33.1 Purpose:

Occasionally, a DNA typing result that was developed using a protocol that is no longer in use may need to be further evaluated for comparison to newly submitted samples or to assess a candidate match. Please see DNA SOP-15 CODIS Hit Confirmations for information on the need for reinterpretation.

33.2 Responsibility:

DNA Unit Personnel authorized for Legacy Data Interpretation

33.3 Procedure:

For Profiler Plus® and COfiler®, the analyst will refer to Section 33.4 below.

For autosomal data generated using AmpFℓSTR Identifiler®, the analyst will refer to Section 33.9 below.

For autosomal data generated using AmpFℓSTR Identifiler® Plus, the analyst will refer to Section 33.9 below.

For autosomal data generated using AmpFℓSTR Minifiler, the analyst will refer to Section 33.16 below.

For autosomal data generated using PowerPlex® Fusion 6C, the analyst will refer to Section 33.17 below.

For Y-STR data generated using Yfiler, the analyst will refer to Section 33.18 below.

33.4 Profiler Plus® and COfiler®:

This procedure pertains to both Perkin Elmer AmpF*l*STR Profiler Plus and AmpF*l*STR CO*f*iler PCR amplification kits. When Profiler Plus and CO*f*iler are employed, the original thirteen core CODIS STR loci are typed in two amplifications (two overlapping STRs + Amelogenin) according to manufacturer's protocols. These STR loci are D3S1358, vWA, FGA, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, CSF1PO, D7S820.

Amplification product from these kits were analyzed using an ABI 377 DNA Sequencer and the supplied GeneScan and Genotyper software.

33.5 GeneScan Analysis:

Following the electrophoretic run, all data is automatically sent to GeneScan (data is saved in a run folder with the date and time of the run) and all software programs are closed. Analysis of the data is outlined below. The gel image is opened. An approved matrix is installed. (Note: A new matrix is generated approximately once a month or when a major reagent is changed, the new matrix is logged into the matrix notebook). Gel lanes are auto-tracked. The top of the gel is cut off above the 400 bp MW standard (Profiler Plus) or the 350 bp MW standard (CO*f*iler). The gel bottom is cut off between the 75 bp standard and the primers. The tracking is inspected and any changes necessary are made. Extract data from the tracked lanes. A GeneScan project is created and stored in the run folder.

33.5.1 GeneScan gel preference defaults are as follows:

Auto-track gel	on
Scan range	0-10,000
Image generation defaults	0-12,000
Multi-component gel image	on
Estimated maximum peak height	3500
3 channel averaging	

33.5.2 GeneScan analysis parameters are as follows:

Analysis parameters (Q sample):
Analysis Range: full range
Data Processing: baseline
Multi-component on
Light smoothing
Peak Detection:
Minimum peak amplitude thresholds B: 50 Y: 50 G: 50 R: 50
Size call range: full
Size calling method: Local Southern
Split Peak correction: none

33.5.3 DNA samples are first analyzed using the standard parameters listed above. To address issues such as shoulder effects, the samples may be analyzed using different parameters provided that the change (e.g. to sample 100) does not remove true alleles. Both the primary and the second analyst must confirm that the new parameters do not adversely affect the results.

33.5.4 When the cases on a gel are analyzed individually, the negative controls for the case in question (on a particular gel) are analyzed at the same parameters as the evidentiary samples for that case. When multiple cases are analyzed on one gel (at the same time) using the same set of controls, the negative controls are analyzed using the parameters of the most stringent case, i.e, the lowest sample parameters of the evidence.

33.5.5 Check the analysis log to ascertain that size calling was successful in the selected range. If standard ROX peaks were missed, check the tracking to make sure they were included. Re-analyze at lower threshold as needed.

33.5.6 Look at the negative control and the reagent blank.

33.5.7 Look at the ladder. Ensure that one ladder has all allele peaks above the threshold. If not, the gel must be rerun.

33.5.8 Look at the positive controls.

33.5.9 Look at the size standards--aligned by size. Verify that all size standards are called within the appropriate size range for the STR system.

33.5.10 Look at each sample. Check for the number of peaks at each locus, poorly formed peaks, or any other feature that may be important in the interpretation of the data. Samples that show background noise >50 RFU should be noted. In Results Control, look for pull-up peaks and raised baselines. Look at maximum peak intensities and level of sample degradation.

33.5.11 Save the project in the run folder.

33.6 Genotyper:

33.6.1 Launch the appropriate template alias (Profiler Plus or CO*f*iler) for the amplifications being analyzed.

33.6.2 Import the appropriate GeneScan sample files including controls (RB, NEG, KJL/TMP, POS) and at least one ladder. At least one set of controls (for each amplification set) must give the expected results*.

*RB and NEG samples should not have any callable profiles. If minor peaks are detected and the evidentiary samples are not affected, the set of extracted samples may be interpreted with caution.

33.6.3 Control Profiles: Look at each color in the controls individually. Ensure that all alleles were included and that the positive controls give the expected genotypes.

See DNA SOP-29 for profiles for the kit positive control (POS) and the laboratory positive control (KJL)

33.6.4 Allelic Ladder: Look at each color individually. Ensure that all alleles that are in the allelic ladder are called.

33.7 Interpretation of DNA Profiles in Genotyper:

The results are evaluated for each locus. However, considering the data from all loci tested will assist in the composite interpretation of the results.

33.7.1 Off-Scale Data: If too much DNA is added to the amplification reaction or is loaded onto the gel the fluorescent intensity for the PCR products may exceed the linear dynamic range for detection by the instrument. Samples with off-scale peaks may exhibit raised baselines, split peaks, increased –A peaks, excessive pull-up and/or higher than expected stutter levels in one or more of the colors. Examine the peak heights in GeneScan software. Any peaks greater than 3500 RFU may be off-scale. These samples should be interpreted with care.

33.7.2 Peak identification: Only allele peaks with a height >50 RFU are reported. The identity of all peaks called by Genotyper, is assigned to one of the following categories:

a) Allele Peak: A called allele has a peak height greater than 50 RFU, a fragment size that falls within the base pair range, and has the appropriate dye color for the locus. However, not all peaks greater than 50 RFU are typed as alleles. Stutter peaks, shoulders, pull-up peaks, and background noise could fall above 50 RFU, especially if the allele peaks are high. Given factory macro parameters, peaks above 50 RFU that are called by GeneScan but removed by Genotyper may be called by the analysts on a case-by-case basis. Such peaks are interpreted with caution. Peaks between 30 and 50 RFU are not reported, but should be noted when appropriate. For these reasons, scientist experience is critical in reporting STR results.

b) Detection of variant alleles: Variant alleles have been identified from many STR loci. These alleles may contain full or partial repeats. Variant alleles are labeled as Off Ladder Alleles (OLA) in Genotyper. If a particular variant has been observed before by the CT DSS, Life Technologies, STRbase, NIST, or in published literature, the sample does not need to be repeated. In Genotyper change the OLA designation to the proper allele call.

c) Stutter peaks (generally N-4): A stutter peak has a fragment length typically four base pairs smaller than the true allele. If the peak height in the stutter

position is less than the PE stutter threshold, the peak is considered a stutter peak and is filtered out by Kazam. CT Forensic Laboratory stutter thresholds are determined by evaluating the PE and CT Forensic Laboratory empirical stutter data on an allele-by-allele basis (see Appendix 8.4). If the height of the N-4 peak is greater than the CT stutter threshold, this indicates a possible mixture, an overloaded sample, or a higher than normal stutter percentage. Additional information provided by other loci may be of assistance in these cases. For offender database samples, Kazam 20% will filter out all N-4 peaks that are less than 20% of the N peak.

d) -A Peaks: AmpF*l*STR kits are optimized to add an extra nucleotide to the 3' end of the PCR product. The nucleotide is predominantly adenosine (A). Lack of a full A addition may be observed especially when the amount of input DNA is greater than approximately 2.5 ng. However, sample degradation can increase the effective concentration of a small amplicon relative to the larger amplicons, leading to -A product at the smaller loci. Data from other loci may be of assistance in these cases. The level of –A product may be reduced by incubating the sample at 60 ^{o}C for an additional 30-90'. When appropriate, a smaller quantity of sample may be re-amplified.

e) Pull-up Peaks: Pull-up peaks are generally seen when excess amplification product is loaded onto the gel or when the matrix is sub-optimal for the gel. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-up peaks are often displayed in several colors. Pull-up peaks should have approximately the same fragment size in each of the colors as the true allele peak. Additional information provided by other loci may be of assistance in these cases.

f) Peaks < 50 RFU: Low-level peaks are those less than 50 RFU but > 30 RFU that are clearly distinguishable from background noise. These peaks cannot be called but their presence is noted when appropriate. This information may be used in overall sample interpretation when appropriate.

33.8 Interpretation of Mixed Samples for Profiler Plus and CO*f*iler**:**

Generally, the detection of more than two alleles per locus indicates a mixed sample. Variation in peak height between alleles in a single locus may assist in the interpretation of such results. Peaks below the 50 RFU threshold may assist in the interpretation of mixed DNA samples. The following guidelines pertain to the interpretation of mixtures:

33.8.1 A DNA mixture is conclusively identified by (a) the detection of three or more alleles at two or more loci or (b) 4 or more alleles detected at one or more loci. Other results are consistent with (but not proof of) a mixture as discussed below. Three peak patterns (at a single locus) have been observed from single sources, but they are rare.

33.8.2 Peak imbalance involving the stutter position, e.g., where the N-4 peak is 15-25% of the N peak, may indicate a mixture. With the possibility of allele sharing among individuals, 3 alleles may not be detected especially with degraded samples or partial profiles.

33.8.3 General peak imbalance: Heterozygous peak imbalance greater than 40% may indicate a mixture, i.e., the smaller peak is less than 60% of the larger peak. However, microvariants in the primer-binding domain may cause either PCR enhancement or suppression.

33.8.4 Highly Degraded/Low Template Samples:

Highly degraded/low template samples are interpreted with caution. Degraded samples may "appear" to exhibit high stutter and greater –A at the smaller amplicons with off-scale data. In addition, degraded/low template samples may display unbalanced heterozygous alleles and locus/allele dropout. Partial profiles may be called as determined by the data present for each locus. Given the possibility of stochastic allele sampling, especially with low quantity and/or degraded samples, single peaks less than approximately 150 RFU are interpreted with caution (pertains to putative genotype assignment at that locus). To maximize the number of loci with peaks above the threshold, different amount of amplification product from one sample may be run on analytical gels. However, any off-scale data at the smaller amplicons are interpreted with caution.

33.8.5 Inhibited Samples:

Inhibited samples may exhibit allele or locus drop out and unbalanced heterozygous alleles.

33.9 Identifiler/Identifiler Plus:

33.9.1 This procedure is a general guideline for the analysis and interpretation of STR profiles when using the Identifiler/Identifiler Plus Kits (ID/IDP). These procedures generally follow those outlined in ABI Prism GeneMapper ID Software v3.2 User Guide.

33.9.2 When the Identifiler/Identifiler Plus Kit is employed, the non-expanded, original thirteen core CODIS STR loci (prior to January 1, 2017), two of the expanded core CODIS STR loci (D2S1338 and D19S433), and Amelogenin are typed in a single PCR amplification reaction according to the manufacturer's and laboratory's protocols.

33.9.3 Identifiler/Identifiler Plus amplification products are separated and detected using an ABI 3130xl Genetic Analyzer and the supplied Run 3130xl Data Collection v4.0 and GeneMapper ID v3.2.1 (GMID) softwares.

33.9.4 For the GMID software, the Analysis Method is as outlined in the user's manual except for the following: Under the Allele Tab, confirm that the Bin set is for the appropriate kit and that Marker Specific Stutter Ratio is used. Under the Peak Detector Tab, the Peak Detection Algorithm is Advanced, the Analysis Range is Partial Range but shall include the full range of required size standard peaks, the Sizing Range is Partial Sizes with the Start Size set to 75 for Identifiler/Identifiler Plus and the Stop Size set to at least 450 for ID/IDP. Smoothing is set to Light for all three kits and the Size Calling Method is set to Local Southern Method for ID/IDP. For the Panel use Identifiler_Plus_Panels_v1 for IDP and AmpFLSTR_Panels_v1.1_Panels for ID. For the Size Standard, use CE_G5_Identifiler_Plus_GS500 for Identifiler Plus.

