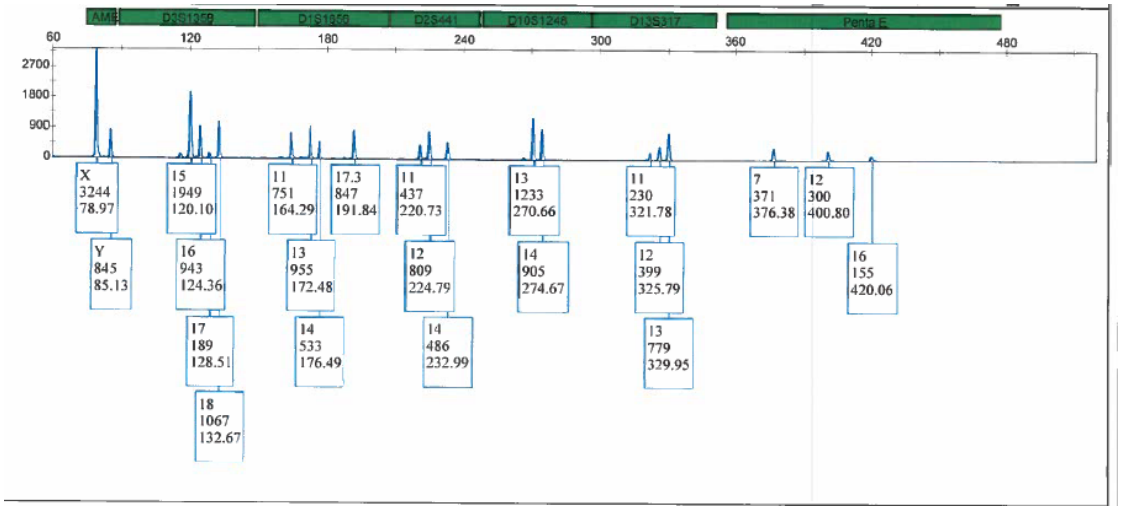
3.2.2 A minimum of one break out locus is required for a URM deconvolution.

3.2.2.1 Considerations may be made regarding referral for probabilistic genotyping as opposed to calculating a non-informative URM in certain cases.

3.2.3 Alleles included in the URM deconvolution that are at or below STH will be designated with an x (allele, x) to account for a possible missing sister allele. However, if full-breakout (4 alleles for a 2 person mixture, 6 alleles for a 3 person mixture) is observed at a locus, no x will be used.

3.2.4 All possible combinations of all alleles above STH will be considered for the calculation regardless of perceived dosage.

3.2.5 Examples of deconvoluted loci are below:



This is a 2 person mixture URM deconvolution. The STH for this sample is 300 RFU.

D3S1358:	URM				
D1S1656:	URM				
D2S441:	URM + H				
D10S1248:	---				
D13S317:	11x	12,12	12,13	13,13	(OR: URM 12,13; 11x)
Penta E:	7,7	12x	16x		

3.3 Deconvolutions for Profiles Different from an Assumed Known

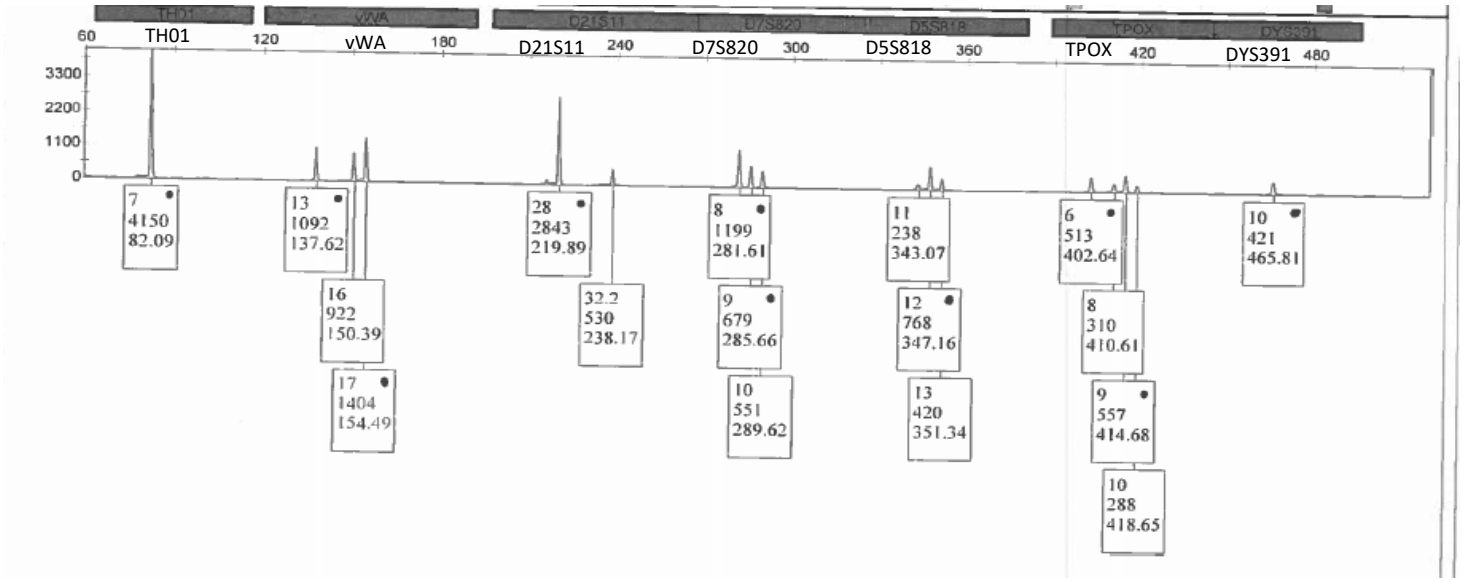
3.3.1 In most instances, this deconvolution will only be used for 2 person mixtures.

