31.1 Purpose:

The primary objective reflected in this section is for the analyst to render the best interpretation of the data.

31.2 Responsibility:

DNA Unit personnel.

31.3 STR Analysis and Interpretation:

This procedure is a general guideline for the analysis and interpretation of STR profiles when using the PowerPlex® Fusion 6C STR kit (F6C) and STRmix™ (see WI-34 for F6C/STRmix™ work flow overview and WI-35 for GeneMarker overview). However, it is not an exhaustive list of all possible casework scenarios. Analyst training, experience, and judgment must be considered when reporting STR profiles. STR data are interpreted by evaluating the results at all loci.

31.4 PowerPlex Fusion F6C System:

31.4.1 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.

31.4.2 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.

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

31.5 PowerPlex® Fusion 6C Analysis:

31.5.1 <u>Analysis of DNA Profiles in GM-HID</u>

31.5.1.1 The standard analysis parameters for forensic unknowns, knowns, ladders, and controls are B,G,Y,R,P,O = 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.

31.5.1.2 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.

31.5.1.3 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. These samples may need to be reinjected for a shorter time or diluted and re-amplified.

31.5.1.4 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:

31.5.1.5 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.

31.5.1.6 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. If a particular variant has been observed before by the DESPP CT Division of Scientific Services (CT_DSS), Promega, NIST, or in published literature, the sample does not need to be repeated. In GM-HID, change the OL to the proper allele call. However, if the repeat has not been observed, the sample may be re-amplified and/or reinjected to confirm the OB/OL allele as warranted.

31.5.1.7 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 were determined by evaluating the CT_DSS empirical stutter data on an allele-by-allele basis (see DNA SOP-6; Section 3). 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™ (see SOP 32).

31.5.1.8 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.

31.5.1.9 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).

31.5.1.10 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.

31.5.1.11 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.

31.5.1.12 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 Fusion 6C Appendices: Appendix 3: Fusion 6C Common dye artifacts).

31.5.1.13 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.

31.5.1.14 High Background: Background fluorescence may reach an RFU level above the analytical threshold. Generally such high background peaks exhibit atypical peak morphology. When appropriate, re-injection may assist with interpretation.

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

31.5.1.16 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. Whenever a non-specific amplification peak is suspected the TL should be consulted for interpretational input and for tracking of common artifact peaks to watch for.

31.5.2 Control Requirements

31.5.2.1 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.

31.3.2.1.1 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.

31.5.2.2 If the required control results are not obtained, the amplification set may be re-injected, re-amplified, or re-extracted as warranted with input from the TL.

31.5.2.3 Note that the RB may be omitted for re-amplifications using the same volume (or less) of template, provided the RB is not the control with the unexpected result. The EP1 may be omitted for re-amplifications if expected results were previously generated for the extraction set. All re-amplifications must include amplification negative and positive controls. POS/EP1 may be omitted for re-injections. The RB and NEG may be omitted for re-injections of equal or lesser time/sensitivity.

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

31.5.2.5 The F6C profiles for the Kit Positive Control DNA 2800M (POS) and the laboratory Extraction Positive Control (EP1) are listed in Fusion 6C Appendices: Appendix 2: Fusion 6C control profiles.

31.5.3 <u>Contamination Assessment</u>

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

31.5.3.2 RB and NEG samples should not have any callable peaks. If callable peaks are detected, the set of samples may be re-run, re-amplified, or re-extracted as warranted with input from the TL. If callable peaks are detected and the evidentiary samples are not affected, bring to the TL for further guidance and notify the Quality Manager for tracking. If a neg/RB is re-amplified (with TL approval) to assess possible contamination (called peaks ≥50 rfu and 25-49 rfu peaks, see below), all evidentiary samples associated with the impacted controls must also be amplified.

31.5.3.3 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. If peaks between 25 and 49 rfu are present in a NEG or RB, bring to the TL for further guidance and notify the Quality Manager for tracking.

31.5.3.4 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.

31.5.3.5 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.

31.5.3.6 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).

31.6 Mixed Samples in F6C:

31.6.1 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 31.7 for detailed guidance on determining the number of contributors to a DNA profile).

31.6.2 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.

31.6.3 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.

31.6.4 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.

31.6.5 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". CT DSS Stochastic thresholds are 150 rfu for Standard and Low injection times and 300 rfu for Maximum injection times. (Refer to SOP 30.5.2.4 for Low, Standard and Maximum injection times on each 3130 instrument for evidentiary samples.)

31.6.6 Known and database samples can be 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 will be 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 will be 85 RFU for 3kV5sec or less and 125 RFU for anything greater than 3kV5sec. The CT-DSS Knowns panel will be used for analysis.

31.6.7 Mixtures are deconvoluted for CODIS entry purposes as warranted by the results. See DNA SOP-13 (13.5.3).

31.7 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).

