

FBS15 – Identifiler Plus Interpretation Guidelines

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1. Scope

- 1.1. This procedure outlines the guidelines used to interpret the results obtained from the GeneMapper® *ID-X* analysis software when samples are amplified with Identifiler Plus.

2. Background

- 2.1. To establish the practices for documenting the examination of evidence to conform to the requirements of the Department of Forensic Sciences (DFS) Forensic Science Laboratory (FSL) *Quality Assurance Manual*, the accreditation standards under ISO/IEC 17025:2005, and any supplemental standards.
- 2.2. The interpretation of results in casework is a matter of professional judgment and expertise. Not every situation can, or should be, covered by a pre-set rule; nor is it expected that competent analysts will always be in full agreement in a particular case. However, it is important that the Laboratory develop and adhere to basic criteria for the interpretation of analytical results. The principles will be based on validation studies, literature references, and casework experience and will be developed with maximum input from analysts. As the collective experience of the Laboratory with various analytical procedures grows, it is to be expected that interpretation guidelines will also continue to evolve.
- 2.3. Once the testing controls have been evaluated and confirmed, the results of the individual samples can be interpreted and compared using the established guidelines. Utilizing the data obtained from the GeneMapper® *ID-X* (GMID-X)

analysis, the sample's results are interpreted through a three-step process that determines the outcome of a profile. First, the overall profile is evaluated to resolve the quality and number of contributors associated with the sample. Due to their reciprocal nature, this process must occur in conjunction with the peak evaluation. Secondly, artifacts both inherent to the PCR process as well as those generated by the detection instrumentation can be isolated and eliminated during the analysis and interpretation of individual peaks, validating the authenticity of a genuine allele. And finally, the interpreted profile is compared to the known profiles jointly submitted in the case to make a forensic conclusion regarding the contributor(s) of each sample. In the case of multiple contributors, deducing guidelines may also be applied to assist in distinguishing between the individuals associated with the profile.

- 2.4. The purpose of these guidelines is to establish a general framework outlining minimum standards to ensure that:
 - 2.4.1. Conclusions in casework reports are scientifically supported by the analytical data, including data from appropriate standards and controls.
 - 2.4.2. Interpretations are made as objectively as possible, consistently from analyst to analyst, and within previously agreed limits.
 - 2.4.2.1. Note: The AmpFISTR[®] Identifiler[®] Plus User's Manual provides further details on the process of interpretation.

3. Safety

- 3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures.
- 3.2. Read Material Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.

4. Materials Required

- 4.1. Not applicable

5. Standards and Controls

- 5.1. AmpFISTR Identifiler Plus Loci

Locus	Dye Label	Dye Color	Size Range	Number of Alleles	Alleles included in Identifiler Ladder	Other Known	Control DNA
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District of Columbia Department of Forensic Sciences

			(bp)	in the Ladder		Alleles	9947A
D8S1179	6-FAM	Blue	128-172	12	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	-	13
D21S11	6-FAM	Blue	187-243	24	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	24.3, 25.3, 28.3, 29.1, 29.3, 30.1, 30.3, 31.1, 32.1, 33.1, 33.3, 34.1, 36.2	30
D7S820	6-FAM	Blue	258-294	10	6, 7, 8, 9, 10, 11, 12, 13, 14, 15	6.3	10, 11
CSF1PO	6-FAM	Blue	307-343	10	6, 7, 8, 9, 10, 11, 12, 13, 14, 15	10.3	10, 12
D3S1358	VIC	Green	114-142	8	12, 13, 14, 15, 16, 17, 18, 19	9, 11, 15.2, 20	14, 15
THO1	VIC	Green	165-204	10	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3	6.1, 7.1, 7.3, 8.3, 14	8, 9.3
D13S317	VIC	Green	218-246	8	8, 9, 10, 11, 12, 13, 14, 15	5	11
D16S539	VIC	Green	257-297	9	5, 8, 9, 10, 11, 12, 13, 14, 15	-	11, 12
D2S1338	VIC	Green	305-357	14	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28	-	19, 23
D19S433	NED	Yellow	106-140	15	9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2	-	14, 15
vWA	NED	Yellow	157-209	14	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24	15.2	17, 18
TPOX	NED	Yellow	225-253	8	6, 7, 8, 9, 10, 11, 12, 13	-	8
D18S51	NED	Yellow	265-345	23	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	9.2, 15.2, 17.2, 19.2	15, 19
Amelogeni	PET	Red	107-113	2	X, Y	-	X

n							
D5S818	PET	Red	135-171	10	7, 8, 9, 10, 11, 12, 13, 14, 15, 16	-	11
FGA	PET	Red	215-353	28	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	15, 16, 16.2, 18.2, 19.2, 20.2, 21.2, 22.2, 22.3, 23.2, 24.2, 25.2, 27.2, 28.2, 34.2	23, 24