3.3.1.1 If 2 assumed knowns are submitted for an intimate sample from which a 3 person mixture is obtained, a similar approach may be used. Discretion should be used in making this determination. It may be more appropriate to use a general mixture approach (M/m, URM or referral for probabilistic genotyping).

3.3.2 Dosage may be considered in assigning alleles as different from the assumed contributor, but should be used with caution when the alleles different are not clearly discernable

3.3.2.1 Exceptions to drop a locus for statistical purposes due to the use of dosage when the more conservative and complete deconvolution includes a person of interest's DNA types will not be considered.

3.3.3 Examples of deconvoluted loci are below:



The dots indicate alleles attributable to the assumed known. The below deconvolution results are for the contributor different from the assumed known:

TH01:	---			
vWA:	16,16	13,16	16,17	(OR: 16 + ACA)
D21S11:	32.2,32.2	28,32.2		
D7S820:	8,10 (this includes use of dosage – another, often preferable, option is [10,10; 8,10; 9,10])			
D5S818:	11,13			
TPOX:	8,10			

ACA = Any Called Allele
If the 32.2 allele at D21S11 had an RFU value of 200, the deconvolution at D21S11 would have been 32.2x.

4 STATISTICAL CALCULATIONS

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

Qualitative (attribution) statements may be used in lieu of a statistical calculation for assumed knowns on samples for which an assumed known approach was used.

The statistical approach applied will depend upon the circumstances of the case and the criteria addressed previously in this manual.

Statistical calculations will use the population allele frequencies provided by NIST. An Excel file of the allele frequencies can be downloaded from the NIST website at the following link:

https://strbase.nist.gov/Information/NIST_Population_Data

Statistical calculations are routinely calculated using the Caucasian, Hispanic, and African American population databases and reported for all three. It is acceptable to include, in addition to the Caucasian, Hispanic, and African American population databases, a calculation using the Asian database, as applicable or when requested by the submitting Agency/Attorney(s). In these instances, all four statistical results will be reported.

Loci for which a known reference profile not eliminated from an evidence profile demonstrates a tri-allelic pattern will be dropped from the statistical calculation(s).

When providing a statistical calculation on evidence analyzed with PowerPlex® Fusion due to a non-elimination of a known reference profile analyzed with PowerPlex® 16, only the applicable PowerPlex® 16 loci will be used.

4.1 Procedure for Rounding Frequencies When Calculating by Hand

4.1.1 Allele and genotype frequencies will be carried out to 3 digits. If the fourth digit is 4 or less the third digit will remain the same. If the fourth digit is 5 or greater the third digit will be rounded up.

Example: 0.3464 would be truncated to 0.346
0.3467 would be rounded to 0.347

4.1.2 Mutation rate frequencies will be carried out to the number of digits in the actual mutation rate.

Example: 0.0002 will be carried out to 4 digits
0.0020 will be carried out to 3 digits

4.1.3 The frequency for the overall DNA pattern, termed a DNA profile, can be determined by multiplying together the genotype frequency (carried out to 3 digits as described in 4.1.1) obtained from each locus. The overall match probability/Likelihood Ratio/CPI (combined probability of inclusion/combined paternity index) will be truncated to two significant figures.

Example: $8.169738341 \times 10^{-11} = 1$ in 12,240,294,100
The reported match probability: 1 in 12 billion

4.1.4 When incorporating a mutation rate calculation for a locus or a Y-STR haplotype frequency, the overall match probability/LR/CPI for the profile (excluding the mutation locus, as applicable) prior to this incorporation will be truncated to two significant figures. The match probability/LR/PI for the mutation locus and/or the Y-STR haplotype frequency will be truncated to two significant figures. These values will then be multiplied together for the final overall match probability/LR/CPI, which will then also be truncated to two significant figures.

4.2 Random Match Probability (RM)

4.2.1 This calculation will be used for:

- Single source profiles
- Deduced major/minor profiles
- Deduced single source profiles different from an assumed contributor

4.2.2 For any homozygous allele below STH, $2p\text{-}p^2$ will be applied.

4.2.3 ArmedXpert™ Software will be used for this calculation.

4.2.3.1 Theta must be ON and set to 0.01.

4.2.3.2 Profile data may be imported or hand entered into the ArmedXpert™ Software.

4.2.3.3 Either the Single Source function or the RMP function in the software may be used.

4.2.3.3.1 If using the RMP function, multiple combinations of alleles may be selected. This function must be chosen if the deconvoluted profile includes multiple possibilities (e.g., 12,12 or 12,13 are both viable options for a deconvoluted profile different from an assumed contributor).