33.9.5 The analysis and interpretation of STR results are listed below.

33.9.5.1 ID/IDP Analysis:

33.9.5.2 A GMID project is created by importing all of the pertinent data files. The standard analysis parameters for forensic unknowns, knowns, ladders, and controls are B, G, Y, R = 50 RFU, O = 150 RFU (IDP-50). Samples originally analyzed with a prior analysis method, (i.e. IDP-75, where B, G, Y, R = 75), will need reanalysis if the comparison shows a positive association to a newly submitted known sample, as well as those comparisons thought to possibly be inconclusive. Please refer to WI-36 for a more detailed explanation on how to reanalyze unknown samples for use with STRmix. Evidentiary profiles (forensic unknowns) with peaks >5000 RFU are interpreted with caution with approval from the DNA TL. With known samples and controls, any peaks > 6500 are interpreted with caution. For known samples, including positive controls, the thresholds for B, G, Y, & R may be raised where appropriate, and the RFU threshold for O may be lowered to 50 RFU. See below:

33.9.5.3 Ladders and any samples with ILS peaks lower than the standard values are interpreted with caution, with the restriction noted in #2.

33.9.5.4 The minimum analysis parameters for ladder peaks and the internal lane standards are: Ladder: B, G, Y, R, and O > 49 RFU (including 250 peak (and 340 peak for IDP)).

33.9.5.5 With all samples and controls, the ILS peaks must be >49 RFU (including 250 peak (and 340 peak for IDP)).

33.9.5.6 Ensure that all required internal lane size standard peaks are called in all samples (75 – 450 nt, GS500-LIZ; 60 – 400 nt for IDP). If a sample's internal size standard fails due to a low sizing quality (e.g. pull-up peaks into the size standard), the option to override the sample's size quality is available and shall be noted on the analysis worksheet. Both the primary and the second analyst must confirm that the size quality failure was not due to the loss of internal lane size standard peaks. The sample may then be analyzed as normal.

33.9.5.7 Ensure that each ladder in each injection has all allele peaks detected above the minimum threshold. If a ladder does not meet these requirements, change the sample type from allelic ladder to sample and reanalyze set. One ladder in each injection must meet the requirements.

33.9.5.8 GMID Analysis-- Control Requirements:

33.9.5.8.1 Import the appropriate GMID sample data files including controls (RB, NEG, EP1, POS) and at least one ladder. Controls for each extraction/amplification set must give the expected results.

33.9.5.8.2 Expected results of controls will be verified and documented on DNA QR-4a which is maintained in the case file.

33.9.5.8.3 Potential contamination is assessed by evaluating the evidentiary samples, RB, NEG, and positive controls. All samples within a batch will be cross-checked against each other to detect potential contamination. This is not necessary for samples reanalyzed for STRmix analysis. Analysts should use their experience and judgment during this process. An excel macro is available to assist with batch cross-comparisons. The appropriate response for a contamination event (including how the sample(s) are reported) is determined on a case-by-case basis with approval of the TL. RB and NEG samples should not have any callable peaks If callable peaks are detected and the evidentiary samples are not affected, the set of extracted samples may be interpreted with caution with TL approval.

33.9.5.8.4 For those samples reanalyzed for STRmix, negative controls associated with those samples must be reassessed for peaks below the analytical threshold, down to 25

RFU. If peaks in this range are present, and the evidentiary samples are not affected, those samples may be interpreted with caution with TL approval.

33.9.5.8.5 Depending on factors such as the type and extent of contamination, and whether the source of the contamination can be identified, the contaminated sample(s) and/or amplification sets may be interpreted with caution in the report or deemed unsuitable for comparison following TL review and approval.

33.9.5.8.6 See SOP-29 for ID/IDP profiles for the Kit Positive Control DNA 9947A (POS) and the laboratory Extraction Positive Control (EP1):

33.10 Interpretation of DNA Profiles in GMID:

The results are evaluated for each locus. However, considering the data from all loci tested will assist in the overall interpretation of the results for each sample.

33.10.1 Off-Scale Data: If too much DNA is added to the amplification reaction or is loaded onto the 3130, the fluorescent intensity for the PCR products may exceed the linear dynamic range for detection by the instrument. Samples with off-scale peaks may exhibit raised baselines, split peaks, increased minus A peaks, excessive pull-up and/or higher than expected stutter levels, and/or amplification artifacts in one or more of the colors. Examine the peak heights in GMID software. Any peaks greater than 5000 RFU may be off-scale.

33.10.2 Peak identification: Only allele peaks with a height of ≥ 50 RFU in GMID are reported. The identity of peaks called by GMID is generally assigned to one of the following categories:

33.10.3 Allele Peak: A called allele has a peak height (≥50 RFU for those samples reanalyzed for STRmix analysis), a fragment size that falls within the base pair range, and has the appropriate dye color for the locus. However, not all peaks are typed as alleles. Stutter peaks, pull-up/pull-down peaks, background noise, and amplification or injection artifacts could fall above the RFU threshold, especially if the allele peaks are high. Peaks that do not meet the Laboratory's calling criteria/thresholds (< 50 RFU) are not analyzed by STRmix. Peaks that are not called by GMID may be called by the analysts on a case-by-case basis with TL approval. Such peaks are interpreted with caution.

33.10.4 Peaks (GMID Peaks): Sub analytical threshold (AT) peaks are those less than 50 RFU that have good peak morphology and are clearly distinguishable from background noise and other artifacts. Any sub AT peaks observed are considered only to evaluate the number of contributors to the profile if STRmix analysis is required. Sub AT peaks in the evidentiary samples or controls are noted on the

appropriate worksheets.. With those samples reanalyzed for use with STRmix analysis software, there are no * peaks, as the calling threshold is 50 RFU. Also, peaks less than 50 RFU are evaluated in the negative controls, and in the evidentiary samples to determine number of contributors.

33.10.5 Detection of variant alleles: Variant alleles have been identified from many STR loci. These alleles may contain full or partial repeats. Variant alleles are labeled as Off Ladder (OL) alleles in GMID. If a particular variant has been observed before by the DESPP CT Division of Scientific Services (CTDSS), Applied Biosystems, NIST, or in published literature, the sample does not need to be repeated. In GMID, change the OL to the proper allele call.

33.10.6 Stutter peaks (generally N-4 bases, but ID/IDP also has N+4 bases for all STR loci): A stutter peak has a fragment length typically four base pairs smaller than the true allele. If the peak height in the stutter position is less than the user-defined stutter threshold, the peak is considered a stutter peak and is filtered out by the GMID program. Any peaks < the CT stutter threshold are manually edited as required. CT DSS stutter thresholds are listed in DNA SOP-29. Peaks in the stutter position that are below the Laboratory's stutter thresholds are not listed on the report and are not used for statistical purposes.

33.10.7 Minus A Peaks: AmpF*l*STR kits are optimized to add an extra nucleotide to the 3' end of the PCR product. The nucleotide is predominantly adenosine (A). Lack of a full A addition may be observed especially when the amount of input DNA is greater than approximately 2.5 ng. However, sample degradation can increase the effective concentration of a small amplicon relative to the larger amplicons, leading to minus A product at the smaller loci. Data from other loci may be of assistance in these cases. When appropriate, for known samples, a smaller quantity of sample may be re-amplified.

33.10.8 Pull-up Peaks: Pull-up peaks are generally seen when excess amplification product is injected on the CE or when the spectral calibration is sub-optimal. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-up peaks are often displayed in several colors. Pull-up peaks should have approximately the same fragment size in each of the colors as the true allele peak. Additional information provided by other loci may be of assistance in these cases. When evaluating allelic peaks that may contain a pull-up (PU) contribution, an estimate of the PU contribution is subtracted from the height of the potential allelic peak to determine if the remaining peak height is sufficient for allele or asterisk designation. The estimated pull-up contribution is determined by evaluating the height of unambiguous pull-up peaks (pull-up with e.g., no stutter or allelic contribution) at each locus where pull-up is detected for a particular color.

33.10.8.1 Example: The percentage of pull-up peaks as compared to the height of the peaks causing the pull-up is calculated. (PU percentage = PU peak height/height of the peak causing the PU). This percentage is converted to an RFU value (multiply percent by height of peak causing the pull-up) which is then subtracted from the potential allelic peak in question.

33.10.8.2 The resulting "subtracted" peak is called where DNA Section allele calling criteria are met. The same methodology would be applied to potential allelic peaks with a pull-down component (see below).

33.10.9 Pull-down Peaks: Pull-down peaks are generally seen when excess amplification product is run on the CE or when the spectral calibration is sub-optimal for the run. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-down peaks can be displayed in several colors. Pull-down peaks can have approximately the same fragment size, but often the pull-down peak will be slightly a larger or smaller size as compared to the true allele. Additional information provided by other loci may be of assistance in these cases.

33.10.10 Spikes: fluorescent spikes can be seen within GMID data. Spikes are typically sharp peaks caused by air bubbles or polymer crystals in a capillary, which can be displayed in different colors, and have approximately the same "fragment size". Additional inspection of the raw data or re-injection may assist in cases where spikes are believed to be present.

33.10.11 Dye Artifacts/Dye Blobs: Broad peaks may be the result of dissociated dyes or fluorescent particles that are trapped within the polymer matrix and then fluorescently excited by the laser. Additional inspection of other samples within the module may assist in interpretation in cases where dye blobs are believed to be present.

33.10.12 Amplification Artifact Peaks: Nonspecific amplification product peaks, generally with good peak morphology, may result when excessive template DNA is amplified. When appropriate, a smaller quantity of sample may be re-amplified.

33.10.13 High Background: Background fluorescence may reach an RFU level above the calling threshold. Generally such high background peaks exhibit atypical peak morphology. When appropriate, re-injection (only of known samples) may assist with interpretation.

33.10.14 Raised Baseline: Elevated baseline of one color over a broader (multiple bin) scan range may occur. Note: pull-down from two adjacent peaks may cause raised baseline between the pull-down peaks; the "pull-down" designation should be used for the editing notation when raised baseline is caused by pull-down.

33.11 Mixed Samples in Identifiler/Identifiler Plus:

Generally, the detection of more than two alleles per locus indicates a mixed sample. Variation in peak height between alleles in a single locus may assist in the interpretation of such results. Peaks below the 50 RFU threshold for GMID may assist in the interpretation of mixed DNA samples. The following guidelines pertain to the interpretation of mixtures.

33.11.1 A DNA profile is consistent with being a mixture by the following; (a) the detection of three or more alleles at one or more loci or (b) or as discussed below in 33.11.2 and 33.11.3.

33.11.2 Allelic peak imbalance involving the stutter position, e.g., where the N-4 peak is 15-25% of the N peak, may indicate a mixture. With the possibility of allele sharing among individuals, 3 alleles may not be detected especially with degraded samples or partial profiles.

33.11.3 General allelic peak imbalance: Heterozygous peak imbalance greater than 40% may indicate a mixture, i.e., the smaller peak is less than 60% of the larger peak. However, microvariants in the primer-binding domain may cause either PCR enhancement or suppression.

33.11.4 Per the "SWGDAM Guidelines on STR Interpretation" A Stochastic Threshold is defined as the "value above which it is reasonable to assume that allelic dropout has not occurred within a single-source sample". The below table lists the applicable stochastic thresholds for CT DSS based on 3130xl instruments and injection times.

	Injections Time Ranges				
Stochastic Threshold	**3130-1**	**3130-2**	**3130-3**	**3130-4**	**3130-6**
150 RFU	2 – 18 sec	2 – 10 sec	2 – 10 sec	2 – 10 sec	2 – 10 sec
200 RFU	19 – 28 sec	11 – 16 sec	11 – 16 sec	11 – 16 sec	11 – 16 sec
250 RFU	29 – 35 sec	17 – 20 sec	17 – 20 sec	17 – 20 sec	17 – 20 sec

33.12 CODIS Entries

Profiles are entered into CODIS as warranted by the results. See DNA SOP-13 CODIS Profile Entry.