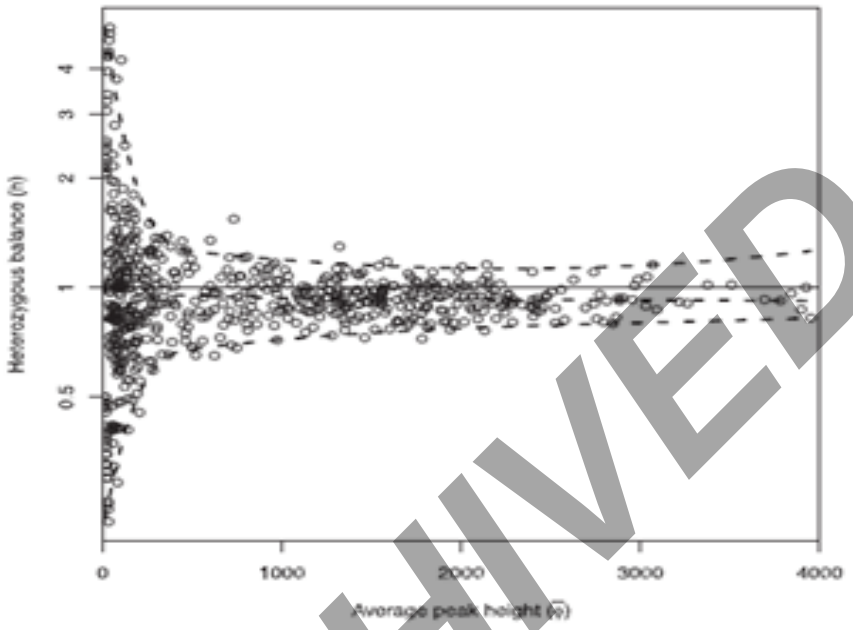

Fig. 1. Heterozygous balance versus average peak height.

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

31.7.1 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 both the technical and administrative reviewers, 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. 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 (see DNA SOP-32) contributors without a clear major contributor being present (see 31.15), the profile is reported as too complex to interpret. Report language:

"Due to the complexity of the DNA profile for item #xx, no comparisons will be made."

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.

31.7.2 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 50rfu, 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 <50rfu 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.

31.8 Highly Degraded/Low Template Samples:

31.8.1 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. To maximize data for a profile, the sample may be injected as appropriate at the standard or maximum time on the 3130xl.

31.9 Inhibited Samples:

31.9.1 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. If this is the case, multiple quantities or dilutions of a sample may be amplified for the original amplification.

31.10 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 report templates, DNA SOP-6; Section 2 and DNA Report Template)

31.10.1 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.

31.10.2 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:

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

31.10.2.2 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.

31.10.3 Positive Associations

31.10.3.1 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.

31.10.3.2 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.

31.10.3.3 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 report templates). An LR ≥ 10,000 is obtained using STRmix.

31.10.4 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:

31.10.4.1 The mixture too complex. With > 4 unknown (unconditioned) contributors, or if the analyst is unable to determine the number of contributors with confidence, report the following statement: "Due to the complexity of the DNA profile for item #xx, no comparisons will be made". An exception to this is when a mixture has a clear major contributor (see 31.15).

31.10.4.2 Insufficient data is obtained for the questioned DNA Profile. Report the following statement: "Due to limited data detected from item #1, the comparison to J. Smith is inconclusive." This statement 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.

31.11 Interpretation of single source DNA profiles

31.11.1 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.

31.11.2 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.

31.11.3 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).

31.12 Interpretation of DNA mixtures

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

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, 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.

31.12.2 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.

31.12.3 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).

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

31.12.5 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).

31.12.6 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.

31.12.7 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.

31.12.8 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.

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

31.13 Statistics – Likelihood ratios

Determining hypotheses for calculating the likelihood ratio (LR)

A LR is calculated using STRmix™ Analysis software for forensic unknown DNA profiles when the known individual(s) cannot be eliminated as the source of or a contributor to the evidence (or is consistent with being the source or included as a contributor) by a manual comparison. (see STRmix™ SOP32) LRs are calculated according to the following equation:

$$LR = \frac{\Pr(E \mid H_1)}{\Pr(E \mid H_2)} \text{ where}$$

Pr = Probability
E = Evidence
| = Given

The likelihood ratio assesses the probability of the evidentiary profile given two alternate (mutually exclusive) hypotheses; (H_1 and H_2). H_1 is typically where the data is explained by an inclusion of the person of interest (POI). H_2 is typically where the data is explained by a person selected at random from the general population.

In general, a LR is calculated for 1 set of hypotheses (see examples below) based on the nature of the DNA profile (single source, mixture, # of contributors, etc.), the scenario of the crime, and any relevant information supplied to the DSS Laboratory. LRs may be calculated based on new information where appropriate and/or upon request by the prosecution or the defense. At times this new information may lead to a repeat of the deconvolution itself (i.e. submission of elimination knowns after a LR to the POI has already been calculated).

Examples:

1. Scenario: a single source profile from evidence at scene matching J. Smith.

H_1: DNA profile originates from J. Smith.

H_2: DNA profile originates from an unknown person selected at random from the general population.

2. Scenario: a two person mixture from an intimate swab collected from Person A. The DNA profile can be explained by a mixture of J. Smith and A.

H_1: DNA profile originates from A and J. Smith.

H_2: DNA profile originates from A and an unknown person selected at random from the general population.

