

## FBS21 – 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 (GMID-X) analysis software when samples are amplified with Identifiler® Plus.

### 2. Background

- 2.1. These practices set forth the FSL FBU's approach for conducting and documenting the interpretation of DNA profiles generated from evidence in conformance with 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 the supplemental standards set by the FSL's accrediting body, as well as the guidance provided in the *Scientific Working Group on DNA Analysis Methods (SWGDM) Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Laboratories* and the Federal Bureau of Investigation *Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories*.
- 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 all instances. However, in an effort to achieve uniformity in approach, the Laboratory has developed and adheres to these basic guidelines for the interpretation of analytical results. The principles are based on the manufacturer's user guides and manuals, internal validation studies, scientific literature references, casework experiences, SWGDAM documents, the FBI's

- QAS, guidance from the DFS Science Advisory Board, and input from the forensic community at large.
- 2.3. Once the testing controls have been evaluated and confirmed, the results of the individual samples can be interpreted and subsequently compared using these established guidelines. Utilizing the data obtained from the GeneMapper® ID-X analysis, each sample's results are interpreted through a four-step process. For evidence profiles, the first three interpretation steps are carried out without knowledge of the known/reference profile(s).
- 2.3.1. First, the overall profile is evaluated to assess its quality and the potential number of contributors associated with the sample. Due to their reciprocal nature, this process occurs in conjunction with the peak evaluation.
- 2.3.2. Second, artifacts inherent to the PCR process as well as those generated by the detection instrumentation are isolated and eliminated, as appropriate, during the analysis and interpretation of individual peaks, validating the authenticity of genuine alleles.
- 2.3.3. Third, the analyst applies information regarding sample quality and the potential number of contributors garnered during the first phase of the interpretation process to make a final determination regarding which alleles are likely to be associated, particularly with respect to mixture profiles, when applicable. When appropriate, the analyst will make a determination regarding which loci meet the criteria for use in comparisons and/or statistical analysis.
- 2.3.4. Fourth, all interpreted profile alleles are compared to the known profiles submitted in connection with the case to make a forensic conclusion regarding the potential for exclusion or inclusion of each individual as a contributor(s) of/to each sample. In the case of intimate samples with multiple contributors, deducing guidelines may also be applied to assist in distinguishing between foreign alleles and those contributed by an individual(s) associated with the profile.
- 2.3.4.1. At the FSL FBU, intimate samples are defined as biological evidence samples taken directly from an individual's body. It is forensically valid to assume that the individual's genotype (either in full or in part) will be present in the DNA typing results. Examples of intimate samples include vaginal swabs, swabs from a bite mark on skin, fingernail scrapings, and hand swabs collected in connection with a sexual assault physical evidence recovery kit.
- 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 objectively, are consistent from analyst to analyst, and are within previously agreed limits.

### 3. Safety

3.1. Not applicable

### 4. Materials Required

4.1. GeneMapper® ID-X (GMID-X) analysis software

### 5. Standards and Controls

5.1. AmpF/STR Identifiler® Plus Loci

Locus designation	Chromosome location	Alleles included in Identifiler® Plus Allelic Ladder	Dye label	Control DNA 9947A
D8S1179	8	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	6-FAM™	13‡
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§
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#
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
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™
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##	
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§§
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

‡ For CODIS purposes, profile reported as 13, 13.

§ For CODIS purposes, profile reported as 30, 30.

# For CODIS purposes, profile reported as 11, 11.

## For CODIS purposes, profile reported as 8, 8.

§§ For CODIS purposes, profile reported as 11, 11.

- 5.2. GeneScan™-500 LIZ® Size Standard – contains 16 single-stranded dye-labeled nucleotide fragments of the following lengths (in bases): 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490, and 500.
- 5.2.1. The 250bp peak is sensitive to small temperature variations during electrophoresis and migrates at approximately 246bp. Accordingly, the 250 base fragment is used only as an indicator of precision within an injection and a run.

## 6. Calibration

- 6.1. Not applicable

## 7. Procedures

- 7.1. **Analytical Controls** – 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 will be evaluated and included in the case file to document that the required/expected results were obtained:
- 7.1.1. **Internal Lane Size Standard (ILS)** – this standard contains DNA fragments (LIZ) of known sizes that are used in-lane to estimate sizes (bp) of STR products, as noted in Section 5. (above).
- 7.1.1.1. Each sample tested must have the correct peak sizes assigned for its in-lane size standard before proceeding further with interpretation. Results of this evaluation will be recorded on the appropriate worksheets. Refer to *FBS14 – Data Analysis Using GeneMapper® ID-X* for more details on evaluating the size standard and its 250bp peak.
- 7.1.1.2. Samples requiring interpretation with failed ILS/insufficient ILS peak heights must be re-injected or re-run to obtain a suitable result. Documentation of re-injection or re-run will be made on the appropriate pages in the case file.
- 7.1.1.2.1. When the ILS in a corresponding reagent blank or negative control meets requirements, the reagent blank does not have to be re-injected or re-run.
- 7.1.2. **Allelic Ladder(s)** – the allelic ladder provided in the kit is used to ascertain the genotypes of the samples. While the ladder consists of the common alleles in the population for each locus, additional atypical alleles

may also be included. See Section 5. (above) for allelic ladder characterization. Results of the allelic ladder evaluation will be recorded on the appropriate worksheets.

- 7.1.2.1. Sample profiles require at least one valid/passing allelic ladder in a run to be considered for interpretation. All passing ladders used in a run that pertain to a case (i.e., are in injections with case samples and/or controls) should be included in the case file.
- 7.1.2.2. Allelic ladders missing any alleles, having off-ladder alleles, insufficient peak heights or are consistent with a poor injection will be marked as failed/invalid in the case file.
- 7.1.2.3. In the event an allelic ladder is determined to have failed, the injection may be reanalyzed using another/other ladder(s) within the injection.
- 7.1.2.4. In the event another ladder is not available for use within the injection, a ladder from another injection in the same run may be used for reanalysis **IF** the ILS 250bp value for the ladder selected for reanalysis is within +/- 0.5bp of the estimated average ILS 250bp value for the remainder of the samples in the injection with the failed ladder.
- 7.1.2.5. In the event all ladders in the run fail or no ladder in the run meets the migration criteria, all samples must be re-injected or re-run. Documentation of re-injection or re-run will be made on the appropriate page(s) in the case file.

### 7.1.3. **Negative Controls:**

- 7.1.3.1. Reagent Blank (extraction control): 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 processed in the same manner as the corresponding sample(s) through PCR amplification and run on along with the test samples.
  - 7.1.3.1.1. The appearance of typeable test results in the reagent blank may indicate that a sample preparation reagent(s) has(have) been contaminated, cross-contamination between samples occurred during testing, or human DNA or STR product got into a sample(s) from some other source.
  - 7.1.3.1.2. When a reagent blank performs appropriately and passes analysis but a corresponding sample(s) needs to be re-injected or re-run, the reagent blank does not have to be re-injected or re-run.