33.13 General Interpretation of STR Results: (without STRmix interpretational software)

When a known is submitted to the laboratory for comparison to samples previously amplified with Identifiler/Identifiler Plus, the analyst can visually eliminate that person as the source of, or a contributor to, the questioned DNA sample(s). The following is a general description of the standard DNA report conclusions. Other conclusions may be reported as warranted by the results with approval of the technical leader. (See DNA SOP-6)

33.13.1 Elimination: used when the known sample is not the source of, or a contributor to, the DNA profile detected from item xx. The known sample has alleles that are not detected in the evidentiary sample and the lack of detection is not reasonably explainable by degradation/inhibition/allele dropout (stochastic effects) within the evidentiary sample.

33.13.2 If the previously amplified and analyzed sample only had reported * peaks (peaks between 50 & 74 RFU analyzed when the laboratory's calling and analytical thresholds were 75 RFU) present in a profile, the result can be used for elimination. If those results suggest an inclusion, the sample must be reanalyzed with a calling threshold of 50 RFU.

33.13.3 If the previously amplified and analyzed sample only has results (whether it be * or called) at one locus, the sample can be reported out as an elimination, or an inconclusive result. If unable to eliminate, the comparison to J. Smith is inconclusive.

If the sample is not being reanalyzed for STRmix analysis, a conclusion as to the number of contributors is not given.

33.13.4 Interpretation of single source DNA profiles: The use of "undetermined" in the Report Conclusions Summary table (in the "Type" column): Applies to the detection of male vs female DNA. Used when the analyst is unable to determine whether a profile that appears to be single source is male or female. In this situation, follow the single source interpretation protocol.

33.14 Interpretation of DNA mixtures (without STRmix interpretational software)

These general approaches do not account for all possible mixture scenarios and no two mixtures are exactly the same.

33.14.1 The analyst will consider the minimum number of persons in the mixture; the minimum number of persons in a mixture is determined by assessing the

maximum number of peaks detected at any locus (total number of peaks above the AT). For example, if the maximum number is 5 or 6 peaks, it is indicative of a 3 or more person mixture; 7 or 8 is indicative of a 4 or more person mixture.

33.14.2 The analyst will also consider the quantity of DNA amplified, the peak heights of the alleles, the extent of degradation/inhibition, the impact of stutter (particularly if there is above average stutter [mean + ~1.5 SD for that locus/allele (see Appendix 1)]), potential carryover between fractions in a differential extraction, and if a known profile compared to the mixture can be objectively determined to be a contributor (e.g. intimate samples).

33.14.3 Factors taken into consideration when interpreting mixtures:

33.14.3.1 If one person can be objectively determined to be included in the mixture given an intimate sample origin, the mixture is evaluated by a consideration of what loci have DNA profile results (above the AT) that could not be from the known source.

33.14.3.2 Incomplete separation. In mixtures from samples that were subjected to the differential extraction protocol, the mixture is evaluated by a consideration of whether the results are consistent with incomplete separation from the other fraction (e.g., A vs B fractions).

33.14.3.3 The ratio of male: female DNA and the extent of degradation in sample as determined by Quantifiler Trio and the STR results.

33.14.3.4 Peak height consistency, i.e. the peak heights in the evidentiary profile potentially attributable to the known DNA profile should generally be consistent at all loci applicable.

33.14.3.5 The "zygosity" of the known: homozygous peaks are generally higher than heterozygous peaks of the same contributor. Therefore, homozygous peaks are less likely to dropout as compared to one allele from a heterozygote.

33.14.3.6 While allele dropout (below the stochastic threshold), can occur at any locus, extensive validation and case experience has shown that smaller amplicons amplify more efficiently and are less affected by degradation than are larger amplicons. Therefore, the results at the smaller amplicons can be particularly diagnostic.

33.14.3.7 Mixture DNA profiles that involve relatives are interpreted with caution.

33.15 Deducing a Clear Major Contributor in Profiles with ≥ 5 Contributors

33.15.1 A clear major contributor may be deduced from Identifiler/Identifiler Plus profiles containing ≥ 5 contributors as outlined below (requires TL & Assistant Director approval). The clear major contributor must be deduced without consideration of any known profile.

1. Where the allele(s) of the major contributor are at least 500 RFU at ≥10 autosomal loci, the heterozygous peak balance of the major profile is ≥ 65% at ≥10 autosomal loci, and the following conditions are met:

A. **Apparent heterozygous major**: the height of the highest minor allele is ≤ 33% of the height of the lowest major allele. If not, the locus does not qualify for statistics.

B. **Apparent homozygous major**: the height of the highest minor allele is ≤ 25% of the height of the major allele. If not, the locus does not qualify for statistics.

2. The allele(s) of the major profile should be called at the remaining loci that do not qualify for statistics.

Notes: The impact of stutter on the height of a minor peak should be addressed by evaluating how much of the peak height would be the result of average stutter from the major allele. Given that the PCR biological model shows continuous peak height variation, exceptions to the binary criteria listed above may be made with TL & Assistant Director approval as warranted.

3. If conditions listed above are met, CODIS entry of the deduced major contributor is permitted with CODIS Administrator/designee approval.

33.15.2 The exported allele table text file will be imported into the Deconvolution Workbook, "CT DSS" is selected as the laboratory, the sample selected, and "5+" selected for contributors. Click "Calculate". If a clear major contributor is present at 10 or more loci, the "Major from 5P+ mixture" button becomes active. Clicking on that button will bring you to DNA-QR-307d Five or More Person Mixture Major Deconvolution.

33.15.3 Print DNA-QR-307d Five or More Person Mixture Major deconvolution, and keep as part of the case record. TL and AD approval will be recorded on this QR. The presence of a clear major profile will be noted on DNA-QR-302 Contributor Estimation Worksheet.

33.15.4 Statistics for Clear Major Profiles

31.15.4.1 If a known sample is positively associated with a clear major profile, a binary LR (1/RMP) for the major contributor (using the Popstats database with posterior means distribution, NRC 4.2, and a factor of 10 lower bound confidence interval) is calculated. The population with the lowest LR is reported.

31.15.4.2 Repeat 33.14.4.1, and click on "LR for Major". DNA-QR-307h, Likelihood Ratio, is printed out for the case jacket. "Min LR/10" will be reported.

33.16 Minifiler:

Minifiler DNA profiles are analyzed and interpreted as described in Section 33.13 above. See SOP-29 for the Minifiler Kit Positive Control DNA 007 (mPOS) and the laboratory Extraction Positive Control (RKO).

33.17 Fusion 6C Analysis and Interpretation:

33.17.1 PowerPlex Fusion F6C System:

33.17.2 When the F6C Kit is employed, the twenty core CODIS STR loci, plus three additional loci (Penta D, Penta E, SE33), 3 Y-STRs (DYS391, DYS576, DYS570) and Amelogenin are typed in a single PCR amplification reaction according to the manufacturer's and laboratory's protocols.

33.17.3 PowerPlex® Fusion 6C System amplification products are separated, detected, and analyzed using an ABI 3130xl Genetic Analyzer, the supplied 3130xl Data Collection v4.0 and GeneMarker HID STR Software (GM-HID) v.2.9.0.

33.17.4 These procedures generally follow those outlined in the GeneMarker HID (GM-HID) Software User Manual for the version in use.

33.17.5 PowerPlex® Fusion 6C Analysis:

33.17.5.1 Analysis of DNA Profiles in GM-HID

33.17.5.2 The standard analysis parameters for forensic unknowns, knowns, ladders, and controls are B,G,Y,R,P = 50 RFU. Evidentiary profiles (forensic unknowns) with peaks > 4000 RFU, and known samples with peaks > 6000 RFU, at loci other than Amelogenin, are not used unless necessary with TL or Lead approval.

33.17.5.3 The results are evaluated for each locus. However, considering the data from all loci tested will assist in the overall interpretation of the results for each profile.

33.17.5.4 Overloaded Data: If too much DNA is added to the amplification reaction or is injected into the 3130, the fluorescent intensity for the PCR products may result in various interpretational challenges. Samples with off-scale peaks may exhibit raised baselines, split peaks, increased minus A peaks, excessive pull-up and/or higher than expected stutter levels, and/or amplification artifacts in one or more of the colors. Examine the peak heights in GM-HID software. Peaks greater than 4000 RFU (6000 RFU for knowns and database samples) but not off-scale, may also lead to artifacts peaks being detected ≥ 50 RFU.

33.17.5.5 Peak identification: Only allele peaks with a height of ≥50 RFU in GM-HID are called/reported and evaluated using STRmix™. This is also the laboratory's analytical threshold (50 RFU). Peaks < 50 RFU may be evaluated to address issues such as whether a sample has been contaminated or whether a sample should be re-amped/re-injected, and also in contributor number assessment. The identity of peaks called by GM-HID is generally assigned to one of the following categories:

33.17.5.6 Allele Peak: A called allele has a peak height ≥50 RFU, a fragment size that falls within the base pair range, good peak morphology, and has the appropriate dye color for the locus. However, not all peaks ≥50 RFU are typed as alleles. Stutter peaks, pull-up/pull-down peaks, background noise, and amplification or injection artifacts could fall above the RFU threshold, especially if the allele peaks are high. Peaks that do not meet the Laboratory's calling criteria are not used by STRmix™ with the exception of the N-1 and N+1 stutter peaks that are modeled. Peaks ≥ 50 RFU that are not called by GM-HID may be called by the analysts on a case-by-case basis with TL approval. Such peaks are interpreted with caution.

33.17.5.7 Detection of variant alleles: Variant alleles have been identified for many STR loci. These alleles may contain full or partial repeats. Variant alleles are labeled as Out of Bin (OB) or Off Ladder (OL) alleles in GM-HID.

33.17.5.8 Stutter peaks (N-1, N-0.5, N+0.5, and N+1 repeats, but N-2 is also possible for Fusion 6C data if over-amplified/injected): A stutter peak has a fragment length typically 1 repeat smaller than the true allele (see exceptions above). If the peak height in the stutter position is less than the CT_DSS allele-specific stutter thresholds, the peak is filtered out by the GM-HID program. CT_DSS stutter thresholds are listed in DNA SOP-29. Peaks in the stutter position that are below the Laboratory's stutter thresholds are not listed on the electropherograms or the case report. Note: N-1 and N+1 peaks are evaluated by STRmix™

33.17.5.9 Minus A Peaks: F6C kits are optimized to add an extra nucleotide to the 3' end of the PCR product. The nucleotide is predominantly adenosine (A). Lack of a full A addition may be observed especially when the amount of input DNA generates peaks ≥ 4000 RFU. However, sample degradation can increase the effective

concentration of a small amplicon relative to the larger amplicons, leading to minus A product at the smaller loci. Data from other loci may be of assistance in these cases. When appropriate, a smaller quantity of sample may be re-amplified.

33.17.5.10 Pull-up Peaks: Pull-up peaks are generally seen when excess amplification product is injected on the CE or when the spectral calibration is sub-optimal. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-up peaks can be displayed in several colors. Pull-up peaks should have approximately the same fragment size in each of the colors as the true allele peak. Additional information provided by other loci may be of assistance in these cases. When evaluating allelic peaks that may contain a pull-up (PU) contribution, an estimate of the PU contribution is subtracted from the height of the potential allelic peak to determine if the remaining peak height is sufficient for allele designation. The estimated pull-up contribution is determined by evaluating the height of unambiguous pull-up peaks (pull-up with e.g., no stutter or allelic contribution) at each locus where pull-up is detected for a particular color.

Example: The percentage of pull-up peaks as compared to the height of the peaks causing the pull-up is calculated. (PU percentage = PU peak height/height of the peak causing the PU). This percentage is converted to an RFU value (multiply percent by height of peak causing the pull-up) which is then subtracted from the potential allelic peak in question. The resulting "subtracted" peak is called where DNA Section allele calling criteria are met. The same methodology would be applied to potential allelic peaks with a pull-down component (see below).

