Department of Forensic Science

FORENSIC BIOLOGY PROCEDURES MANUAL

INTERPRETATION OF POWERPLEX® 16 CE DATA

TABLE OF CONTENTS

1 General Interpretation Workflow

2 Interpretation of PowerPlex® 16 CE Data

2.1 Examining Internal Lane Standard Results
2.2 Examining Allelic Ladder Results
2.3 Examining the Reagent Blank(s)
2.4 Examining the Negative Control (Negative Amplification Control)
2.5 Examining the Positive Control (Positive Amplification Control)
2.6 Examining Casework Samples
2.7 Microvariant / Off Ladder Variant Interpretation and Nomenclature
2.8 Limit of Detection and Stochastic Thresholds
2.9 Expected Minimum Peak Height Ratios for Heterozygous Loci
2.10 General Interpretation of Single Source Samples
2.11 General Interpretation of 2 Person Mixtures
2.12 General Interpretation of 3 Person Mixtures
2.13 General Interpretation of 4 Person Mixtures
2.14 General Interpretation of Profiles from Intimate/Ownership Samples (Use of an Assumed Known)
2.15 Interpretation of Criminal Paternity/Maternity and Missing Person Cases

3 Mixture Deconvolution Procedures

3.1 Major/minor or Major Deconvolutions
3.2 Unrestricted Random Match Deconvolutions (URM)
3.3 Deconvolutions for Profiles Different from an Assumed Known

4 Statistical Calculations

4.1 Procedure for Rounding Frequencies When Calculating by Hand
4.2 Random Match Probability (RM)
4.3 Traditional Likelihood Ratio (LR)
4.4 Combined Probability of Inclusion
4.5 Unrestricted Random Match Probability (URM)
4.6 Likelihood Ratio Generated by TrueAllele®
4.7 Paternity/Relationship Statistical Calculations
4.8 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 Procedure for Calculating Allele and Genotype Frequencies

Appendix A Code of Virginia – Paternity

Appendix B References

Appendix C STR Population Frequencies

Appendix D Traditional Likelihood Ratio Calculation Formulas

1 GENERAL INTERPRETATION WORKFLOW

The raw PowerPlex® 16 CE data obtained from the 3130*xl* is to be analyzed using the GeneMapper® ID (GMID) or GeneMapper® ID-X (GMID-X) software prior to following these interpretation steps. Refer to FB PM, Analysis of CE Results using GeneMapper® ID or 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® 16 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 TrueAllele® (URM, CPI, RM, traditional LR using POPSTATS).
- M/m = Major/minor
- Probative Evidence = Evidence through testing that demonstrates the proposition that a biological fluid/material may or may not have been deposited by a specific "individual of interest" who is believed to be associated with the evidence in question.
- 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 or 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 Using the following procedures, an evidence profile may fall within any of the following categories.

1.1.2 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 2.10-2.15 below in mind in making this determination.

1.1.2.1 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 1.1.2 need all be considered.

1.1.2.2 Assumed known reference samples may be evaluated as 1.2 describes and used to aid in this assessment.

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

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

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

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

1.1.4 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 modeling (i.e., TrueAllele®) on the same mixture. If a mixture is

developed for which it is clear a TrueAllele® referral will be made, the referral should be made to address all non-eliminations, 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.

1.1.4.1 Mixture profiles for which a CPI calculation will be conducted or that will be referred to TrueAllele® due to the case type or a lack of breakout loci for a URM will not be manually deconvoluted; however the assumption of the number of contributors and the intent to perform a CPI or refer to TrueAllele® will be documented on the electropherogram or landscape.

1.1.4.2 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. The unselected mixtures, if this option is chosen, will remain available for further interpretation, if necessary or if requested.

1.1.4.2.1 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.

1.1.4.2.2 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 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.

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

1.2.1 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.

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

1.2.1.1 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.

1.2.1.2 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.

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

1.3.1 The results of a comparison of a known 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.
- Because no traditional statistical calculations can be conducted, no conclusions will be made. (This generally applies to samples from non-person cases for which 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 is made with regard to a mixture profile that will be referred to TrueAllele® for statistical calculations, no conclusions will be reported by the primary examiner. The conclusion and statistical analysis, if applicable, will be reported by the TrueAllele® examiner.

1.4 Once conclusions are drawn, applicable statistics will be calculated or samples referred to TrueAllele® or for kinship statistics for conclusions and statistics.

1.4.1 All conclusions of not eliminated require a statistical calculation within the same Certificate of Analysis.

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

1.5 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.

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

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

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

1.5.1.1.2 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.

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

1.5.2 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 technical 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® 16 CE DATA

Amelogenin is used only to indicate gender and does not apply to any locus/allele counts or other interpretation rules detailed in this manual.

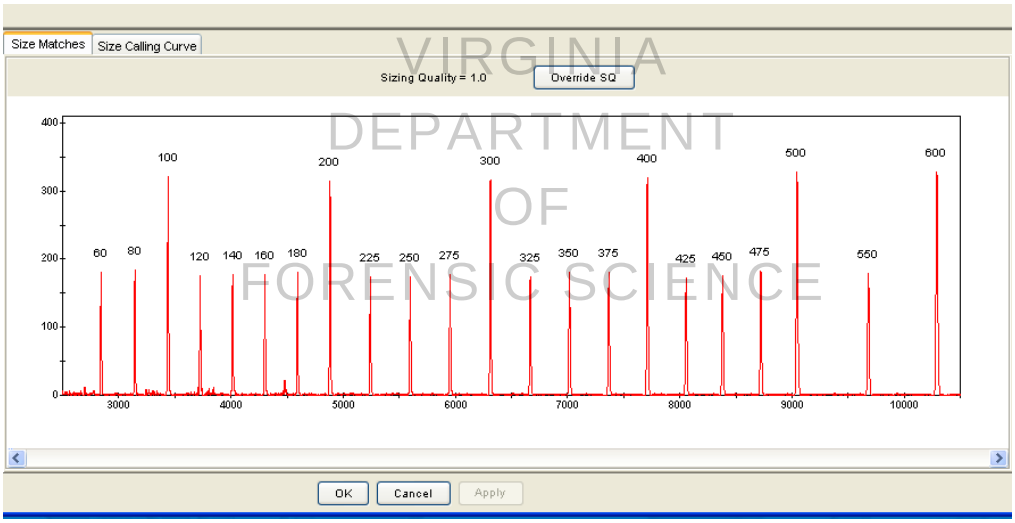
Amelogenin will not be used for any statistical purposes in any case.

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 ILS 600 Size Standard peaks should appear as follows:

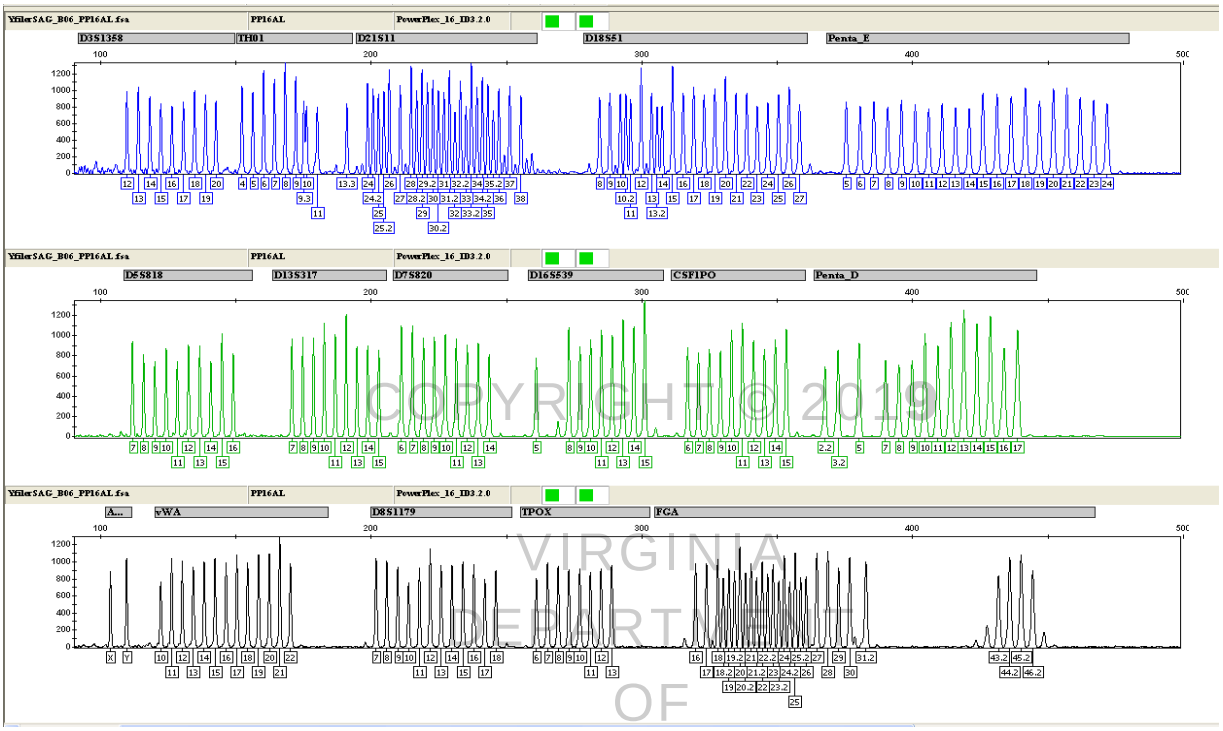


2.1.2 To assess the ILS size standard for individual samples, highlight the sample(s) and select Tools→ Size Match Editor or click on the following icon:

