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1. Scope

The interpretation guidelines are intended to be a general guide for the evaluation of the typing results for DNA amplified with the AmpFlSTR™ Identifiler™ PCR Amplification Kit and analyzed with the Applied Biosystems (AB) 3130 Genetic Analyzer and GeneMapper® ID v3.2 analysis software. The guidelines will ensure consistency in the analysis and evaluation of the typing results between DNA analysts. These guidelines cannot encompass the entire range of samples or circumstances that will be encountered in forensic casework. Exceptions may be made based on the analyst's training and experience and must be approved by the DNA Technical Leader. The guidelines were based on the manufacturer's recommendations, manufacturer's user's manuals, internal validation studies, interpretation guidelines published by the Scientific Working Group on DNA Analysis Methods (SWGDAM), and the scientific literature.

2. References

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3. Equipment

3.1. Computer with GeneMapper® ID analysis software

4. Safety / Quality Assurance

4.1. Safety: Not applicable

4.2. Quality Assurance: See Procedure Section

5. Procedure

5.1 General DNA Profile Interpretation Steps

5.1.1 Evaluation of the standards, allelic ladders and controls

- Internal Size Standard for each sample
- Allelic Ladder(s)
- Negative controls (extraction reagent blank, amplification reagent blank, formamide blank)
- Positive amplification control

5.1.2 Evaluation of evidence sample DNA profiles

- Designate allelic peaks, mark artifacts if present.
- Determine if each sample contains sufficient data for interpretation and/or comparison.
- For those profiles containing sufficient data for interpretation and/or comparison, determine if each sample profile is from a single contributor or multiple contributors.
- If the sample is determined to originate from a single contributor, follow the steps listed under the "Single Contributor DNA Profile Interpretation" listed below.
- If the sample is determined to originate from multiple contributors, follow the steps listed under the "Multiple Contributor DNA Profile Interpretation" listed below.

5.1.3 Analysis of known DNA sample profiles

- After the interpretation of the evidentiary samples, the results of the known samples can then be reviewed and interpreted. The interpretation of each evidentiary DNA profile will be documented prior to the review of any known samples associated with the case, where possible. This may not be possible in all circumstances, for example, if additional evidence is submitted after the evidentiary and known samples have been analyzed.

5.1.4 Comparing known DNA profiles to the Evidentiary DNA profiles

- After the interpretation and documentation of the evidentiary profiles, the known DNA profiles can then be compared to the evidentiary profiles. In addition to comparing the evidentiary samples to known DNA profiles, comparisons should be made to the other evidentiary samples associated with the case, if present. Two conclusions can be drawn from this comparison:
  - Excluded: the individual associated with the known DNA sample cannot be a contributor to the evidentiary sample.
  - Not Excluded: the individual associated with the known DNA sample can be a contributor to the evidentiary sample.

5.1.5 Statistical Analysis

- If it is determined that an individual associated with a known DNA profile cannot be excluded as a possible contributor to the evidence profile, a statistical calculation will be performed to lend weight to the meaning of inclusion except in cases as noted in the "Statistical Calculation" section below.

5.1.6 CODIS

- All profiles suitable for comparison purposes will be compared to the Staff Index, Profiles Generated Index, and LDIS database prior to being reported as a way of detecting possible contamination. See CODIS Manual for instructions on entering and searching profiles in CODIS.

5.1.7 Report

- The results of the DNA analysis are then detailed in a laboratory report.

5.2 Evaluation of the Controls

Verification of Internal Size Standard (GS-500)

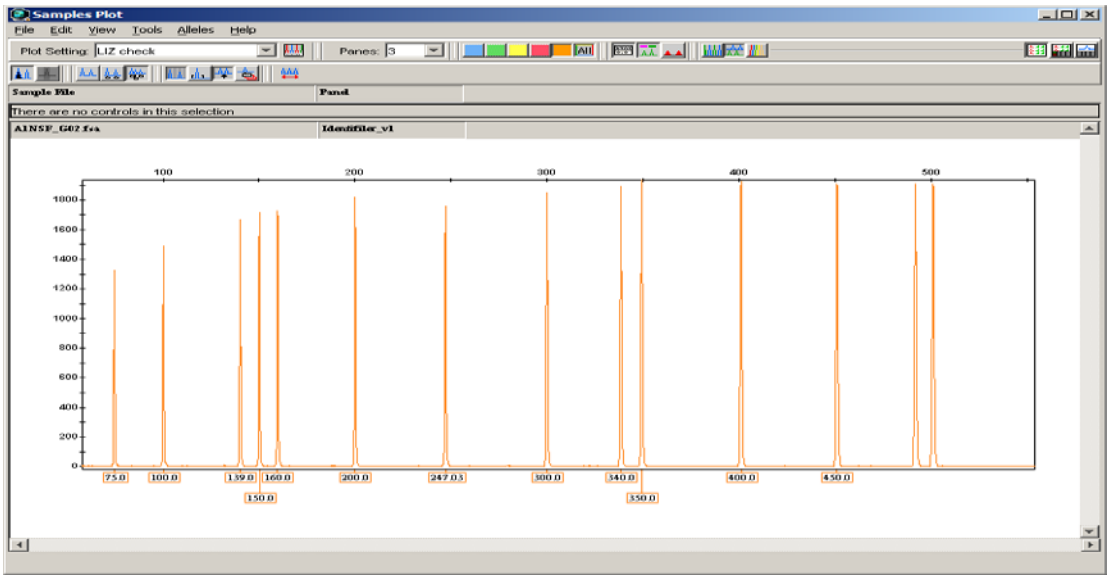
The Internal Size Standard (GS-500, LIZ dye labeled) consists of twelve peaks of known base pair size ranging from 35 base pairs to 500 base pairs.

All Internal Size Standard peaks ranging from 75 to 450 base pairs must be present, have the expected peak morphology, have good peak balance across the range of sizing, and be labeled correctly with peak heights greater than 50 RFU for the associated profile to be acceptable.

Extraneous peaks observed in the Internal Size Standard can be disregarded as long as they are not incorrectly labeled as peaks of the Internal Size Standard.

	Internal Size Standard (GS-500) peaks necessary for the analysis of AB AmpFlSTR™ Identifiler™ amplified products
Base Pair Sizes (bp)	75, 100, 139, 150, 160, 200, 250*, 300, 340, 350, 400, 450

** The electrophoresis of the 250 base pair peak is not automatically assigned since fluctuations in the laboratory's temperature will result in variations in the electrophoretic mobility of the peak. It can be used as an additional control. The calculated size of the 250 peak should not vary by more than one base pair across the run. If the variation is greater than 1 base pair, the run may need to be broken up into separate projects. If an individual sample or ladder is the only sample that falls outside the acceptable range and it can be reasonably concluded that it was an individual capillary event, the sample can be disregarded and the rest of the run analyzed.*



Typical Internal Size Standard (GS-500)

Verification of the AmpFlSTR™ Identifiler™ Allelic Ladder

The presence of all alleles of the allelic ladder must be verified (See *Characterization of the AmpFlSTR™ Identifiler™ Loci* table below).

- Allelic ladders missing one or more alleles or containing allele peaks that are marked off-ladder are not valid.
- Sample profiles must be associated with one or more valid allelic ladders to be considered for interpretation.
- The Sample Type of allelic ladders that are found not to be valid may be changed from “Allelic Ladder” to “Sample.” If this is done, the project must be re-analyzed.

Locus Designation	Chromosome Location	Alleles Included in Identifiler Allelic Ladder	Dye Label	Control DNA 9947A
D8S1179	8	8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	6-FAM	13[a]
D21S11	21q11.2-q21	24, 24.2, 25, 26, 27, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 37, 38		30[b]
D7S820	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		10, 11
CSF1PO	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		10, 12
D3S1358	3p	12, 13, 14, 15, 16, 17, 18, 19	VIC	14, 15
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3		8, 9.3
D13S317	13q22-31	8, 9, 10, 11, 12, 13, 14, 15		11[c]
D16S539	16q24-qter	5, 8, 9, 10, 11, 12,13, 14, 15		11, 12
D2S1338	2q35-37.1	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28		19, 23

Locus Designation	Chromosome Location	Alleles Included in Identifiler Allelic Ladder	Dye Label	Control DNA 9947A
D19S433	19q12-13.1	9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2	NED	14, 15
vWA	12p12-pter	11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24		17, 18
TPOX	2p23-2per	6, 7, 8, 9, 10, 11, 12, 13		8[d]
D18S51	18q21.3	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27		15, 19
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y	PET	X
D5S818	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16		11[e]
FGA	4q28	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2		23, 24

Characterization of the AmpFlSTR® Identifiler™ Loci. AmpFlSTR® Identifiler™ PCR Amplification Kit User's Manual, 2005. a. For CODIS purposes, profile reported as 13, 13. b. For CODIS purposes, profile reported as 30,30. c. For CODIS purposes, profile reported as 11,11. d. For CODIS purposes, profile reported as 8,8. e. For CODIS purposes, profile reported as 11,11.