- 7.1.3.2. Negative Amplification Control: 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.
- 7.1.3.2.1. 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.
- 7.1.3.2.2. When a negative amplification control performs appropriately and passes analysis but a corresponding sample(s) needs to be re-injected or re-run, the negative amplification control does not have to be re-injected or re-run.
- 7.1.3.3. If any typeable peak (a reproducible peak above the analytical threshold) is present in the reagent blank or negative amplification control in the defined analysis range of 75 – 450bp that is not attributable to an artifact, then follow the steps listed below. The ultimate determination regarding repeated testing or use of the control/affected sample results will be clearly noted on the corresponding electropherograms and on the appropriate worksheets, when applicable.
- 7.1.3.4. Typeable peaks occurring in a negative control may indicate a critical contamination problem. When possible, steps must be taken to attempt to determine the source of contamination before further testing is conducted. When necessary, at the direction of the Technical Leader (or designee), all testing in the Unit will cease and steps will be taken to ensure the contamination event has been contained/resolved.
- 7.1.3.5. The appearance of a peak(s) in a negative control 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. It is noted, however, that further testing may be necessary to support these hypotheses.
- 7.1.3.6. The analyst will ensure the Technical Leader (or designee) has been apprised of the contamination event as soon as practicable and initiate an evaluation to determine the source of the contamination. The analyst will ensure the Technical Leader/designee remains apprised of the status of the contamination event evaluation until such a time as the matter

has been resolved. The initial evaluative step should include a determination regarding whether a sample/tube switch may have occurred or whether DNA from a sample in the run may have inadvertently gotten into the negative control resulting in the contamination event. This assessment will help to inform whether cessation of testing in the Unit needs to be considered.

- 7.1.3.7. If possible, the samples associated with the contaminated reagent blank or negative amplification control should be reanalyzed. Reanalysis should occur unless there are consumption issues. This reanalysis may initially consist of re-injecting or re-running all associated samples, followed by re-amplifying and/or re-extracting all associated samples, as needed and when possible. Results of the reanalysis will be used to determine if the contamination was most likely isolated or may have been due to a contaminated reagent, consumable, work surface, or piece of equipment.
- 7.1.3.8. When re-injection, re-running, and/or re-amplification have not resolved the situation, and it is not possible to re-extract a sample associated with a contaminated reagent blank or negative amplification control, the original typing results may be used for exclusionary purposes if the results differ from the DNA profile (partial or otherwise) observed in the control sample. The results may also be used for inclusionary purposes when all of the following requirements are met:
  - 7.1.3.8.1. Extraction or amplification cannot be repeated due to consumption issues;
  - 7.1.3.8.2. The contamination results are explained/their source has been determined, no FBU employee is the source of the contamination, the contamination results are accounted for in the case file and FBU Environment Monitoring Log (EML), and it is clear the sample results are not compromised by the contamination event; and
  - 7.1.3.8.3. Approval has been received from the Technical Leader and documented in the case file and notification has been provided to the FSL Quality Specialist for inclusion in the EML.
- 7.1.3.9. If potential peaks below the analytical threshold are noted in a negative control, the corresponding sample results may be reported. The electropherogram for the negative control will be clearly marked to document that the potential peaks were noted. In addition, the analyst will notify the Technical Leader and the

FSL Quality Specialist to ensure the necessary information and documentation is entered into the EML.

7.1.3.9.1. For the copy of the electropherogram prepared for the EML, the analyst may be asked to analyze the negative control at a reduced threshold(s) solely for the purpose of assessing the potential source of the contamination.

7.1.3.10. The raw data for all negative controls, including those with no signs of typeable peaks, must be examined for the presence of primer peaks to ensure the samples were properly amplified. The results of this examination will be recorded on the appropriate worksheets.

7.1.3.11. In the event a reagent blank associated with a set of samples to be re-amplified cannot be re-amplified due to limited volume, if it was determined that the reagent blank was free from contamination from a previous analysis, the samples associated with the reagent blank may be analyzed.

7.1.4. **Positive Amplification Control (AmpF/STR<sup>®</sup> Control DNA 9947A)** – the positive amplification control is a human female DNA sample with known autosomal STR genotypes (see Section 5. above) included in the Identifiler<sup>®</sup> Plus amplification/typing kit. The positive amplification control ensures that the amplification and typing processes are working properly. Each positive control will be assessed to ensure it contains the appropriate allele calls. The results of this evaluation will be recorded on the appropriate worksheets.

7.1.4.1. Each injection in a run will include a positive control.

7.1.4.2. Sample profiles require at least one valid/passing positive control in a run to be considered for interpretation. All positive controls in a run that pertain to a case (i.e., are in injections with case samples and/or controls) should be included in the case file. If no injection in a run yields a passing positive amplification control, the results for all of the corresponding samples will be considered inconclusive.

7.1.4.3. In the event a positive control in an injection is determined to have failed, no action is needed if another positive control within the injection was deemed valid/passing (with the exception of troubleshooting the reason for the failed injection).

7.1.4.4. In the event another passing positive control is not available within the injection, a positive control from another injection in the same run may be used **IF** the ILS 250bp value for the positive control selected is within +/- 0.5bp of the estimated average ILS 250bp value for the remainder of the samples in the injection.

- 7.1.4.5. In the event all positive controls in the run fail or no positive control in the run meets the migration criteria, all samples may be re-injected or re-run when the analyst determines that doing so will be beneficial. Documentation of re-injection or re-run will be made on the appropriate page(s) in the case file.
  - 7.1.4.5.1. For re-injections in general and re-runs in particular, a positive amplification control must be included to demonstrate that the typing procedure is working properly.
- 7.1.4.6. If the positive control still fails, the entire batch of samples must be re-amplified. When it is not possible to re-amplify a corresponding sample(s), the sample(s) should be re-extracted whenever possible.
- 7.1.4.7. If it is not possible to re-amplify or re-extract a sample associated with the positive control, and all indications are that the amplification process was successful for the associated samples, the original typing results will still be considered inconclusive but may **only** be used for exclusionary purposes.

7.2. **Peak Evaluation** – as a profile is evaluated, each peak identified by the GMID-X 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.

7.2.1. Alleles – a reportable, true allele peak is defined as a distinct, triangular section of an electropherogram that is equal to or higher than the analytical threshold (AT). The analytical threshold is the minimum signal at which a peak can reliably be distinguished from noise.

7.2.1.1. Based on the laboratory's internal validation studies, the following dye-specific ATs have been established for samples amplified with Identifier<sup>®</sup> Plus, injected for 10 seconds at 3kV on a 3130x/ instrument, and analyzed by GMID-X:

7.2.1.1.1. For profiles containing at least one allele at or above 1000 rfus\*:

Dye Color	AT
Blue	60
Green	50
Yellow	70
Red	60
Orange	70

*\*At the discretion of the analyst and with documented approval of the Technical Leader, a*

*sample profile may be reanalyzed using the analysis parameters set forth in 7.2.1.1.2.*

7.2.1.1.2. For profiles in which all alleles are below 1000 rfus:

Dye Color	AT
Blue	20
Green	50
Yellow	40
Red	40
Orange	70

7.2.1.2. When both the <1000 rfus and >1000 rfus analysis methods are used for samples in a case, the negative control and affected sample associated reagent blank(s) must be included in the <1000 rfu analysis project; the control electropherograms generated using the <1000 rfus analysis method will be retained in the case file.

7.2.1.2.1. The X-axis on all electropherogram printouts depicting a full profile will be from 70 to 460bp.

7.2.1.2.2. The Y-axis on negative control and reagent blank electropherogram printouts will be equivalent to the analytical threshold in each dye color.

7.2.1.2.3. The Y-axis on sample, ladder and positive control electropherograms will be slightly above the highest peak in each dye color.