5.2. GeneScan-500 [LIZ] Denatured Fragment Molecular Lengths

35 bp	139 bp	250 bp	400 bp
50 bp	150 bp	300 bp	450 bp
75 bp	160 bp	340 bp	490 bp
100 bp	200 bp	350 bp	500 bp

5.2.1. Note: The 250bp peak migrates at approximately 246bp.

6. Calibration

6.1. Not applicable

7. Procedures

7.1. Analytical Controls

7.1.1. A variety of controls are required to assess the quantity and quality of each sample as well as the effectiveness, accuracy and precision of the analytical procedures. Evaluation of the controls is essential to the proper interpretation of the data. The following controls should be evaluated and printed to document that proper results were obtained.

7.1.2. Negative Controls:

7.1.2.1. Reagent Blank (extraction control)

7.1.2.1.1. The reagent blank is a check for possible contamination of the extraction reagents by human DNA or by amplified STR product. The reagent blank is created by carrying out the DNA extraction in a tube containing no sample. This “blank” extract is then amplified and typed along with the test samples.

7.1.2.1.2. The appearance of typeable test results in the reagent blank may indicate that:

7.1.2.1.2.1. The sample preparation reagents have been contaminated.

7.1.2.1.2.2. Cross-contamination between samples occurred during preparation.

7.1.2.1.2.3. Human DNA or STR product is getting into samples from some other source.

7.1.2.2. Negative Amplification Control

7.1.2.2.1. The negative amplification control is a check for contamination during set up of the PCR amplification reaction. It essentially monitors the “environment” in that process for possible sources of contamination. If typing results reproducibly occur in this control, then the PCR reaction set up area, equipment and amplification reagents should be considered possible sources of contamination. Testing will resume when it is concluded that the procedure is free of contamination. The test set where the contamination is noted should be evaluated closely to establish if the results are reliable. If feasible and warranted, the original results may be considered inconclusive and the analysis may need to be repeated.

7.1.2.3. Typeable signals (reproducible signals above the analytical threshold) occurring in a negative control may indicate a critical contamination problem and corrective action(s) must be taken. When possible, steps must be taken to determine the source of contamination before further testing can be conducted. Examples of various corrective actions can include the testing of all reagents (e.g., extraction, amplification, typing), a thorough cleaning of all work surfaces and equipment, and the evaluation of procedures to rule out other possible avenues of contamination (e.g., sample-to-sample mixing, switched samples, etc.). Testing will resume when it is concluded that the entire procedure is free of

contamination. The test set where the contamination is noted should be evaluated closely to establish if the results are reliable.

7.1.2.4. The appearance of signals in the negative controls does not necessarily mean that the types obtained from the test samples are incorrect. The contamination might be due to a single event limited to the control. Alternatively, even if a reagent used to prepare all the samples was contaminated, the level of contamination might be inconsequential compared to the amount of DNA being amplified and typed in the test samples. However, further testing may be necessary to support these hypotheses.

7.1.2.5. When the signals in a negative control are not typeable (below analytical threshold) and the test signals are clearly typeable, the contamination may not be considerable but should be noted. Furthermore, the appearance of signals in a negative control does not automatically mean that the types obtained from the samples are incorrect. In certain situations the sample types may be reported. Depending on the nature of the contaminant and sample, the original results may be considered inconclusive and the analysis may need to be repeated.

7.1.2.6. If a negative control shows no signs of typeable signals, the raw data must be examined for the presence of primer peaks to ensure that these samples have been properly amplified. The results of this examination shall be recorded on the appropriate Reader Worksheet.