Evaluation of Negative Controls

Reagent Blank

The reagent blank is initiated at the extraction step and is processed in parallel with the associated evidentiary sample(s). It consists of all the reagents used during the extraction. The reagent blank is used to detect DNA contamination from the reagents, disposables, or environment that may affect the evidentiary samples. Contamination detected in the reagent blank may be systemic in nature or a randomly occurring event. If contamination is detected, an effort should be made to identify the source to prevent future contamination events.

An acceptable reagent blank will contain no peaks above the analytical threshold (50 RFU).

A reagent blank containing a peak above the analytical threshold (50 RFU) between 100 base pairs and 362 base pairs, which is believed to be an artifact such as a spike or spectral pull-up from the internal size standard, shall be re-injected. If, upon re-injection, no peaks above the analytical threshold are present, the reagent blank is acceptable.

- Artifacts outside the 100 to 362 base pair window are not significant and do not necessitate the re-injection of the control.

If at least one non-artifact peak above the analytical threshold or a recognizable pattern of peaks below the threshold is present in the reagent blank, all samples associated with the reagent blank must be re-analyzed, if possible.

- Based on the results of the other controls (e.g. amplification blank, positive control, etc.) associated with the evidentiary samples, the re-analysis may initially consist of re-injecting all samples, re-amplification of all samples, or re-extraction of all associated samples, if possible.
- If it is not possible to re-extract a sample associated with a contaminated reagent blank and it is reasonable to assume that the integrity of the DNA analysis was not affected, the results shall be reported as described in the Report Wording protocol. The issues shall be noted in the case file.
- For evidentiary samples associated with a contaminated reagent blank where it is reasonable to assume that the integrity of the DNA analysis may have been affected, the results shall be reported as described in the Report Wording protocol (ATF-LS-FB18). In addition, a note shall be made in the report briefly describing the issue and stating the results should be interpreted with caution.
- If a reagent blank associated with a set of samples to be re-amplified cannot be re-amplified due to limited volume, and it was determined to be valid from a previous analysis, the samples associated with the reagent blank can still be analyzed as long as the sensitivity of the re-amplified samples does not exceed the original sensitivity of the reagent blank.
- A printout of the raw data demonstrating the presence of the primer peaks will be included with the case material along with a printout of the electropherogram with a maximum Y-axis of 200 RFU.

Amplification Negative Control

The amplification negative control is initiated at the amplification step and consists of all the reagents used in the amplification, except TE^{-4} is used in the place of the template DNA.

An acceptable amplification negative control shall contain no peaks above the analytical threshold (50 RFU).

An amplification negative control containing a peak above the analytical threshold between 100 base pairs and 362 base pairs which is believed to be an artifact, such as a spike or spectral pull-up from the internal size standard, shall be re-injected. If, upon re-injection, no peaks above the analytical threshold are present, the amplification negative control is acceptable.

- Artifacts outside the 100 to 362 base pair window are not significant and do not necessitate the re-injection of the control.

If at least one non-artifact peak above the analytical threshold or a recognizable pattern of peaks below the threshold is present in the amplification negative control, all samples associated with the amplification negative control must be re-analyzed.

- The re-analysis may consist of re-injection or re-amplification, if possible.
- If it is not possible to re-amplify a sample associated with a contaminated amplification negative control and it is reasonable to assume that the integrity of the DNA analysis was not affected, the results shall be reported as described in the Report Wording protocol. The issues shall be noted in the case file.
- For evidentiary samples associated with a contaminated amplification negative control, where it is reasonable to assume that the integrity of the DNA analysis may have been affected, the results shall be reported as described in the Report Wording protocol. In addition, a note shall be made in the report briefly describing the issue and stating the results should be interpreted with caution.
- A printout of the raw data demonstrating the presence of the primer peaks will be included with the case material along with a printout of the electropherogram with a maximum Y-axis of 200 RFU.

Formamide Blank

A formamide blank consists of the formamide solution with ILS, but without amplified DNA product.

At least one formamide blank will be analyzed with each set of samples run on the Genetic Analyzer.

An acceptable formamide blank shall contain no peaks above the analytical threshold (50 RFU).

- It is typical for the formamide blank to contain spectral pull-up from the ILS due to the lack of primers and amplified product during the electrokinetic injection which results in a significant increase of ILS fragments entering the capillary. The pull-up peaks detected will mimic the ILS pattern and can be marked as such. These peaks do not preclude the interpretation of the associated samples nor does their presence require the samples to be re-injected.

Formamide blanks containing a peak above the analytical threshold between 100 base pairs and 362 base pairs which is believed to be an artifact other than that mentioned in the preceding paragraph, such as a spike, shall be re-injected. If, upon re-injection, no peaks above the analytical threshold are present (other than possible ILS pull-up), the formamide blank is acceptable.

- Artifacts outside the 100 to 362 base pair window are not significant and do not necessitate the re-analysis of the control.

If at least one non-artifact peak above the analytical threshold or a recognizable pattern of peaks below the threshold is present in the formamide blank, all samples associated with the formamide blank, must be re-analyzed.

- The re-analysis may consist of re-injection from the same plate or setting up a new plate for the Genetic Analyzer.

Amplification Positive Control (9947A)

The amplification positive control is initiated at the amplification step and is processed in parallel with the evidentiary samples. It consists of all the reagents used in the amplification reaction and template DNA of a known profile (9947A). The amplification positive control monitors the amplification reaction and ensures the reliability of the results for the associated evidentiary samples.

The typing results for the positive control should match the expected profile (see below).

Locus	Genotype	Locus	Genotype
D8S1179	13,13	D2S1338	19,23
D21S11	30,30	D19S433	14,15
D7S820	10,11	vWA	17,18
CSF1PO	10,12	TPOX	8,8
D3S1358	14,15	D18S51	15,19
TH01	8,9.3	Amelogenin	X,X

D13S317	11,11	D5S818	11,11
D16S539	11,12	FGA	23,24

Expected typing results for Control DNA 9947A

If the allelic peak heights are greater than the relevant thresholds (analytical and stochastic, 50 and 200 RFU, respectively), match the expected profile and meet the minimum expected peak height ratio (See Appendix A), the positive control is valid.

- On rare occasions, the heterozygote peak height balance may fall outside expectations. The positive control is still valid in this situation. If multiple loci demonstrate greater than expected imbalance, this may be an indication of an issue with amplification or capillary electrophoresis.

If the typing results do not match the expected profile (incorrect profile, additional alleles due to possible contamination or partial/no results), the positive control is not valid and all evidentiary samples associated with the positive control shall be re-analyzed. The re-analysis may consist of re-injection or re-amplification.

If it is not possible to re-analyze a sample associated with a failed positive control, where it is reasonable to assume that the integrity of the DNA analysis was not affected, the results shall be reported as described in the Report Wording protocol (*ATF-LS-FB18*). The issues shall be noted in the case file.

For evidentiary samples associated with a failed positive control where it is reasonable to assume that the integrity of the DNA analysis may have been affected, the results shall be reported as described in the Report Wording protocol. In addition, a note shall be made in the report briefly describing the issue and stating the results should be interpreted with caution.

Single Injection/Capillary Events

In general, if a single capillary or single injection event occurs that results in a poor injection as evidenced by the ILS peaks, the sample or samples specifically affected may be re-injected alone and the entire run does not have to be re-injected and analyzed. This is true for all samples except for the positive control. Each run must have a valid positive control.

5.3 Evaluation of Sample Profiles

Analytical Threshold (AT)

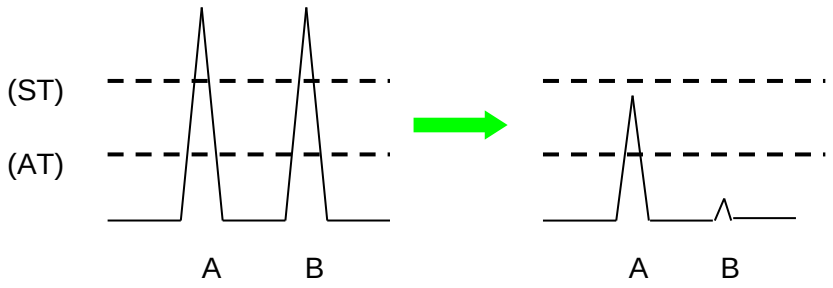
The Analytical Threshold is the relative fluorescent unit (RFU) value that, when exceeded by peaks that display the expected peak morphology, allows those peaks to be considered "real" products of amplification.

The Analytical Threshold is set at 50 RFU.