7.2.1.2.4. As needed, a portion(s) of an electropherogram with a 'zoomed in' view of an area(s) will be included in the case file.

7.2.1.3. 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.

7.2.1.4. Based on an initial review of results, when appropriate and quantity of sample allows, the analyst may opt to concentrate the extract and re-amplify, re-amplify with more and/or less template DNA, and/or re-extract. When a sample is analyzed more than one time, the first page of the electropherogram used for reporting purposes will be clearly marked.

7.2.1.4.1. When appropriate, a composite profile for a sample may be generated. When a composite profile is

generated it will clearly marked as such in the case file, clearly reported as such, and will be the profile used for comparisons.

7.2.1.4.1.1. A composite profile is a DNA profile generated by combining typing results from different loci obtained from multiple injections of the same amplified sample and/or multiple amplifications of the same DNA extract.

7.2.2. Off Ladder Alleles (OL): An off ladder allele occurs when an allele, in relationship to the allelic ladder, is between two allelic ladder peaks or before or after the peaks in the allelic ladder and not thought to be due to migration effects. In this instance, the peak is designated as “OL” by the GMID-X software.

7.2.2.1. When an OL allele occurs between two allelic ladder peaks, this allele will be considered to be a microvariant. The analyst will assign an allele designation based on the base pair value of the allele. The allele will be assigned a designation of the lower complete repeat value followed by the number of bases in the incomplete repeat (e.g., an allele which migrates two bases above the D7S820 11 allele will be designated as D7S820 11.2).

7.2.2.2. When an allele is seen between two loci and either the locus to the left or the right contains two peaks, the allele will be considered to belong to the locus containing the single peak. Based on the base pair value of the allele, when possible, an allele designation will be assigned (e.g., an allele is noted between D8S1179 and D21S11 and a two allele pattern is noted only at the D21S11 locus, the allele is assigned to the D8S1179 locus. Further, given that the base pair value for the allele in this example is approximately 4 base pairs longer than the longest allele in the D8S1179 ladder (allele 19), the allele in question may be designated as 20). The allele may also be designated as > or < the closest allele in the allelic ladder.

7.2.2.2.1. If an allele is detected between FGA 33.2 and 42.2, the allele will be designated as >33.2.

7.2.2.3. When an allele is seen above the largest or below the smallest allele of an allelic ladder, the allele will be designated as either > or < the respective ladder allele (e.g., an allele which migrates below the D3S1358 12 allele will be designated as D3S1358 <12).

7.2.2.4. A portion of the electropherogram with a ‘zoomed in’ view of the locus with the off ladder allele and the corresponding locus in

the ladder (i.e., ‘blow up with ladder’) will be printed showing the microvariant designation.

- 7.2.2.5. The sample will be re-injected or re-run to confirm the allele designation of the microvariant. The additional view of the re-injected/re-run sample electropherogram will be printed as stated in 7.2.2.4.
- 7.2.2.6. If an allele is seen between two loci and the surrounding loci each contain only a single peak (or two peaks), both loci will be deemed inconclusive.

### 7.2.3. Artifacts/Non-Allelic Peaks

7.2.3.1. *Stutter Products* – 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 when present, the reverse stutter peak will be four bases shorter (or N-4), while the forward stutter peak will be four bases longer (or N+4). In general for each locus, the percent reverse stutter increases with allele length and each allele within a locus displays a reverse stutter percent that is consistent.

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

7.2.3.1.2. The following table lists the reverse stutter percentages expected for each locus as determined by the internal validation studies for the Identifiler® Plus kit:

Locus	N-4 Stutter %	Locus	N-4 Stutter %	Locus	N-4 Stutter %	Locus	N-4 Stutter %
D8S1179	10.48	D3S1358	11.22	D19S433	11.47	Amelogenin	—
D21S11	9.87	TH01	4.04	vWA	10.63	D5S818	8.46
D7S820	7.93	D13S317	9.07	TPOX	6.92	FGA	11.86
CSF1PO	8.50	D16S539	9.76	D18S51	13.60		
		D2S1338	11.90				

7.2.3.1.3. The following table lists the forward stutter percentages expected for each locus as determined by the internal validation studies for the Identifiler® Plus kit:

Locus	N+4 Stutter %	Locus	N+4 Stutter %	Locus	N+4 Stutter %	Locus	N+4 Stutter %
D8S1179	1.81	D3S1358	2.13	D19S433	2.90	Amelogenin	—
D21S11	2.84	TH01	2.17	vWA	2.85	D5S818	2.66
D7S820	2.52	D13S317	3.01	TPOX	2.27	FGA	4.54
CSF1PO	2.67	D16S539	1.82	D18S51	4.64		
		D2S1338	4.74				

7.2.3.1.4. The stutter cut-off values for each locus determined during the internal validation studies are included in the filtering step in GMID-X. A peak in a stutter position that is above the stutter cut-off value will not be filtered. Minor peaks in a stutter position that have not been filtered will remain labeled and should be further evaluated. These peaks may be called elevated stutter at the analyst's discretion based on the specific circumstances.

7.2.3.1.4.1. Stutter peaks are often elevated in profiles with higher rfu values. In particular, the percent stutter for peaks that are off-scale may be unusually high.

7.2.3.1.4.2. Likewise, samples with stochastic effects due to very low input DNA may also have stutter peaks that are elevated.

7.2.3.1.5. If there is no indication of a mixture in a profile, the labeled minor peaks in N-4 and N+4 stutter positions can most often be attributed to elevated stutter. If a mixture is observed, labeled minor peaks in N-4 and N+4 stutter positions must be carefully evaluated. When they cannot be attributed to elevated stutter, they will be taken into consideration during the mixture interpretation process.

7.2.3.1.6. When a peak is present between a parent peak and its possible stutter peak, the stutter filter will not be applied by the software. Accordingly, any such peaks in stutter position must be assessed to determine if they may be unfiltered stutter (e.g., when the result at D21S11 is 33.2, 34, 34.2, the 33.2 peak must be assessed to see if it meets the criteria for stutter corresponding to the 34.2 peak, or when minus A is present a peak in the stutter position must be assessed).

- 7.2.3.2. *Spikes* – a spike is a random non-reproducible artifact usually observed in one or more dye colors at the same base pair location, generally seen as a tall thin peak that does not display proper peak morphology. Peak height (rfu) usually varies between dye colors. Samples and positive controls containing spikes within the defined analysis range (75 – 450bp) do not need to be re-injected or re-run if the spike does not interfere with the sizing of the sample. When spikes occur in analytical regions or negative controls, the sample will typically be re-injected and/or re-run.
- 7.2.3.3. *Dye Blobs* – dye blobs generally occur between 60 and 90 base pairs, but can occasionally occur outside this range. Dye blobs usually appear as a broad peak in a single color. When dye blobs occur in analytical regions or negative controls, the sample should be re-injected or re-run. If the dye blob is still present after re-injection or being re-run, the sample may need to be re-amplified using an Identifiler® Plus kit with a new lot number. When a dye blob is seen and documented during QC of the kit and/or known to occur and/or observed over many samples, re-injection or re-running may not be necessary.
- 7.2.3.4. *Pull-up (Bleed-through)* – pull-up peaks are caused by one peak “bleeding” into another color. 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 (typically +/- 0.25bp) the same data point or evaluating the sample’s raw data. Samples and controls exhibiting pull-up peaks do not need to be re-injected or re-run if it does not interfere with the sizing of the samples. When an ongoing matrix problem exists, a new spectral must be run, applied and the samples re-injected/re-run.
- 7.2.3.5. *Split Peaks/Minus A/Non-template Nucleotide Addition* – split or minus A peaks may be seen in a sample if amplification did not go to completion. 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). Split or minus A peaks 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. Samples with

apparent excess PCR product may be re-amplified using less input DNA.