33.17.5.11 Pull-down Peaks: Pull-down peaks are generally seen when excess amplification product is run on the CE or when the spectral calibration is sub-optimal for the run. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-down peaks can be displayed in several colors. Pull-down peaks can have approximately the same fragment size, but often the pull-down peak will be a slightly larger or smaller size as compared to the true allele. Additional information provided by other loci may be of assistance in these cases.

33.17.5.12 Spikes: fluorescent spikes can be seen within GM-HID data. Spikes are typically sharp peaks caused by air bubbles or polymer crystals in a capillary, which can be displayed in different colors, and have approximately the same "fragment size". Additional inspection of the raw data or re-injection may assist in cases where spikes are believed to be present.

33.17.5.13 Dye Artifacts/Dye Blobs: Broad peaks may be the result of dissociated dyes or fluorescent particles that are trapped within the polymer matrix and then fluorescently excited by the laser. Additional inspection of other samples (especially negative controls) within the module may assist in interpretation in cases where dye blobs are believed to be present (see SOP-29.

33.17.5.14 Amplification Artifact Peaks: Nonspecific amplification product peaks, generally with good peak morphology, may result when excessive template DNA is amplified.

33.17.5.15 High Background: Background fluorescence may reach an RFU level above the analytical threshold. Generally such high background peaks exhibit atypical peak morphology.

33.17.5.16 Raised Baseline: Elevated baseline of one color over a broader (multiple bin) scan range may occur.

33.17.5.17 Non-specific amplification peaks: With some samples, primers may bind to non-human DNA or human DNA other than the target region, and amplification may occur. Resulting peaks do not exhibit stutter but typically have good morphology otherwise. Such peaks may be observed at characteristic base pair sizes. Most often they fall outside of bins or at fractional repeat numbers. More than one non-specific amplification peak may be seen in the same sample.

33.17.6 Control Requirements

33.17.6.1 For evidentiary sample (forensic unknown) amplifications, all controls (RB, NEG, EP1, POS) for each extraction/amplification set must give the expected results. For known or database samples, only one positive control (EP1 or POS) is required to give the expected results; all other controls must give the expected results.

33.17.6.2 If either the NEG or the RB for a database plate does not inject properly (ex. size standard fails during multiple re-preps/re-injections), results from the plate may be used with TL approval.

33.17.6.3 Expected results of controls will be verified and documented on DNA-QR-4a DNA QA/QC Case Review Checklist which is maintained in the case file.

33.17.6.4 The F6C profiles for the Kit Positive Control DNA 2800M (POS) and the laboratory Extraction Positive Control (EP1) are listed in SOP-29.

33.17.7 Contamination Assessment

33.17.7.1 Potential contamination is assessed by evaluating the evidentiary samples, RB, NEG, and positive controls.

33.17.7.2 RB and NEG samples should not have any callable peaks. If callable peaks are detected, the profile may be used if the evidentiary sample is not affected.

33.17.7.3 Since peaks between 25 and 49 RFU may be used in contributor number assessment for any evidentiary samples in the set, the potential for contamination at this level that could impact contributor number determination should be assessed using the negative controls.

33.17.7.4 All samples within a batch will be cross-checked against each other to detect potential contamination. Analysts should use their experience and judgment during this process. An excel macro is available to assist with batch cross-comparisons.

33.17.7.5 The appropriate response for a contamination event (including how the sample(s) are reported) is determined on a case-by-case basis with approval of the TL.

33.17.7.6 Depending on factors such as the type and extent of contamination, and whether the source of the contamination can be identified, the contaminated sample(s) and/or amplification sets may be interpreted with caution or deemed unsuitable for comparison following TL review and approval (see Alternate Report Template Statements located within the DNA Report Template).

33.17.8 Documentation of controls and standards on DNA QR-300:

The following key should be used as a guideline to determine how controls and standards should be documented on DNA-QR-300. This list is not all encompassing, and other situations may arise not fully documented here. Any questions on how to document controls and standards on DNA QR-300 should be brought to the TL.

Did the ILS perform as expected?

Sizing quality score less than 88	No
Not all 21 peaks called*	No
Score 88 or more and all 21 peaks called	Yes
Broad peaks, but score is 88 or more and all 21 peaks are called	Yes

* The 500 peak does need a green arrow in the "Calibration Chart" window. However, it does not need a call in the "All Color Browser", as the settings only call peaks up to 500 BP in size, and this peak may size at say, 500.2.

Did the negative controls perform as expected?

Called peaks in Neg or RB*	No
Peaks below AT in Neg or RB*	Yes

Broad peaks in WEN, but no called peaks or peaks below AT	Yes

* Need TL initials in comments section showing that no affect to samples

Did the positive controls perform as expected?

Broad peaks, but genotype still correctly called	Yes
Broad peaks causing peaks not to be called	No
Large peak height imbalance, but genotype still correctly called	Yes
Large peak height imbalance causing dropout	No
High peaks, needing reinjection at low, but genotype still correctly called	Yes
Extremely high peaks (i.e. overamplified sample), sample needs re-amplification	No
Low peaks, needing reinjection at MAX, peaks not called	No

33.17.9 Mixed Samples in F6C:

33.17.9.1 Generally, the detection of more than two alleles per locus indicates a mixed sample. Variation in peak height between alleles in a single locus may assist in the interpretation of such results. Peaks below the 50 RFU threshold for HID may assist in the determining the number of contributors. (see section 33.17.10 for detailed guidance on determining the number of contributors to a DNA profile).

33.17.9.2 A DNA profile is generally consistent with being a mixture when three or more alleles are detected at any locus and as discussed below. Note that three peak patterns (at a single locus) have been observed from single sources, but they are rare.

33.17.9.3 Allelic peak imbalance involving the stutter position, e.g., where the N-1 repeat peak is outside the expected range, may be indicative of a mixture. With the possibility of allele sharing among individuals, 3 alleles may not be detected especially with degraded samples or partial profiles.

33.17.9.4 General allelic peak imbalance: Heterozygous peak imbalance greater than expected for a set of parameters may indicate a mixture. However, microvariants in the primer-binding domain may cause either PCR enhancement or suppression.

33.17.9.5 CT DSS Stochastic thresholds are 150 RFU for Standard and Low injection times and 300 RFU for Maximum injection times. (see below for Low, Standard, and Maximum injection times on each 3130 instrument for DNA samples.)

33.17.9.6 The following injection times were validated for use on specific 3130 instruments that are online for casework:

3130-1	2sec – 35sec (CO/knowns only)
3130-2	5sec, 11sec, 22sec
3130-3	5sec, 10sec, 20sec
3130-4	5sec, 11sec, 22sec
3130-6	5sec, 8sec, 16sec

33.17.9.7 Known and database samples are injected at 3kV for 2-35 seconds. For the 29 cycle Fusion 6C amplification of knowns and database samples using extract from EZ1 normalization, the stochastic threshold is 150 RFU for 3kV5sec or less and 300 RFU for anything greater than 3kV5sec. For the 26 cycle Fusion 6C amplification of knowns and database samples using extract from DNA IQ or Fusion 6C Direct Amplification, the stochastic threshold is 85 RFU for 3kV5sec or less and 125 RFU for anything greater than 3kV5sec. The CT-DSS Knowns panel is used for analysis.

33.17.9.8 Mixtures are deconvoluted for CODIS entry purposes as warranted by the results. See DNA SOP-13.

33.17.10 Determination of Single Source or Mixture Profile & Number of Contributors

Single Source Profile Attributes: A single source profile will generally have no more than 2 alleles at any locus. Expected heterozygote peak balance (HPB) decreases as a function of peak height (see Fig. 1). In rare instances, an individual may exhibit a tri-allelic pattern at a locus. When a suspected tri-allelic pattern is observed but not confirmed by multiple observations in a case, the sample would typically be re-amplified to confirm its presence.

Note: If an electropherogram has only an X at Amelogenin then it is considered a No Result.

When a tri-allelic pattern is noted, the results must be documented in the case file, but the locus is not included in the statistical calculation (see STRmix™ DNA SOP-32).

General Mixture Profile Attributes: Use ‘consistent with a mixture’ for profiles with multiple contributors. A profile with no more than 2 alleles can be best explained as a mixture if there is significant peak height imbalance at one or more loci (see Fig. 1 re: expected heterozygous peak balance vs peak height).

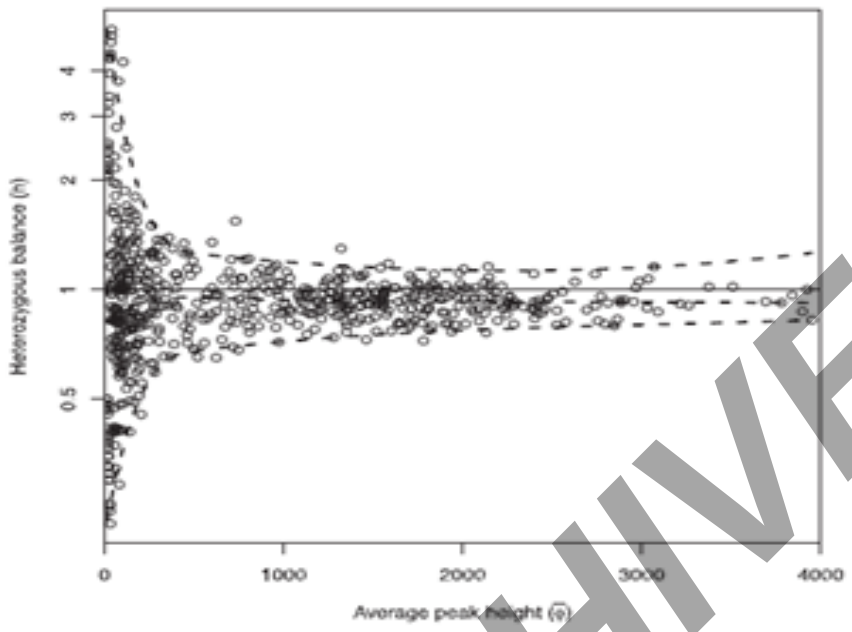


Fig. 1. Heterozygous balance versus average peak height.

"Modelling heterozygote balance in forensic DNA profiles"
Kelly et al, FSI Genetics 6 (2012) 729-734

33.17.10.1 Determination of the number of contributors to the DNA profile

Determination of the number of contributors to a profile begins with assessing the minimum number of contributors from the locus that exhibits the greatest number of allelic peaks. For example, if at most five alleles are observed at a locus, then the DNA results are consistent with having arisen from at least three individuals. Proceed with caution when only one additional allele at a locus would lead to an increased number of possible contributors, as peaks in stutter positions, and the potential for a tri-allele can complicate mixture interpretation. While counting allele peaks is useful in determining a minimum number of contributors, the analyst must also consider that allele sharing between individuals may result in an underestimation of the actual number of contributors. The potential of peaks in stutter positions should be considered. The number of contributors chosen for STRmix™ analysis reflects the analyst's assessment of the most likely number of contributors required to reasonably explain the observed profile.

A determination of the number of contributors to a profile is generally made by the DNA analyst prior to STRmix™ deconvolution and prior to the comparison of

the profile to any reference samples in STRmix™. However, when scientifically warranted, the comparison of an evidentiary profile to a conditioned elimination known might lead the analyst to alter the original number of contributor determination. This change needs to be reviewed by the technical reviewer, and technical lead approval must be obtained prior to STRmix deconvolutions or report writing. A detailed explanation of the reasoning for the change must be documented on DNA QR-308 Changed and/or Not Reported Interpretations. The original DNA QR-302 Contributor Estimate for the sample shall be DNR'd with initials and date of the reassessment.

Sometimes the number of contributors is ambiguous. This could be because the profile is too complex and may contain putative indications of additional contributors. DNA analysts will use their professional judgement to assess the number of contributors, and may evaluate peaks less than 50 RFU that are above the 3130 limit of detection (non-artifact peaks 25-49 RFU with no raised baseline issue) and high stutter as appropriate.