4.3 Traditional Likelihood Ratio (LR)

4.3.1 This calculation will be used for:

Intimate samples with a mixture of 2 individuals (the assumed known and one other contributor) where the entire DNA profile different from the assumed known cannot be determined by subtracting the contribution of the known contributor and all alleles different from the assumed known are above STH.

NOTE: If any alleles from either the assumed known or compared person of interest are missing from the mixture profile, this calculation will not be used.

4.3.2 When the assumed known and person of interest share alleles at a locus, the shared allele(s) may be accounted for as either unknown or known in the likelihood ratio calculation.

4.3.2.1 An example when both the shared allele(s) would be counted as unknown is if the assumed known's contribution to the mixture is the minor component throughout the mixture profile and the type(s) that are shared by the assumed known and the person of interest are consistent with the predominant contributor (based upon the peak heights of the alleles in relationship to the rest of the DNA profile). In this instance, the shared allele(s) can be used as unknown in the calculation.

4.3.2.2 An example when the shared allele(s) would be counted as known is if the assumed known's contribution to the mixture is the predominant component throughout the mixture profile and the shared type(s) are therefore also consistent with the predominant contributor (based upon the peak heights of the alleles in relationship to the rest of the DNA profile). In this instance the shared allele(s) will not be used as unknown in the calculation.

4.3.2.3 If four alleles are observed at a locus, the alleles different from the assumed known will be used in the calculation.

4.3.3 POPSTATS Software will be used for this calculation.

4.4 Combined Probability of Inclusion

4.4.1 This calculation will be used for:

- Complete mixtures of 2 people in which all alleles are above STH.
  - If a traditional likelihood calculation, a major/minor deconvolution, or a deconvolution of a profile different from an assumed known can be performed, it may be more appropriate to use one of these approaches.
- Complete mixtures of 3 people in which all alleles are above STH.
  - If a major deconvolution can be performed and used for a statistical calculation, it may be more appropriate to use this approach.

NOTE: Cases involving mixtures from which biological relatives (typically first- or second-order relatives) are not-eliminated will be referred to TrueAllele® for statistical calculations in lieu of calculating a CPI. Consultation with a TrueAllele® team member may be necessary.

Rare exceptions to the rule that all alleles must be above STH 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.

4.4.2 POPSTATS Software will be used for this calculation.

4.5 Unrestricted Random Match Probability (URM)

4.5.1 This calculation will generally be used for:

- 2 person mixture profiles that do not qualify for a CPI and contain at least one break out locus (3 or 4 alleles at the locus).
  - If a Major/minor can be determined, the RM on the major and/or minor may be used in lieu of the URM.
  - If the sample is intimate and qualifies for a traditional LR, the traditional LR may be used in lieu of the URM.
  - If the sample is intimate or a non-intimate ownership sample and a deconvolution of a profile different from the assumed known can be performed, the RM may be used in lieu of the URM.
- 3 person mixture profiles that do not qualify for a CPI and contain at least one break out locus (5 or 6 alleles).
  - If a Major can be determined, the RM on the major may be used in lieu of the URM.

NOTE: Cases involving mixtures from which biological relatives (typically first- or second-order relatives) are not-eliminated will be referred to TrueAllele® for statistical calculations in lieu of calculating a URM. Consultation with a TrueAllele® team member may be necessary.

4.5.2 ArmedXpert™ Software will be used for this calculation.

4.5.2.1 Theta must be OFF.

4.5.2.2 Profile data may be imported or hand entered into the ArmedXpert™ Software

4.5.3 For any alleles below STH the ‘allele, any’ will be chosen for the potentially missing sister allele for the calculation.

EXAMPLE: If a mixture locus contains alleles 12, 13, 15 and is deconvoluted as: 12,12; 12,13; 13,13; 15x, the “15, Any” option will be chosen in the ArmedXpert™ Software.

4.6 Likelihood Ratio Generated by the STRmix™ System

4.6.1 This calculation will be used for:

- PowerPlex® Fusion mixtures that do not qualify for a traditional statistical calculation or for TrueAllele® analysis.
- PowerPlex® Fusion mixtures that do not qualify for TrueAllele® analysis and for which a URM discards too much information.

NOTE: Mixtures involving biological relatives (typically first- or second-order relatives – consultation with a TrueAllele® team member may be necessary) and mixtures for which statistics will address the comparison to a person for whom insufficient information exists to draw a conclusion regarding elimination/non-elimination are not eligible for STRmix™ analysis.

A member of the STRmix™ team will be responsible for conducting this statistical analysis and reporting the statistical results and conclusions. Refer to the STRmix™ System Manual for the applicable procedures.

4.6.1.1 In some cases, STRmix™ analyses may not be performed if other equally probative samples are subjected to traditional statistics or TrueAllele® analysis. Consultation with the STRmix™ team may be necessary.