- 7.2.3.6. *Shoulders* – shoulders peaks are approximately 1 – 4bp smaller, but occasionally larger than the main allele, caused by a flat decline of the fluorescence of the main peak. Shoulders are easily recognized because they do not have proper peak morphology. Samples and controls exhibiting shoulders do not need to be re-injected or re-run if it does not interfere with the sizing of the samples.
- 7.2.3.7. *Elevated Baseline* – raised baseline will appear as non-specific elevation of the baseline causing signal(s) to be labeled. These labeled signals do not typically display proper peak morphology. Samples and controls exhibiting elevated baseline do not need to be re-injected or re-run if it does not interfere with the sizing of the samples.
- 7.2.3.8. *Identifiler<sup>®</sup> Plus Kit Artifacts* – artifacts and anomalies have been seen in data produced using the Identifiler<sup>®</sup> Plus Kits, in general, and during QC of specific kit lot numbers. The shape of these artifacts and anomalies is not consistent with the shape of labeled DNA fragments as seen on the 3130x/ Instrument. Artifacts are typically reproducible while anomalies (such as spikes or baseline noise) are not observed consistently and are typically not reproducible. Analysts should consider these artifacts and anomalies when interpreting data. In general, samples and controls exhibiting these types of non-specific artifacts and/or anomalies do not need to be re-injected or re-run.
- 7.2.3.9. *Off-scale Peaks/Data* – an off-scale peak occurs when the fluorescent signal exceeds the dynamic detection range of the CCD camera in the 3130x/ Genetic Analyzer. Capillary electrophoresis instruments are limited in the amount of fluorescence they can detect. Signal saturation will be indicated by the presence of a pink line through the allele(s) and the corresponding GMID-X quality flag. The GMID-X software is unable to determine the true peak heights for these alleles. Their values should not be used for quantitative evaluation (e.g., peak height ratio calculations or stutter calculations). In addition, off-scale data may cause many of the above listed artifacts. As needed, samples containing off-scale data should be re-amplified at a lower target concentration.
- 7.2.3.9.1. Some samples may appear to be overloaded without displaying a GMID-X quality flag. At the discretion of the analyst, these may be either edited or the sample re-amplified at a lower concentration.

#### 7.2.4. Peak Height Ratio (PHR)/Minimum Expected Peak Height Balance

- 7.2.4.1. Use of peak height ratio is a method of quantifying the degree of balance or potential association between possible sister alleles in order to determine if alleles originated from a single source. With Identifiler<sup>®</sup> Plus, the expected peak height ratio for autosomal heterozygous alleles from samples amplified for 28 cycles is between 55-100%. Occasionally, the PHR for sister alleles in a non-mixed sample will be lower than 55%, especially if alleles are below the stochastic threshold.
- 7.2.4.2. PHR is calculated by dividing the rfu value of the lower allele of the heterozygote pair by the rfu value of the higher allele and multiplying by 100. (i.e., [(lower peak height)/(higher peak height)]\*100).
- 7.2.4.3. In the target range of approximately 0.5 ng to 1.0 ng of input DNA, the majority of the time the PHR of sister alleles across loci with the Identifiler<sup>®</sup> Plus Kit is 70% or greater as determined by the internal validation studies. Given the lower bound observation during the validation studies of a 55% PHR, the laboratory uses a 55% PHR to assess the potential for sister alleles originating from the same source.
- 7.2.4.4. A sequence mutation in the primer region of template DNA may cause the allele to not be amplified (i.e., resulting in a null allele), be significantly lower than the expected PHR for the allele pair, or have no discernable effect.
- 7.2.4.5. In a single source sample, if the PHR is significantly outside the minimum expected range at a single locus when the concentration of template DNA is within or above the optimal target range, at the analyst's discretion, the sample may be re-amplified to assess whether or not the imbalance is reproducible. Refer to the guidelines below for information regarding how loci with alleles that fall below the minimum 55% PHR requirement are denoted.
- 7.2.4.6. In a single source sample with an input concentration below the optimal target range, it is not uncommon to encounter sister alleles with a PHR below 55%, particularly when the rfu values are below 1000.
- 7.2.4.7. The 55% PHR cut-off value is only applicable to allelic peaks that meet or exceed the stochastic threshold.

7.3. **Overall Profile Evaluation** – the overall condition of each profile, as well as the potential number of contributors and any relevant source information, will be assessed prior to any in-depth determinations about the data observed. The presence of apparent degradation, inhibition, or significant stochastic effects in a profile will be considered during the interpretation process and may influence the assessments made at each locus. In addition, the number of potential contributors to a sample will affect how the sample is interpreted. It is also possible that knowledge or perceived knowledge regarding a potential contributor(s) to a mixture sample may affect sample interpretation (e.g., an intimate swab with semen positive results). These observations will be noted on the appropriate worksheet.

7.3.1. Degradation: DNA degradation is a process in which DNA molecules randomly break down 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, sister peak height imbalance, abnormally high stutter product(s), allele dropout, and/or locus dropout may be observed. Typically, degraded profiles 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. It is important to note that it is possible for the profile from one or more donors in a multiple contributor profile to exhibit degradation in the absence of observed profile degradation of the other donor(s).

7.3.2. Inhibition: DNA samples may contain one or more of the PCR inhibitors commonly encountered in forensic casework. The presence of inhibitors may manifest itself by the failure to produce results at some or many loci and may mimic results seen when degradation is present. Samples containing inhibitors often produce partial profile results in which the smaller loci drop out before the larger loci. Since real-time quantitative PCR can be used to assess the presence of inhibitors, the presence of a partial DNA profile from a sample with input DNA of greater than 0.5 ng and/or qPCR results indicative of inhibition will be noted as potentially inhibited.

7.3.3. Stochastic Effects: Reduced amounts of input DNA may result in observable stochastic effects at multiple loci. Stochastic effects consist of the observation of intra-locus peak imbalance and/or allele dropout resulting from random, disproportionate amplification of alleles in low-quantity template samples and/or abnormally high stutter products. Observation of intra-locus peak imbalance and/or allele dropout and/or locus dropout and/or elevated stutter at multiple loci will be considered to be indicative of stochastic effects and will be noted as such. At a minimum potential stochastic effects will be taken into account during interpretation by use of the stochastic threshold.

7.3.3.1. When interpreting low-intensity autosomal STR genotypes, care must be taken to accurately distinguish a homozygote peak from a heterozygote peak whose sister allele has dropped out. The stochastic threshold defines the peak height below which a second allele of a heterozygous pair may not be detected above the analytical threshold. Based on internal validation studies, for a 10 second 3kV injection from a sample amplified for 28 cycles, the stochastic threshold is set at 200 rfus for 3130x/-A and 3130x/-B. As a general guideline, in a single source sample, a single peak at a locus should not be considered a homozygous allele unless the peak is  $\geq 200$  rfus. Details regarding the use of peaks below the stochastic threshold in interpretations, comparisons and statistical calculations are addressed in detail below.