If a profile is generated that contains one called allele but there are peaks between 25-49 RFU that would show evidence of a mixture; the profile will be reported as consistent with being a mixture. The report would state insufficient for STRmix analysis but may be used for comparison (inconclusive or elimination). The peaks between 25-49 RFU shall be used to determine the assumption of the number of contributors.

Note: the limit of detection is the average amount of machine noise plus 3 standard deviations.

In circumstances when the number of contributors cannot be adequately assessed, or when there are greater than 4 unknown/ unconditioned contributors without a clear major contributor being present, the profile is reported as too complex to interpret.

After analysis of the STRmix™ MCMC (Markov Chain Monte Carlo) output, it may be determined that the deconvolution does not conform to scientific expectations and may be re-run under a different number of contributors/iterations. In this event and with TL approval, the analyst's assumptions are documented on DNA QR-308 Changed and/or Not Reported Interpretations, the original STRmix™ printout is DNR'd and kept in the case jacket, and the electronic file of both deconvolutions is retained in the STRmix™ server's casework folder.

33.17.10.2 Method for assigning the number of contributors for a profile

The following steps are followed to assign the likely number of contributors to a profile:

1. Review the profile as a whole, assessing the level of degradation, presence of low level peaks, noisy or clean baseline and general quality (template) of the profile.
2. Identify likely stutter peaks (both forward and back) by reference to DSS allele stutter ratio (SR) expectations (plots of SR per each Allele).
3. Find the locus with the highest number of unambiguous allelic peaks, A. If A is an odd number, add 1. A/2 gives the initial assessment of the number of contributors to the profile.
4. Review peak height imbalances at the most informative locus (greatest number of alleles). Taking into account potential allele sharing among contributors, visually attempt to 'pair' alleles and assign them to contributors. If there is too much imbalance between alleles, this may mean the presence of an additional contributor(s) above that indicated by allele count alone. Refer to figure 1 for an indication of expected heterozygote balance at varying peak heights.
5. If one or more contributors at this locus are either minor or a clear major, check that this pattern is represented at other loci.
6. Apply the general pattern of contributors (number and proportion) to other loci in the profile. If it holds, assign this number of contributors to the profile; otherwise consider the addition or subtraction of one.
7. Analysts may utilize the Contributor Estimator Macro to assist in determining the number of contributors to a profile. (See WI-34 Step 16 under "Analyst".)

Notes

1. Discriminating loci such as FGA and SE33 are particularly informative for determining the number of likely contributors to a profile; however important information can be gleaned at any locus.
2. The presence of one or two minor peaks can sometimes be indicative of drop-in and not a true additional contributor.
3. Non-artifact peaks below 50 RFU, but above the limit of detection, may be useful to identify the presence of low level/minor additional contributors where genotype assignment of contributor(s) by STRmix would be impacted. Peaks < 50 RFU are not used for NOC determination where there is a reasonable expectation that all genotypes associated with the contributor(s) of the reportable alleles can be identified by STRmix, i.e., reported alleles for contributors are all above the ST and genotype assignment is unambiguous. When peaks <50 RFU are used to infer an extra contributor there should be data elsewhere in the profile to support this. The effects of the mis-

assignment of the number of contributors are typically restricted to false exclusions of true contributors and false inconclusive LRs.

33.17.11 Highly Degraded/Low Template Samples:

33.17.11.1 Highly degraded/low template samples are interpreted with caution. Degraded samples may "appear" to exhibit high stutter and greater –A at the smaller amplicons with off-scale data. In addition, degraded/low template samples may exhibit unbalanced heterozygous alleles and locus/allele dropout. Partial profiles are interpreted as determined by the data present for each locus. Given the possibility of stochastic effects, especially with low quantity and/or degraded samples, results from low template amplifications are interpreted with caution. Note that stochastics effects can be different for each contributor to a mixture based on the quantity/quality of the template.

33.17.12 Inhibited Samples:

33.17.12.1 Inhibited samples may exhibit allele, locus, or complete profile dropout, unbalanced heterozygous alleles, and inter-locus peak imbalance. To possibly overcome an inhibitor, smaller quantities of sample may be re-amplified. Quantitation data may aid in determining whether re-amplification is warranted as well as the amount of sample to be re-amplified. Before the genotyping amplification is performed, an evaluation of the Quantifiler Trio IPC for each sample is useful. The Quantifiler Trio IPC result for a sample being undetected or having an elevated C_T may indicate that a sample has an inhibitor present.

33.17.13 Qualitative Interpretation of STR Results: The following is a general description of the standard DNA report conclusions. Other conclusions may be reported as warranted by the results with approval of the technical leader (see DNA SOP-6.

33.17.13.1 Elimination: Conclusion reported in two situations: (1) When it has been determined by manual comparison that the known sample is not the source of, or a contributor to, the DNA profile detected from item #xx. The known sample has alleles that are not detected in the evidentiary sample and the lack of detection is not reasonably explainable by degradation/inhibition/allele dropout (stochastic effects) within the evidentiary profile. (2) When the LR is less than 1 by STRmix™ analysis.

33.17.13.2 Inconclusive: No conclusion can be drawn from the comparison between the known sample and the evidentiary sample due to uncertainty. There are 2 reasons for inconclusive results:

33.17.13.2.1 The likelihood ratio obtained to a STRmix deconvolution is greater than or equal to 1 but less than 10,000.

33.17.13.2.2 MCMC secondary diagnostics did not perform as expected: "the MCMC deconvolution process did not meet scientific expectations; therefore the comparison to Known A is inconclusive."

Note: If a known individual can be manually eliminated as a source of/contributor to a profile that has sub-par MCMC secondary diagnostics, this elimination is reported.

33.17.13.3 Positive Associations

33.17.13.3.1 Cannot be eliminated (CBE): An individual cannot be eliminated (CBE) as the source of (or a contributor to) a DNA profile if there is a strong positive association between the known sample and the evidentiary sample (see calculation of likelihood ratios) but some of the alleles present in the known sample are not detected in the evidentiary sample above the analytical threshold. In this instance, STRmix considers the probability of dropout taking into consideration peak height data, overall quantity of template DNA, information regarding the extent of degradation, inhibition, stochastic effects, and potential masking by stutter or contributor ratio. An LR ≥ 10,000 is obtained using STRmix.

33.17.13.3.2 Consistent with Source: An individual is consistent with being the source of a single-source DNA profile if all alleles consistent with the known sample are detected (≥50 RFU) in the evidentiary profile at all loci where results are generated (see report templates). An LR ≥ 10,000 is obtained using STRmix.

33.17.13.3.3 Inclusion: An individual is included as a potential contributor to a mixture DNA profile if all alleles consistent with the known sample are detected (≥50 RFU) in the evidentiary profile at all loci where results are generated (see SOP-6. An LR ≥ 10,000 is obtained using STRmix.

33.17.13.4 No STRmix Interpretation: STRmix is unable to be run to obtain a likelihood ratio to a deconvolution from a questioned sample. There are 2 reasons for this:

33.17.13.4.1 The mixture too complex. With > 4 unknown (unconditioned) contributors, or if the analyst is unable to determine the number of contributors with confidence. An exception to this is when a mixture has a clear major contributor (SOP-33.17.18).

33.17.13.4.2 Insufficient data is obtained for the questioned DNA Profile. This determination is made for low-level, partial DNA profiles when there is insufficient data from

the evidentiary DNA profile for comparison to a particular known using STRmix™. DNA profiles with only one resulting allele are unable to be run through STRmix. This includes a low level contributor(s) associated with a clear major contributor. With TL approval, other low level profiles can be deemed insufficient for comparison to a reference sample without being run through STRmix. If this determination is made after a STRmix likelihood ratio has already been calculated, document the non-reporting of the likelihood ratio on DNA QR-308 Changed and/or Not Reported Interpretations. The STRmix electronic files shall be maintained in the case folder, and the STRmix printouts shall be DNR'd, and remain in the case jacket.

Note: If a known individual can be manually eliminated as a source of/contributor to a profile insufficient for STRmix™ interpretation, this elimination is reported.

33.17.14 Interpretation of single source DNA profiles

33.17.14.1 For single source partial profiles with peaks ≥ 50 RFU, report a positive association with potential dropout when the observed allele(s) are consistent with the known source and the LR ≥ 10,000.

33.17.14.2 Note: for low-level profiles in situations where no loci have more than 2 peaks above the AT [e.g. such as if there is unexpected "heterozygous" peak imbalance], it is important to consider if the profile could be a partial composite of 2 or more individuals.

33.17.14.3 Note: Report "undetermined" in the "Type" column of the Testing or Conclusions Summary table when the analyst is unable to determine whether a single source profile is male or female if the Amelogenin X peak is below the stochastic threshold and no Y peaks are detected. For single source profiles where the individual is assumed, the "Type" would be reported as "Male" or "Female" in the Conclusions Summary table as appropriate (peaks above or below the stochastic threshold).

33.17.15 Interpretation of DNA mixtures

33.17.15.1 The analyst considers the quantity of DNA amplified, the peak heights of the alleles, the extent of degradation/inhibition, the impact of stutter, potential carryover between fractions in a differential extraction, and if a known profile compared to the mixture can be objectively determined to be a contributor such as for intimate samples.

33.17.15.2 If one person can be objectively determined to be included in the mixture given an intimate sample origin, the mixture may be evaluated by a consideration of what

loci have DNA profile results (above the AT) that could not be from the known source.

33.17.15.3 Incomplete separation: In mixtures from samples that were subjected to the differential extraction protocol, the mixture is evaluated by a consideration of whether the results are consistent with incomplete separation from the other fraction (e.g., A vs B fractions).

33.17.15.4 The ratio of male:female DNA and the extent of degradation in a sample as determined by Quantifiler Trio and the STR results.

33.17.15.5 Peak height consistency, i.e. the peak heights in the evidentiary profile potentially attributable to the known DNA profile should generally be consistent at all loci applicable. If not, this may not optimally fit the CBE conclusion (note: this may not apply to degraded profiles).

33.17.15.6 The "zygosity" of the known: homozygous peaks are generally higher than heterozygous peaks of the same contributor. Therefore, homozygous peaks are less likely to dropout as compared to one allele from a heterozygote.

33.17.15.7 The total number of dropouts (overall and at each locus) that would be required to still be consistent with a CBE conclusion: While allele dropout (below the stochastic threshold), can occur at any locus, extensive validation and case experience has shown that smaller amplicons amplify more efficiently and are less affected by degradation than are larger amplicons. Therefore, the results at the smaller amplicons can be particularly diagnostic.

33.17.15.8 To what degree (at how many loci) would a putative low-level contributor coincidently be included by a high-level contributor due to overlap? This is particularly important to consider with mixtures involving relatives.

33.17.15.9 Mixture DNA profiles that involve relatives are interpreted with caution.

33.17.16 Statistics – Likelihood ratios

Determining hypotheses for calculating the likelihood ratio (LR)

An LR is calculated using STRmix™ Analysis software for forensic unknown DNA profiles as discussed in SOP-39 (see also STRmix™ SOP-32).

33.17.17 Reporting

See DNA SOP-6 for a general discussion regarding reporting profile results.

33.17.17.1 Likelihood Ratio

The 99% 1-sided lower HPD (highest posterior density) LR value is reported for F6C DNA profiles using STRmix™ for the population with the lowest LR. Exceptions can be made if the scenario indicates an alternate LR is more appropriate. Likelihood ratios are rounded down when reported as listed in the table below:

<1	Elimination, no LR reported
1-9,999	Comparison inconclusive, no LR reported
10,000 – 100 billion	LR rounded down—use up to two significant figures
>100 billion	Ceiling—report LR is at least 100 billion

33.17.17.2 Reporting Comparisons using STRmix™ (see DNA SOP-6 for Report Templates)

33.17.17.2.1 LR obtained is less than 1:

The likelihood of obtaining the profile is greater under H_2 than H_1); reported as an exclusion of the individual considered in H_1.

Note that reference samples may also be eliminated through manual comparison to the questioned sample.