7.1.3. Positive Controls:

7.1.3.1. Positive Amplification Control (AmpFISTR® Control DNA 9947A)

7.1.3.1.1. This is a human female DNA sample with predetermined autosomal STR genotypes and is included in the AmpFISTR® amplification/typing kits. The positive amplification control ensures that the amplification and typing processes are working properly. If the positive amplification control fails to give the correct results, then the analysis must be repeated. For re-injections, a positive amplification control should be included to ensure that the typing procedure is working properly. For analysis, if the positive control fails for a specific set, only that set needs to be reanalyzed. Results of this

evaluation shall be recorded on the appropriate Reader Worksheet.

7.1.4. Other Controls:

7.1.4.1. Internal Lane Size Standard

7.1.4.1.1. This standard contains DNA fragments (LIZ) of known sizes (bp) that are used in-lane to estimate sizes (bp) of STR products. Each sample tested must have the correct peak sizes assigned for the in-lane size standard. Results of this evaluation shall be recorded on the appropriate Reader Worksheet. Please refer to FBS21 for more details on evaluating the size standard.

7.1.4.2. Allelic Ladders

7.1.4.2.1. The allelic ladders provided in the kits are used to ascertain the genotypes of the samples. While the ladders consist of the common alleles in the population, additional atypical alleles may also be identified for many loci. See Appendix I for allelic ladder characterization. Results of the allelic ladder evaluation shall be recorded on the appropriate Reader Worksheet.

7.2. Overall Profile Evaluation

7.2.1. Condition of the profile

7.2.1.1. The overall condition and number of possible contributors to a profile should be considered prior to making determinations about the data observed. A profile which appears degraded or inhibited can influence the assessments made at each locus and a mixed profile will be interpreted differently than a sample which appears to be from a single source. Once this evaluation is complete, each allele can be individually examined.

7.2.2. Degradation

7.2.2.1. DNA degradation is a process which randomly breaks down DNA molecules into smaller pieces. This becomes problematic for forensic DNA typing when it occurs within the target DNA sequences of PCR. If the DNA has been degraded or damaged at these locations, an inconclusive or no result may be obtained at

a locus. Typically, samples which are degraded will display a downward sloping or “ski-slope” pattern from left to right for each dye. This pattern is due to the larger/longer loci being more likely to contain a break due to degradation than the smaller loci. Degradation may result in peak imbalance at these large loci to the extent of drop out or loss of an allele.

- 7.2.2.2. Little can be done at this point to remedy DNA degradation. “MiniSTR” kits may help recover information from degraded DNA samples that typically produce partial profiles and a total loss of information from larger STR amplicons. These kits are currently not in use but will be evaluated in the near future.

7.2.3. Inhibition

- 7.2.3.1. DNA samples of various origins may contain organic and inorganic compounds that prevent the amplification of nucleic acids by PCR. Inhibitors can interfere with the cell lysis necessary for DNA extraction, cause nucleic acid degradation, and/or inhibit polymerase activity thus preventing amplification. Some known DNA inhibitors often encountered in forensic casework include hematin, indigo dyes, melanin, iron, tannins, urea, bile salts, and humic acid.
- 7.2.3.2. The presence of inhibitors can manifest itself by incomplete PCR amplification or failure of some or all loci. Samples containing PCR inhibitors often produce partial profile results in which the smaller loci drop out before the larger loci. However, some common patterns in the electropherograms of inhibited samples may also show characteristics of degradation and are therefore hard to differentiate. These samples may need to be re-amplified using both a larger quantity of DNA and a smaller quantity of DNA than originally amplified.
- 7.2.3.3. Since real-time quantitative PCR incorporates an internal positive control (IPC) in each reaction, the possible presence of inhibitors can be determined by evaluating C_T values obtained from the quantitation.
- 7.2.3.4. In some cases inhibition can be overcome by minimizing the amount of template DNA amplified and/or adding BSA to the amplification mixture. Alternatively, the DNA extract can be subjected to additional TE buffer washes and concentration using a microcon.

7.2.4. Partial Profiles

7.2.4.1. Partial profiles do not have alleles at every locus. This situation usually occurs in samples that are degraded, have low template DNA, and/or contain PCR inhibitors. Partial profiles are interpreted with care and are reported only when there is consensus between the analyst, second reader, and technical reviewer.