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 or FB PM, Analysis of CE Results using GeneMapper® ID-X as appropriate.

2.2 Examining Allelic Ladder Results

2.2.1 The PowerPlex® 16 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 display plots icon.

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

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

2.3 Examining the Reagent Blank(s)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2.4 Examining the Negative Control (Negative Amplification Control)

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

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

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

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

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

2.5 Examining the Positive Control (Positive Amplification Control)

2.5.1 The positive amplification control DNA supplied with the PowerPlex® 16 kit is 2800M. The correct types are as follows:

Locus	Genotype
D3S1358	17,18
TH01	6, 9.3
D21S11	29, 31.2
D18S51	16,18
Penta E	7, 14
D5S818	12
D13S317	9, 11
D7S820	8, 11

D16S539	9, 13
CSF1PO	12
Penta D	12, 13
Amelogenin	X, Y
VWA	16, 19
D8S1179	14, 15
TPOX	11, 11
FGA	20, 23

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 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 or FB PM, Analysis of CE Results using GeneMapper® ID-X as needed.

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

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 4 nucleotides smaller than the amplicon size (true peak) 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.

2.6.2.3.1 Peaks in the N-1 or N+1 position that fall below the percentages listed below must be edited out as stutter. The, the N-1 stutter percentages are included in the GMID analysis method in use and are automatically applied. 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 or 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	2.4	13	4.3
TH01	1.5	5	N/A
D21S11	3.3	14	6.8
D18S51	2.7	14	9.7
Penta E	N/A	8	1.0
D5S818	1.5	11	3.9
D13S317	1.4	11	3.8
D7S820	3.1	10	5.0
D16S539	1.4	11	2.4
CSF1PO	N/A	11	6.6
Penta D	N/A	4	1.0
vWA	13.5	18	8.2
D8S1179	2.2	11	2.1
TPOX	N/A	6	N/A
FGA	1.3	13	2.4

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.

2.6.3 Indication of Gender Using the Amelogenin Locus

2.6.3.1 A single source sample exhibiting a peak only at ~106 bp (X allele) will generally be considered to have originated from a female.

2.6.3.2 A single source sample exhibiting a peak at both ~106 bp (X allele) and ~112 bp (Y allele) 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) will be considered to have inconclusive amelogenin results and will be inconclusive with regard to gender.

2.6.3.4 A sample known to have originated from a male but exhibiting only a peak at ~106 bp (X allele) will be considered to have inconclusive amelogenin results.

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 or GMID-X software or labeled "OMR" for out of marker range by the GMID-X software, review the data to determine that it is a true microvariant (MV) or off-ladder (OL) allele. True OL or MV peaks may be confirmed through re-injection or re-amplification, if necessary.

NOTE: Peaks outside of a locus whether unlabeled by GMID or labeled OMR by GMID-X and determined to be real OL peaks should be addressed as described in 2.7.2-2.7.6. Once those instructions have been followed, they can be 'assigned' to their proper locus in GMID-X only 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), 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.

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

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

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

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.

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.

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 of the lower molecular weight locus and to the smallest allelic ladder peak 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). If it is within an appropriate size distance from 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.

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.

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 or GMID-X software and will not be used as a called allele in the resulting profile.

2.8.1.2 The limit of detection (LOD) for each dye channel is shown below, with the exception of the red (ILS) dye channel. The red LOD default will be set to 52 RFU, but can be adjusted as needed to capture all ILS peaks in a sample.

Channel Color	LOD (RFU)
Blue	73
Green	84
Yellow	75

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 10 second injection is 460 RFU. The STH for a 5 second injection is 320 RFU. The STH for a 2 second injection is 210 RFU.

EXAMPLES: If a homozygous peak in a single source sample injected at 5 seconds is ***at or below*** 320 RFU, 2p will be used in any associated RM statistical calculation for that locus.

If a 12 allele observed in a mixture injected at 10 seconds is ***at or below*** 460 RFU, the "12, any" (12x) option will be used for any associated URM calculation.

2.9 Expected Minimum Peak Height Ratios for Heterozygous Loci

These values were derived from internal validation by the Department.

2.9.1 Heterozygous loci should generally meet the following minimum peak height ratios for peaks from the same contributor:

Locus	Minimum PHR
D3S1358	69%
TH01	53%
D21S11	72%
D18S51	61%
Penta E	33%
D5S818	75%
D13S317	55%
D7S820	59%
D16S539	51%
CSF1PO	61%
Penta D	53%
Amelogenin	64%
VWA	60%
D8S1179	78%
TPOX	59%
FGA	50%

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 expectations listed above.
  - 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 7 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
- At least 4 of the 7 loci must be heterozygous OR have a homozygous allele above the STH (restricted genotypes).
- Allelic drop out is not counted at loci where no DNA typing results are obtained.
- Possible allelic drop out can occur for data that is detected below the STH.
- A maximum of 3 instances of allelic drop out can occur for data below the STH when compared to a reference sample for a conclusion of not eliminated to 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 with the exception of amelogenin.
  - Statistical calculations will incorporate 2p for homozygous alleles below the STH.

2.11 General Interpretation of 2 Person Mixtures

- 1-4 alleles are detected per locus.
- DNA typing results are required at 7 or more loci before conclusions can be reached.
- A maximum of 3 loci with drop out may occur when compared to a reference samples for a conclusion of not eliminated to be reached.
  - Exceptions may be considered by the Program Manager (Technical Leader) or Assistant Technical Leader and, if granted, must be documented in the case file.
- Allelic 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.
  - 4 or more loci 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.
  - If CPI cannot be calculated for a non-person case, a URM will be calculated at loci where breakout has occurred (3 or 4 alleles at a locus).
    - URM may be calculated at a minimum of 1 locus.
  - If neither a CPI nor a URM can be calculated (all alleles are not >STH and no breakout loci are available for the URM) for a non-person case, no comparisons will be conducted for that sample.
    - Exceptions may be considered on a case by case basis by the Program Manager (Technical Leader) or Assistant Technical Leader.
  - If CPI cannot be calculated for a person case, the profile will be will be forwarded to TrueAllele® for statistical analysis.
    - TrueAllele® analyses may not be performed for cases in which other equally probative samples are subjected to traditional statistics. Consultation with the TrueAllele® team may be necessary. In these cases, a URM may be calculated on the mixture, if possible.

2.12 General Interpretation of 3 Person Mixtures

- 1-6 alleles are detected per locus.
- DNA typing results are required at 7 or more loci before conclusions can be reached.
- A maximum of 3 loci with drop out may occur when compared to a reference samples for a conclusion of not eliminated to be reached.
  - Exceptions may be considered by the Program Manager (Technical Leader) or Assistant Technical Leader and, if granted, must be documented in the case file.

- Allelic 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.
  - 4 or more loci are required for statistics to be calculated for major contributors.
  - A person of interest 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)
  - 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.
  - If a CPI cannot be calculated for a non-person case, a URM will be calculated at loci where breakout has occurred (5or 6 alleles at a locus).
    - URM may be calculated at a minimum of 1 locus.
  - If neither a CPI nor a URM can be calculated (all alleles are not >STH and no breakout loci are available for the URM) for a non-person case, no comparisons will be conducted for that sample.
    - Exceptions may be considered on a case by case basis by the Program Manager (Technical Leader) or Assistant Technical Leader.
  - If a CPI cannot be calculated for a person case, the profile will be forwarded to TrueAllele® for statistical analysis.
    - TrueAllele® analyses may not be performed for cases in which other equally probative samples are subjected to traditional statistics. Consultation with the TrueAllele® team may be necessary. In these cases, a URM may be calculated, 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 clearly discernable from noise but below 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.