Stochastic Threshold (ST)

Due to the inherent nature of PCR amplification of low levels of DNA, the results may contain dramatic peak height imbalance and allele drop-out. The Stochastic Threshold is the RFU value that, when exceeded by a single allelic peak, the DNA analyst can be confident that the sister peak of a heterozygous pair would be detected (i.e. would be above the Analytical Threshold). The Stochastic Threshold listed below refers to a single source sample. The use of the Stochastic Threshold must be modified to account for possible allele sharing during the interpretation of mixed DNA profiles due to the possible additive effects of allele sharing.

The Stochastic Threshold is set at 200 RFU.



Off-Scale Peaks

An Off-Scale Peak is a peak which exceeds the linear dynamic range of the instrument. Off-Scale Peaks may result in raised baselines and/or excessive "pull-up" in one or more colors.

Analyzed data from Off-Scale Peaks should not be used for quantitative comparisons since the true quantity of signal is not known. For example, this can result in disproportionably high stutter calculations.

Typically, Off-Scale Peaks will be associated with other artifacts such as –A peaks, increased n-4 peaks, n-8 peaks, and n+4 peaks which cause difficulties during interpretation.

Sample profiles containing Off-Scale data may be re-analyzed at the discretion of the analyst.

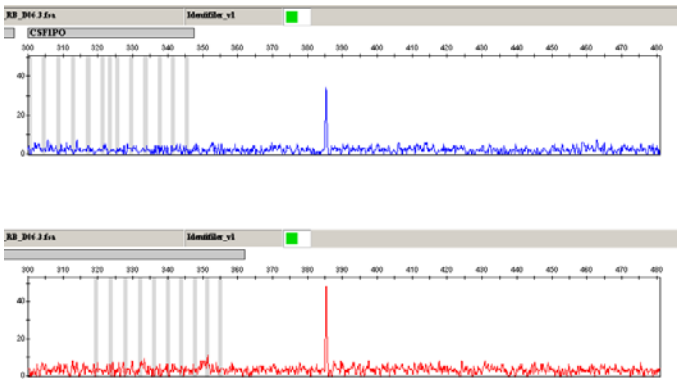
- Re-analysis may consist of adding less amplified product to the AB 3130 set-up tray, diluting the amplified product in formamide prior to adding it to the 3130 set-up tray, or re-amplifying the sample. Re-amplification is preferred and may be done at the analyst's discretion.

Artifacts

Spikes

A spike is defined as a non-specific, non-reproducible, and non-allelic peak, which is typically observed in one or more colors and is an artifact of the electrophoresis and signal detection. A spike does not typically display the same peak morphology as an allelic peak with respect to peak width, starting slope and ending slope.

If a spike is present in the analytical range (100 – 362 base pairs), the spike may be confirmed to be an artifact by re-injection if it interferes with interpretation. Upon re-injection, the same spike should not be present.



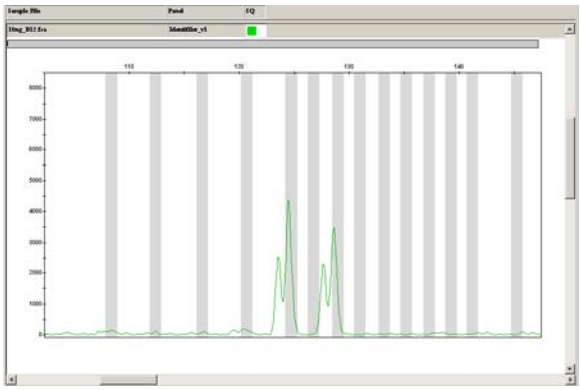
Example of a spike exhibiting the typical characteristics (narrow peak width, detected in multiple channels, similar peak height in different channels).

Non-Template Nucleotide Addition

The Taq polymerase has the inherent property of non-template directed nucleotide addition. This nucleotide addition is reverse primer dependent. In the AmpFlSTR™ Identifiler™ reaction, the non-template nucleotide addition is favored to the extent that, under normal conditions, the majority of the amplified product contains the additional nucleotide.

In extreme conditions, a portion of the amplified product will not contain the additional nucleotide resulting in a fragment one base pair shorter than the expected allele. Since the nucleotide typically

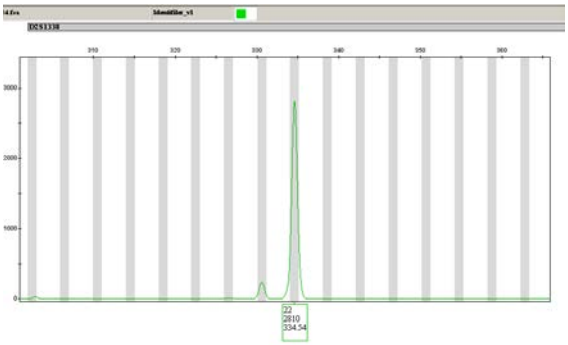
added is adenine (A), the peak representing amplified product in which the non-template directed nucleotide addition did not occur is oftentimes referred to as the –A peak. One extreme circumstance occasionally encountered is the addition of excess template DNA.



Example of –A peak

Stutter

Stutter peaks are an artifact of the Polymerase Chain Reaction when amplifying repeat regions of DNA. The primary peak is representative of the actual repeat number contained in the template DNA. The stutter peak is an artifact usually one repeat shorter in length than the primary peak. For tetrameric repeats, the stutter peak is n-4 base pairs where n is the base pair length of the primary peak. Occasionally n+4 peaks may be observed.



Example of a stutter peak (unlabeled) one repeat in length shorter than the primary peak (labeled as "22" allele).

Stutter peaks can be characterized both by their size and their peak height proportion when compared to the associated primary peak expressed as the percentage of the stutter peak height compared to the primary peak. The expected maximum stutter percentage for each locus will vary and can be

experimentally defined. The table below lists the maximum stutter percentages expected for each locus.*

Locus	Maximum Expected Stutter %*	Locus	Maximum Expected Stutter %*
D8S1179	8.2	D2S1338	11.1
D21S11	9.4	D19S433	13.3
D7S820	8.2	vWA	12.6
CSF1PO	9.2	TPOX	4.8
D3S1358	10.7	D18S51	17.0
TH01	5.1	Amelogenin	---
D13S317	8.0	D5S818	6.8
D16S539	10.4	FGA	14.7

**AB Maximum Stutter Values are taken from the AmpFlSTR® Identifiler™ PCR Amplification Kit User's Manual, 2005. It is a compilation of data from the analysis of 1,187 samples*

Minor peaks cannot be absolutely identified as stutter peaks even though they meet both the size and stutter peak percentage criteria.

- If there is no other indication of a mixture in a profile, the minor peaks meeting the stutter peak criteria can be attributed to stutter.
- If evidence of a mixture is observed in a profile, minor peaks falling within the stutter peak criteria should be taken into consideration during the interpretation.

Under some circumstances, stutter peaks may exceed the maximum expected stutter percentages. These peaks may be called stutter at the analyst's discretion based on the specific circumstances.

Stutter peak calculations associated with off-scale allele peaks will not be reflective of the true percentage.

Stutter peaks between two alleles that differ by two repeats (e.g. 15 and 17 allelic peaks with a stutter peak at the 16 position) may exceed the maximum expected stutter percentages due to the n-4 contribution from the 17 allele and the n+4 contribution from the 15 allele.

Stutter peak percentages may increase at lower levels of input template DNA.

Matrix Failure (Spectral Pull-Up)
Matrix Failure, also known as spectral pull-up, results in peaks from one color "bleeding" into another color when the wavelength ranges emitted by the fluorescent tags overlap. The software is designed to use a matrix algorithm to separate the fluorescent signal given off by each of the fluorescent tags. This separation is not always complete resulting in minor peaks being detected in the adjacent spectral colors. These pull-up peak heights are typically less than 5% of the peak height of the source peak. The pull-up peak is usually sized within ±0.25 base pairs of the source peak.

If pull-up peaks are observed in a sample, the sample may be re-analyzed at the analyst's discretion based on the specific circumstances.

Raised Baseline
Raised baseline may be observed in samples containing excess template DNA or due to instrument failure. The raised baseline appears as non-specific elevation along the x-axis of the electropherogram.

If excess raised baseline is observed in a sample, the sample may be re-analyzed at the analyst's discretion.

Other Artifacts
Other peaks that do not represent genetic data from the template DNA may be observed in the sample electropherogram. These peaks or series of peaks may result from dissociated fluorescent dyes or other causes due to the instrument, capillary electrophoresis, or amplification kit. For example, a series of peaks is routinely observed between 90 and 100 base pairs in the VIC dye (green). If these artifacts appear within the analytical range (100 – 362 base pairs) and are excessive, the sample should be re-analyzed.

Off-Ladder Alleles
Most common alleles are represented by the allelic ladder or virtual bins set by the software. Occasionally, non-artifact peaks will be observed outside of the allele bins and will be labeled as "OL."