7.2.5. Apparent Single Source Profiles

7.2.5.1. Autosomal STR Loci

7.2.5.1.1. An apparent single source profile consists of no more than two relatively balanced alleles at each locus. (Single source samples with three allele peaks at a single locus have been reported, however they are rare.)

7.2.6. Mixture Profiles

7.2.6.1. Evidence samples may contain DNA from more than one individual. The possibility of multiple contributors should be carefully considered using all the data available when interpreting the results.

7.2.6.2. Each of the following can aid in determining whether a sample is a mixture (assuming any peak >70 rfu):

7.2.6.2.1. For autosomal STR analysis, more than two alleles at some or all of the loci.

7.2.6.2.2. The presence of a peak at a stutter position that is significantly greater in percentage than what is typically observed in a single source sample. (See discussion of Stutter.)

7.2.6.2.3. Significantly imbalanced alleles for a heterozygous autosomal STR genotype.

7.2.6.2.4. **Samples amplified for 28 cycles:**

7.2.6.2.4.1. For autosomal STR loci, peak height ratios less than 55% are rare in normal, unmixed samples. Other possible causes of imbalance at a locus are degraded DNA, presence of inhibitors, extremely low amounts of input DNA, extreme size difference between two alleles in a heterozygous pair, and/or the presence of an allele containing a rare sequence that does not

amplify as efficiently as the other allele. Amplification and analysis of additional loci may assist in the interpretation of the sample.

7.2.6.2.4.2. Note: A peak height ratio imbalance may occur at less than 200 rfu due to stochastic effects. Therefore, a heterozygote may be miscalled as a mixture if the peak height ratio is less than 55%.

7.2.6.2.5. Samples amplified for 29 cycles:

7.2.6.2.5.1. For autosomal STR loci, peak height ratios less than 50% are rare in normal, unmixed samples. Other possible causes of imbalance at a locus are degraded DNA, presence of inhibitors, extremely low amounts of input DNA, extreme size difference between two alleles in a heterozygous pair, and/or the presence of an allele containing a rare sequence that does not amplify as efficiently as the other allele. Amplification and analysis of additional loci may assist in the interpretation of the sample.

7.2.6.2.5.2. Note: A peak height ratio imbalance may occur at less than 300 rfu due to stochastic effects. Therefore, a heterozygote may be miscalled as a mixture if the peak height ratio is less than 50%.

7.2.6.2.5.3. For autosomal STR analysis, a sample containing DNA from two sources can be comprised (at a single locus) of any of the following seven genotype combinations (excluding amelogenin):

<i>Heterozygote + heterozygote</i>	<i>no overlapping alleles</i>	<i>(4 peaks)</i>
<i>Heterozygote + heterozygote</i>	<i>one overlapping allele</i>	<i>(3 peaks)</i>
<i>Heterozygote + heterozygote</i>	<i>two overlapping alleles</i>	<i>(2 peaks)</i>
<i>Heterozygote + homozygote</i>	<i>no overlapping alleles</i>	<i>(3 peaks)</i>
<i>Heterozygote + homozygote</i>	<i>one overlapping allele</i>	<i>(2 peaks)</i>
<i>Homozygote + homozygote</i>	<i>no overlapping alleles</i>	<i>(2 peaks)</i>
<i>Homozygote + homozygote</i>	<i>overlapping alleles</i>	<i>(1 peak)</i>

- 7.2.7. Specific genotype combinations and input DNA ratios of the samples contained in a mixture determine whether it is possible to resolve the genotypes of the major and minor component(s) at a single locus. Ultimately, the likelihood that any sample is a mixture must be determined by the analyst in the context of each particular case.
- 7.2.8. When the profile obtained from a sample is a mixture of two contributors and it is reasonable to assume that one of the contributors is a person whose profile is known, it may be possible to deduce information about the profile of the second contributor based on the quantitative data in the profile (peak height in rfu) for each observed peak.

7.3. Peak Evaluation

- 7.3.1. As a profile is evaluated, each peak identified by the software must be checked to confirm it is a valid allele call. Typical characteristics and requirements related to the shape, size and location of a true allele are listed below along with a variety of recognizable artifacts.