Exceptions to allow the use of a 4 person mixture for elimination only may be considered 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 from Intimate/Ownership Samples (Use of an Assumed Known)

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

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

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

- 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's should generally be within peak height ratio.
  - 4 or more loci with results different from the assumed known's are required for statistics to be calculated.

- A maximum of 3 loci with drop out may occur when compared to a reference sample for a conclusion of not eliminated to be reached.
- 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 at loci where a 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.
  - 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).
  - Non-intimate samples/ownership samples for which two assumed knowns are submitted (steering wheel with 2 drivers submitted, etc.) will be interpreted using a general mixture approach.
    - Rare exceptions may be considered on a case by case basis by the Program Manager (Technical Leader) or Assistant Technical Leader.

2.15 Interpretation of Criminal Paternity/Maternity and Missing Person Cases

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

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

2.15.2 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.

2.15.2.1 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.

2.15.2.2 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.

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 to TrueAllele®. Input from a supervisor, the Program Manager (Technical Leader) or Assistant Technical Leader may be sought.

Examples 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 or another person 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 a 12, 13, 17, 19.
  - Examples of possible documentation for this locus are URM, URM_2, and listing all possible combinations of the four alleles (12,13; 12,17; 12,19; 13,17; 13,19; 17,19).
- A 2 contributor URM is conducted on a locus with a 12, 13, 17 – all above STH.
  - Examples of possible documentation for this locus are URM + H, URM_2 + H, URM + homozygotes, URM, URM_2 and listing all possible combinations (12; 12,13; 12,17; 13; 13,17; 17).
- 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 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 to TrueAllele®. 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.

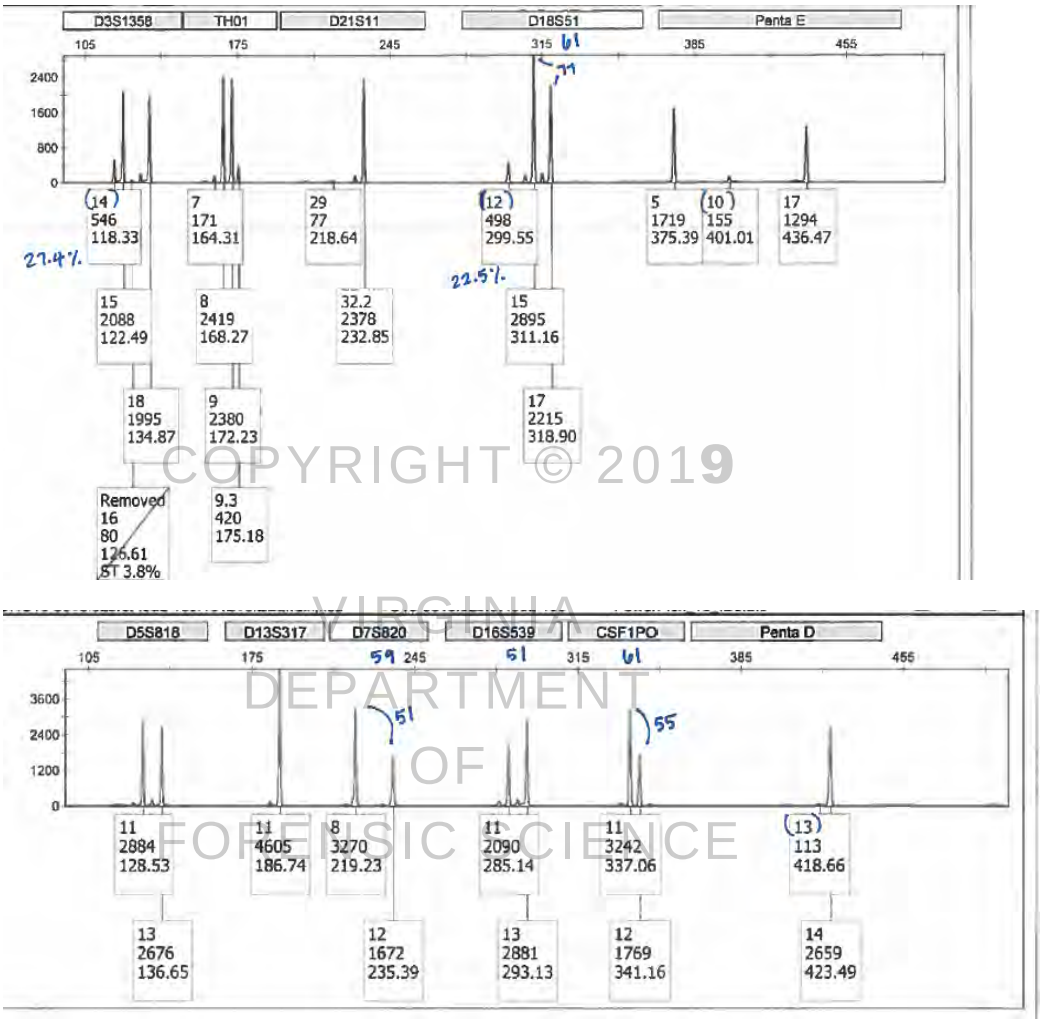
3.1.4 At least one allele must be above STH in order for M/m to be determined at that locus.

3.1.5 For 2 person mixtures, the major alleles for a locus must be within expected peak height ratio 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 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.

3.1.8 Examples of deconvoluted loci are below:



The STH for this sample is 460 RFU.

This is a 2 person mixture with a M/m. The minor deconvolution will be shown for this example, but was deemed to be of no value due to the limited results.

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 by hand.

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

D3S1358:	M: 15,18	/	m: [14,14; 14,15 or 14,18]
TH01:	M: 8,9	/	m: 7,9.3
D21S11:	M: 32.2,32.2	/	m: 29x
D18S51:	M: 15,17	/	m: [12,12; 12,15 or 12,17]
Penta E:	M: 5,17	/	m: 10x
D5S818:	M: 11,13	/	no minor
D13S317:	M: 11,11	/	no minor
D7S820	no major	/	no minor

D16S539:	M: 11,13	/	no minor
CSF1PO:	no major	/	no minor
Penta D:	M: 14,14	/	m: 13x

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.

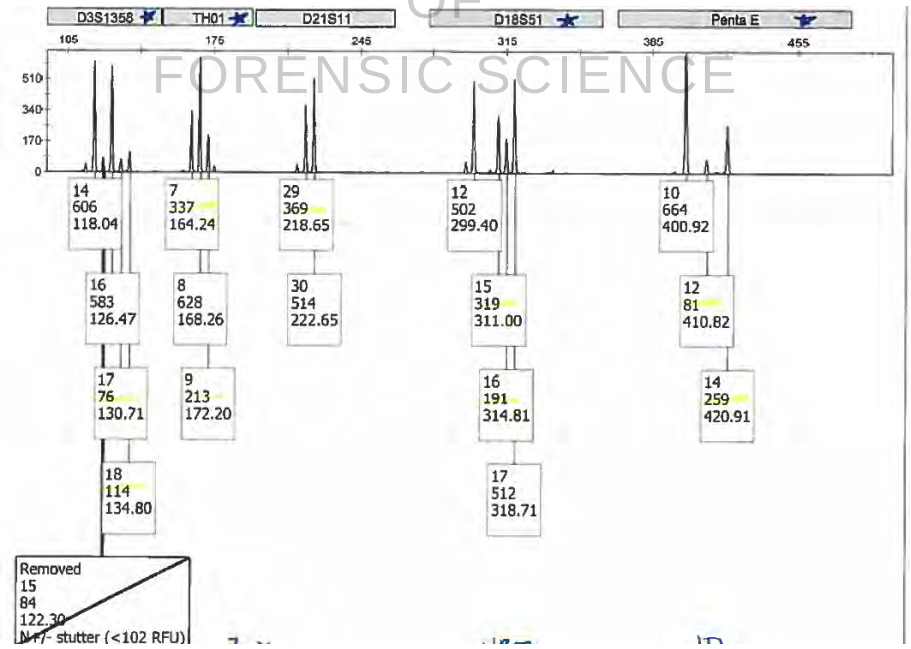
3.2.2.1 Considerations may be made regarding referral to TrueAllele® as opposed to conducting a non-informative URM in certain cases.