Off-ladder alleles may result from temperature fluctuations in the instrument's environment during the course of the electrophoresis. The change in temperature may cause a migration shift of allelic peaks that consequently fall outside the ±0.5 base pair bins even though the alleles are represented in the ladder.

Micro-variant and rare variant alleles may also be observed that fall between alleles within a locus or the range of alleles between two loci. Micro-variant and rare variant alleles shall be re-injected to confirm the sizing.

Off-ladder allelic peaks (OL) observed between alleles within a locus should be labeled as a variant of the smaller flanking allele.

- If the OL peak is approximately one base pair longer than the smaller flanking allele (X), the OL peak will designated as X.1.
- If the OL peak is approximately two base pairs longer than the smaller flanking allele, it will be designated as X.2.
- If the OL peak is approximately three base pairs longer than the smaller flanking allele, it will be designated as X.3.
- For example, an OL peak falling between the 8 and 9 alleles that is approximately two base pairs longer than the 8 allele would be designated as an 8.2.

An OL allelic peak that falls between the allele range of two loci first should be associated with one of the loci and then be labeled as described below.

- To associate the OL peak with one of the flanking loci, the alleles present in the flanking loci along with the base pair sizes of the alleles need to be considered.
- If one of the flanking loci contains two alleles and the other locus contains one, the OL peak will be associated with the latter locus.
- If both loci contain one allele, the base pair size of the OL peak should be compared to the base pair sizes of the flanking allelic ladders. If the OL peak is a “perfect repeat” of one of the allelic ladders, it will be associated with that locus. If it is not a “perfect repeat” of either flanking allelic ladder, then both loci will be considered inconclusive and may only be used for exclusionary purposes.
- If the OL peak has been associated with a locus, it will then be designated as “<” or “>” the last allele of the associated allelic ladder.

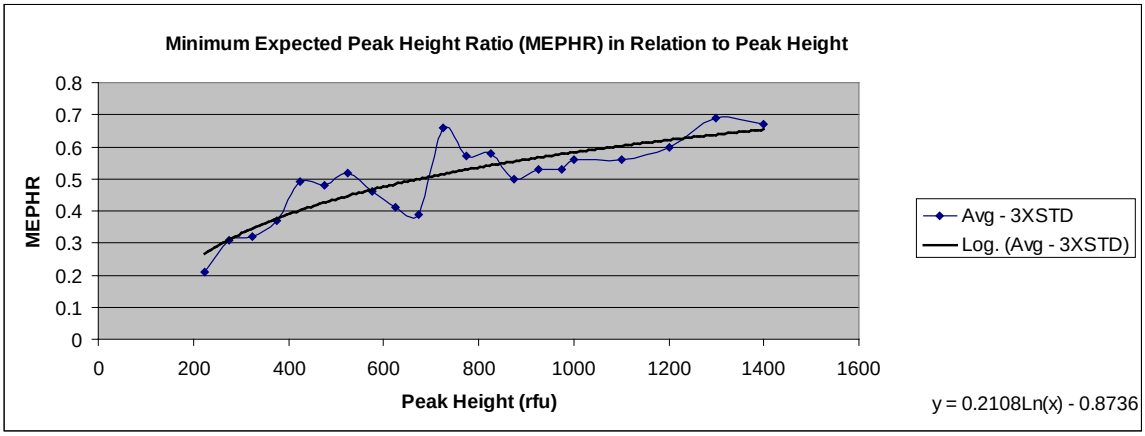
Peak Height Imbalance

Expected Variation in Peak Height Balance

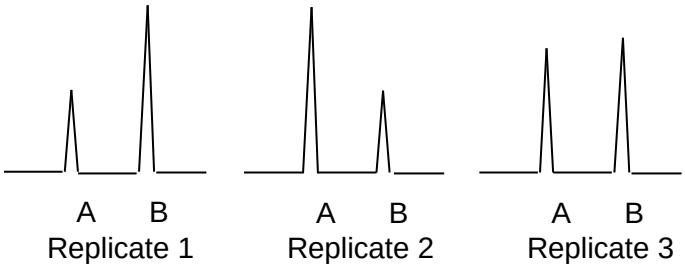
It is not unusual for PCR to produce imbalanced peak heights for heterozygous loci. As the amount of template DNA decreases, the imbalance may become more dramatic.

The following chart displays the minimum expected peak height balance within a locus for a single source sample. The ranges are based on the peak height of the allele with the greatest peak height. The balance is calculated as a percentage of the lesser peak height as compared to the greater peak height. The chart displays a smooth curve based on the average peak height balance minus three Standard Deviations. Therefore by definition, it would be expected that 99.7% of the heterozygous peaks examined would meet these expectations. This chart and the supporting data are contained in Appendix A. Alleles with peaks below 200 RFU would be have a minimum expected peak height ratio of <25% and

could result in sister peaks below the analytical threshold. For alleles with peaks above 1,400 RFU, the minimum expected peak height ratio is 65%.



For heterozygous loci with alleles separated by relatively few repeats, the imbalance is not significantly affected by allele size. Hence, the imbalance will affect the larger allele as much as the smaller allele. Therefore, if a sample is amplified multiple times using the same template quantity, the imbalance may vary and may favor the larger allele one time and the smaller allele another time (see below).



For heterozygous loci with alleles separated by a larger number of repeats (such as the locus FGA), the imbalance may be greater than the expectations defined by the chart below and may favor the smaller allele. This imbalance will not be reproducible.

Peak Height Imbalance due to Primer Site Mutation

A sequence mutation in the primer region may have one of three effects. If the mutation causes significant destabilization, the allele will not be amplified and therefore not detected (null allele). If the mutation does not affect the stability of the primer binding, no effect will be observed. If, however, the mutation causes some destabilization of the primer binding but not to the extent to prevent amplification, the resulting peak balance at the locus may be outside the expected range. This form of imbalance is reproducible.

If the peak height imbalance falls outside the expected range at a single locus in an assumed single source profile, the sample can be re-amplified to attempt to reproduce the imbalance.

- If the second amplification displays a similar peak imbalance, then the locus can be called.
- If the second amplification produces peak balance within the expected range, the locus can be called.
- If the second amplification does not demonstrate the presence of the second allele or if the samples cannot be re-amplified, the locus may be used for comparison purposes at the discretion of the analyst based on training and experience. Stochastic effects can cause imbalance outside of expectations, but so can a low level second contributor.

Expected Peak Height Balance in Mixed DNA Profiles
The above chart displays the Minimum Expected Peak Height Balance for single source samples. If a DNA profile is the result of DNA from multiple contributors, the additive effects of allele sharing must be considered (see Mixed Sample Interpretation section below for more details).

Composite Profiles
Combining data from multiple injections/amplifications resulting from a single DNA extract is called a composite profile. For example, if a sample is severely degraded, the initial amplification using 1ng of template DNA may have produced interpretable data at a few of the smaller molecular weight loci. However, when the same DNA extract was re-amplified using 2ng of template DNA, additional interpretable data was obtained from a few of the higher molecular weight loci. The smaller molecular weight loci in the second amplification are now not interpretable due to saturation. The data from the two amplifications can be combined into a single composite profile.

In some instances, it may be useful to re-inject or re-amplify a sample to obtain interpretable data for additional loci.

- Composite profiles must be the product of data generated from a single DNA extract.
- Data obtained from a second cutting from the same stained area cannot be combined with the data from the initial cutting of the stained area.

Interpretation/Statistical Calculation Documentation
An Interpretation/Statistical Calculation (ISC) worksheet shall be completed for each unique evidentiary DNA profile suitable for comparison.

- The ISC documents the possible contributing genotypes to the observed profile, and calculates the random match probability.
- This includes two or more DNA profiles that are consistent with each other but may be lacking data at one or more loci.
- This ISC worksheet and accompanying information (if necessary) will document the interpretation of the DNA profile and results in a list of possible contributing genotypes and the Random Match Probability for the profile.

- Major and minor components shall have separate ISC worksheets. If the minor component is not suitable for comparison purposes, it is not necessary to complete the ISC worksheet.
- The ISC worksheet shall be completed prior to viewing the DNA profile from relevant reference DNA profiles.
    - There will be some circumstances where this is not feasible (e.g. subsequent submissions, etc.).

Preliminary Evaluation of Sample DNA Profile

The entire DNA profile should be evaluated. Several conclusions can be drawn at this point.