7.3.1.1. Alleles

- 7.3.1.1.1. An allele peak is defined as a distinct, triangular section of an electropherogram.
- 7.3.1.1.2. The minimum peak height threshold for recognition of an allele is established at 70 relative fluorescence units (rfu) for GMID-X. Peaks below 70 rfu will not be interpreted.
- 7.3.1.1.3. For autosomal STR analysis, homozygote allele peak heights are approximately twice that of heterozygotes as a result of a doubling signal from two alleles of the same size.
- 7.3.1.1.4. When interpreting autosomal STR genotypes for samples of low intensity, care must be taken to accurately distinguish a homozygote peak from a heterozygote peak whose sister allele has dropped out. As a general guideline, single peaks from samples amplified for 28 cycles should not be called as homozygote alleles unless the peak is ≥ 200 rfu. As a general guideline, single peaks from samples amplified for 29 cycles should not be called as homozygote alleles unless the peak is ≥ 300 rfu. It is important to assess the peak heights of alleles at both smaller and larger loci to aid in this type of interpretation.
- 7.3.1.1.5. The expected peak height ratio for autosomal heterozygote alleles from samples amplified for 28 cycles

is 55-100%. Occasionally, a non-mixed sample will be lower than 55%.

7.3.1.1.6. $[\text{lower peak height}] \div [\text{higher peak height}] \times 100 = \text{peak height ratio}$

7.3.1.1.7. The expected peak height ratio for autosomal heterozygote alleles from samples amplified for 29 cycles is 50-100%. Occasionally, a non-mixed sample will be lower than 50%.

7.3.1.1.8. $[\text{lower peak height}] \div [\text{higher peak height}] \times 100 = \text{peak height ratio}$

7.3.1.1.9. Genotypes are determined from the diagnostic peaks (allelic ladder) of the appropriate dye color (blue, green, yellow, red) and size range (bp) for a particular STR marker system. Peaks not aligning with those in the allelic ladders have been detected both within and outside the range of the ladders (off-ladder alleles). Some peaks may represent variant alleles containing incomplete repeats. GMID-X will accurately label many of the alleles not present in the allelic ladders; these variants have been termed "other known alleles" or "virtual alleles." Those not labeled, will be typed manually by the analyst based on the base pair size of the peak and the base pair size of the closest allelic ladder peak (s). A base pair (bp) size will be reported for those samples that cannot be genotyped by the typing software or by peak size comparison.

7.3.1.1.10. Depending on the situation, it may be necessary for the analyst to reamplify, reprepare, and/or reinject the sample to verify the allele in question. Each variant will be closely examined by the analyst and the technical reviewer and will be handled on a case-by-case basis.

7.3.1.1.11. Peaks other than the target alleles may be detected on the electropherogram displays. The following is a description of various causes for the appearance of extra peaks.

7.3.1.2. Stutter Products

7.3.1.2.1. A stutter peak is a well-characterized PCR artifact that refers to the appearance of a minor peak one repeat unit smaller (or less frequently, one repeat larger) than the major STR product. All of the loci in the Identifiler® Plus kit are tetranucleotide repeat units, thus the stutter peak

would be four bases shorter, or $n-4$. It is important to note that stutter four bases longer ($n+4$), were also observed in the validation studies and may be observed in casework samples. These peaks will not be filtered using the recommended stutter filter and must be carefully evaluated prior to elimination.

7.3.1.2.2. Stutter products for autosomal STR have many of the same characteristics:

7.3.1.2.2.1. For each AmpFISTR kit locus, the percent stutter generally increases with allele length.

7.3.1.2.2.2. Each allele within a locus displays a percent stutter that is consistent.

7.3.1.2.2.3. The highest observed percent stutter ($n+4$) for each locus is included as the filtering step in GMID-X. Peaks in the stutter position that are above the highest observed stutter will not be filtered. Peaks in the stutter position that have not been filtered will remain labeled and can be further evaluated.

7.3.1.2.2.4. The measurement of percent stutter for peaks that are off-scale may be unusually high. Likewise, samples with stochastic effects due to very low input DNA, may also have stutter peaks that are unnaturally high.

7.3.1.2.2.5. The proportion of the stutter product relative to the main allele (percent stutter) is measured by dividing the height of the stutter peak by the height of the main allele peak.