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.

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:

EXAMPLE 1 – 2 contributor URM

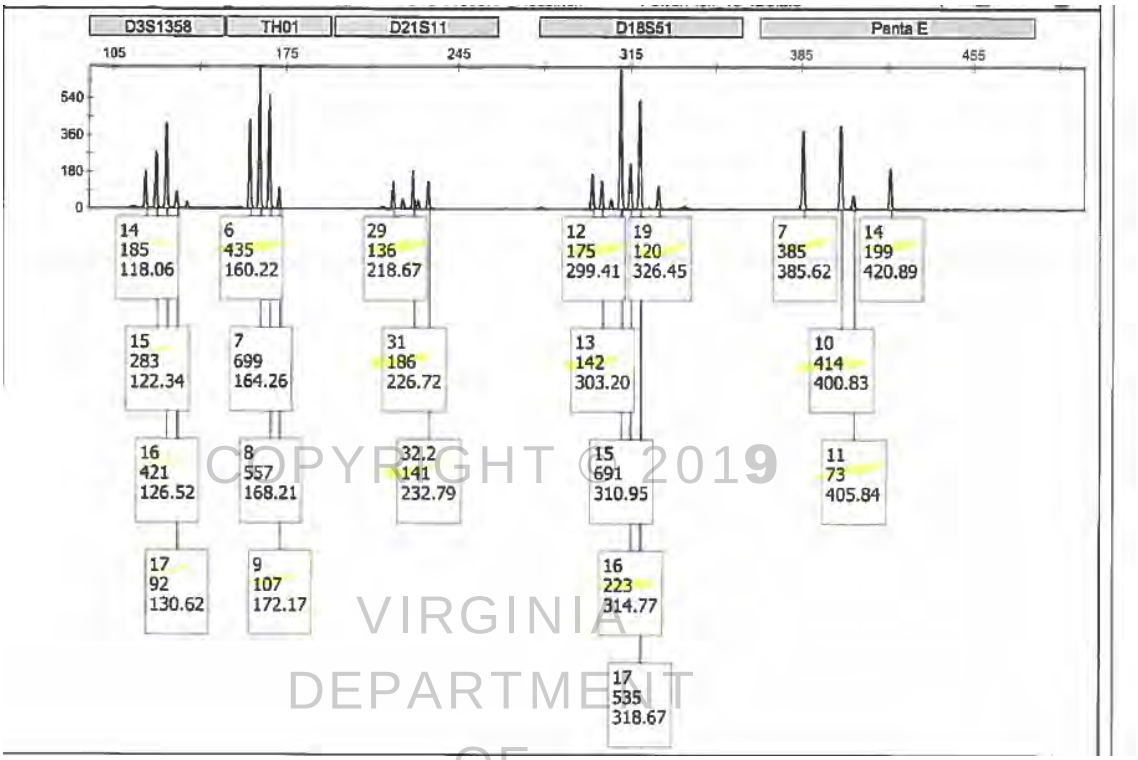


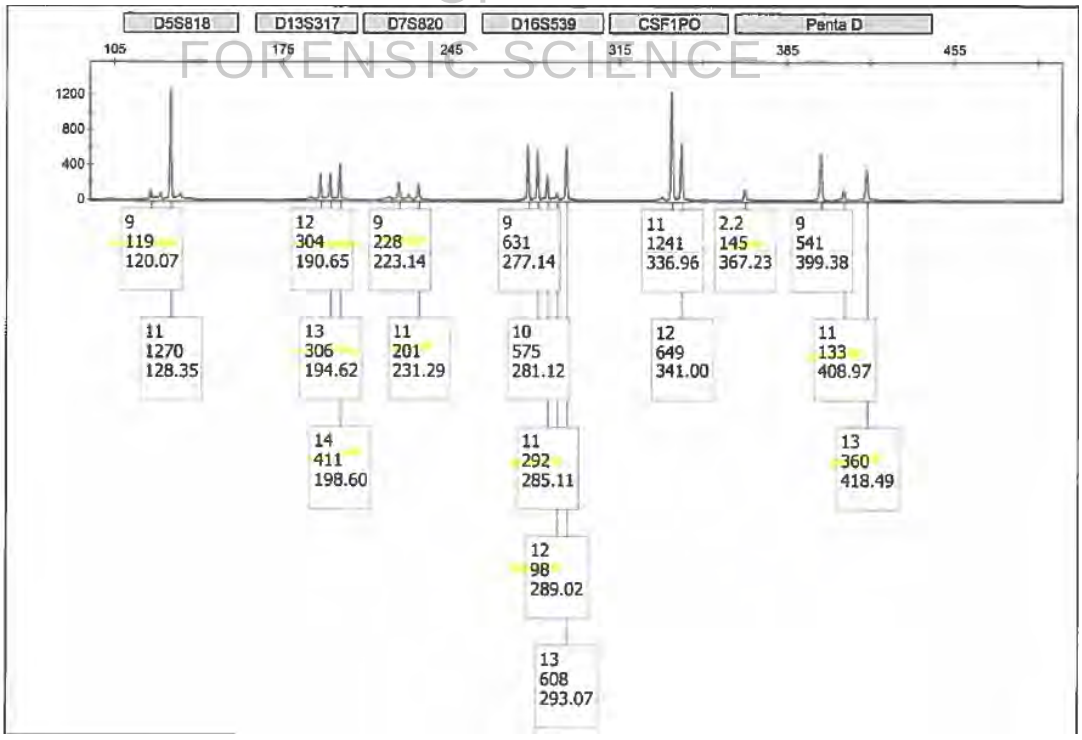
The STH for this sample is 460 RFU.

The stars indicate break out loci at which a URM can be designated.

D3S1358:	URM		
TH01:	7x	8,8	9x
D18S51:	URM		
Penta E:	10,10	12x	14x

EXAMPLE 2 – 3 contributor URM





The STH for this sample is 460 RFU.

D18S51:	URM							
D16S539:	9,9	9,10	9,13	10,10	10,13	13,13	11x	12x

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 to TrueAllele®).