7.3.4. Potential Number of Contributors Determination: Each profile will be assessed as to the potential number of contributors. The entire profile must be taken into account when determining if a profile is from a single contributor or contains a mixture of DNA from two or more contributors. In assessing the number of contributors to a profile, all data at and above the analytical threshold (AT) will be taken into account.

7.3.4.1. For data analyzed using the >1000 rfus analysis method, peaks below the >1000 rfus AT parameters that are  $\geq$  to the <1000 rfus AT parameters should be considered in the determination of whether a sample is most likely single source or a mixture (i.e., they will be designated as 'other alleles to consider'). Given that these peaks fall below the analysis method AT parameters they do not meet the criteria for reportable/true alleles since they cannot definitively be distinguished from noise. Inasmuch, peaks at or above the <1000 rfus AT parameters that are less than the >1000 rfus analysis method AT parameters may only be used to assess the number of contributors to a profile and they will not be reported or treated as 'true alleles'. These peaks between the <1000 rfus AT parameters and the >1000 rfus analysis method AT parameters may also be taken into consideration when assessing suitability of a locus for inclusion in a statistical calculation.

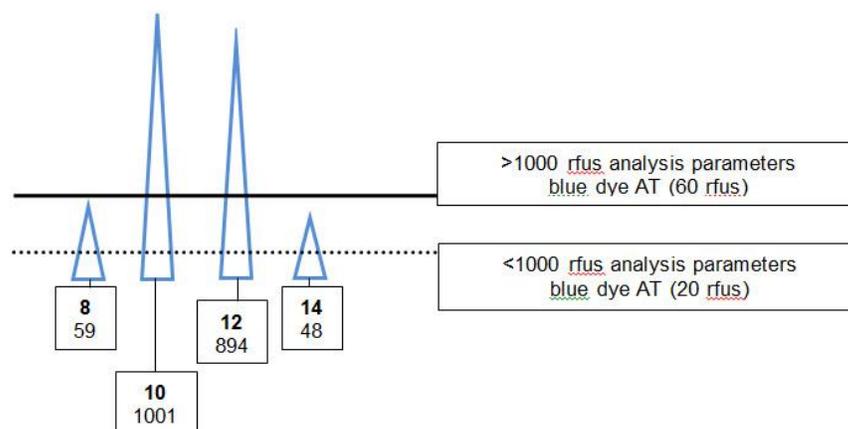


Figure 1 – depiction of gap between the >1000 rfus analysis parameters and the <1000 rfus analysis parameters (at D7S820 in the 5-FAM™ (blue) dye) where peaks below the analysis parameter AT are considered for assessment of the number of contributors for samples analyzed using the >1000 rfus analysis parameters.

- 7.3.4.2. **General Single Source Profile Attributes:** A single source profile will typically have no more than two relatively well-balanced alleles at all loci. In rare instances, an individual may exhibit a tri-allelic pattern at a locus. When a tri-allelic pattern is noted and not confirmed by multiple observations in a case, the sample will typically be re-amplified to confirm its presence. Refer to Single Source Profile Interpretation Guidelines below.
- 7.3.4.3. **General Mixture Profile Attributes:** A mixture profile typically has more than two alleles at one or more loci (with a tri-allelic pattern as the exception) and/or exhibits significant imbalance between peak heights at one or more loci. The presence of more than two peaks at one locus or imbalanced peak heights does not, in and of itself, necessitate a profile being designated as a mixture. While it may be possible to infer a sample is a mixture based on > 2 alleles/peaks and/or significant peak height imbalance at a single locus, it is preferable to observe characteristics consistent with a mixture at no fewer than two loci. Refer to the Minimum Number of Contributors Assessment section immediately below, as well as the Two Person Mixture Interpretation Guidelines and the Three Person or More Mixture Interpretation Guidelines below.
- 7.3.4.4. **Minimum Number of Contributors Assessment:** The minimum number of contributors to a mixture will generally be determined by examining the locus demonstrating the largest number of peaks  $\geq$  the analysis method AT parameters. The peaks are counted at that locus and then divided by two. The resulting quotient is rounded up to the nearest integer for the minimum number of contributors.

7.3.4.4.1. In general, a profile containing a mixture of DNA from two people will have no more than four alleles at all loci; a profile containing a mixture of DNA from three people will have no more than six alleles at all loci; and so on (i.e., there will be no more than  $2 \times$  [the number of contributors] alleles at all loci).

7.3.4.4.2. Ultimately, however, the number of alleles and their ratios will be used to more accurately assess the number of potential contributors to a mixed profile.

7.3.4.4.2.1. Low-level profiles (especially at loci with alleles below the stochastic threshold and with evidence of stochastic effects) may not exhibit more than two alleles at any locus but information gleaned from the profile as a whole may allow for the profile to be characterized as a mixed sample.

7.3.4.4.2.2. Conversely, significant peak height imbalance at multiple loci in samples amplified with the target amount of input DNA may provide information regarding the potential for an additional contributor(s) to a mixture profile (e.g., as in mixtures containing DNA from two or more biologically related individuals).

7.3.4.4.2.3. It is noted that it may not be possible to accurately assess the potential number of contributors to mixture profiles exhibiting characteristics consistent with having at least 3 or more contributors.

### 7.3.5. Perceived Probative Value/Forensic Intelligence

7.3.5.1. Decisions regarding use of a result in comparisons and/or in a statistical calculation may be influenced by the quality of the profile as a whole coupled with the perceived probative value of the result and/or information regarding a probable contributor (e.g., as with an intimate sample). As noted above, a forensically valid assumption for intimate samples presumes a person's DNA may be present in a mixture; this assumption can be used to support subtracting that individual's genotype from a mixture profile in the appropriate ratio/proportion in an effort to deduce the foreign contributor component. Additional specific case circumstances may be considered for use of a forensically valid assumption to utilize the known sample of an individual for

deduction purposes with documented approval of the Technical Leader.

7.3.5.1.1. With respect to intimate samples, failure to detect the full genotype of the assumed contributor at all loci or at a locus will not be automatic cause for exclusion. Indeed, for intimate samples potential assumed contributor genotype information/results will be critically reviewed to ensure interpretations 'make sense'. In other words, more leeway will be allowed with respect to not excluding an individual from their intimate sample profile/locus result when only a portion of their genotype is detected. In general, detection of an individual's genotype on a sample obtained from their body is considered a non-probative result. Inasmuch, a statistic will typically not be calculated.

#### **7.4. Single Source Profile Interpretation Guidelines**

7.4.1. Following determination that a profile is single source, all of the profile data, as well as the data at each locus, is assessed to determine how the allele calls will be denoted, whether the result(s) may be used for comparisons, and how the results will be addressed with respect to a random match probability (RMP) statistical calculation based on PHR and ST information.

7.4.1.1. The closer the alleles are/allele is to the AT may affect determinations regarding suitability for comparisons and/or inclusion in the statistical calculation. A notation(s) of 'other peaks to consider' (denoted by the double dagger symbol (‡) when necessary) may also have an effect.

7.4.2. When two alleles are detected at a locus, the peak height ratio is above 55%, and the rfu values are  $\geq 200$  for both alleles, the results are denoted with no notations (e.g., 8,9). The result is suitable for comparisons and inclusion in the statistical calculation.

7.4.3. When two alleles are detected at a locus, the peak height ratio is above 55%, and the rfu values of one or both alleles are  $< 200$  rfus, the peak(s) below the ST are indicated by an asterisk (e.g., 8\*,9 when the rfu value for the 8 allele is below 200 rfus or 8\*,9\* when the rfu values for both alleles are below 200 rfus). The result is suitable for comparisons and is typically suitable for inclusion in the statistical calculation.