7.3.1.2.3. The following table summarizes the highest percent stutter observed for the Identifiler[®] Plus kit:

STR Locus	Percent Stutter Observed (ABI Recommended)
6-FAM (blue)	
<i>D8S1179</i>	10.3155%
<i>D21S11</i>	10.6714%

<i>D7S80</i>	9.6926%
<i>CSF1PO</i>	9.2041%
<i>VIC (green)</i>	
<i>D3S1358</i>	12.2719%
<i>TH01</i>	4.0813%
<i>D13S317</i>	9.9348%
<i>D16S539</i>	10.3945%
<i>D2S1338</i>	12.4409%
<i>NED (yellow)</i>	
<i>D19S433</i>	11.2096%
<i>VWA</i>	12.446%
<i>TPOX</i>	6.3832%
<i>D18S51</i>	13.6799%
<i>PET (red)</i>	
<i>D5S818</i>	10.0599%
<i>FGA</i>	13.028%

7.3.1.2.4. The manufacturer's recommended stutter filters for Identifiler[®] Plus loci will be employed until an in-house stutter study is complete.

7.3.1.3. Spikes

7.3.1.3.1. Spikes are random events that may be observed on an electropherogram. They are generally tall, thin peaks observed in most or all of the dye colors at the same base pair location. Peak height (rfu) usually varies between dye colors. If spikes occur in diagnostic regions or negative controls, the sample should be re-injected and/or retested. Note that artifacts and spikes can occur in a single dye color.

7.3.1.4. Dye Blobs

7.3.1.4.1. Dye blobs generally occur between 60 and 90 base pairs, but can occasionally occur outside this range. Dye blobs generally appear as a broad peak in a single color. If dye blobs occur in diagnostic regions or negative controls, the sample should be re-injected and/or retested. If the dye blob is still present after re-injection or retesting, the sample may need to be reamplified using a new AmpFISTR[®] kit. In the instance that a dye blob is seen and known to occur over many samples, re-injection or retesting may not be necessary.

7.3.1.5. Pull-up (bleedthrough)

7.3.1.5.1. Unusually high pull-up (>3%) or extra peaks can be caused by oversaturation of the system or a problem with the matrix. Pull-up peaks are a result of the matrix not correcting for all the spectral overlap. These peaks are easily recognized by overlaying the colors and observing the alignment of peaks at the same data point or evaluating the sample's raw data. If an ongoing matrix problem exists, a new spectral must be run, applied and the samples retested.

7.3.1.6. Artifacts

7.3.1.6.1. Artifacts, or anomalies, have been seen in data produced using the Identifiler[®] kit. The shape of these artifacts is not consistent with the shape of labeled DNA fragments as seen on the CE Instrument. Artifacts may or may not be reproducible. Analysts should consider these artifacts when interpreting data. See the AmpFISTR[®] Identifiler[®] Plus User's Manual for examples of reproducible artifacts.

7.3.1.7. Split Peaks/Minus A

7.3.1.7.1. Taq polymerase catalyzes the addition of a single nucleotide (predominantly adenosine) to the 3' ends of double stranded PCR products. This non-template addition results in a PCR product that is one base pair longer than the actual target sequence (+A). Split peaks and minus A are a result of the incomplete addition of an

adenosine, where each allele is represented by two peaks one base pair apart (-A and +A).

7.3.1.7.2. Lack of full A nucleotide addition may be observed when the amount of input DNA is greater than recommended. This occurs because more time is needed for the Taq polymerase to add the A nucleotide to all molecules with each cycle. Amplification of too much input DNA will also result in off-scale data. Sample tubes with excess PCR product may be diluted, or reamplified using less input DNA and then reinjected.

7.3.1.7.3. Note: Many times, the appearance of a minus A peak will result in the software not recognizing a stutter peak. Take care when evaluating these instances. If minus A is present at a locus, be sure to hand calculate any peaks which may be unfiltered stutter.