4.7 Likelihood Ratio Generated by TrueAllele®

4.7.1 This calculation will be used for:

- PowerPlex® Fusion mixtures that are forwarded by a STRmix™ team member based upon criteria listed in the FB PM STRmix™ System.
- PowerPlex® 16 mixtures that do not qualify for traditional statistical calculations or for which a URM discards too much information.
- unresolved PowerPlex® 16 or PowerPlex® Fusion mixtures involving the potential non-elimination of biological relatives (typically first- or second-order relatives – Consultation with a TrueAllele® team member may be necessary.).
- PowerPlex® 16 or PowerPlex® Fusion mixtures for which insufficient information exists to draw a conclusion regarding a particular individual (due to drop out rules), as described in this manual or the FB PM Interpretation of PowerPlex® 16 Data.

NOTES: Conclusions of INC re: an individual may be addressed with TrueAllele® analysis on the most informative/probative sample in a case for which an individual is INC, if that individual is not addressed with a statistic due to a non-elimination on any other sample in the case.

If a mixture is referred for TrueAllele® analysis, all statistical analyses regarding that mixture profile will be conducted using TrueAllele®.

A member of the TrueAllele® team will be responsible for conducting this statistical analysis and reporting the statistical results and conclusions. Refer to the TrueAllele® Manual for the applicable procedures.

4.7.1.1 In some cases, TrueAllele® analyses may not be performed if other equally probative samples are subjected to STRmix™ analysis or traditional statistics. Consultation with the STRmix™ and/or TrueAllele® team may be necessary.

4.8 Paternity/Relationship Statistical Calculations

Cases for which multiple samples are submitted for body identification purposes will include all of only the most informative calculation(s).

EXAMPLES: An alleged parent sample and an alleged sibling sample are submitted – only the single parent calculation will be conducted and reported.

Two separate alleged sibling samples are submitted – both sibling calculations (with regard to the unidentified remains) will be conducted and reported.

Two separate alleged child samples and one alleged sibling sample are submitted – both single parent/child calculations will be conducted and reported.

In general, the Paternity/Maternity Index for body identification purposes should meet or exceed 100. If a Single Parent-Offspring or Trio calculation results in a LR of less than 100, consultation with the Program Manager (Technical Leader) or Assistant Technical Leader is required.

4.8.1 POPSTATS Software will be used for these calculations.

4.8.2 Any locus at which a single allele is observed and that allele is at or below STH will be dropped from the calculation.

EXCEPTION: If, in a rare case, the only reasonable explanation for the result is a mutation, refer to 4.8.12.

4.8.3 For cases involving an apparent mutation at D12S391, vWA will be dropped from the calculation, and the mutation calculation for the D12S391 locus will be included.

4.8.3.1 If the rare instance occurs in which both D12S391 and vWA include an apparent mutation, both calculations/sets of calculations (one including only vWA and one including only D12S391) will be conducted and considered. The lower of the two results will be reported.

NOTE: The METHODS statement in the Certificate of Analysis will be adjusted accordingly.

4.8.4 For paternity trio cases involving mixtures developed from products of conception, if D12S391 includes an obligate paternal allele while vWA does not, vWA will be dropped from the calculation, and D12S391 will be included.

NOTE: The METHODS statement in the Certificate of Analysis will be adjusted accordingly.

4.8.5 Random Man Not Excluded (RMNE): the frequency with which men selected at random from the same racial background as the Alleged Father would not be excluded as the Biological Father in a given test which includes the Mother-Child Combination.

Mother	P	R
Child	P	Q
Alleged Father	Q	N

i. $RMNE = 2q - q^2$ (Where q equals the obligatory allele frequency)

Mother	P	Q
Child	P	Q
Alleged Father	Q	N

ii. $RMNE = 2(p + q) - (p + q)^2$ (Where p and q equal the obligate allele frequency)

4.8.6 Paternity Index (PI): This is the ratio of the chance that the mother (M) and a man of the Alleged Father's (AF) phenotype produced the child (passed the obligate allele) compared to the chance that the mother and a random man produced the child (passed the obligate allele).

H_0 = Alleged father is the biological father
H_1 = Alleged father is not the biological father

$P(R|H_0)$ = Probability of the child (with R genetic information of M and AF) given H_0.
$P(R|H_1)$ = Probability of the child (with R genetic information of M and AF) given H_1.

When the numerator and denominator are divided by $P(R|H_0)$

$$P(H_0|R) = P(R|H_0) / P(R|H_0) + P(R|H_1)$$

Let $P(R|H_0) = X$
$P(R|H_1) = Y$

$$P(H_0|R) = 1 / 1 + Y/X$$

PI = X/Y or 1 / Y/X, also known as a likelihood ratio (L)

PI compares two hypotheses or scenarios – informally they are 1.) paternity, and 2) non-paternity. PI provides a measure of how many times more characteristic of (1) the genetic evidence is than of (2).