- Is the profile suitable for comparison purposes?
    - If only a few alleles are detected across the entire profile and the peak heights are relatively low, the analyst may determine that the DNA profile is not suitable for comparison purposes. A specific number of alleles are not listed due to the wide variation in results that may be observed. For example, only two loci may demonstrate results with two alleles at each, all above the stochastic threshold, for a total of four alleles detected. This profile may be deemed interpretable. Another DNA profile may also have four alleles at four different loci, all below the stochastic threshold. This profile may be deemed uninterpretable.
    - If the DNA profile is the result of four or more contributors and the peak heights are relatively low, the mixed DNA profile is not be suitable for comparison purposes.
- Is the DNA profile consistent with resulting from a single contributor?
- Is the DNA profile consistent with resulting from multiple contributors?
    - Peaks below the analytical threshold may be used to aide in the determination of the number of contributors as it affects the subsequent interpretation at the analyst's discretion based on training and experience.
    - The interpretation of an apparent single source profile with allelic peaks significantly above the stochastic threshold (e.g. 1000 RFU) will not be affected by one or more possible peaks below the analytical threshold.
    - The interpretation of a low level apparent single or multiple source profile may be affected by the presence of possible allelic peaks just below the analytical threshold.

5.4 Single Contributor DNA Profile Interpretation

A DNA profile may be considered to be from a single source under the following conditions:

- No more than two alleles are present at each locus (excluding stutter and other explainable artifacts).
    - Tri-allele patterns are not common, but can be observed. If three alleles are observed at a single locus and all other loci display no more than two alleles, the possibility of a tri-allele pattern should be considered. However, it is also possible that the third allele is the product of a second low level contributor only detected at a single locus (especially at the low molecular weight loci).
- Loci containing two allelic peaks meet the minimum expected peak height ratio.
- Typically, peaks below the Analytical Threshold can be ignored. However, numerous peaks detected with the expected allelic peak morphology and falling within allelic bins just below the analytical threshold may be considered when interpreting a low level sample. While these peaks cannot be considered true allelic peaks, their presence may be an indication of additional contributors which could influence the interpretation of low levels samples.
- A sufficient number of alleles and loci are detected from which a conclusion as to the number of contributors can be reliably drawn. For example, a partial DNA profile in which only three loci have one or two alleles above the analytical threshold is not sufficient to determine the number of contributors reliably. This determination will be made based on the analyst's training and experience.
    - Information specific to the case and the item of evidence should be taken into account when making this determination. For example, a small blood stain collected from a sidewalk may be assumed to be from a single contributor. A swab from a doorknob in a public building will most likely contain DNA contributions from multiple individuals.
- If a profile is determined to be from a single contributor, this assumption will be documented in the notes.
- The possible contributing genotypes will be documented in the notes.

<u>Assumed Single Source – Two Alleles</u>

Loci containing two peaks above the stochastic threshold may be used for comparison and statistical purposes.

If the profile is of sufficient quality and the assumption has been made that it is the result of a single contributor, then loci containing two alleles with either one or both peaks between the analytical threshold and the stochastic threshold, may be used for comparison and statistical purposes.

- This assumption will be noted in the report preceding any statistical analyses.

The two peaks detected at a locus for an assumed single source profile should fall within the minimum expected peak height balance range (See Appendix A).

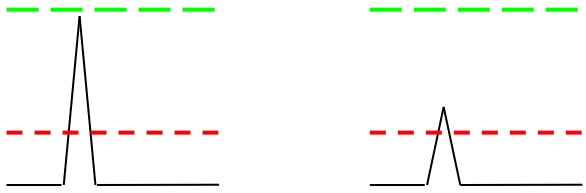
- If the peak height balance is outside the range, this could be an indication of a mixture or a primer binding site mutation. If this imbalance is observed at multiple loci, it is most likely the result of a mixture and the sample cannot be treated as a single source.

Assumed Single Source – One Allele

Loci containing one peak above the stochastic threshold may be used for comparison and statistical purposes.

If the profile is of sufficient quality and the assumption has been made that it is the result of a single contributor, loci containing one peak below the stochastic threshold but above the analytical threshold may be used for comparison and statistical purposes under the following conditions:

- Due to the potential loss of allelic data due to drop-out of a sister allele to the allele detected, all genotypes associated with the detected allele must be considered a possible contributor to the DNA profile. For example, if the A allele is detected at locus X and the peak height is 120 RFU, all genotypes associated with the A allele must be considered a possible contributor to the DNA profile. If the alleles at locus X are A through E, then the possible contributing genotypes are AA, AB, AC, AD and AE.
- The probability that drop-out occurred decreases as the peak height of the detected allele approaches the stochastic threshold. For example, the probability that the sister allele of an allele with a peak height of 190 RFU dropped-out is much less than the probability that the sister allele of an allele with a peak height of 53 RFU dropped-out. This should be taken into account when making the comparison of the evidentiary sample DNA profile to a known DNA profile. If multiple loci of an assumed single source profile demonstrate the presence of a single peak with a peak height of 195 RFU and the known DNA reference is heterozygous at each of these loci; the possibility of excluding the individual associated with the known DNA sample should be considered.



A
Probability of drop-out approaches zero

A
Probability of drop-out >>0

Comparison and Conclusions

Excluded

The individual associated with the known DNA sample cannot be a contributor to the evidentiary sample DNA profile.

If the reference sample profile contains a genotype not consistent with the possible genotypes documented as possible contributors for the evidentiary DNA profile, the individual associated with the reference sample is excluded as a possible contributor.

Not Excluded

The individual associated with the known DNA sample can be a contributor to the evidentiary sample.

If the reference sample genotypes are consistent with the genotypes observed at all loci present in the evidentiary sample, then the individual associated with the reference sample cannot be excluded as a possible contributor.

Profiles may also be deemed "not suitable for comparison or statistical purposes" due to limited results or the complexity of the mixture (e.g. four or more contributors).

If during the comparison process, the determination that a specific genotype which was previously determined not to meet expectations is changed, then the reason shall be sufficiently documented. This would only be expected to occur due to a rare event such as a primer site mutation or tri-allelic pattern.

If a Y allele peak is detected above the analytical threshold at the Amelogenin locus, even in the absence of the X allele, the presence of male DNA can be established.

Statistical Analysis of Assumed Single Contributor DNA Profiles

Values for p and q represent allele frequencies for the identified alleles at each locus and are obtained from reference population data. If the profile is assumed to be the result of a single contributor, loci with alleles that meet the interpretation expectations as described above may be used for statistical purposes.

A Random Match Probability shall be calculated using the appropriate software and the formulae listed below to estimate the rarity of the profile in the relevant populations.

- Heterozygous locus:

  $$\mathbf{2pq}$$ (*NRCII formula 4.1b*)
- Homozygous locus, allelic peak above the stochastic threshold:

  $$\mathbf{p^2 + p(1-p)\theta}$$

  where θ = 0.01 for most populations. A θ value of 0.03 may be used for small isolated populations (*NRCII formula 4.4a).*
- Homozygous locus, allelic peak below the stochastic threshold:

  $$\mathbf{2p - p^2}$$ (*NRCII recommendation 4.1)*
  - This formula is the sum of all genotype frequencies associated with the single allele detected and is equal to $a^2 + 2ab + 2ac + 2ad + 2ae$.

The Random Match Probability for the entire profile is the product of the Random Match Probabilities calculated for each locus.

See Appendix B for examples.

5.5 Multiple Contributor DNA Profile Interpretation

The general flow to mixture interpretation is listed below (Clayton, et al., 1998):

Identify the presence of a mixture
Designate the allele peaks
Identify the minimum number of contributors
Estimate the relative ratio of the individual contributors to the mixture
Consider all possible genotype combinations
Compare reference samples
Statistical analysis (if applicable)

Identify the presence of a mixture

A DNA profile may be considered to be from two or more sources under the following conditions:

- Three or more alleles are present at one or more loci (excluding stutter and other explainable artifacts). Tri-allele patterns are not common but can be observed. If three alleles are observed at a single locus and all other loci display no more than two alleles, the possibility of a tri-allele pattern should be considered. However, it is also possible that the third allele is the product of a second low level contributor only detected at a single locus.
- The peak balance at one or more loci fall significantly outside the expected range. A primer binding site mutation may result in a null allele or significant heterozygous peak imbalance. While this should be considered if only a single locus does not meet the expected peak height balance, primer mutations would not be expected to occur at multiple loci and the imbalance is reproducible.
- Stutter peaks significantly exceed the expected range.

Designate allele peaks

Differentiate between allelic peaks and artifact peaks. See Artifacts section for description of potential artifact peaks.

Identify the minimum number of contributors

The minimum number of contributors to a mixed DNA profile can be estimated using the locus/loci demonstrating the largest number of alleles.

- For example, if the maximum number of alleles at one locus is four, it can be inferred that the minimum number of contributors is two. It is possible in this case that more than two individuals contributed to the biological material, and no more than four alleles were detected at a locus due to homozygosity or allele sharing, but the minimum number of contributors is two. In addition, more than two individuals could have contributed to the biological material, but due to the ratio of contributions from each individual, only the genetic information from two of the individuals is being detected.