7.4.4. When two alleles are detected at a locus, when the peak height ratio is below 55% and both alleles are  $\geq 200$  rfu, the results at that locus are denoted in curly brackets (e.g., {8,9}). The result is suitable for comparisons and may still be used for statistical purposes.

- 7.4.5. When two alleles are detected at a locus, when the peak height ratio is below 55% and only one allele height is  $\geq 200$  rfu, the results at that locus are denoted in curly brackets, with the allele falling below 200 rfu indicated by an asterisk (e.g., {8\*,9}). The result is suitable for comparisons and may still be used for statistical purposes.
- 7.4.6. When two alleles are detected at a locus, the peak height ratio is below 55%, and both allele heights are  $< 200$  rfu, the results are denoted in curly brackets with the rfu values for both alleles indicated by asterisks (e.g., {8\*,9\*}). The result is suitable for comparisons. When the analyst and the Technical Reviewer have documented their agreement, the locus may be used for statistical purposes.
- 7.4.7. When a single allele is detected at a locus that is  $\geq 200$  rfu and there is no indication of another contributor, the results are denoted with no notations (e.g., 8,8). The result is suitable for comparisons and for inclusion in the RMP statistical calculation.
- 7.4.8. When a single allele is detected at a locus that is  $< 200$  rfu, the result will be denoted as the allele plus --- to indicate the potential for allelic dropout (e.g., 8,---). The result is suitable for comparisons. When the analyst and the Technical Reviewer have documented their agreement, the locus may be used for statistical purposes.
- 7.4.9. When a tri-allelic pattern is noted, the results will be denoted, though the locus will not be included in the statistical calculation. While statistics will not be generated for a tri-allelic locus result, the frequency of a three allele profile is very rare, thereby greatly increasing the significance of an association.

## 7.5. General Mixture Interpretation Guidelines and Statistical Implications

- 7.5.1. The number of alleles, allele peak heights, origin of samples and use of known sample profiles (when allowable and appropriate) can yield information regarding individual donors to mixture profiles.
- 7.5.2. Significant differences in peak heights can be used to assign certain alleles to certain contributors. In essence, peak heights can be used to associate sister alleles to a particular donor. As noted above, a minimum peak height ratio of approximately 55% will be used as a guideline for association of sister alleles when analyzing mixture samples.
- 7.5.3. When sister alleles can be assigned to particular donors, calculation of a mixture proportion or ratio (also called a mass ratio) is a means to compare the relative amount of DNA contributed by each donor. The mixture proportion and ratio use quantitative peak information to assess the relative proportion or ratio of the DNA contributions of multiple individuals to a mixed DNA typing result.

- 7.5.3.1. The mixture proportion is generated by dividing the allele peak heights associated with the single major contributor by all of the allele peak heights from the single major contributor and the allele peak heights associated with the minor contributor or contributors.
  - 7.5.3.2. The mixture ratio is generated by dividing the allele peak heights associated with the single major contributor by the allele peak heights associated with the minor contributor or contributors.
  - 7.5.3.3. A mixture proportion of 75% or ratio of 3:1 for a two person mixture would indicate there is 3 times more DNA present from the major contributor relative to the minor contributor. Refer to Section 9. for additional information regarding calculation of mixture proportions and ratios.
  - 7.5.3.4. When mixture proportions or ratios of various loci fall within a consistent range, confidence is increased that a deconvolution or deduction can be performed. In this instance, a lower mixture proportion or ratio (e.g., 75% or 3:1) may be informative.
  - 7.5.3.5. When there is a wide range of mixture proportions or ratios between loci, confidence is reduced in the ability to conduct a successful deconvolution or deduction. In this instance, it may be more appropriate to consider a higher mixture proportion or ratio (e.g., 83% or 5:1) as a decision point.
  - 7.5.3.6. When appropriate and of assistance in mixture deconvolution, peaks below the stutter cut-off (i.e., peaks in stutter position that have been filtered out) with rfu values close to those obtained for labeled allelic peaks may be taken into account for mixture proportion or ratio calculation purposes.
- 7.5.4. General information – for a mixture from two individuals:
- 7.5.4.1. An overall minimum mixture proportion or ratio of approximately 75% or 3:1 will act as a threshold regarding whether a distinct major contributor can be deconvolved/deduced. This determination may be made on a locus by locus basis.
  - 7.5.4.2. When the major contributor can be deconvolved, then a random match probability (RMP) calculation will be performed using the criteria set forth in Section 7.4 to describe the rarity of the major contributor's profile. Loci with a mixture proportion or ratio of approximately 66% or 2:1 may still qualify for the RMP statistic as long as the overall profile mixture proportion or ratio of 75% or 3:1 is met.
    - 7.5.4.2.1. When possible, a modified RMP calculation will be performed on the minor contributor profile.

- 7.5.4.3. If a distinct major contributor cannot be deconvolved and the profile is not from an intimate sample enabling use of a known profile to deduce the profile of the foreign contributor, the profile will be deemed indistinguishable. A combined probability of inclusion (CPI) calculation will be utilized to describe the rarity of the indistinguishable mixture profile. Only those loci with no indication of allelic dropout and with all alleles above the ST may be included in the CPI calculation.
- 7.5.4.4. When the profile is from an intimate sample and the DNA profile of the foreign contributor can be fully deduced as the major contributor as noted above, the RMP calculation will be utilized to describe the rarity of the foreign/major contributors profile using the criteria set forth in Section 7.4.
  - 7.5.4.4.1. When the foreign contributor's profile is deducible as a minor contributor, when possible, a modified RMP calculation will be performed on the minor contributor profile.
  - 7.5.4.4.2. In some instances it may be appropriate to (also) perform a CPI calculation to describe the significance of the deduced minor contributor profile.
- 7.5.4.5. For an intimate sample, when the foreign profile cannot be deduced (nor deconvolved), the profile will be deemed indistinguishable. A CPI calculation will be utilized to describe the rarity of the indistinguishable mixture profile. Only those loci with no indication of allelic dropout and with all alleles above the ST may be included in the CPI calculation.
- 7.5.5. General information – for a mixture determined to be from three or more people:
  - 7.5.5.1. The profile will be examined to determine if a distinct major(s) can be deconvolved/deduced. Caution will be used when attempting a mixture deconvolution on a mixture of three people. Due to dosing/sharing of alleles, in most instances it is not appropriate to attempt to perform a mixture deconvolution on a mixture of more than three individuals.
  - 7.5.5.2. Overall, a mixture proportion or ratio of approximately 80% or 4:1 will serve as a threshold as to whether a distinct major contributor(s) can be deconvolved/deduced from a mixture of three individuals. If a single major contributor can be deconvolved/deduced, as needed, a RMP will be calculated to describe the rarity of the major contributor's profile using the criteria set forth in Section 7.4.
    - 7.5.5.2.1. As needed and when possible, a CPI calculation will be conducted to describe the rarity of the full profile

(that includes the minor contributors). Only those loci with no indication of allelic dropout and with all alleles above the ST may be included in the CPI calculation.

7.5.5.3. When a major contributor(s) cannot be fully deconvolved/deduced from a mixture of three (or more) persons, the profile will be deemed indistinguishable or, as needed, uninterpretable. When appropriate, a CPI calculation will be used to describe the rarity of the indistinguishable mixture profile. Only those loci with no indication of allelic dropout and with all alleles above the ST may be included in the CPI calculation.