33.17.17.2.2 LR obtained is ≥ 1 but less than 10,000; the comparison is inconclusive.

33.17.17.2.3 LR obtained is ≥ 10,000; the comparison is a positive association.

33.17.17.2.4 For samples that are identical to one another, there is no need to run STRmix again.

33.17.18 **5+ Person Mixtures with a Clear Major Profile**

33.17.18.1 During analysis and number of contributor estimation, an analyst can determine (with TL and AD approval) that a 5 or more person mixture has a clear major contributor. The following criteria for this must be met at at least 10 loci. If any of these criteria are not met, the locus does not qualify for statistics:

33.17.18.1.1 All of the alleles of the major contributor are at least 500 RFU.

33.17.18.1.2 The heterozygous peak balance of the major profile ≥ 65%

33.17.18.1.3 For an apparent heterozygous major, the height of the highest minor allele is ≤ 33% of the height of the lowest major allele

33.17.18.1.4 For an apparent homozygous major, the height of the highest minor allele is ≤ 25% of the height of the major allele

33.17.18.2 After determining the number of contributors to be 5 or greater, the analyst will determine if a clear major profile is present.

33.17.18.3 The exported allele table text file will be imported into the Fusion 6C Deconvolution Workbook, "CT DSS" is selected as the laboratory, the sample selected, and "5+" selected for contributors. Click "Calculate". If a clear major contributor is present at 10 or more loci, the "Major from 5P+ mixture" button becomes active. Clicking on that button will bring you to DNA-QR-307d Five or More Person Mixture Major Deconvolution.

33.17.18.4 Print DNA-QR-307d Five or More Person Mixture Major deconvolution, and keep a part of the case record. TL and DD approval will be recorded on this QR. The presence of a clear major profile will be noted on DNA-QR-302 Contributor Estimation Worksheet.

33.17.18.5 Qualitative comparisons to known profiles: Known profiles are compared to the clear major profile as described in 33.17.13.

33.17.18.5.1 The alleles of the major profile should typically be called at the remaining loci that do not qualify for statistics. Note: this is not checked by the deconvolution macro.

33.17.18.6 Statistics for Clear Major Profiles:

33.17.18.6.1 If a known sample is positively associated with a clear major profile, a binary LR (1/RMP) for the major contributor (using the Popstats database with posterior means distribution, NRC 4.2, and a factor of 10 lower bound confidence interval) is calculated. The population with the lowest LR is reported.

33.17.18.6.2 Repeat 33.15.2.1, and click on "LR for Major". DNA-QR-307h, Likelihood Ratio, is printed out for the case jacket. "Min LR/10" will be reported.

33.17.18.6.3 Since statistics are able to be calculated, profiles that have a clear major are eligible for CODIS, with CODIS Administrator approval.

33.17.18.7 Reporting Out Complex Mixtures with Clear Majors

33.17.18.7.1 Reports without comparisons to knowns: Major profile will not be included in the Testing Summary table, or the Appendix. Remarks section will not discuss complexity of this mixture.

33.17.18.7.2 Reports with comparisons to knowns: Both profile and major profile will be included in Testing Summary table. If there is a known consistent with the source of the major profile, the comparison to the full profile is N/A. If there is a known eliminated as the source of the major profile, the Testing Summary table will note "No Comparison" to the full profile.

33.17.18.7.3 5+ person mixture wording: see DNA SOP-6.

33.17.19 Criminal Parentage Testing:

For criminal parentage testing, standard statistical methods for autosomal STR results will be used as described below:

33.17.19.1 The statistic calculated (for inclusions) is the expected frequency (parentage inclusion probability) of individuals who could contribute the paternally (or maternally) transmitted alleles. The formula used is: $p^2 + 2p(1-p) = 2p-p^2$, where p is the frequency of the obligatory allele.

33.17.19.2 No statistics are required for non-matches. Due to the occurrence of mutations between generations, an individual must be excluded at more than two loci in order to be eliminated as a potential parent. Regarding apparent mutations using STR systems, the repeat # difference of the putative mutation (child's obligatory allele vs. alleged parent's alleles typically varies by +/- 1 repeat) may be relevant to the final conclusion. In the event of apparent mutations, additional testing may be conducted as appropriate.

33.17.19.3 Statistics statement for paternity (RMNE): "The expected frequency of individuals who could be the father of NAME is less than 1 in xx in the general male population" (using the population with the most common match probability). The RMNE ceiling that is reported for Fusion 6C criminal parentage testing is 1 in 100 billion.

33.17.9.4 In the event that the evidentiary sample is a maternal/child mixture, the fetal/child component is deconvoluted as described in DNA SOP-21using the CT DSS analytical and stochastic thresholds.

33.18 Yfiler Analysis:

33.18.1 This procedure is a general guideline for the analysis and interpretation of Y-STR profiles when using the Yfiler (YF) STR kits. These procedures generally follow those outlined in ABI Prism GeneMapper ID Software v3.2 User Guide.

33.18.2 When the Yfiler kit is employed, seventeen Y-STR loci; DYS391, DYS389I, DYS439, DYS389II, DYS438 (5 base repeat), DYS437, DYS19, DYS392 (3 base repeat), DYS393, DYS390, DYS385 a/b (2 loci amped with one set of primers), DYS456, DYS458, DYS635, Y-GATA H4, and DYS448 (6 base repeat) are typed in a single PCR amplification reaction according to manufacturer's and laboratory protocols.

33.18.3 Yfiler amplification products are separated and detected using an ABI 3130xl Genetic Analyzer and the supplied Run 3130xl Data Collection v4.0 and GeneMapper ID v3.2 (GMID) softwares.

33.18.4 For the GMID software for YF, the Analysis Method is as outlined in the user's manual except for the following: Under the Allele Tab, confirm that the Bin set is for the appropriate kit and that Marker Specific Stutter Ratio is used. Note: The Yfiler Kit contains one locus that is a 3 base repeat, one locus that is a 5 base repeat, and one locus that is a 6 base repeat, so there are some extra parameter values needed for the Analysis Method under the Allele Tab, Marker Repeat Type. Under the Peak Detector Tab, the Peak Detection Algorithm is Advanced, the Analysis Range is Partial Range but shall include the full range of required size standard peaks, the Sizing Range is Partial Sizes with the Start Size set to 75 and the Stop Size set to at least 400. Smoothing is set to Light and the Size Calling Method is set to Local Southern Method. For the Panel, use Yfiler_v2. For the Size Standard, use CE_G5_HID_GS500. In panel manager, confirm that the marker specific stutter thresholds match those listed in DNA SOP-29 for the Yfiler amplification kit.

33.18.5 Analysis of Y-STR DNA Profiles using GMID:

33.18.5.1 The results are evaluated for each locus. However, considering the data from all loci tested will assist in the overall interpretation of the results for each sample. See DNA SOP-6 for standard report conclusions.

33.18.5.2 Off-Scale Data: If too much DNA is added to the amplification reaction or is loaded onto the 3130, the fluorescent intensity for the PCR products may exceed the linear dynamic range for detection by the instrument. Samples with off-scale peaks may exhibit raised baselines, split peaks, increased minus A peaks, excessive pull-up and/or higher than expected stutter levels, and/or amplification artifacts in one or more of the colors. Examine the peak heights in GMID software. Peaks greater than 5000 RFU may be off-scale.

33.18.5.3 Peak identification: The laboratory's analytical threshold (AT) is 50 RFU. The identity of peaks called by GMID is generally assigned to one of the following categories:

33.18.5.4 Allele Peak: A called allele has a peak height ≥ 50 RFU, a fragment size that falls within the base pair range, and has the appropriate dye color for the locus. However, not all peaks ≥ 50 RFU are typed as alleles. Stutter peaks, pull-up/pull-down peaks, background noise, and amplification or injection artifacts could fall above the RFU threshold, especially if the allele peaks are high. Peaks ≥ 50 RFU that are not called by GMID may be called by the analysts on a case-by-case basis. Such peaks are interpreted with caution.

33.18.5.5 Detection of variant alleles: Variant alleles have been identified from many STR loci. These alleles may contain full or partial repeats. Variant alleles are labeled as Off Ladder (OL) alleles in GMID. If a particular variant has been observed before by the DESPP CT Division of Scientific Services (CTDSS), ThermoFisher (Life Technologies), NIST, or in published literature, the sample does not need to be repeated. In GMID, change the OL to the proper allele call.

33.18.5.6 Stutter peaks (N-2, N-3, N-4 N-5, N-6, N+2, and N+3—depending on the locus): A stutter peak has a fragment length typically four base pairs smaller than the true allele (see exceptions above). If the peak height in the stutter position is less than the user-defined stutter threshold, the peak is considered a stutter peak and is filtered out using the GMID program. Any peaks < the CT stutter threshold are manually edited as required. (see DNA SOP-29. Peaks in the stutter position that are below the Laboratory's stutter thresholds are not listed on the report and are not used for statistical purposes. If the height of the peak in the stutter position is greater than the CT stutter thresholds, this indicates a possible mixture, an overloaded sample, or a higher than normal stutter ratio. Additional information provided by other loci may be of assistance in these cases.

33.18.5.7 Minus A Peaks: AmpF*l*STR kits are optimized to add an extra nucleotide to the 3' end of the PCR product. The nucleotide is predominantly adenosine (A). Lack of a full A addition may be observed especially when the amount of input DNA is greater than approximately 2.5 ng. However, sample degradation can increase the

effective concentration of a small amplicon relative to the larger amplicons, leading to minus A product at the smaller loci. Data from other loci may be of assistance in these cases.

33.18.5.8 Pull-up Peaks: Pull-up peaks are generally seen when excess amplification product is injected on the CE or when the spectral calibration is sub-optimal. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-up peaks are often displayed in several colors. Pull-up peaks should have approximately the same fragment size in each of the colors as the true allele peak. Additional information provided by other loci may be of assistance in these cases. When evaluating allelic peaks that may contain a pull-up (PU) contribution, an estimate of the PU contribution is subtracted from the height of the potential allelic peak to determine if the remaining peak height is sufficient for allele or asterisk designation. The estimated pull-up contribution is determined by evaluating the height of unambiguous pull-up peaks (pull-up with e.g., no stutter or allelic contribution) at each locus where pull-up is detected for a particular color.

Example: The percentage of pull-up peaks as compared to the height of the peaks causing the pull-up is calculated. (PU percentage = PU peak height/height of the peak causing the PU). This percentage is converted to an RFU value (multiply percent by height of peak causing the pull-up) which is then subtracted from the potential allelic peak in question. The resulting "subtracted" peak is called where DNA Section allele calling criteria are met. The same methodology would be applied to potential allelic peaks with a pull-down component (see below).

33.18.5.9 Pull-down Peaks: Pull-down peaks are generally seen when excess amplification product is run on the CE or when the spectral calibration is sub-optimal for the run. The greater fluorescent intensity causes uncompensated spectral overlap. Pull-down peaks can be displayed in several colors. Pull-down peaks can have approximately the same fragment size, but often the pull-down peak will be slightly a larger or smaller size as compared to the true allele. Additional information provided by other loci may be of assistance in these cases.

33.18.5.10 Spikes: fluorescent spikes can be seen within GMID data. Spikes are typically sharp peaks caused by air bubbles or polymer crystals in a capillary, which can be displayed in different colors, and have approximately the same "fragment size". Additional inspection of the raw data or re-injection may assist in cases where spikes are believed to be present.

33.18.5.11 Dye Artifacts/Dye Blobs: Broad peaks may be the result of dissociated dyes or fluorescent particles that are trapped within the polymer matrix and then fluorescently excited by the laser. Additional inspection of other samples within

the module may assist in interpretation in cases where dye blobs are believed to be present.

33.18.5.12 Amplification Artifact Peaks: Nonspecific amplification product peaks, generally with good peak morphology, may result when excessive template DNA is amplified.

33.18.5.13 High Background: Background fluorescence may reach an RFU level above the calling threshold. Generally such high background peaks exhibit atypical peak morphology.

33.18.5.14 Raised Baseline: Elevated baseline of one color over a broader (multiple bin) scan range may occur. Note: pull-down from two adjacent peaks may cause raised baseline between the pull-down peaks; the "pull-down" designation should be used for the editing notation when raised baseline is caused by pull-down.