Example: Mother (M) = 10, 11
Child (C) = 10, 11
Alleged Father (AF) = 10, 12

$$\text{Paternity Index} = \frac{(M_{10})(AF_{11}) + (M_{11})(AF_{10})}{(M_{10})(RM_{11}) + (M_{11})(RM_{10})}$$

$$= \frac{(0) + (AF_{10})}{(RM_{11}) + (RM_{10})}$$

$$= (AF_{10})/[(RM_{11}) + (RM_{10})]$$

Child	Mother	Alleged Father	Paternity Index
AA	AA	AA	$1/P_A$
AA	AB	AA	$1/P_A$
AB	AA	AA	$1/P_A$
AB	BB	AA	$1/P_A$
AB	BC	AA	$1/P_A$
AA	AB	AB	$1/2P_A$
AA	AA	AB	$1/2P_A$
AB	BB	AB	$1/2P_A$
AB	BB	AC	$1/2P_A$
AB	BC	AD	$1/2P_A$
AB	BC	AB	$1/2P_A$
AA	AB	AC	$1/2P_A$
AB	AA	BB	$1/P_B$
AB	AC	BB	$1/P_B$
AB	AA	AB	$1/2P_B$
AB	AA	BC	$1/2P_B$
AB	AC	BD	$1/2P_B$
AB	AA	BC	$1/2P_B$
AB	AC	AB	$1/2P_B$
AB	AB	AA	$1/P_A + P_B$
AB	AB	AB	$1/P_A + P_B$
AB	AB	BB	$1/P_A + P_B$
AB	AB	BC	$½(P_A + P_B)$
AB	AB	AC	$½(P_A + P_B)$

4.8.7 Combined Paternity Index (CPI): The combined paternity index is calculated by multiplying together each individual paternity index.

$CPI = PI_1 \times PI_2 \times PI_3 \ldots\ldots\ldots PI_n$

4.8.8 Probability of Paternity: The probability of paternity is expressed as a frequency (or percentage), incorporating the combined paternity index and a prior probability (which is most typically set at 0.5) which compares the likelihood that the tested man may pass the required genes to the likelihood that an untested, unrelated random man of the same race may pass these genes.

Probability of Paternity = (CPI)(Pr) / (CPI)(Pr) + (1-Pr)
Pr = Prior Probability and CPI = Combined Paternity Index
With a Pr = 0.5, the formula reduces to:
Probability of Paternity[2,3,4] = CPI / CPI + 1

4.8.9 Paternity/Maternity Relationship Calculations from a Single Parent:

Where:

p = frequency in the population
q = frequency in the population
AF_p = chance of passing p
AF_q = chance of passing q

$PI = (p)(AF_q) + (q)(AF_p) / 2pq = (p)(0.5) + (q)(0) / 2pq = 1/4q$

Person other		
Child	P	Q
Alleged Father	Q	R

$PI = (p)(AF_q) + (q)(AF_p) / 2pq = (p)(1) + (q)(0) / 2pq = 1/2q$

Person other		
Child	P	Q
Alleged Father	Q	Q

$PI = (p)(AF_q) + (q)(AF_p) / 2pq = (p)(0.5) + (q)(0.5) / 2pq = (p + q) / 4pq$

Person other		
Child	P	Q
Alleged Father	P	Q

$PI = (q)(AF_p) / q^2 = (q)(0.5) / q^2 = 1 / 2q$

Person other		
Child	Q	Q
Alleged Father	Q	R

$PI = (q)(AF_p) / q^2 = (q)(1) / q^2 = 1 / q$

Person other		
Child	Q	Q
Alleged Father	Q	Q

4.8.10 Missing Person Calculations Where the Mother's and Father's genotypes are known:

Prob(EIM, F, Q) is the probability that the evidence would be observed given that the mother and the father were the parents of the evidence sample (Q).

Prob(EIM, F, U) is the probability that the evidence would be observed given that a random member of the population was the questioned sample (U).

LR is the ratio of the two probabilities = Prob(EIM, F, Q) / Prob(EIM, F, U)
$P_{A,}$ etc., is the estimated frequency of the "A" allele in the population