7.3.1.8. Off-Ladder Alleles

7.3.1.8.1. An off-ladder allele may be the result of one of the artifacts listed above, overloading, or may occur if a sample has gone through temperature changes while running. A true off-ladder allele occurs when a person's genotype at a particular locus is not documented by the ladder. Typically, these samples are re-injected or retested to confirm the sizing and allele designation except when the off-ladder allele observed in the known sample matches another sample taken from the same individual. To estimate the size of an off-ladder allele, compare the base pair sizes of the closest ladder fragments with the base pair size of the off-ladder allele. If an off-ladder allele falls outside of the range of the allelic ladder, it may be reported as greater than the highest allele or less than the lowest allele in the ladder at that locus.

7.4. Conclusions and Profile Comparison

7.4.1. The report should state whether a sample contains a mixture or possible mixture of DNA and, if possible, the minimum number of individuals who could be possible contributors to the mixture.

- 7.4.1.1. **Single Contributor:** A sample is considered to be from a single contributor when the number of alleles at each autosomal locus and peak height ratios are consistent with a profile from a single contributor (e.g., for samples amplified for 28 cycles, one or two alleles at each autosomal locus, $\text{PHR} \geq 55\%$; for samples amplified for 29 cycles, one or two alleles at each autosomal locus, $\text{PHR} \geq 50\%$).
 - 7.4.1.2. **Mixtures with Major/Minor Components:** A sample is considered a mixture of major and minor contributors when there is a large difference in peak heights between a major and minor component and the genotypes of the major component are easily inferred. In this situation, the major contributor is considered to be from a single contributor. For autosomal STR analysis, the minor genotypes can only be determined if three or four alleles are present at a locus (depending on whether the major component is a homozygote or heterozygote) because other alleles may be masked by the major component alleles.
 - 7.4.1.3. **Mixtures with a Known Contributor:** When a sample is a mixture of two components and the source of one component is known (e.g., vaginal epithelial cell carryover into a sperm fraction), the genetic profile of the unknown contributor may be inferred. This can be accomplished by subtracting the contribution of the known donor from the mixed profile.
 - 7.4.1.4. **Mixtures with Indistinguishable Contributors:** When major and minor contributors cannot be distinguished because of similarity in signal intensities or the presence of shared or masked alleles, individuals are simply included or excluded as possible contributors. A statistical calculation may be performed to supplement the inclusion/exclusion and provide weight (or lack thereof) to the evidence.
- 7.4.2. Where applicable, evidence DNA profiles are then compared to reference DNA profiles. The following are possible conclusions:
- 7.4.2.1. Inclusion (“match”) – the evidence and reference samples have the same genotypes at every locus tested.
 - 7.4.2.2. Exclusion (“nonmatch”) – the evidence and reference samples have different genotypes at some or every locus tested.
 - 7.4.2.3. Inconclusive – the test results from the evidence sample are ambiguous making a determination of inclusion or exclusion difficult.
 - 7.4.2.4. No results – no results are obtained from an evidence sample.

8. Sampling

8.1. Not applicable

9. Calculations

9.1. Not applicable

10. Uncertainty of Measurement

10.1. When quantitative results are obtained, and the significance of the value may impact the report, the uncertainty of measurement must be determined. The method used to determine the estimation of uncertainty can be found in the *FSL Quality Assurance Manual – Estimation of Uncertainty of Measurement (Section 5.4.6)*.

11. Limitations

11.1. Text

12. Documentation

- 12.1. 1st Reader Worksheet
- 12.2. 2nd Reader Worksheet
- 12.3. Batch File Review Checklist
- 12.4. FBU Report of Results

13. References

- 13.1. Applied Biosystems GeneMapper *ID-X*[®] software. Applied Biosystems.
- 13.2. Applied Biosystems. AmpFISTR[®] Identifiler[®] Plus PCR Amplification Kit User's Manual.
- 13.3. DNA Advisory Board. Quality Assurance Standards for Forensic DNA Testing Laboratories. Forensic Science Communications (2000) July 2(3).
- 13.4. National Research Council. The Evaluation of Forensic DNA Evidence, Washington, DC: Academy Press, 1996.

- 13.5. Technical Working Group on DNA Analysis Methods. Guidelines for a quality assurance program for DNA analysis. *Crime Laboratory Digest* (1995) 22(2):21-43.
- 13.6. *Forensic Science Laboratory Quality Assurance Manual* (Current Version)
- 13.7. *FSL Departmental Operations Manuals* (Current Versions)
- 13.8. *FSL Laboratory Operations Manuals* (Current Versions)