Largest # of alleles at a locus	Minimum # of contributors
2	1
3	2
4	2
5	3
6	3
7	4
8	4

<u>Estimate the relative ratio of the individual contributors to the mixture</u>

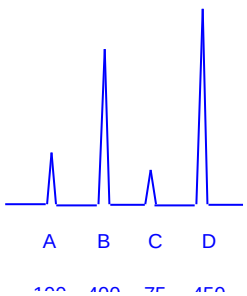
The ratio can be estimated by examining loci with the maximum number of alleles expected for the assumed number of contributors. For example, loci with four alleles would be used for a two person mixture.

The Mixture Ratio (M_r) can be calculated using the following formula:

$$M_r = \frac{PH(a) + PH(b)}{PH(a) + PH(b) + PH(c) + PH(d)}$$

Where PH(a) = Peak Height of Allele A, etc.

Example:



$$M_r \text{ Contributor 1 (A,C)} \quad \frac{100 + 75}{100 + 400 + 75 + 450} = 0.17$$

$$M_r \text{ Contributor 2 (B,D)} \quad \frac{400 + 450}{100 + 400 + 75 + 450} = 0.83$$

Ratio of Contributor 1 : Contributor 2 = 0.17 / 0.83 = 1:4.8

- The major and minor components of a mixture may be distinguished if the average estimated ratio is at least 4:1 (M_r = 80%).
  - In these cases, the major component can be treated as a separate profile.
  - For a single source major component, the major component can be treated as described in the Single Contributor DNA Profile Interpretation section.
  - For a multiple contributor major component, the major component can be treated as an indistinguishable mixture for those alleles associated with the major component.
- Because the mixture ratio may vary from locus to locus across the profile outside of any influences such as differing states of degradation, the calculated mixture ratio is considered an estimate. In general, a range of the mixture ratio is ± 1 (e.g. the range for a 3:1 ratio becomes 2:1 to 4:1) for high RFU DNA profiles. This range may increase as the amount of template DNA decreases or the amount of degradation increases. An increase in the mixture ratio range may also be observed in mixtures with a large difference between the individual contributions (e.g. 15:1). It may be beneficial to look at the range of the mixture ratio across the entire profile to estimate the range to be used during interpretation.
  - For example, if the mixture ratio range across the profile varies from 3:1 to 7:1 and the genotype combination being considered would result in a 15:1 ratio, the combination should be excluded. If the genotype combination would result in an 8:1 mixture ratio, then it may be more conservative to include it as a possibility.
  - It may also be necessary to take into account other factors, such as stutter, when making determinations based on mixture ratio.
    - For example, if a minor component allele is in the stutter position of the major component allele, a portion of the minor component's peak height could be attributed to stutter. This would falsely increase its peak height and lower the mixture ratio.
- The M_r may change across the entire profile due to differing states of degradation of the DNA contributions in the original sample. For example, if fresh blood was deposited on the handle of a knife on top of older, more degraded biological material, the M_r of the smaller loci may be

significantly different than those of the larger loci where the effects of the degradation are more apparent.

or

- If the average mixture ratio for the stronger component is less than 80% (i.e. 4:1), the mixture must be treated like an indistinguishable mixture.

<u>Consider all possible genotype combinations</u>

Each locus should be evaluated individually at this step. All possible genotype combinations should be examined to determine if they meet the interpretation expectations. During this step, the analyst may use the assumptions from *Steps 3 and 4* (the number of contributors and the estimated mixture ratio, respectively) to derive the peak height balance expectations.

The possibility of allele drop-out must be taken into consideration during the interpretation step if an allele or alleles fall below the stochastic threshold.

It is important to note that just because all alleles detected exceed the stochastic threshold does not implicitly mean that there is no potential allele drop-out associated with one of the alleles. The additive effects of a shared allele may cause the peak height of one allele of a heterozygous pair to exceed the stochastic threshold even though the sister allele was not detected. If this is the case, then the possibility of allele drop-out must be a consideration during the interpretation step.

See the table below for the genotype combinations for a two person mixture given the number of alleles detected.

Two Person Mixture Genotype Combinations*							
Four alleles (a,b,c,d)		**Three alleles (a,b,c)**		**Two alleles (a,b)**		**One allele (a)**	
a,b	c,d	a,a	b,c	a,a	a,b	a,a	a,a
a,c	b,d	b,b	a,c	a,b	a,b	a,a	a,x
a,d	b,c	c,c	a,b	a,a	b,b	a,x	a,x
		a,b	a,c	a,b	b,b		
		b,c	a,c	a,x	b,b		

			a,b	b,c		a,x	a,b		
			a,x	b,c		b,x	a,a		
			b,x	a,c		b,x	a,b		
			c,x	a,b		a,x	b,x		

**Modified from Clayton, et al., 1998 x = drop-out of sister allele of heterozygous pair*

Due to inhibition, degradation or other effects on the detection of the alleles, it is possible that at least a portion of the contributor's genotypes is not detected at all loci. As described on page 29, differing states of degradation between the two components of a mixture may cause the apparent contribution of one of the components to decrease as the loci increase in molecular weight. If it can be reasonably assumed that this has occurred and it has progressed to the point at which the component's genotype is completely undetected, then this observation will be documented in the analyst's notes and no genotype can be excluded as a possible contributor to the results at this locus.

If the minor contributor's contribution to a mixed DNA profile results in peaks similar in height to the stutter peaks, the stutter peaks must be considered potential true alleles even if the analysis software has filtered the peaks out. Assumptions about the mixture (number of contributors, mixture ratio, etc.) can be used to logically discount the incorporation of peaks in the stutter position as true alleles in the interpretation.

- For example, in an assumed two person mixture where four alleles are displayed at a locus, it is not necessary to consider peaks in the stutter position as possible true alleles.

The genotype combinations that do or do not meet expectations and the assumptions used to make the determinations must be documented in the analyst's notes.

These conclusions must be drawn prior to any comparison to reference sample profiles where possible. Exceptions to this include the submission of additional evidence subsequent to the analysis of the original evidentiary and reference samples.

The genotype combinations associated with three person mixtures can be much more complex, especially if four or fewer alleles are detected. In these cases, it is important to ensure that at least a portion of the genetic information from each of the three contributors is represented and the potential for allele drop-out associated with each of the alleles is examined.

It may not always be possible to draw reliable conclusions for mixtures containing four or more individuals due to their complexity.

For examples of interpretation, see Appendix C.

Compare reference samples

If a reference sample is available, the reference sample profile can be compared to the genotypes documented as possible contributors to the mixed DNA profile from the evidence sample.

Excluded

The individual associated with the known DNA sample cannot be a contributor to the evidentiary sample.

If the reference sample profile contains a genotype not consistent with the possible genotypes documented as possible contributors for the evidentiary DNA profile, the individual associated with the reference sample is excluded as a possible contributor.

Not Excluded

The individual associated with the known DNA sample can be a contributor to the evidentiary sample.

If the reference sample genotypes are included as possible contributing genotypes (previously determined) at all loci present in the evidentiary sample, then the individual associated with the reference sample cannot be excluded as a possible contributor to the mixed DNA profile and the appropriate statistical calculation can be performed.

- In lower level mixtures, it is sometimes necessary to consider the possibility of allele drop-out to explain the inclusion of a genotype as a potential contributor to the mixture when only one of the known profile's heterozygous pair of alleles is present. The probability that drop-out occurred decreases as the peak height of the detected allele approaches the stochastic threshold. For example, the probability that the sister allele of an allele with a peak height of 190 RFU dropped-out is much less than the probability that the sister allele of an allele with a peak height of 53 RFU. This should be taken into account when making the comparison of the evidentiary sample DNA profile to a known DNA profile. If the occurrence of allele drop-out is necessary to accommodate the inclusion of an individual associated with a known DNA profile at multiple loci where the detected allelic peak approaches the stochastic threshold, the possibility of excluding the individual associated with the known DNA sample should be considered.

Profiles deemed "not suitable for comparison or statistical purposes" due to limited results or the complexity of the mixture (e.g. four or more contributors) will not be compared to reference samples.

If during the comparison process, the determination that a specific genotype which was previously determined not to meet expectations is changed, then the reason shall be sufficiently documented. This would only be expected to occur in complex mixtures or due to a rare event such as a primer site mutation or tri-allelic pattern.

If a Y allele peak is detected above the analytical threshold at the Amelogenin locus, even in the absence of the X allele, the presence of male DNA can be established.

If a mixed DNA profile has been obtained from an intimate sample, the known contributor may be subtracted out by comparing the mixed DNA profile to the known DNA profile. Examples of intimate samples include but are not limited to vaginal swabs and breast swabs.

- In these cases, depending on the remaining alleles, the remaining profile may be treated as a single source profile or a minor component of a distinguishable mixture if the potential for allele masking exists.