33.18.6 Highly Degraded/Low Template Samples:

Highly degraded/low template samples are interpreted with caution. Degraded samples may "appear" to exhibit high stutter and greater –A at the smaller amplicons with off-scale data. In addition, degraded/low template samples may display unbalanced heterozygous alleles and locus/allele dropout. Partial profiles may be interpreted as determined by the data present for each locus. Given the possibility of stochastic allele sampling, especially with low quantity and/or degraded samples, single peaks (at DYS385) less than the stochastic threshold are interpreted with caution (pertains to putative haplotype assignment at that locus).

33.18.7 Inhibited Samples:

33.18.7.1 Inhibited samples may exhibit allele, locus, or complete profile dropout, unbalanced alleles at DYS385, and interlocus peak imbalance.

33.18.7.2 Input of excessive female DNA may result in inhibition of Y-STR amplification.

33.18.8 X-homologous peaks:

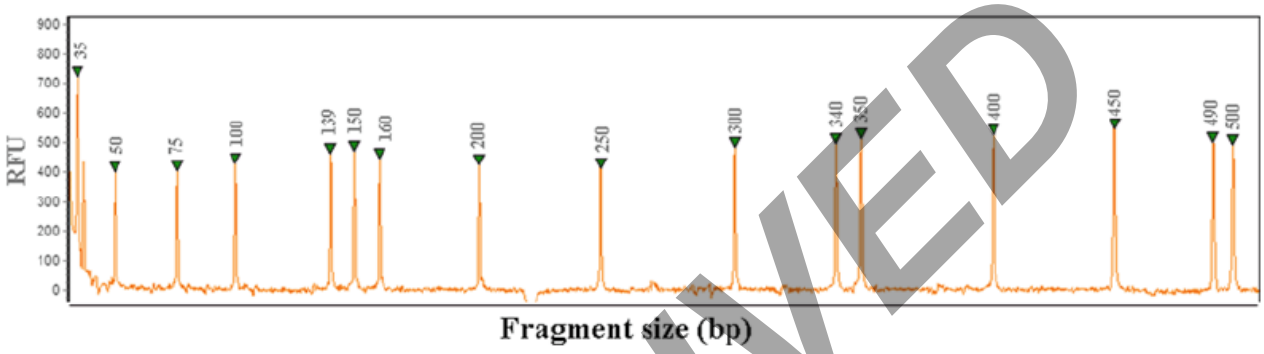
Rarely, (especially with large quantities of female DNA) the Y primers may generate X-homologous or non-specific peaks. If the known female sample is amplified, any X-homologous peaks may be removed from the evidentiary DNA profile.

33.18.9 General Analysis of Yfiler Results

33.18.9.1 The standard analysis parameters for samples and controls are B,G,Y,R, O = 50 RFU. Profiles (forensic unknowns) with peaks >3000 RFU are interpreted with

caution with approval of the TL. Known profiles > 5000 RFU are interpreted with caution with approval of the TL.

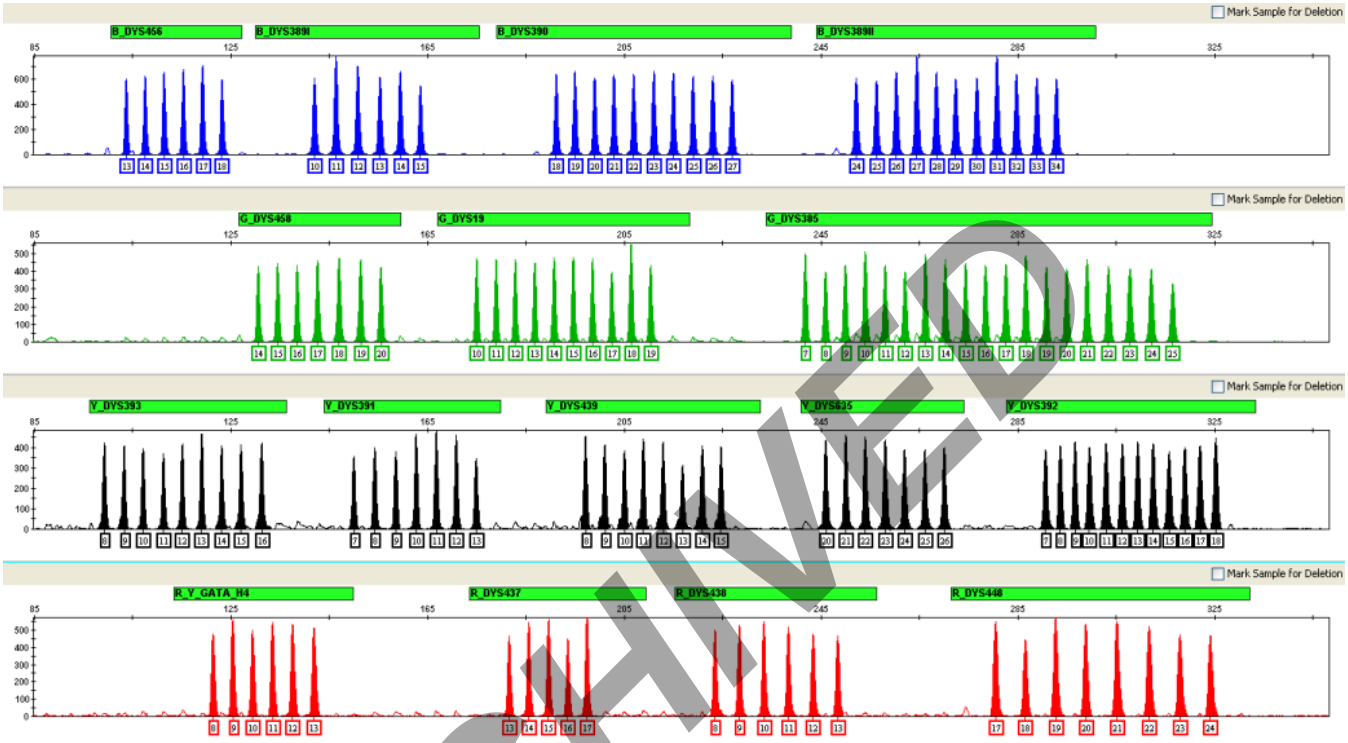
33.18.9.2 Ensure that all required internal lane size standard (75-400) peaks are called in all samples.



33.18.9.3 If a sample's internal size standard fails due to a low sizing quality (e.g. pull-up peaks into the size standard), the option to override the sample's size quality is available and shall be noted on the analysis worksheet. Both the primary and the second analyst must confirm that the size quality failure was not due to the loss of internal lane size standard peaks. The sample may then be analyzed as normal.

33.18.9.4 Ensure that each ladder in each injection has all allele peaks detected above the minimum threshold. If a ladder does not meet these requirements, change the sample type from allelic ladder to sample. One ladder in each injection must meet the requirements.

33.18.9.5 The Yfiler Allelic Ladder should appear as follows:



33.18.10 Y-STR Control Requirements:

33.18.10.1 Import the appropriate sample files including controls (RB, NEG, EP1*, POS control DNA 007 for YF) and at least one ladder. At least one set of controls (for each amplification set) must give the expected results (see DNA SOP-29).

*With extraction sets where a male laboratory control (EP1) is not available, using the YF kit positive control is sufficient.

33.18.10.2 Potential contamination is assessed by evaluating the evidentiary samples, RB, NEG, and positive controls. All samples within a batch will be cross-checked against each other to detect potential contamination. An excel macro is available to assist with batch cross-comparisons.

RB and NEG samples should not have any callable peaks. If callable peaks are detected and the evidentiary samples are not affected, the set of extracted samples may be interpreted with caution with TL approval. If sub AT peaks are detected and the evidentiary samples are not affected, the set of extracted samples may be interpreted with caution.

If contamination is detected, depending on factors such as the type and extent of contamination and whether the source of the contamination can be identified, the contaminated sample(s) and/or amplification sets may be interpreted with caution in the report or deemed unsuitable for comparison following TL review and approval. Contaminated profiles that are deemed unsuitable for comparisons are reported out as such.

33.18.10.3 Positive Control Profiles: Ensure that all alleles were included.

33.18.10.4 If a peak is labelled as off ladder (OL) or is outside the ladder region and therefore not labelled by the GMID software, review the data to determine that it is a true microvariant or off-ladder allele.

33.18.10.5 The analytical threshold is 50 RFU and the stochastic threshold (ST) is 150-250 RFU for DYS385a/b (see table below).

33.18.10.6 For Yfiler, the stochastic threshold is the peak height above which one can expect that both peaks at DYS385a/b would be observed.

Injections Time Ranges

Stochastic Threshold	3130-1	3130-2	3130-3	3130-4	3130-6
150 RFU	2 – 18 sec	2 – 11 sec	2 – 10 sec	2 – 11 sec	2 – 8 sec
200 RFU	19 – 29 sec	12 – 18 sec	11 – 16 sec	12 – 18 sec	9 – 13 sec
250 RFU	30 – 35 sec	19 – 22 sec	17 – 20 sec	19 – 22 sec	14 – 16 sec

33.18.11 Determination of Single Source or Mixture Profile & Number of Contributors

Single Source Profile Attributes: A single source profile will generally have no more than 1 allele at any locus except DYS385a/b (typically 2 alleles). Expected peak balance at DYS385a/b decreases as a function of peak height similar to that seen with autosomal loci. In rare instances, an individual may exhibit a bi/tri-allelic pattern at a locus.

When an extra-allelic pattern is identified, the results must be documented in the case file. The locus is able to be searched in the YHRD database.

33.18.11.1 Determination of the number of contributors (different Y lineages) to the DNA profile.

33.18.11.1.1 Evidence profiles are assessed for the number of possible contributors. Data above/below the Stochastic Threshold at the DYS385a/b locus, the amount of visible drop out (peaks observed below analytical threshold but clearly discernable from noise (25-49 RFU), peak heights of called alleles, etc., are evaluated when making this determination as discussed below:

The following steps are followed to assign the likely number of lineages in the profile:

1. Review the profile as a whole, assessing the level of degradation, presence of low level peaks, noisy or clean baseline and general quality (template) of the profile.
2. Identify likely stutter peaks by reference to DSS allele stutter ratio (SR) expectations (plots of SR per each allele).
3. Find the locus with the highest number of unambiguous allelic peaks, A. A gives the initial assessment of the likely number of contributors to the profile.
4. Review peak height balance at DYS385.

The detection of more than one allele per locus (other than the DYS385 locus) is generally indicative of a mixed sample. Variation in peak heights between alleles between loci and at DYS385 may assist in the interpretation of such results. Typically, if a maximum of 2 alleles are observed at a locus (except for DYS385), the DNA results are consistent with a mixture of two male lineages. Two peak patterns (at a single locus other than DYS385) have been observed from single source samples, but they are rare. The analyst should consider the possibility of high stutter peaks, which can complicate interpretation. While counting allele peaks is critical in determining the number of contributors, the analyst must also consider that allele sharing between different haplotype lineages may result in an underestimation of the actual number of contributors. The number of lineages identified reflects the analyst's assessment of the most likely number of different haplotypes required to reasonably explain the observed profile.

A Y-STR mixture simulation tool has been validated by the Division of Scientific Services to assist in the determination of the number of haplotypes that are present in Yfiler profiles. This tool may be used by analysts to demonstrate the expected amount of allele sharing at each locus per contributor number in the absence of dropout.

Note: Non-artifact peaks below 50 RFU (25-49 RFU) are evaluated when assessing the number of contributors to the profile.

33.18.12 Qualitative Assessment of Yfiler Profiles—Comparisons to Knowns

33.18.12.1 Single source haplotypes. If 1 peak was called ≥ 50 RFU) (except at DYS385) with nothing else detected above 25 RFU at any locus), and "Known A" has that allele, the results would be consistent with "Known A" being the source. One would consider the issue of "heterozygous" allelic peak imbalance at DYS385. Therefore, with good peak balance: the results would be consistent with "Known A" being the source of the DNA profile for item #xx. Significant peak imbalance (> 50%) at DYS385, may be indicative of a mixture.