3.3.2 Dosage may be considered in assigning alleles as different from the assumed contributor.

3.3.3 Examples of deconvoluted loci are below:

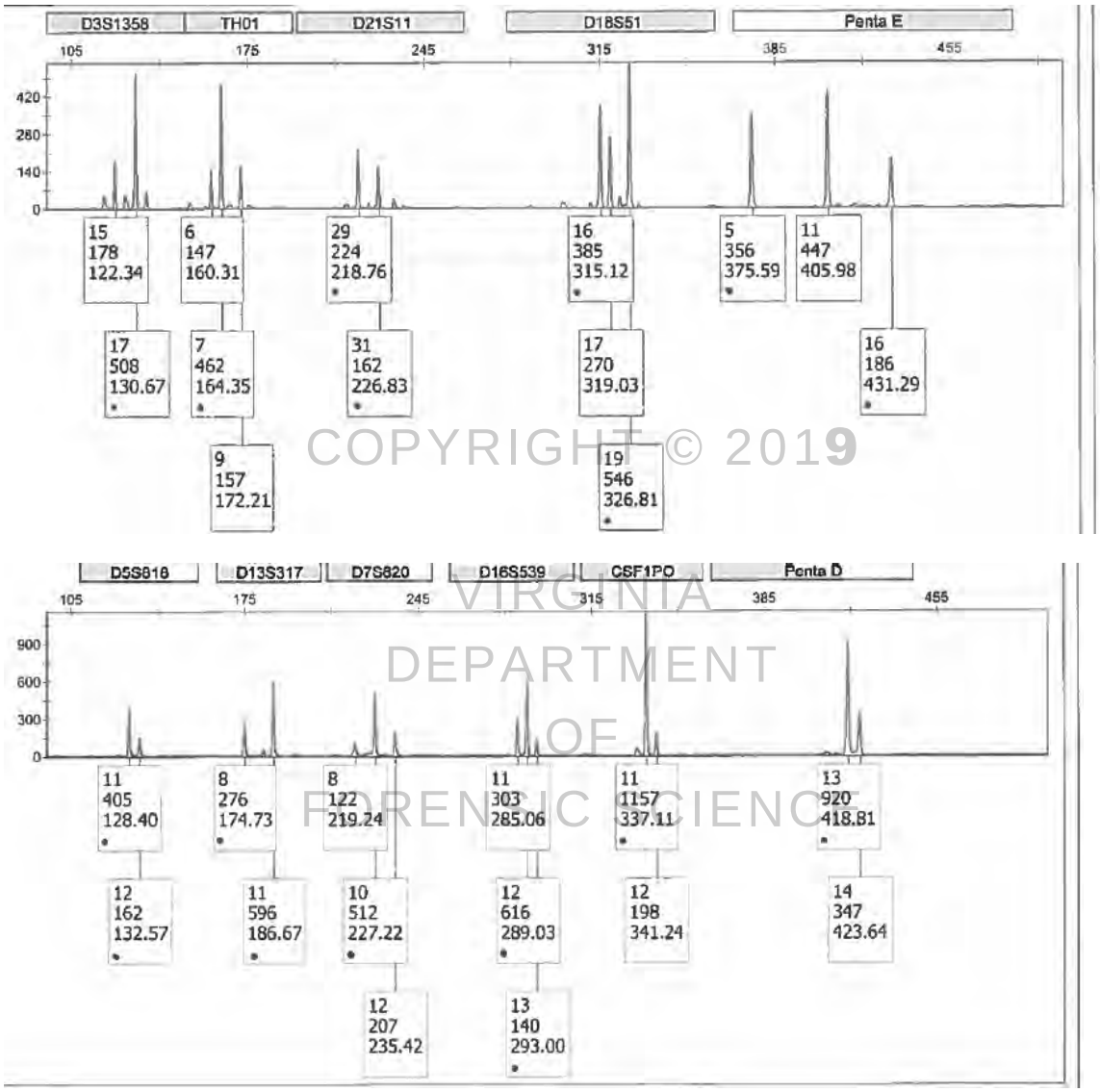
EXAMPLE 1

The STH for this sample is 210 RFU.

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

D3S1358:	17,18
TH01:	8,9
D21S11:	27,31
D18S51:	14,18 (this includes the use of dosage – another option is: [14,14; 10, 14; 14, 18]
Penta E:	11,13

EXAMPLE 2



The STH for this sample is 460 RFU.

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

D3S1358:	15x
TH01:	6, 9
D21S11:	---
D18S51:	17x
Penta E:	11x
D5S818:	---
D13S317:	---
D7S820	8, 12
D16S539:	11x
CSF1PO:	12x
Penta D:	14x

4 STATISTICAL CALCULATIONS

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

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

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 generated by the Virginia Department of Forensic Science (see Appendix C for the VA DFS population allele frequencies).

If statistical calculations using a population database different from Caucasian, Hispanic or African American are requested, discuss with the Biology Program Manager.

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).

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 of the person of interest's alleles 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 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 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 or a major/minor deconvolution 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.

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 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.
- 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.

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 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 for the 15 allele.

4.6 Likelihood Ratio Generated by TrueAllele®

4.6.1 This calculation will be used for mixtures in person cases that do not qualify for a traditional statistical calculation. 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.

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

Exceptions for non-person case mixture samples or mixtures for which the traditional statistical calculation is limited in its use of the available data and therefore not informative may be considered by the Program Manager (Technical Leader) and or Assistant Technical Leader.

4.7 Paternity/Relationship Statistical Calculations

4.7.1 POPSTATS Software will be used for these calculations.

4.7.2 Any locus at which a single allele is observed and that allele is at or below STH will be dropped from the calculation except in cases addressed in 4.7.10.

4.7.3 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)

4.7.4 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	$½(P_A + P_B)$

4.7.5 Combined Paternity Index (CPI)

The combined paternity index is calculated by multiplying together each individual paternity index.

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

4.7.6 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

4.7.7 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

4.7.8 Paternity/Maternity/Relationship Calculations from a Single Grandparent

Child	Grandmother	Grandfather	Paternity Index
AA	AA	Unknown	(1 + a) / 2a
AA	AB	Unknown	(1 + 2a) / 4a
AA	BC	Unknown	1 / 2
AA	BB	Unknown	1 / 2
AB	AA	Unknown	(1 + 2a) / 4a
AB	AB	Unknown	(a + b + 4ab) / (8ab)
AB	BC	Unknown	(1 + 4b) / (8b)
AB	BB	Unknown	(1 + 2b) / (4b)
AB	CC	Unknown	1 / 2
AB	CD	Unknown	1 / 2

4.7.9 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	$2P_AP_B \times 2P_CP_D \times ½ \times ½$	$2P_AP_B \times 2P_CP_D \times 2P_AP_C$	$1 / 8P_AP_C$

4.7.10 When performing a Missing Person or Paternity Index calculation on a partial DNA profile it is possible that an individual or an evidence sample may be identified that cannot be eliminated as a possible offspring/parent or have originated from the offspring/parent. However, the individual's DNA profile or the evidence sample 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. Therefore, to account for both of these situations two probabilities will be provided in the Certificate of Analysis and will be calculated in the following manner:

4.7.10.1 To account for the possibility of allele drop out, the locus will not be used in the overall calculation.

4.7.10.2 To account for the possibility of a mutation, the formula addressed in 4.7.11 will be used for the locus and factored into the overall calculation.

EXCEPTION: 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 may be omitted.

4.7.11 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 reported by the American Association of Blood Banks (AABB)

$PI = \mu / 2P_A$ 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 Established by the AABB

Locus	Paternal	Maternal
FGA	0.0029	0.0005
TPOX	0.0002	<0.0001
D8S1179	0.002	0.0003
VWA	0.0032	0.0003
Penta E	0.0012	0.0002
D18S51	0.002	0.0006
D21S11	0.0015	0.0011
TH01	0.0001	0.0001
D3S1358	0.0015	0.0003
Penta D^{12}	0.00066	0.00064
CSF1PO	0.0014	0.0002

D16S539	0.0011	0.0002
D7S820	0.0013	0.0002
D13S317	0.0015	0.0005
D5S818	0.0014	0.0002

4.7.12 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

4.7.13 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.

4.7.14 Alleged Half-Sibling Formulas (also Grandparent-Child or Uncle/Aunt-Niece/Nephew)

Half-Sibling Genotype		**Probability of S2 genotype if two persons are:**		
S1	**S2**	**Truly Related**	**Are Unrelated**	**Half-Sibling Index**
AA	AA	$(a+a)^2/2$	a^2	(1+a)/2a
AA	AB	(b+2ab)/2	2ab	(1+2a)/4a
AA	BB	$b^2/2$	b^2	½
AA	BC	bc	2bc	½
AB	AB	(a+b+4ab)/4	2ab	(a+b+4ab)/8ab
AB	AA	$(a+2a^2)/4$	a^2	(1+2a)/4a
AB	AC	(c+4ac)/4	2ac	(1+4a)/8a
AB	CD	cd	2cd	½

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

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

4.8.2 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 coeffiecient 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	$(1 + pA + pB + 2(pA)(pB))/4$

4.8.3 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.

4.9 Procedure for Calculating Allele and Genotype Frequencies

4.9.1 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

4.9.2 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:

$$(\text{Frequency of the 7 allele})^2 + \text{Frequency of the 7 allele}(1-\text{Frequency of the 7 allele})\theta$$
$$=$$
$$(0.141)^2 + 0.141(1-0.141)0.01 = 0.021$$

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® 16 System Technical Manual.

2. 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.

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

4. Promega Technical Support, personal communication.

5. 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.

6. Virginia Department of Forensic Science study, "Validation of the PowerPlex® 16 Amplification System: Heterozygous Peak Balance".

7. 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.

8. 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.

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

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

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

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

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

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

15. 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).

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

17. 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.

18. 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.

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

20. 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.

21. 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.

22. 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.