Statistical Analysis of Multiple Contributor DNA Profiles

The assumed number of contributors used during the interpretation and statistical calculation steps shall be noted in the laboratory report preceding the results of the statistical analysis.

Distinguishable Components of a Mixed DNA Profile

Major Component of a Distinguishable Mixed DNA Profile

A Random Match Probability shall be calculated using the appropriate software and the formulae as described in the Statistical Analysis of an Assumed Single Contributor DNA Profile section.

Minor Component of a Distinguishable Mixed DNA Profile

The statistical analysis of the minor component of a distinguishable mixture shall be performed in a similar manner as the statistical analysis of an indistinguishable mixed DNA profile.

All alleles detected shall be considered in the statistical analysis of the minor component, not just the minor alleles. Other alleles associated with the minor component may be masked by the major component alleles.

Genotypes excluded during the interpretation step may be excluded in the statistical analysis.

See Appendix C for examples of statistical analyses of mixed DNA profiles.

Indistinguishable Components of a Mixed DNA profile

The statistical analysis of a mixed DNA profile with indistinguishable components shall be conducted using the Random Match Probability.

The Random Match Probability at a specific locus is the sum of all the genotype frequencies documented as possible contributors to the mixed DNA profile.

The frequency of each genotype shall be calculated using the appropriate formulae listed below. Values for p and q represent allele frequencies for the identified alleles at each locus and are obtained from reference population data.

- Heterozygous genotype:

  2pq (*NRCII formula 4.1b*)
- Single allelic peak, no zygotic ambiguity (i.e. no potential allele drop-out associated with the allele) as determined during the interpretation step:

  $\mathbf{p^2 + p(1-p)\theta}$ (*NRCII formula 4.4a)*

  where θ = 0.01 for most populations. A θ value of 0.03 may be used for small isolated populations.
- Single allelic peak, zygotic ambiguity exists (i.e. potential allele drop-out associate with the allele) as determined during the interpretation step:

  $\mathbf{2p - p^2}$ (*NRCII formula 4.1)*

  This formula is the sum of all genotype frequencies associated with the single allele detected and is equal to $a^2 + 2ab + 2ac + 2ad + 2ae$.

Due to the conservative nature of the calculations used, it is possible to obtain a Random Match Probability greater than 1. In these cases, the Random Match Probability assigned to the locus will be 1.

The Random Match Probability for a two-person mixture at loci displaying one, two, or three alleles where there is no indication of potential allele drop-out and no genotype combinations can be excluded based on peak height ratio or mixture ratio expectations is identical to the Combined Probability of Inclusion (CPI).

- Random Match Probability is the square of the sum of the allele frequencies of the alleles detected.
- Given the following results for Locus X, where A, B, and C are the alleles detected and a, b, and c are the corresponding allele frequencies:

  $$\text{Random Match Probability} = (a + b + c)^2$$

The Random Match Probability for a two-person mixture at a given locus displaying four alleles is similar to that described immediately above, except the homozygous frequencies may be subtracted out.

$$\text{Random Match Probability} = (a + b + c + d)^2 - a^2 - b^2 - c^2 - d^2$$

Random Match Probability calculations for three person mixtures are more complex.

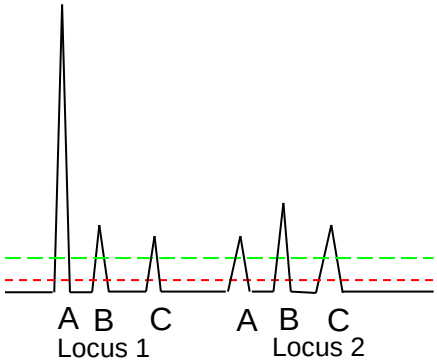
- At lower levels, it is more conservative to treat each allele as if the potential exists for allele drop-out associated with each allele.
- Allele drop-out does not need to be considered at loci displaying six alleles in an assumed three person mixture.

Loci at which possible complete drop-out of a component's genetic information has occurred shall be given a value of "1" (i.e. no genotype is excluded as a possible contributor). This determination will be made during the interpretation step.

The Random Match Probability for the entire profile is the product of the Random Match Probabilities calculated for each locus.

If the Mixture Ratio (M_r) of the components of the mixture vary significantly across the profile in a defined pattern (e.g. one component is more degraded than the other), then the analyst may choose one the following ways to interpret the mixture:

- A Random Match Probability for the distinguishable major component may be calculated at the loci of similar molecular weight which exceed the 4:1 average mixture ratio.
- The entire profile can be considered an indistinguishable mixture and the statistical calculation performed accordingly.
- A combination of the two methods list above may be employed (see example below). In this example, the major contributor at Locus 1 is an AA. At Locus 2, the mixture is indistinguishable, but the major contributor must still be one of the possible contributing genotypes at this locus (AA, AB, AC, BB, BC, or CC).



- The most discriminating profile shall be reported.

See Appendix C for examples of statistical analyses of mixed DNA profiles.

For three person mixture interpretation, see Appendix D.

Unique Statistical Situations

Use of Non-Standard Populations

In some instances, it may be desirable to use a population database that more accurately reflects the region of interest or person(s) of interest. In these cases, the analyst may select an appropriate population database from a peer-reviewed journal to perform the statistical calculations in addition to the standard population databases described above.

Statistical Calculations for Familial Relationships

Statistical calculations for familial relationships shall be performed by an external qualified expert.

Body Identification and Criminal Paternity Interpretation

The ATF Laboratory may be requested to aid in the identification of a body or criminal paternity through DNA analysis. In these instances, the following guidelines should be followed.

- The reference profiles shall be evaluated by the criteria described above.
- These profiles shall be provided to an external qualified expert to perform comparisons, draw conclusions and, if appropriate, perform the statistical analysis.

Staff Contamination

During the evaluation and comparison of the evidence, reference, and control DNA profiles, it may be determined that contamination from a "staff" member was detected. "Staff" includes laboratory personnel and some visitors, other ATF personnel, and other members of law enforcement.

- If the contamination occurred in one of the control samples (reagent blank, amplification positive control, or amplification negative control), the contamination shall be treated in the manner previously described.
- If a "staff" profile is detected on an item of evidence, the report shall make note of it, however the staff member name shall not appear in the report. If the DNA profile is a mixture and a portion of the mixture is consistent with a staff member, the remaining portion may be reported following the normal process described above. For example, an ATF agent may be detected on a weapon recovered during an arrest or a member of the laboratory staff may be detected on an exhibit that had been analyzed in the past. See ATF "Standard Approach to Forensic Biology Examination" for information on handling a "staff" contamination event.
- If there is information that the evidence was not handled properly and the integrity of the DNA results may be affected, a cautionary statement will be added to the note describing the staff contamination event in the report. If the circumstances are sufficiently significant, the evidentiary profile(s) shall be determined to be not suitable for comparison purposes.

Comparison to Reference DNA Profiles Generated by Another Laboratory

In some instances, it may be necessary to compare an evidentiary DNA profile to a reference DNA profile generated by another laboratory. For example, the individual is deceased and there is no reasonable method to obtain a direct sample.

In these cases, a laboratory report containing the allele table for the reference DNA profile that has undergone a technical and administrative review or the allele table from the bench notes that has undergone a technical and administrative review can be used for comparison purposes. This information will be maintained in the case notes and a note shall be made in the report describing the source of the information.

Multiple DNA Profiles from the Same Exhibit

A statistical calculation to lend weight to a comparison where an individual cannot be excluded must be performed for each DNA profile, even when multiple consistent profiles that differ slightly due to allele drop-out, etc. are obtained from the same exhibit of evidence.

- The statistical weight does not have to be calculated for items taken directly from an individual that are consistent with that individual (intimate samples such as fingernail scrapings, vaginal swabs, etc.).
- If multiple DNA profiles are obtained from the same exhibit, but each has a different statistical weight, the group of profiles may be reported together using the lowest statistical weight to describe the entire group. See Report Wording Guidelines.

Documentation

Data Interpretation Sheet

A Data Interpretation Sheet shall be completed for each sample analyzed.

The Data Interpretation Sheet includes exhibit number, description of evidence, assumptions made during interpretation, brief summary of interpretation of profile (e.g. number of contributors in a mixture, completeness of profile, etc.), and the result of comparison, if applicable.

Allele Summary Table

An Allele Summary Table listing the alleles detected in the DNA profile following the guidelines listed below shall be included in the case notes for reference DNA profiles (this includes secondary references). It is not necessary to complete an ISC worksheet for reference samples. The allele table can then be used to compare the reference DNA profiles to the list of documented possible contributing genotypes for the evidentiary samples.

- Each peak above the analytical threshold at a locus shall be listed in the table.
- Alleles at heterozygous loci shall be listed in ascending order (e.g. 15,20).
- The allele detected at a homozygous locus will be listed as a genotype (e.g. 15,15).
- If a single peak is detected at a locus and the allele's peak height is less than 200 RFU, the results for the locus shall be designated as "A,---" where A is the single allele.