33.18.12.2 For single source partial profiles with peaks ≥ 50 RFU, report a CBE interpretation when partial results are detected at DYS385, as long as the observed alleles are consistent with the known source.

Note: in situations where no loci have more than 1 peak above the AT (except 2 peaks at DYS385 [e.g. such as if there is significant peak imbalance (> 50%)], it is important to consider if the profile could be a mixture (partial composite of 2 or more individuals).

33.18.13 Interpretation of DNA mixtures

33.18.13.1 The analyst considers the quantity of DNA amplified, the peak heights of the alleles, the extent of degradation/inhibition, the impact of stutter, potential carryover between fractions in a differential extraction, and if a known profile compared to the mixture can be objectively determined to be a contributor (e.g. intimate samples).

33.18.13.2 Factors taken into consideration when interpreting mixtures:

33.18.13.2.1 If one person can be objectively determined to be included in the mixture (e.g. consensual partner), the mixture is evaluated by a consideration of what loci have DNA profile results (above the AT) that could not be from the known source.

33.18.13.2.2 The total number of dropouts (overall and at each locus) that would be required to still be consistent with a CBE conclusion. While allele dropout (including peaks below the stochastic threshold at DYS385), can occur at any locus, extensive validation and case experience has shown that smaller amplicons amplify more

efficiently and are less affected by degradation than are larger amplicons. Therefore, the results at the smaller amplicons can be particularly diagnostic.

33.18.13.2.3 To what degree would a putative low-level contributor coincidently be included by a high-level contributor due to overlap.

33.18.13.2.4 If the analyst and reviewers feel that the above rules do not adequately apply to a specific mixture comparison, consult the TL.

33.18.13.2.5 Y-STR profiles will be searched against the Staff Index and documented on DNA QR-20 Staff Search Worksheet.

33.18.14 General Report Conclusions for Y-STR Results:

33.18.14.1 Y-STR report templates are described in DNA SOP-6.

33.18.14.2 Elimination: used when the known sample is not the source of, or a contributor to, the DNA profile detected from item xx. The known sample has alleles that are not detected in the evidentiary sample and the lack of detection is not reasonably explainable by degradation/inhibition/allele dropout (stochastic effects) within the evidentiary sample.

33.18.14.3 Inconclusive: No conclusion can be drawn from the comparison between the known sample and the evidentiary sample due to uncertainty. There are two reasons for inconclusive results. See 33.18.14.3.1 and 33.18.14.3.2 below:

33.18.14.3.1 Based on limited data: This statement is made when the data does not provide sufficient support for any conclusion regarding a comparison of a mixture to a particular known.

33.18.14.3.2 Mixture too complex: This statement is made for mixtures of 3 or more male lineages where the known is not eliminated.

33.18.14.4 Cannot be eliminated (CBE) as the source of, or a contributor to a DNA profile: This conclusion is used when there is a strong correlation between the known sample and the evidentiary sample, but some of the alleles present in the known sample are not detected in the evidentiary sample above the analytical threshold. In this instance, dropout is a reasonable explanation taking into consideration peak height data, overall quantity of template DNA, information regarding the extent of degradation, inhibition, stochastic effects, and masking by stutter or contributor ratio.

33.18.14.5 Inclusion: All alleles consistent with the known sample are detected (≥ 50 RFU) in the evidentiary profile at all loci where results are generated (See 33.18.15 for statistics).

33.18.15 Y-STR Statistics: (see 33.18.16 - YF Workflow and qualification of loci for statistics)

33.18.15.1 For single source Y-STR profiles statistics are calculated at all loci where DNA results are obtained above the analytical threshold (except for DYS385). The Y-STR random match probability is calculated using the counting method. A haplotype frequency is generated by counting the number of times a haplotype has been observed in the population database divided by the total number of haplotypes in the Database. Haplotype frequencies are given an upper bound 95% confidence interval (See DNA SOP-6. The Y-Chromosome STR Haplotype Reference Database (YHRD) Y-STR population database (available at https://yhrd.org) is used.

33.18.15.2 On the YHRD website homepage, click "Search the Database" on the upper left of the site. From there, click on "Manually enter the haplotype/haplotypes to search for". "Yfiler" is the default for the order the loci are listed in, which can be adjusted if needed. Enter the haplotype, with a comma separating the two alleles (even if a homozygote) at DYS385, then click "Search". When search is complete, click "Add feature to this report" and select "National Database (with Subpopulations, current SWGDAM-compliant version). Click the "X" to close the "worldwide" results before printing page for the case jacket.

33.18.15.3 An exact upper bound 95% confidence interval (Clopper-Pearson method) is calculated from the results of a database search in order to estimate the population frequency of a haplotype using the population (United States data set: African American, Caucasian, or Hispanic) with the most common haplotype frequency. The upper bound estimate is dependent on the size of the database, and these estimates may change as the database size changes.

33.18.15.4 The formula used to calculate the 95% upper bound frequency estimate is:

$$\sum_{k=0}^{x}\binom{n}{k}p_0^k(1-p_0)^{n-k}=0.05$$

https://strbase.nist.gov/pub_pres/SWGDAM-Jan2012-YSTRs.pdf

Clopper, C. and Pearson, E. *The use of confidence or fiducial limits illustrated in the case of binomial,* Biometrika (1934) 26:404-413.

33.18.15.5 Y-STR haplotypes (and mtDNA haplotypes) are inherited independently from autosomal loci. Therefore, Y haplotype (and cumulative haplotype) frequencies may be combined with autosomal STR RMPs or CPIs using the product rule to generate an overall profile frequency estimate (applies to outsourced cases only with no STRmix analysis).

33.18.15.6 Y-STR Mixtures: (see 33.18.16--YF Workflow and qualification of loci for statistics)

33.18.15.6.1 The expected frequency of haplotypes that could contribute to a Y-STR mixture is calculated using a combined counting method (CCM). A cumulative haplotype frequency is generated by counting how many times each of the possible haplotypes are observed in the population Database, summing them and dividing by the total number of haplotypes in the database (i.e. cumulative haplotype frequency = x/N, where x is the number of potentially contributing haplotypes observed in a population and N is the total number of haplotypes searched in that population). An exact upper bound 95% confidence interval is then applied using the population (African American, Caucasian, or Hispanic) with the most common CCM frequency. Note: The frequency and confidence interval for a mixture profile are calculated using the software tool Y-Mix, which contains the U.S. portion of the YHRD database.

The cumulative haplotype frequency (with an upper bound 95% confidence interval) is calculated for samples with an additional allele at any locus, i.e., 2 alleles at DYS393, DYS19, DYS389II, DYS390, DYS391, or 3 alleles at DYS385.

33.18.15.6.2 The File for the Y-Mix software tool is located on the S drive. The loci in the Excel folder are in alphanumeric order. Enter the mixture profile under the appropriate loci, with one row per allele. Defaults (Limit database to samples with all the loci entered above?: Yes, Treat this profile as a single source sample?: No, Desired UCI: 95%, Use (x+1)/(N+1)?: No) are to be unchanged.

Click "compare the profile to the database" when profile entry is complete. Print page when results are obtained.

33.18.16 **YF Workflow and Qualification of Loci for Statistics**

33.18.16.1 Evaluate data at all loci (≥ 25 RFU) to determine if profile is a single haplotype or a mixture (see 33.18.11).

33.18.16.2 Qualify results for statistics at DYS385 using a ST of 150-250 RFU (based on injection time) and at other loci as discussed below. Statistics are calculated as described in 33.18.15 for single lineage YF profiles and 2 lineage mixtures only.

33.18.16.3 Compare known(s) to evidentiary profiles as in 33.18.12-15.

33.18.16.4 Single Haplotype Profile:

33.18.16.4.1 2 alleles detected at DYS385: use data (above & below ST) at DYS385 and other loci where included.

33.18.16.4.2 1 allele detected at DYS385: (A) Qualify locus if allele is above ST and use data for statistics at other loci where included. (B) Disqualify locus for statistics if allele is below ST and use data at remaining loci where included. Note: the known would be eliminated as the source if the known does not have the allele found in the evidence profile.

33.18.16.5 Mixture of 2 Haplotypes (CCM): Evaluate results at all loci as described in 33.18.16.1-3.

33.18.16.5.1 Mixture evident at DYS385 with < 4 alleles and all alleles above ST at DYS385: Qualify locus for stats and use data for statistics at other loci where the mixture is detected.

33.18.16.5.2 Mixture evident at DYS385 with < 4 alleles and some allele(s) below ST at DYS385: Disqualify locus for stats and use data at other loci where the mixture is detected. Note: the known (2 alleles at DYS385) would be eliminated as a contributor if the known does not have at least 1 allele found in the evidence profile. If the known has only l allele at DYS385, the known may not be eliminated depending on the results at other loci.

33.18.16.5.3 Mixture with 4 alleles at DYS385 and allele(s) above or below ST at DYS385: qualify locus for statistics and use data for statistics at other loci where the mixture is detected.

33.18.16.5.4 Mixture not evident at DYS385: Disqualify locus for statistics. Use data for statistics at other loci where the mixture is detected.

33.18.17 Deconvolution of 2 Male Lineage Mixtures

33.18.17.1 A major contributor to mixtures of 2 male lineages may be deconvoluted in lieu of the combined counting method (CCM) discussed in 33.18.16.5 with TL/DD approval as follows:

33.18.17.2 Deconvolution of mixtures at all loci except DYS385: A major contributor may be deconvoluted where the height of the major allele is at least 5X greater than the minor allele (minor is ≤ 20% of major). Example: if the minor allele is 100 RFU, the major allele must be ≥ 500 RFU.

33.18.17.3 Where only 1 allele is detected at any locus except DYS385, the "major contributor allele" must be greater than 150-250 RFU depending on the injection time (see 33.18.10.7). If not, the locus is not eligible for deconvolution.

33.18.17.4 To qualify the results at DYS385 for deconvolution: where 1 allele is present at DYS385, the major contributor may be deduced if the peak height is above the stochastic threshold and the mixture ratio at all loci where the mixture is detected is at least 5:1.

33.18.17.5 To qualify the results at DYS385 for deconvolution: where 2 alleles are present at DYS385, the "heterozygous" major contributor may be deduced if the peaks are above the stochastic threshold, the mixture ratio at all loci where the mixture is detected is at least 5:1, and "heterozygous" peak balance is at least 50%.

33.18.17.6 To qualify the results at DYS385 for deconvolution: where 2 alleles are present at DYS385 ("homozygous" major with significant peak imbalance), the major contributor may be deduced if the major peak is above the stochastic threshold and the mixture ratio at DYS385 is at least 5:1 (and at all other loci where the mixture is detected).

33.18.17.7 To qualify the results at DYS385 for deconvolution: where 3 alleles are present at DYS385 ("heterozygous" major): the major contributor may be deduced if both peaks are above the stochastic threshold, the mixture ratio of the lowest major allele to the minor allele is at least 5:1 (and at all other loci where the mixture is detected), and "heterozygous" peak balance is at least 50%.

33.18.17.8 To qualify the results at DYS385 for deconvolution: where 3 alleles are present at DYS385 ("homozygous" major): the major contributor may be deduced if the major allele is above the stochastic threshold and the mixture ratio of the major allele to the highest minor allele is at least 5:1 (and at all other loci where the mixture is detected).

33.18.17.9 To qualify the results at DYS385 for deconvolution: where 4 alleles are present at DYS385: the major contributor may be deduced if the two highest peaks are above the stochastic threshold, the mixture ratio is at least 5:1 (lowest major allele must be at least 5X greater than the highest minor allele), and "heterozygous" peak balance is at least 50%.

33.18.17.10 Once deconvolution is completed, the known(s) are compared to the profile of the deconvoluted major contributor (see 33.18.13 & 33.18.14).

33.18.17.11 If an individual is eliminated as a potential source of the major contributor but cannot be eliminated from the overall mixture (i.e. a potential minor contributor), the CCM statistic is calculated as discussed in 33.18.16.5.