APPENDIX C – STR POPULATION FREQUENCIES

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
BLACK POPULATION DATA
CSF1P0, TPOX, TH01 AND vWA ALLELE FREQUENCY TABLE

DATE: JUNE 1, 1998 N = 194

ALLELE CSF1P0	OBSERVATION	FREQUENCY		ALLELE TH01	OBSERVATION	FREQUENCY
15	0*	0.01289		11	0*	0.01289
14	2*	0.01289		10	4*	0.01289
13	24	0.06186		9.3	34	0.08762
12	107	0.27577		9	50	0.12886
11	90	0.23196		8	96	0.24742
10	92	0.23711		7	154	0.39690
9	14	0.03608		6	47	0.12113
8	29	0.07474		5	3*	0.01289
7	30	0.07732				
6	0*	0.01289				
n = 388				n = 388		
ALLELE TPOX	OBSERVATION	FREQUENCY		ALLELE VWA	OBSERVATION	FREQUENCY
13	1*	0.01289		21	1*	0.01289
12	8	0.02062		20	11	0.02835
11	88	0.22680		19	28	0.07216
10	32	0.08247		18	44	0.11340
9	86	0.22165		17	61	0.15722
8	138	0.35567		16	110	0.28351
7	6	0.01546		15	96	0.24742
6	29	0.07474		14	29	0.07474
				13	6	0.01546
				12	0*	0.01289
				11	2*	0.01289
n = 388				n = 388		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 388, or 0.01289. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
BLACK POPULATION DATA
D16S539, D7S820, D13S317 AND D5S818 ALLELE FREQUENCY TABLE

DATE: JUNE 1, 1998 N = 194

ALLELE D16S539	OBSERVATION	FREQUENCY		ALLELE D13S317	OBSERVATION	FREQUENCY
15	1*	0.01289		15	1*	0.01289
14	12	0.03093		14	24	0.06190
13	56	0.14433		13	54	0.13918
12	73	0.18814		12	158	0.40722
11	113	0.29124		11	127	0.32732
10	37	0.09536		10	7	0.01804
9	81	0.20876		9	10	0.02577
8	15	0.03866		8	7	0.01804
5	0*	0.01289		7	0*	0.01289
n = 388				n = 388		
ALLELE D7S820	OBSERVATION	FREQUENCY		ALLELE D5S818	OBSERVATION	FREQUENCY
14	1*	0.01289		15	0*	0.01289
13	7	0.01804		14	10	0.02577
12	41	0.10567		13	89	0.22938
11	90	0.23196		12	141	0.36340
10	132	0.34021		11	99	0.25515
9	41	0.10567		10	26	0.06701
8	73	0.18814		9	8	0.02062
7	3*	0.01289		8	15	0.03866
6	0*	0.01289		7	0*	0.01289
n = 388				n = 388		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 388, or 0.01289. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
BLACK POPULATION DATA
D18S51 AND D21S11 ALLELE FREQUENCY TABLE

DATE: MAY 19, 2000 N = 192

ALLELE D18S51	OBSERVATION	FREQUENCY		ALLELE D18S51 cont.	OBSERVATION	FREQUENCY
17	60	0.15625		27	0*	0.01302
16	68	0.17708		26	0*	0.01302
15	66	0.17188		25	0*	0.01302
14	25	0.06510		24	0*	0.01302
13.2	1*	0.01302		23	1*	0.01302
13	27	0.07031		22	4*	0.01302
12	28	0.07292		21	6	0.01563
11	0*	0.01302		20	18	0.04688
10.2	3*	0.01302		19	28	0.07292
10	3*	0.01302		18	46	0.11979
9	0*	0.01302				
8	0*	0.01302		n = 384		
ALLELE D21S11	OBSERVATION	FREQUENCY		ALLELE D21S11 cont.	OBSERVATION	FREQUENCY
31.2	19	0.04948		38	0*	0.01302
31	26	0.06771		37	0*	0.01302
30.2	7	0.01823		36	3*	0.01302
30	74	0.19271		35.2	0*	0.01302
29.2	0*	0.01302		35	11	0.02865
29	79	0.20573		34.2	0*	0.01302
28	97	0.25260		34	4*	0.01302
27	16	0.04167		33.2	11	0.02865
26	1*	0.01302		33	3*	0.01302
25.2	0*	0.01302		32.2	30	0.07813
25	0*	0.01302		32.1	0*	0.01302
24.2	0*	0.01302		32	3*	0.01302
24	0*	0.01302		n = 384		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 384, or 0.01302. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
BLACK POPULATION DATA
Penta E AND D3S1358 ALLELE FREQUENCY TABLE

DATE: MAY 19, 2000 N = 168 (Penta E) and 192 (D3S1358)

ALLELE Penta E	OBSERVATION	FREQUENCY		ALLELE Penta E cont.	OBSERVATION	FREQUENCY
15	18	0.05357		25	0*	0.01488
14	20	0.05952		24	0*	0.01488
13	46	0.13691		23	0*	0.01488
12	33	0.09821		22	0*	0.01488
11	26	0.07738		21	1*	0.01488
10	16	0.04762		20.3	0*	0.01488
9	12	0.03571		20	3*	0.01488
8	64	0.19048		19	0*	0.01488
7	37	0.11012		18	4*	0.01488
6	0*	0.01488		17	12	0.03571
5	34	0.10119		16	10	0.02976
				n = 336		
ALLELE D3S1358	OBSERVATION	FREQUENCY		ALLELE D3S1358 cont.	OBSERVATION	FREQUENCY
16.2	1*	0.01302		21	0*	0.01302
16	103	0.26823		20	0*	0.01302
15	124	0.32292		19	1*	0.01302
14	45	0.11719		18	21	0.05469
13	4*	0.01302		17	81	0.21094
12	3*	0.01302				
11	1*	0.01302		n = 384		

Note: * = Alleles for Penta E with fewer than 5 observations are defaulted to a frequency of 5 per 336,or 0.01488. Alleles for D3S1358 with fewer than 5 observations are defaulted to a frequency of 5 per 384, or 0.01302. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
BLACK POPULATION DATA
FGA AND D8S1179 ALLELE FREQUENCY TABLE

DATE: MAY 19, 2000 N = 192

ALLELE FGA	OBSERVATION	FREQUENCY		ALLELE FGA cont	OBSERVATION	FREQUENCY
25	33	0.08594		46.2	1*	0.01302
24.2	0*	0.01302		45.2	0*	0.01302
24	54	0.14063		44.2	2*	0.01302
23.2	0*	0.01302		43.2	0*	0.01302
23	66	0.17188		42.2	1*	0.01302
22.2	0*	0.01302		31.2	0*	0.01302
22	77	0.20052		31	1*	0.01302
21.2	1*	0.01302		30.2	1*	0.01302
21	39	0.10156		30	0*	0.01302
20.2	1*	0.01302		29	2*	0.01302
20	30	0.07813		28	7	0.01823
19.2	1*	0.01302		27	11	0.02865
19	25	0.06510		26.1	0*	0.01302
18.2	9	0.02344		26	18	0.04688
18	4*	0.01302		25.2	0*	0.01302
17	0*	0.01302				
				n = 384		
ALLELE D8S1179	OBSERVATION	FREQUENCY		ALLELE D8S1179	OBSERVATION	FREQUENCY
12	42	0.10938		18	0*	0.01302
11	19	0.04948		17	7	0.01823
10	11	0.02865		16	23	0.05990
9	2*	0.01302		15	71	0.18490
8	2*	0.01302		14	114	0.29688
7	0*	0.01302		13	93	0.24219
				n = 384		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 384, or 0.01302. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
BLACK POPULATION DATA
Penta D ALLELE FREQUENCY TABLE

DATE: JANUARY 7, 2002 N = 100

ALLELE Penta D	OBSERVATION	FREQUENCY		ALLELE Penta D cont.	OBSERVATION	FREQUENCY
10	22	0.11000		17	0*	0.02500
9	34	0.17000		16	0*	0.02500
8	23	0.11500		15	2*	0.02500
7	9	0.04500		14	4*	0.02500
5	8	0.04000		13	15	0.07500
3.2	3*	0.02500		12	20	0.10000
2.2	27	0.13500		11	33	0.16500
				n = 200		

Note: * = Alleles for Penta D with fewer than 5 observations are defaulted to a frequency of 5 per 200, or 0.0250. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
CAUCASIAN POPULATION DATA
CSF1P0, TPOX, THO1 AND vWA ALLELE FREQUENCY TABLE

DATE: JUNE 1, 1998 N = 174

ALLELE CSF1P0	OBSERVATION	FREQUENCY		ALLELE THO1	OBSERVATION	FREQUENCY
15	0*	0.01437		11	0*	0.01437
14	3*	0.01437		10	4*	0.01437
13	27	0.07759		9.3	108	0.31034
12	125	0.35919		9	50	0.14368
11	97	0.27874		8	35	0.10057
10	83	0.23851		7	66	0.18966
9	11	0.03161		6	83	0.23851
8	1*	0.01437		5	2*	0.01437
7	1*	0.01437				
6	0*	0.01437				
n = 348				n = 348		
ALLELE TPOX	OBSERVATION	FREQUENCY		ALLELE Vwa	OBSERVATION	FREQUENCY
13	0*	0.01437		21	0*	0.01437
12	18	0.05172		20	2*	0.01437
11	86	0.24713		19	29	0.08333
10	23	0.06609		18	81	0.23276
9	31	0.08908		17	96	0.27586
8	190	0.54598		16	73	0.20977
7	0*	0.01437		15	33	0.09483
6	0*	0.01437		14	33	0.09483
				13	1*	0.01437
				12	0*	0.01437
				11	0*	0.01437
n = 348				n = 348		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 348, or 0.01437. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
CAUCASIAN POPULATION DATA
D16S539, D7S820, D13S317, AND D5S818 ALLELE FREQUENCY TABLE