7.5.5.3.1. A restricted CPI calculation may be applied to multiple major contributors to the mixture despite the presence of minor contributor(s) alleles below the stochastic threshold when all major contributor alleles meet or exceed a mixture proportion or mass ratio of 80% or 4:1 and are above ST.

7.5.6. A RMP or modified RMP will be used whenever reasonable as it is the most informative approach.

7.5.7. When a mixture does not have at least six loci qualify for use in a statistical calculation, has not been deemed uninterpretable, and a compared individual cannot be excluded, the report will reflect that no conclusion can be made in relation to the compared individual.

7.5.8. A worksheet(s) will be prepared for profiles, as appropriate, and retained as part of the case file. At the discretion of the analyst, the worksheet will be used to document the profile alleles and peak heights, stutter position adjustments, peak height balance calculations, mixture ratios, deconvolved (or deduced, when applicable) major/minor profiles, and subsequent comparisons, when applicable. When a worksheet is not used, all relevant profile observations will be documented in another way in the case file.

## **7.6. Two Person Mixture Profile Interpretation Guidelines**

7.6.1. Following determination that a DNA profile is consistent with a mixture of at least two individuals/two individuals, all of the profile data, as well as the data at each locus, is assessed to determine:

- whether the profile can be deconvolved/deduced (i.e., the profiles of the two contributors can be separated);
- how the allele calls will be denoted;
- whether the resulting contributor profile(s), or the full profile when deconvolution/deducing is not possible, may be used for comparison purposes; and

- how the results will be addressed with respect to statistical calculations based on AT, PHR, and ST information.

NOTE: It is reiterated here that data below the AT cannot reliably be distinguished from instrument noise. Accordingly, only data at or above the AT is to be reported, considered during inclusionary comparisons, and included in statistical calculations.

7.6.1.1. An effort will be made to deconvolve intimate samples first. If deconvolution cannot result in separation of the contributor profiles, at the next phase of interpretation, prior to comparisons, an effort to deduce the foreign contributor profile will be made using the known contributor's reference sample.

7.6.2. Deconvolution Assessment: Loci peak height ratios and mixture proportions or ratios are evaluated to determine if the profiles of the contributors are distinguishable or indistinguishable. Typically loci with 4 alleles are most informative; however, 3 allele loci with two minor peaks and Amelogenin, may also be of value when making this assessment. When necessary, rfu adjustments will be made for peaks in stutter positions and, along with the potential for shared alleles (i.e., allele 'stacking'), will be taken into account when assessing mixture ratios and all potential allele combinations. As a result of the assessment, the analyst will either separate out a full or partial major contributor along with a full or partial minor contributor profile; separate out a full or partial major contributor profile and determine that no minor contributor profile can be determined based on the data; or find the mixture is indistinguishable. The profile evaluation and calculations (when applicable) will be recorded on the appropriate worksheet. Refer to Section 9. for more information regarding some deconvolution/deducing assessment calculations.

7.6.2.1. When the profiles of the contributors cannot be clearly distinguished and an intimate sample profile is being evaluated, once all other case evidence profiles have been interpreted, the DNA profile of the known/assumed contributor may be used to deduce the foreign contributor profile. In this instance, this subsequent evaluation of the profile will be recorded on the applicable worksheet.

7.6.3. For distinguishable mixtures:

7.6.3.1. With respect to the major contributor profile (full or partial), follow the single source interpretation guidelines set forth in Section 7.4 above.

7.6.3.1.1. When no portion of the major contributor genotype at a locus can be definitively deconvolved/deduced, the locus will be deemed inconclusive for comparisons/inclusion in the statistical calculation.

7.6.3.2. With respect to the minor contributor profile (full or partial), the data is assessed to determine whether or not a single minor contributor genotype can be deconvolved/deduced, how the result will be denoted, whether the result(s) may be used for comparisons, and how the results will be addressed with respect to a modified random match probability (mRMP) statistical calculation. A low mixture proportion or ratio and/or the closer the alleles are/allele is to the AT may affect determinations regarding the ability to fully deconvolve/deduce a locus result, suitability for comparisons, and/or inclusion in the statistical calculation. A notation(s) of 'other peaks to consider' may also have an effect.

7.6.3.2.1. When a single minor contributor genotype is deconvolved/deduced at a locus, each peak for a heterozygous result is denoted in parentheses (e.g., (13),(15)). The peak (adjusted value  $\geq 200$  rfus) for a homozygous result is denoted in parentheses (e.g., 13,13)). The result is suitable for comparisons and inclusion in the mRMP calculation.

7.6.3.2.2. When a single minor contributor genotype cannot be deconvolved/deduced at a locus and a minor obligate allele can be determined, all possible minor genotype combinations will be derived and recorded. The obligate minor allele will be denoted in parentheses along with --- to indicate the potential for allelic dropout (e.g., (13),(---)). The result is suitable for comparisons. When the analyst and Technical Reviewer have documented their agreement, the locus may be used for statistical purposes.

7.6.3.2.3. When a single minor contributor genotype cannot be deconvolved/deduced at a locus and no minor obligate allele can be determined, the result will be deemed inconclusive for comparisons/inclusion in the statistical calculation.

NOTE: When applicable, similar notations to those used for single source alleles/genotypes (asterisk, curly brackets, ---, and double dagger) may be used in addition to the parentheses to denote minor contributor result(s).

#### 7.6.4. For indistinguishable mixtures:

7.6.4.1. When based on the mixture proportion or ratio evaluation a profile is deemed indistinguishable, no curly brackets or parentheses will be used to denote the results. Asterisks may

still be used to denote alleles below ST, and, when applicable --- may be used to denote the potential for allelic dropout and the double dagger symbol may be used to denote 'other peaks to consider'.

7.6.4.1.1. When all alleles at a locus are  $\geq 200$  rfus (prior to adjustment), there are no indications of potential allelic dropout, or any alleles/peaks other than those that are called that need to be considered, the result is typically suitable for comparison purposes and inclusion in the CPI statistical calculation.

7.6.4.1.2. When one (or more than one) allele at a locus is below 200 rfus, there is no indication of potential allelic dropout, or any alleles/peaks other than those that are called that need to be considered, the result is typically suitable for comparison purposes; however, it cannot be included in the statistical calculation.

7.6.4.1.3. When multiple alleles at a locus are below 200 rfus and/or are close to the AT, and/or there is an indication of allelic dropout and/or there are alleles/peaks marked as needing to be considered, caution should be used when using the result for comparisons. When the analyst and Technical Reviewer have documented their agreement, the locus may be used for comparisons; however, it cannot be included in the statistical calculation.

## 7.7. Three Person or More Mixture Profile Interpretation Guidelines

7.7.1. Following determination that a DNA profile is consistent with a mixture of three individuals/at least three individuals (or more), all of the profile data, as well as the data at each locus, is assessed to determine:

- whether one or more contributor component of the profile can be deconvolved/ deduced;
- how the allele calls will be denoted;
- whether the resulting contributor profile(s), or the full profile when deconvolution/deducing is not possible, may be used for comparison purposes; and
- how the results will be addressed with respect to statistical calculations.

7.7.2. Each mixture with more than two contributors will be assessed to determine its suitability for interpretation. The guidance provided in

Sections 7.4., 7.5 and 7.6 above when interpreting these profiles will be used.