Mother	Question	Father	Prob(EIM, F, Q)	Prob(EIM, F, U)	LR
AA	AA	AA	$P_A^2 \times P_A^2$	$P_A^2 \times P_A^2 \times P_A^2$	$1 / P_A^2$
AA	AA	AB	$P_A^2 \times 2P_AP_B \times ½$	$P_A^2 \times 2P_AP_B \times P_A^2$	$1 / 2P_A^2$
AA	AB	BB	$P_A^2 \times P_B^2$	$P_A^2 \times P_B^2 \times 2P_AP_B$	$1 / 2P_AP_B$
AA	AB	AB	$P_A^2 \times 2P_AP_B \times ½$	$P_A^2 \times 2P_AP_B \times 2P_AP_B$	$1 / 4P_AP_B$
AA	AB	BC	$P_A^2 \times 2P_BP_C \times ½$	$P_A^2 \times 2P_BP_C \times 2P_AP_B$	$1 / 4P_AP_B$
AB	AB	BB	$2P_AP_B \times P_B^2 \times ½$	$2P_AP_B \times P_B^2 \times 2P_AP_B$	$1 / 4P_AP_B$
AB	AB	AB	$2P_AP_B \times 2P_AP_B \times (¼ + ¼)$	$2P_AP_B \times 2P_AP_B \times 2P_AP_B$	$1 / 4P_AP_B$
AB	AB	AC	$2P_AP_B \times 2P_AP_C \times ½ \times ½$	$2P_AP_B \times 2P_AP_C \times 2P_AP_B$	$1 / 8P_AP_B$
AB	AA	AA	$2P_AP_B \times P_A^2 \times ½$	$2P_AP_B \times P_A^2 \times P_A^2$	$1 / 2P_A^2$
AB	AA	AB	$2P_AP_B \times 2P_AP_B \times ½ \times ½$	$2P_AP_B \times 2P_AP_B \times P_A^2$	$1 / 4P_A^2$
AB	AA	AC	$2P_AP_B \times 2P_AP_C \times ½ \times ½$	$2P_AP_B \times 2P_AP_C \times P_A^2$	$1 / 4P_A^2$
AB	AC	CC	$2P_AP_B \times P_C^2 \times ½$	$2P_AP_B \times Pc^2 \times 2P_AP_C$	$1 / 4P_AP_C$
AB	AC	BC	$2P_AP_B \times 2P_BP_C \times ½ \times ½$	$2P_AP_B \times 2P_BP_C \times 2P_AP_C$	$1 / 8P_AP_C$
AB	AC	AC	$2P_AP_B \times 2P_AP_C \times ½ \times ½$	$2P_AP_B \times 2P_AP_C \times 2P_AP_C$	$1 / 8P_AP_C$
AB	AC	CD	$2P_AP_B \times 2P_CP_D \times ½ \times ½$	$2P_AP_B \times 2P_CP_D \times 2P_AP_C$	$1 / 8P_AP_C$

4.8.11 When performing a Missing Person or Paternity Index calculation, it is possible that an individual or an evidence sample may be identified that cannot be eliminated as a possible offspring/parent or have originated from the offspring/parent. However, the individual's DNA profile or the evidence sample profile is missing an allele at a particular locus that is observed in the mother/father/child's DNA profile. The allele may be missing as a result of allelic drop out or a mutation.

4.8.11.1 To account only for the possibility of allele drop out (i.e., the most reasonable explanation for the missing allele is drop out due to a single peak at or below STH/there is no indication that a mutation occurred), the locus will not be used in the overall calculation.

4.8.11.2 To account only for the possibility of a mutation (i.e., the locus in question is heterozygous but missing the allele of interest or the locus in question is homozygous and a mutation appears to be the only reasonable explanation for the result), the formula addressed in 4.8.12 will be used for the locus and factored into the overall calculation.

4.8.11.3 To account for both the possibility of drop out and the possibility of a mutation (i.e., the locus is homozygous and either drop out or a mutation could reasonably explain the result), two calculations will be conducted – one without the locus in question and one using the formula addressed in 4.8.10 for the locus in question.

NOTE: If data support that the allele is missing due to drop out (e.g., a peak is observed below LOD in the appropriate position), the calculation for the possibility of a mutation will not be used.

4.8.12 The following formula is used when calculating Paternity/Maternity type calculations involving a potential mutation:

$PI = M_{SR} / 2P_A$ M_{SR} refers to the specific mutation rate for changing allele S to R

$M_{SR} \cong \mu$ μ represents the average mutation rate published by Ge et al.

$PI = \mu / 2P_A$ $P_{A,}$ etc., is the estimated frequency of the "A" allele in the population

NOTE: Depending upon the genotypes exhibited by the child and alleged parent, this formula may need to be modified.

Average Mutation Rates Published in Table 1 in Ge et al. *Investigative Genetics* 2012, **3**.1

Locus	Paternal	Maternal
D3S1358	0.00168	0.000255
D1S1656	0.00154	0.00037
D2S441	0.00154	0.00037
D10S1248	0.00154	0.00037
D13S317	0.00174	0.000403
Penta E	0.00026	0.000253
D16S539	0.00103	0.000525
D18S51	0.00223	0.000793
D2S1338	0.00136	0.000249
CSF1PO	0.00198	0.000319
Penta D	0.000259	0.000253
TH01	0.000052	0.0000603
vWA	0.00325	0.000468
D21S11	0.00175	0.00118
D7S820	0.00137	0.0000723
D5S818	0.00166	0.000269
TPOX	0.000165	0.000105
D8S1179	0.00206	0.000333
D12S391	0.00154	0.00037
D19S433	0.000975	0.000548
FGA	0.00371	0.000493
D22S1045	0.00154	0.000370

4.8.13 Alleged Sibling Formulas

Sibling Genotype		Probability of S2 genotype if two persons are:		
S1	**S2**	**Truly Related**	**Are Unrelated**	**Sibling Index**
AA	AA	$(1+a)^2/4$	a^2	$(1+a)^2/4\ a^2$
AA	AB	(b+ab)/2	2ab	(1+a)/4a
AA	BB	$b^2/4$	b^2	¼
AA	BC	bc/2	2bc	¼
AB	AB	(1+a+b+2ab)/4	2ab	(1+a+b+2ab)/8ab
AB	AA	$(a+a^2)/4$	a^2	(1+a)/4a
AB	AC	(c+2ac)/4	2ac	(1+2a)/8a
AB	CD	cd/2	2cd	¼

4.8.14 A sibling index threshold of 33 is designated based upon the literature, at and above which, it will be concluded that the statistical data support a sibling relationship and below which the evidence in support of a sibling relationship is deemed inconclusive.