DATE: JUNE 1, 1998 N = 174

ALLELE D16S539	OBSERVATION	FREQUENCY		ALLELE D13S317	OBSERVATION	FREQUENCY
15	1*	0.01437		15	0*	0.01437
14	6	0.01724		14	15	0.04310
13	62	0.17816		13	40	0.11494
12	112	0.32184		12	100	0.28736
11	89	0.25575		11	102	0.29310
10	24	0.06897		10	20	0.05747
9	48	0.13793		9	23	0.06609
8	6	0.01724		8	48	0.13793
5	0*	0.01437		7	0*	0.01437
n = 348				n = 348		
ALLELE D7S820	OBSERVATION	FREQUENCY		ALLELE D5S818	OBSERVATION	FREQUENCY
14	3*	0.01437		15	0*	0.01437
13	8	0.02299		14	8	0.02299
12	54	0.15517		13	55	0.15805
11	66	0.18966		12	121	0.34770
10	108	0.31034		11	128	0.36782
9	47	0.13506		10	20	0.05747
8	52	0.14943		9	15	0.04310
7	10	0.02874		8	1*	0.01437
6	0*	0.01437		7	0*	0.01437
n = 348				n = 348		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 348, or 0.01437. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
CAUCASIAN POPULATION DATA
D18S51 AND D21S11 ALLELE FREQUENCY TABLE

DATE: MAY 19, 2000 N = 173

ALLELE D18S51	OBSERVATION	FREQUENCY		ALLELE D18S51 cont.	OBSERVATION	FREQUENCY
17	51	0.14740		27	0*	0.01445
16	47	0.13584		26	0*	0.01445
15	55	0.15896		25	0*	0.01445
14	55	0.15896		24	0*	0.01445
13.2	0*	0.01445		23	0*	0.01445
13	49	0.14162		22	2*	0.01445
12	43	0.12428		21	1*	0.01445
11	6	0.01734		20	5	0.01445
10.2	0*	0.01445		19	6	0.01734
10	1*	0.01445		18	25	0.07225
9	0*	0.01445				
8	0*	0.01445		n = 346		
ALLELE D21S11	OBSERVATION	FREQUENCY		ALLELE D21S11 cont.	OBSERVATION	FREQUENCY
31.2	27	0.07804		38	0*	0.01445
31	24	0.06936		37	0*	0.01445
30.2	13	0.03757		36	0*	0.01445
30	91	0.26301		35.2	1*	0.01445
29.2	0*	0.01445		35	0*	0.01445
29	73	0.21098		34.2	3*	0.01445
28	40	0.11561		34	0*	0.01445
27	7	0.02023		33.2	13	0.03757
26	0*	0.01445		33	0*	0.01445
25.2	1*	0.01445		32.2	43	0.12428
25	1*	0.01445		32.1	1*	0.01445
24.2	0*	0.01445		32	8	0.02312
24	0*	0.01445		n = 346		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 346, or 0.01445. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
CAUCASIAN POPULATION DATA
Penta E AND D3S1358 ALLELE FREQUENCY TABLE

DATE: MAY 19, 2000 N =120 (Penta E) and 173 (D3S1358)

ALLELE Penta E	OBSERVATION	FREQUENCY		ALLELE Penta E cont.	OBSERVATION	FREQUENCY
15	20	0.08333		25	0*	0.02083
14	11	0.04583		24	0*	0.02083
13	18	0.07500		23	0*	0.02083
12	41	0.17083		22	0*	0.02083
11	30	0.12500		21	0*	0.02083
10	19	0.07917		20.3	0*	0.02083
9	2*	0.02083		20	3*	0.02083
8	2*	0.02083		19	5	0.02083
7	50	0.20833		18	3*	0.02083
6	0*	0.02083		17	11	0.04583
5	16	0.06667		16	9	0.03750
				n = 240		
ALLELE D3S1358	OBSERVATION	FREQUENCY		ALLELE D3S1358 cont.	OBSERVATION	FREQUENCY
16.2	0*	0.01445		21	0*	0.01445
16	89	0.25723		20	2*	0.01445
15	82	0.23699		19	7	0.02023
14	48	0.13873		18	51	0.14734
13	0*	0.01445		17	66	0.19075
12	0*	0.01445				
11	1*	0.01445		n = 346		

Note: * = Alleles for Penta E with fewer than 5 observations are defaulted to a frequency of 5 per 240, or 0.02083. Alleles for D3S1358 with fewer than 5 observations are defaulted to a frequency of 5 per 346, or 0.01445. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
CAUCASIAN POPULATION DATA
FGA AND D8S1179 ALLELE FREQUENCY TABLE

DATE: MAY 19, 2000 N = 173

ALLELE FGA	OBSERVATION	FREQUENCY		ALLELE FGA cont.	OBSERVATION	FREQUENCY
25	31	0.08960		46.2	0*	0.01445
24.2	0*	0.01445		45.2	0*	0.01445
24	43	0.12428		44.2	0*	0.01445
23.2	0*	0.01445		43.2	0*	0.01445
23	45	0.13006		42.2	0*	0.01445
22.2	4*	0.01445		31.2	0*	0.01445
22	56	0.16185		31	0*	0.01445
21.2	0*	0.01445		30.2	0*	0.01445
21	66	0.19075		30	0*	0.01445
20.2	0*	0.01445		29	0*	0.01445
20	58	0.16763		28	0*	0.01445
19.2	1*	0.01445		27	4*	0.01445
19	20	0.05780		26.1	0*	0.01445
18.2	0*	0.01445		26	14	0.04046
18	3*	0.01445		25.2	0*	0.01445
17	1*	0.01445				
				n = 346		
ALLELE D8S1179	OBSERVATION	FREQUENCY		ALLELE D8S1179	OBSERVATION	FREQUENCY
12	49	0.14162		18	0*	0.01445
11	32	0.09249		17	3*	0.01445
10	37	0.10694		16	12	0.03468
9	2*	0.01445		15	31	0.08960
8	5	0.01445		14	68	0.19653
7	0*	0.01445		13	107	0.30925
				n = 346		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 346, or 0.01445 Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
CAUCASIAN POPULATION DATA
Penta D ALLELE FREQUENCY TABLE

DATE: JANUARY 7, 2002 N = 101

ALLELE Penta D	OBSERVATION	FREQUENCY		ALLELE Penta D cont.	OBSERVATION	FREQUENCY
10	27	0.13366		17	0*	0.02475
9	46	0.22772		16	0*	0.02475
8	2*	0.02475		15	2*	0.02475
7	1*	0.02475		14	11	0.05446
5	0*	0.02475		13	43	0.21287
3.2	0*	0.02475		12	41	0.20297
2.2	0*	0.02475		11	29	0.14356
				n = 202		

Note: * = Alleles for Penta D with fewer than 5 observations are defaulted to a frequency of 5 per 202, or 0.02475. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
HISPANIC POPULATION DATA
CSF1P0, TPOX, THO1 AND vWA ALLELE FREQUENCY TABLE

DATE: JUNE 1, 1998 N = 181

ALLELE CSF1P0	OBSERVATION	FREQUENCY		ALLELE THO1	OBSERVATION	FREQUENCY
15	1*	0.01381		11	0*	0.01381
14	3*	0.01381		10	2*	0.01381
13	20	0.05525		9.3	73	0.20166
12	120	0.33149		9	49	0.13536
11	109	0.30111		8	34	0.09392
10	90	0.24862		7	100	0.27624
9	12	0.03315		6	104	0.28729
8	3*	0.01381		5	0*	0.01381
7	4*	0.01381				
6	0*	0.01381				
n = 362				n = 362		
ALLELE TPOX	OBSERVATION	FREQUENCY		ALLELE VWA	OBSERVATION	FREQUENCY
13	2*	0.01381		21	0*	0.01381
12	31	0.08564		20	5	0.01381
11	100	0.27624		19	24	0.06630
10	19	0.05249		18	52	0.14365
9	31	0.08564		17	89	0.24590
8	169	0.46685		16	106	0.29282
7	3*	0.01381		15	53	0.14641
6	7	0.01934		14	31	0.08564
				13	1*	0.01381
				12	0*	0.01381
				11	1*	0.01381
n = 362				n = 362		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 362, or 0.01381. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
HISPANIC POPULATION DATA
D16S539, D7S820, D13S317 AND D5S818 ALLELE FREQUENCY TABLE