Appendix A

Appendix A is a separate Excel workbook that can be found in the Forensic Biology Worksheets folder on Qualtrax (ATF-LS-FB17 Appendix A).

Appendix B

Examples of Interpretation and Statistical Analyses of Assumed Single Contributor DNA Profiles

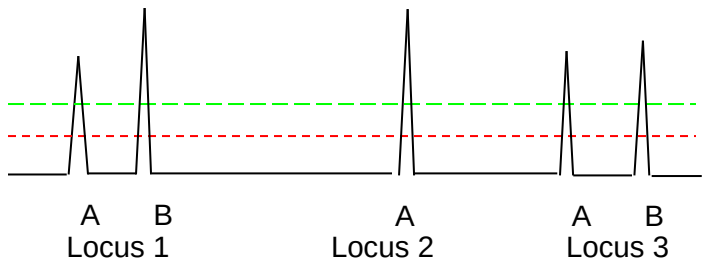
Alleles denoted as A and/or B

Allele frequencies denoted as a and/or b

Analytical Threshold: - - - - - - - - - - -

Stochastic Threshold: – – – – – – –

Example B1



No allelic drop-out possible at any of the three loci

Genotypes included:

Locus 1: A,B

Locus 2: A,A

Locus 3: A,B

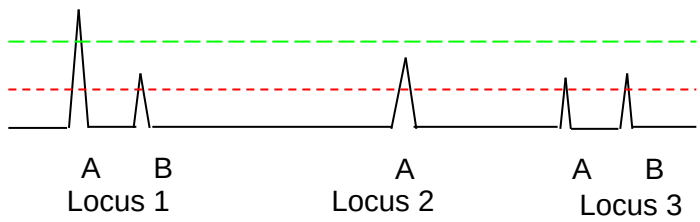
Statistical analysis:

Locus 1: 2ab

Locus 2: $a^2 + a(1-a)\theta$

Locus 3: 2ab

Example B2



Allelic drop-out possible at Locus 2 only. Under the assumption that this is a single source profile, allele drop-out is not expected at loci displaying two alleles.

Genotypes included:

Locus 1: A,B

Locus 2: A,X (where X = any allele associated with allele A including A,A)

Locus 3: A,B

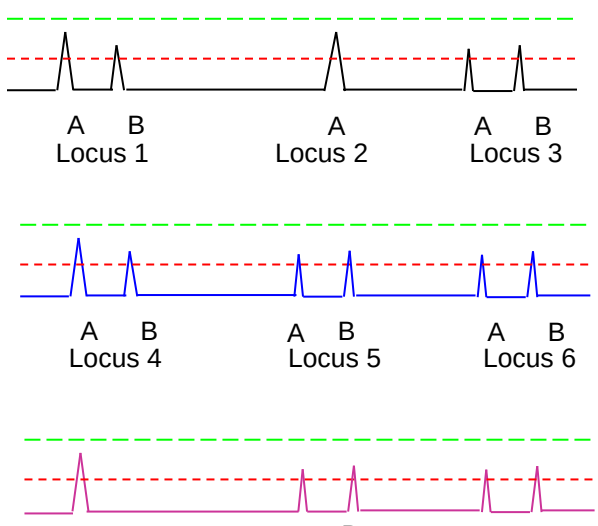
Statistical analysis:

Locus 1: 2ab

Locus 2: $2a - a^2$

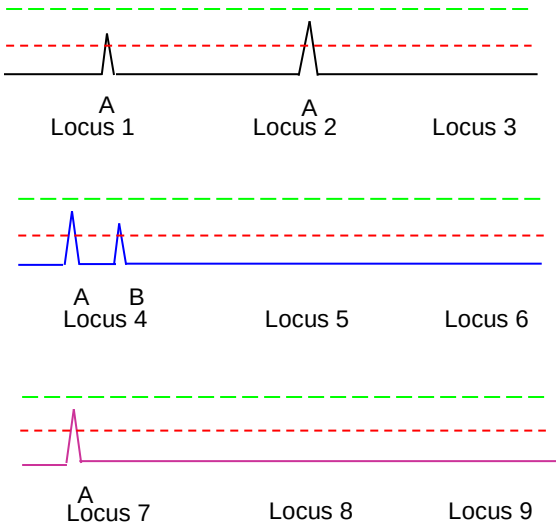
Locus 3: 2ab

Example B3



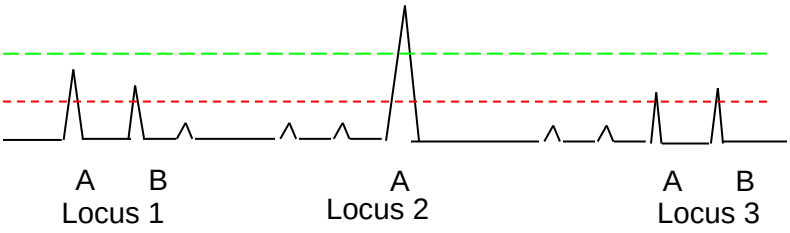
This DNA profile can be assumed to be a single source profile and can be used for matching and statistical purposes as described in Examples 1 and 2 even though none of the loci contain an allele exceeding the stochastic threshold. Sufficient data is present to reasonably make the assumption of a single contributor.

Example B4



While allelic peaks are detected in this DNA profile, there is not enough information to reasonably assume the number of contributors. If the number of contributors cannot be reasonable assumed, then the analyst cannot determine if all components are at least partially represented at a locus and therefore, each locus would be given a value of one (no genotype excluded). This profile may or may not be considered suitable for comparison purposes based on the context of the sample (e.g. drop of blood compared to a swabbing of gun grips).

Example B5



In this case, the maximum number of alleles detected at a locus is two. However, a pattern of peaks below the analytical threshold can be seen. Assuming these peaks cannot be attributed to a form of artifact, have the expected allelic peak morphology, and fall within an allelic bin, the possibility of a mixed DNA profile should be considered during the interpretation. If the peaks detected are significantly greater than the stochastic threshold (e.g. 1000 RFU), the possible minor contribution below the analytical threshold does not need to be considered. If however, the detected alleles are closer to the stochastic threshold or even analytical threshold, the presence of the peaks below the analytical threshold may affect the interpretation of the DNA profile.

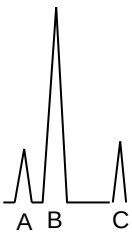
Appendix C

Examples of Interpretation and Statistical Analyses of Assumed Multiple Contributor DNA Profiles

Example C1

Assume the overall DNA profile is consistent with a two person mixture and the peak heights for the alleles are as follows: allele A (900 RFU), allele B (600 RFU), allele C (1000 RFU) and allele D (300 RFU). Since four peaks are present, the locus is a mixture of two heterozygous genotypes. Based on these assumptions, the A,D (Peak Height Ratio of 33%) and C,D genotypes (Peak Height Ratio of 30%) do not meet the Minimum Expected Peak Height Ratio expectations and are therefore excluded as possible genotypes. Because four alleles were detected, the potential for allele drop-out does not exist. This leaves the A,C and B,D genotypes that could be possible contributors.

Example C2



Appendix C

Assume the overall DNA profile is consistent with a two person mixture at an average mixture ratio of 2:1 and the peak heights of the alleles are as follows: allele A (500 RFU), allele B (2000 RFU) and allele C (500 RFU).

Genotype Combinations Examined:

Three alleles (a,b,c)		**Possible?**
a,a	b,c	No
b,b	a,c	Yes
c,c	a,b	No
a,b	a,c	No
b,c	a,c	No
a,b	b,c	No
a,x	b,c	No
b,x	a,c	No
c,x	a,b	No

The following genotypes are excluded because the remaining genotype does not meet the Minimum Expected Peak Height Ratio (MEPHR) based on the listed assumptions: AA, AX, CC, and CX. In these cases, the remaining genotype (B,C or A,B) falls outside the MEPHR.

The genotype combination A,B with a B,C also does not fall within the expected parameters. The maximum expected peak height associated with a peak at 500 RFU is approximately 895 RFU. Even at a 1:1 ration where both genotypes have the 500 RFU to 895 RFU ratio, the combined RFU for the B allele does not equal 2000 RFU.

Appendix C

The 2:1 ratio allows for the combination of an A,C mixed with a B,B. The average mixture ratio is assumed to be 2:1, which provides a range of 1:1 and 3:1. Because three alleles were detected and all exceed the stochastic threshold, no potential for allele drop-out exists.

The statistical calculation is then the sum of the remaining genotype frequencies:

$$B,B = b^2 + b(1-b)\theta$$

$$A,C = 2ac$$

Appendix D

Appendix D is a separate PowerPoint document that can be found in the Forensic Biology Methods of Analysis (ATF-LS-FB17 Appendix D).