4.9 Profiles for Which a Relative of the Included (Not Eliminated) Person of Interest is Suspected to Have Possibly Been the Donor of the Profile

4.9.1 Best practice is to obtain a known reference from the relative, if at all possible.

4.9.2 If a suspected relative sample cannot be obtained, the following formulas may be used to determine the conditional probability that the relative has a particular genotype consistent with that of the included person of interest:

Genotype of the person of interest	Probability of the same genotype in a relative
Homozygote: AA	$pA^2 + 4pA(1-pA)F$
Heterozygote: AB	$2(pA)(pB) + 2(pA + pB – 4(pA)(pB))F$

F represents the kinship coefficient and pA and pB represent the frequency of alleles for the race of the relative in question.

For parents and offspring:	F = 1/4
For half-siblings:	F = 1/8
For uncle/aunt or nephew/niece:	F = 1/8
For first cousins:	F = 1/16

For full siblings the following formulas will be used:

Homozygote: AA	$(1 + 2pA + pA^2)/4$
Heterozygote: AB	$(1 + pA + pB + 2(pA)(pB))/4$

4.9.3 Alternatively, the sample may be referred for TrueAllele® analysis upon consultation with the Program Manager (Technical Leader), Assistant Technical Leader and/or the TrueAllele® team.

4.10 Procedure for Calculating Allele and Genotype Frequencies

The following represents an example of data collected for a STR database and the procedures used to determine the allele and genotype frequencies.

EXAMPLE: TH01 locus in Caucasian population (n = 209)

4.10.1 Allele Frequency

Frequency of allele = Number of times the allele was observed out of all possible alleles for a particular locus/2n.

NOTE: Alleles that contain fewer than 5 events are defaulted to 5 events in order to provide a more conservative frequency.

EXAMPLES:

Allele 5 was observed 3 times out of 418 alleles. Therefore, the allele 5 will default to a total of 5 events (5/418) = 0.012

Allele 6 was observed 100 times out of 418 alleles (100/418) = 0.239

Allele 7 was observed 59 times out of 418 alleles (59/418) = 0.141

Allele 8 was observed 50 times out of 418 alleles (50/418) = 0.120

Allele 9 was observed 64 times out of 418 alleles (64/418) = 0.153

Allele 9.3 was observed 68 times out of 418 alleles (68/418) = 0.163

Allele 10 was observed 74 times out of 418 alleles (74/418) = 0.177

Allele 11 was observed 0 times out of 418 alleles. Therefore, the allele 5 will default to a total of 5 events (5/418) = 0.012

The sum of the individual allele frequencies should equal approximately 1.000. However, because events less than 5 are defaulted to 5, the total frequencies may not total to exactly 1.000:
(0.012) + (0.239) + (0.141) + (0.120) + (0.153) + (0.163) + (0.177) + (0.012) = 1.017

4.10.2 Expected Genotype Frequency

Based on the assumption that the TH01 genetic locus is in Hardy-Weinberg equilibrium, the expected genotype frequencies are calculated from the allele frequencies, as in the following examples:

TH01 Genotype 7, 7:

(Frequency of the 7 allele)^2 + Frequency of the 7 allele(1-Frequency of the 7 allele)θ
=
$(0.141)^2 + 0.141(1-0.141)0.01 = 0.021$

OR

TH01 Genotype 7, 9.3:

2(Frequency of the 7 allele)(Frequency of the 9.3 allele) = 2(0.141)(0.163) = 0.046

APPENDIX A – CODE OF VIRGINIA – PATERNITY

How parent and child relationship established.

Refer to § 20-49.1.

"…Scientifically reliable genetic tests, including blood tests, which affirm at least a ninety-eight percent probability of paternity. Such genetic test results shall have the same legal effect as a judgment entered pursuant to § 20-49.8…."

Administrative establishment of paternity.

Refer to § 63.2-1913.

"…A genetic test result affirming at least a ninety-eight percent probability of paternity shall have the same legal effect as a judgment entered pursuant to § 20-49.8…."

APPENDIX B – REFERENCES

1. PowerPlex® Fusion System Technical Manual.

2. Hill, C., *et al.* (2013) U.S. population data for 29 autosomal STR loci, *Forensic Science International*, Volume 7, Issue 3, pages e82-e83.

3. Bär W. *et al*. (1997) DNA recommendations: further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems, *Int. J. Legal Med.* **110**, 175.

4. Gill, P. *et al*. (1997) Considerations from the European DNA profiling group (EDNAP) concerning STR nomenclature. *Forensic Science International* **87**, 185-192.

5. Promega Technical Support, personal communication.

6. Crouse C. et al. (1999) Analysis and interpretation of short tandem repeat microvariants and three-banded allele patterns using multiple allele detection systems. *J Forensic Sci* **44**,87-94.

7. Virginia Department of Forensic Science studies associated with the internal validation of the PowerPlex® Fusion System.

8. Gill, P. *et al*. (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA, *Forensic Science International* 112, 17-40.