## 7.8. Comparison of Interpreted Profiles to Reference Samples

7.8.1. After the first three phases of interpretation have been completed on all case profiles, comparisons to reference samples submitted in connection with the investigation will be conducted. To ensure the integrity of the comparison, all case profile interpretations are to occur prior to comparisons. The only exception is for intimate samples where the known contributor's reference sample is accessed for deducing purposes after all other case evidence profiles have been interpreted.

7.8.2. When an individual cannot be excluded as a source of a profile or as a contributor to a mixture profile and the result is probative, a statistical calculation is conducted. Refer to *FBS22 – Population Statistics* for additional information.

## 8. Sampling

8.1. Not applicable

## 9. Calculations

9.1. Refer to *FBS22 – Population Statistics* for the equations used to calculate RMP, mRMP and CPI statistics.

9.2. Mixture ratios (or proportions) may be calculated to assist in mixture deconvolution and deduction. The two common calculation methods that follow assume a mixture of two individuals for demonstrative purposes and use a locus with four alleles. It is also possible to calculate mixture ratios/proportions using a three allele locus where a homozygote with high rfus is present with two smaller heterozygote alleles. Amelogenin may also be used. The lower case Greek letter phi ( $\phi$ ) represents peak height in the following equations.

9.2.1. Mixture Ratio ( $M_R$ )

Assuming a 4 allele pattern with major contributor alleles A and B and minor contributor alleles C and D:

$$M_R = \frac{\phi A + \phi B}{\phi C + \phi D}$$

9.2.2. Mixture Proportion ( $M_X$ )

Assuming a 4 allele pattern with major contributor alleles A and B and minor contributor alleles C and D:

$$M_X = \left( \frac{\phi A + \phi B}{\phi A + \phi B + \phi C + \phi D} \right) \times 100$$

9.3. Adjustments to peak heights may be necessary to ensure accurate mixture proportion/ratio and peak height ratio assessments for use in deconvolution and deducing. In most instances only N-4 stutter adjustments will be made; however, at the analyst's discretion N+4 stutter adjustments may be made if it will be of assistance in deconvolving or deducing a mixture profile.

The following example of a D8S1179 result in Figure 2 demonstrates this process. Of note, the N-4 stutter cut-off for D8 is 10.48%.

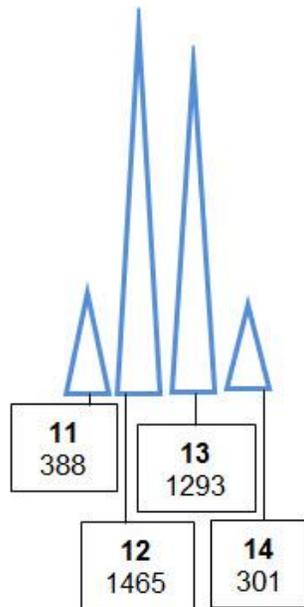


Figure 2 – D8S1179 result.

The maximum amount of N-4 stutter contributing to the 12 allele from the 13 allele is  $(1293 \times 0.1048) = 136$

To obtain the adjusted peak height for the 12 allele, the maximum stutter contribution from the 13 allele is subtracted from the 12 allele peak height  $(1465 - 136) = 1329$

The maximum amount of N-4 stutter contributing to the 11 allele from the 12 allele is (1329 (the adjusted peak height) x 0.1048) = 139

To obtain the adjusted peak height for the 11 allele, the maximum stutter contribution from the adjusted 12 allele is subtracted from the 11 allele peak height (388 – 139) = 249

Using the adjusted peak heights, the effect on the mixture ratio and proportion can be seen:

Before adjustments:

$$M_R = (1465 + 1293)/(388 + 301) = 2758/689 = 4.0 \approx 4:1 \text{ (major/minor)}$$

$$M_X = (1465 + 1293)/(388 + 1465 + 1293 + 301) = 2758/3447 = 0.80 \times 100 = 80\%$$

After adjustments:

$$M_R = (1329 + 1293)/(249 + 301) = 2622/550 = 4.8 \approx 5:1 \text{ (major/minor)}$$

$$M_X = (1329 + 1293)/(249 + 1330 + 1293 + 301) = 2622/3173 = 0.83 \times 100 = 83\%$$

## 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.

## 11. Limitations

11.1. Interpretation of low level samples should be approached with caution due to the limits in sensitivity of the STR testing procedure. Interpretation of low level results must factor in the potential loss of data due to the inability to accurately detect all genotypes present in an effort to ensure a reliable result is obtained as well as the potential for ‘allelic drop-in’. When interpretation of testing results indicates that the limit of detection is being approached, the laboratory will report the results as inconclusive.

11.2. Interpretation of complex mixtures often has limitations due to the inability to adequately assess the number of potential contributors. Inability to properly assess the number of contributors may result in ambiguity in conclusions drawn.

When interpretation of testing results indicates an inability to properly assess the number of relevant contributors, the laboratory will report the results as uninterpretable.

## 12. Documentation

- 12.1. The following documents may be generated during the interpretation process:
  - 12.1.1. Electropherograms
  - 12.1.2. 1st Reader/Verifier Worksheet
  - 12.1.3. 2nd Reader/Verifier Worksheet
  - 12.1.4. Batch File Review Checklist
  - 12.1.5. Profile Interpretation Worksheet(s)
  - 12.1.6. Deducing Calculations Worksheet
  - 12.1.7. STR Summary Sheet
  - 12.1.8. FBU Report of Examination

## 13. References

- 13.1. *Applied Biosystems GeneMapper ID-X<sup>®</sup> software.*
- 13.2. *Applied Biosystems AmpFISTR<sup>®</sup> Identifiler<sup>®</sup> Plus PCR Amplification Kit User Guide* (current revision).
- 13.3. *Quality Assurance Standards for Forensic DNA Testing Laboratories*, Federal Bureau of Investigation (current revision).
- 13.4. National Research Council. *The Evaluation of Forensic DNA Evidence*, Washington, DC: Academy Press, 1996.
- 13.5. *SWGDM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories* (current revision).
- 13.6. *ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories*, International Organization for Standardization, Geneva, Switzerland (current revision)

- 13.7. *Forensic Quality Services Supplemental Requirements for Forensic Testing Including FBI QAS, FQS ANSI-ASQ Accreditation Board, Tampa, FL (current revision)*
- 13.8. *ASCLD/LAB-International® Supplemental Requirements for the Accreditation of Forensic Science Testing and Calibration Laboratories, American Society of Crime Laboratory Directors/Laboratory Accreditation Board, Garner, NC (current revision)*
- 13.9. *Forensic Science Laboratory Quality Assurance Manual (current revision)*
- 13.10. *DFS Departmental Operations Manual (current revision)*
- 13.11. *FSL Laboratory Operations Manual (current revision)*
- 13.12. *FBS14 – Data Analysis Using GeneMapper ID-X (current revision)*
- 13.13. *FBS22 – Population Statistics (current revision)*
- 13.14. *FBS23 – FBU Report Wording Guidelines (current revision)*
- 13.15. Butler, J.M. (2004). *Forensic DNA Typing, 2<sup>nd</sup> Ed.* New York: Elsevier Academic Press. 1<sup>st</sup> book
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- 13.21. STRBase (2015). <http://www.cstl.nist.gov/strbase/> (accessed January 20, 2015).
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