DATE: JUNE 1, 1998 N = 181

ALLELE D16S539	OBSERVATION	FREQUENCY		ALLELE D13S317	OBSERVATION	FREQUENCY
15	0*	0.01381		15	0*	0.01381
14	9	0.02486		14	27	0.07459
13	58	0.16022		13	56	0.15470
12	97	0.26796		12	80	0.22099
11	73	0.20166		11	79	0.21823
10	55	0.15193		10	20	0.05525
9	62	0.17127		9	61	0.16851
8	7	0.01934		8	39	0.10773
5	1*	0.01381		7	0*	0.01381
n = 362				n = 362		
ALLELE D7S820	OBSERVATION	FREQUENCY		ALLELE D5S818	OBSERVATION	FREQUENCY
14	4*	0.01381		15	0*	0.01381
13	6	0.01657		14	3*	0.01381
12	72	0.19890		13	58	0.16022
11	94	0.25967		12	104	0.28729
10	94	0.25967		11	126	0.34807
9	34	0.09392		10	21	0.05801
8	55	0.15193		9	27	0.07459
7	3*	0.01381		8	5	0.01381
6	0*	0.01381		7	18	0.04972
n = 362				n = 362		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 362, or 0.01381. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
HISPANIC POPULATION DATA
D18S51 AND D21S11 ALLELE FREQUENCY TABLE

DATE: MAY 19, 2000 N = 183

ALLELE D18S51	OBSERVATION	FREQUENCY		ALLELE D18S51 cont	OBSERVATION	FREQUENCY
17	55	0.15027		27	0*	0.01366
16	43	0.11749		26	0*	0.01366
15	51	0.13934		25	0*	0.01366
14	60	0.16393		24	0*	0.01366
13.2	0*	0.01366		23	2*	0.01366
13	51	0.13934		22	1*	0.01366
12	37	0.10109		21	1*	0.01366
11	4*	0.01366		20	10	0.02732
10.2	1*	0.01366		19	23	0.06284
10	1*	0.01366		18	26	0.07104
9	0*	0.01366				
8	0*	0.01366		n = 366		
ALLELE D21S11	OBSERVATIONS	FREQUENCY		ALLELE D21S11 cont	OBSERVATION	FREQUENCY
31.2	42	0.11475		38	0*	0.01366
31	30	0.08197		37	0*	0.01366
30.2	4*	0.01366		36	1*	0.01366
30	88	0.24044		35.2	0*	0.01366
29.2	1*	0.01366		35	3*	0.01366
29	78	0.21312		34.2	2*	0.01366
28	42	0.11475		34	1*	0.01366
27	7	0.01913		33.2	16	0.04372
26	0*	0.01366		33	0*	0.01366
25.2	0*	0.01366		32.2	46	0.12568
25	0*	0.01366		32.1	0*	0.01366
24.2	0*	0.01366		32	5	0.01366
24	0*	0.01366		n = 366		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 366, or 0.01366. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed above a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
HISPANIC POPULATION DATA
Penta E AND D3S1358 ALLELE FREQUENCY TABLE

DATE: MAY 19, 2000 N = 181 (Penta E) and 183(D3S1358)

ALLELE Penta E	OBSERVATION	FREQUENCY		ALLELE Penta E cont.	OBSERVATION	FREQUENCY
15	32	0.08840		25	1*	0.01381
14	28	0.07735		24	0*	0.01381
13	29	0.08011		23	1*	0.01381
12	58	0.16022		22	4*	0.01381
11	21	0.05801		21	8	0.02210
10	27	0.07459		20.3	0*	0.01381
9	6	0.01658		20	4*	0.01381
8	21	0.05801		19	12	0.03315
7	34	0.09392		18	14	0.03867
6	0*	0.01381		17	16	0.04420
5	19	0.05249		16	27	0.07459
				n = 362		
ALLELE D3S1358	OBSERVATION	FREQUENCY		ALLELE D3S1358 cont.	OBSERVATION	FREQUENCY
16	93	0.25410		21	1*	0.01366
15	143	0.39071		20	0*	0.01366
14	26	0.07104		19	3*	0.01366
13	1*	0.01366		18	30	0.08197
12	0*	0.01366		17	69	0.18853
11	0*	0.01366		16.2	0*	0.01366
				n = 366		

Note: * = Alleles for Penta E with fewer than 5 observations are defaulted to a frequency of 5 per 362, or 0.01381. Alleles for D3S1358 with fewer than 5 observations are defaulted to a frequency of 5 per 366, or 0.01366. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
HISPANIC POPULATION DATA
FGA AND D8S1179 ALLELE FREQUENCY TABLE

DATE: MAY 19, 2000 N = 183

ALLELE FGA	OBSERVATION	FREQUENCY		ALLELE FGA cont.	OBSERVATION	FREQUENCY
25	47	0.12842		46.2	0*	0.01366
24.2	0*	0.01366		45.2	1*	0.01366
24	58	0.15847		44.2	0*	0.01366
23.2	0*	0.01366		43.2	0*	0.01366
23	46	0.12568		42.2	0*	0.01366
22.2	1*	0.01366		31.2	0*	0.01366
22	36	0.09836		31	0*	0.01366
21.2	1*	0.01366		30.2	0*	0.01366
21	65	0.17760		30	1*	0.01366
20.2	0*	0.01366		29	1*	0.01366
20	40	0.10929		28	2*	0.01366
19.2	0*	0.01366		27	9	0.02459
19	31	0.08470		26.1	1*	0.01366
18.2	0*	0.01366		26	25	0.06831
18	1*	0.01366		25.2	0*	0.01366
17	0*	0.01366				
				n = 366		
ALLELE D8S1179	OBSERVATION	FREQUENCY		ALLELE D8S1179	OBSERVATION	FREQUENCY
12	40	0.10929		18	0*	0.01366
11	17	0.04645		17	0*	0.01366
10	30	0.08197		16	17	0.04645
9	1*	0.01366		15	44	0.12022
8	3*	0.01366		14	96	0.26230
7	0*	0.01366		13	118	0.32240
				n = 366		

Note: * = Alleles with fewer than 5 observations are defaulted to a frequency of 5 per 366, or 0.01366. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE
HISPANIC POPULATION DATA
Penta D ALLELE FREQUENCY TABLE

DATE: NOVEMBER 13, 2001 N = 157

ALLELE Penta D	OBSERVATION	FREQUENCY		ALLELE Penta D cont.	OBSERVATION	FREQUENCY
10	49	0.15556		17	1*	0.01587
9	66	0.20952		16	0*	0.01587
8	9	0.02857		15	1*	0.01587
7	3*	0.01587		14	16	0.05079
5	3*	0.01587		13	56	0.17778
3.2	0	0.01587		12	59	0.18730
2.2	9	0.02857		11	43	0.13651
				n = 315**		

Note: * = Alleles for Penta D with fewer than 5 observations are defaulted to a frequency of 5 per 315, or 0.01587. Therefore, allele frequencies on this page will NOT add to 1. If an allele is observed that is not listed in the above table a frequency of 5/n will be used.

N = Total number of individuals
n = Total number of alleles from N individuals

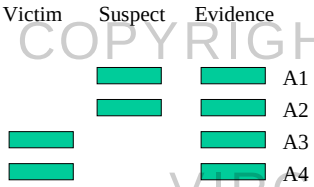
** One of the samples analyzed for the creation of the Hispanic population database contained a 3 banded pattern at Penta D. Therefore, the number of alleles observed in the 157 individuals was 315.

APPENDIX D – 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.

$LR = 1/P_1^2$

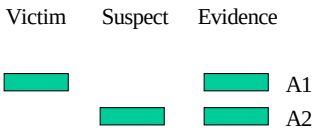
C1 column 0 unknown - leave field blank
C2 column 1 unknown - leave field blank

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 formulas 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.