9. Curran, J.M., *et al*. (2005) Interpretation of repeat measurement DNA evidence allowing for multiple contributors and population substructure, Forensic Science International 148, 47-53.

10. The Evaluation of Forensic DNA Evidence, National Academy Press, Washington D.C., 1996.

11. Evett, Ian W. and Weir, Bruce S., 1998. Interpreting DNA Evidence, Chapter 6.

12. Gürtler, H. 1956. Principles of blood group statistical evaluation of paternity cases at the University Institute of Forensic Medicine, Copenhagen, Acta. Med. Leg. Soc., Liège 9:83-93.

13. Paternity Testing, American Association of Blood Banks, New Orleans, Louisiana, 1979

14. Fung, W., Wong, D.M., and Tsui, P. 1996. Determination of both parents using DNA profiling, Jurimetrics Journal, 36:337-342

15. Wenk, R.E., Traver, M., and Chiafari, F.A., 1996. Determination of sibship in any two person, Transfusion, 36:259-262

16. Gjertson, D.W., Appendix 13. The effect of an isolated single-locus inconsistency in the statistical evaluation of paternity, adapted from presentations given by Debra Endean and David Gjertson at the Promega Sponsored Statistical Workshop (September 1996) and to the English Speaking Working Group of the International Society of Forensic Haemogenetics (October 1996).

17. Endean, D. and Gjertson, D., Suspected DNA mutations: statistical approach.

18. Gill, P. *et al*. (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA, Forensic Science International 112, 17-40.

19. Curran, J.M., *et al*. (2005) Interpretation of repeat measurement DNA evidence allowing for multiple contributors and population substructure, Forensic Science International 148, 47-53.

20. Guidance for Standards for Relationship Testing Laboratories, 8th Edition.

21. Chang EP and Linacre A. Systematic evaluation of sensitivity and specificity of sibship determination by using 15 STR loci. J Forensic Legal Med 2008;15:329-34.

22. Reid TM, Wolf CA, Kraemer CM, Lee SC, Baird ML and Lee RF. Specificity of sibship determination using the ABI Identifiler multiplex system. J Forensic Sci 2004;49:262-4.

23. Pu CE and Linacre A. Systematic evaluation of sensitivity and specificity of sibship determination by using 15 STR loci. J Forensic and Legal Med (2008);15:329-34.

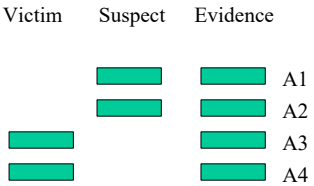
24. Ge et al. *Investigative Genetics.* 2012, **3**:1. http://www.Investigativegenetics.com/content/3/1/1

APPENDIX C – TRADITIONAL LIKELIHOOD RATIO CALCULATION FORMULAS

The following formulas address the question what is the likelihood that the suspect left the DNA that is different from the victim and/or the likelihood that the suspect is a co-contributor of the genetic material identified on the item of evidence. These formulas may also be used to answer the converse question what is the likelihood that the victim left the DNA different from the suspect. However, the appropriate alleles must be plugged into the formulas to obtain this information.

C1 and C2 columns below refer to columns found in the Popstats software and refer to the numerator and denominator, respectively.

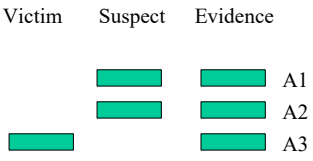
1.



$LR = 1/ 2P_1P_2$

C1 column 0 unknown - leave field blank
C2 column 1 unknown - enter alleles A1 & A2

2.



$LR = 1/ 2P_1P_2$

C1 column 0 unknown - leave field blank
C2 column 1 unknown - enter alleles A1 & A2

3.

$$LR = 1/P_1^2 + 2P_1P_2 + 2P_1P_3$$

C1 column 0 unknown - leave field blank
C2 column 1 unknown - enter allele A1

4.

$$LR = 1/P_1^2 + 2P_1P_2 + P_2^2$$

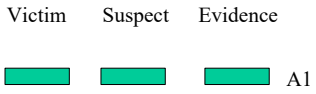
C1 column 0 unknown - leave field blank
C2 column 1 unknown - leave field blank

5.

$$LR = 1/P_1^2 + 2P_1P_2 + P_2^2$$

C1 column 0 unknown - leave field blank
C2 column 1 unknown - leave field blank

6.



$$LR = 1/P_1^2$$

C1 column 0 unknown - leave field blank
C2 column 1 unknown - leave field blank

7.

$$LR = 1/2P_1P_2 + P_2^2$$

C1 column 0 unknown - leave field blank
C2 column 1 unknown - enter allele A2

8.

$$LR = 1/2P_1P_2 + P_2^2$$

C1 column 0 unknown - leave field blank
C2 column 1 unknown - enter allele A2

The formulas provided above were obtained from Ian W. Evett and Bruce S Weir's 1998 book, Interpreting DNA Evidence. These are general formula that cover most of the cases that are handled on a day-to-day basis by the

Virginia Department of Forensic Science. However, depending on the scenario of the case other likelihood ratio formulas not listed above may need to be used.