3. Scenario: a three person mixture from evidence recovered from the scene. One contributor corresponds to J. Smith.

H_1: DNA profile originates from J. Smith and two unknowns.

H_2: DNA profile originates from three unknowns selected at random from the general population.

31.14 Reporting

The following information is described when reporting profile results:

1. A general description of the DNA profile, such as mixed, single source, etc.
2. The hypotheses/assumptions used when the LR has been calculated.

General description: Use the standard terminology regarding single source samples and mixtures per current SOPs.

31.14.2 Assumptions: All assumptions that are made as part of the interpretation will be clearly stated within the report. This includes an assigned number of contributors and any assumed/conditioned contributors.

31.14.3 Conditioning the hypotheses: an assumption that a person can be included in a DNA profile (such as for intimate samples) regarding the person swabbed.

31.14.3.1 To condition an individual in the MCMC, the assumption must be consistent with the DNA profile results (i.e., the known is not eliminated) and there must be objective support for the assumption i.e., there must be a reasonable scientific expectation that an individual's DNA is in the evidentiary profile. In general, this would apply to non-probative comparisons for intimate samples and other items that have been regularly handled or worn by the person conditioned. The conditioned individual appears in both the numerator and the denominator.

A conditioning LR threshold has been established for ambiguous (where there is uncertainty as to whether the known source is contributing) profiles generated from non-intimate samples. The known profile in question is first evaluated using STRmix. The known is assumed to the profile and will be used for conditioning purposes when the resulting LR is ≥ 1000. Since this likelihood ratio will not be reported, proper documentation on DNA QR-

308 Changed and/or Not Reported Interpretations must be maintained in the case jacket. If the resulting LR is less than 1000, the known profile is not assumed/conditioned to the profile, and the comparisons is reported out either as inconclusive or eliminated, based on the likelihood ratio.

31.14.3.2 If a staff search indicates a putative non-random positive association between a staff member and an evidentiary profile (see SOP 13.8.1, documented on QR-20), the staff member may be conditioned in the MCMC on a case-by-case basis with TL/AD approval when the LR to the individual is at least 1000 (conditioning threshold, non-intimate samples). It has been shown in validations studies that conditioning true contributors to a profile lowers LRs for non-contributors and increases LRs for other true contributors. In general, the staff contributor would be conditioned for comparison to submitted known samples and/or for CODIS entry purposes when reprocessing the evidence is not a viable option or when reprocessing has not resolved the issue. The staff contributor may also be conditioned in scenarios where the contamination is trace (e.g., the presence of the contaminant is not expected to significantly impact the LR to the overall profile) or where there is a clear major contributor that is not the staff member.

Notes:

1) Unambiguous contributors to non-intimate profiles do not require STRmix evaluation for conditioning. Both analyst and reviewers must be in agreement. If any uncertainty arises, STRmix will be run and handled as above. Any comparison where the reference sample's DNA profile is present at less than half the loci with results is considered ambiguous, and therefore must be run through STRmix.

2) When there are two or more elimination knowns for consideration, it is appropriate to condition the unambiguous known when evaluating the other. In situations with multiple ambiguous elimination samples, consult TL for further guidance and approval.

3) Likelihood ratios to ambiguous contributors in 5 person mixtures may be calculated using STRmix. If the resulting LR is <1000, the comparison shall be reported out as uninterpretable for STRmix, as in 31.10.4.1, and DNA QR-308 Changed and/or Not Reported Interpretations shall be filled out appropriately.

4) Mixtures containing 6 persons or more may be considered too complex for determination of conditioning depending on the number of unambiguous and ambiguous profiles associated with the sample. These comparisons should be reported out as uninterpretable for STRmix, as in 31.10.4.1.

31.14.4 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

31.14.5 Reporting Comparisons using STRmix™

31.14.5.1 LR obtained is less than 1:

The following is the standard reporting statement used when the likelihood ratio obtained through STRmix is less than 1 (meaning the likelihood of obtaining the results is greater for H_2 than H_1), reported as an exclusion of the individual considered in H_1:

Single source samples: "The results are consistent with the DNA profile from item #xx originating from a single (male/female) individual. The results do not support the

hypothesis that J. Smith is the source of this profile. Assuming one individual, J. Smith is eliminated as the source of the DNA profile from item #XX.

Mixture samples: "The results are consistent with the DNA profile from item #xx being a mixture of xx contributors (with (at least) xx of them being male). The results do not support the hypothesis that J. Smith is a contributor to this profile. Assuming xx individuals, J. Smith is eliminated as a contributor to the DNA profile from item #xx"

Note that reference samples eliminated through visual comparison to the questioned sample: "J. Smith is eliminated as the source of/a contributor to the DNA profile from item #xx"

31.14.5.2 LR obtained is ≥ 1 but less than 10,000; the comparison is inconclusive:

Single source samples: "The results are consistent with the DNA profile from item #xx originating from a single (male/female) individual. Assuming one individual, given the low likelihood ratio(s) calculated, the results are inconclusive as to whether J. Smith could be the source of the DNA profile from item #xx."

Mixture samples: "The results are consistent with the DNA profile from item #xx being a mixture of xx contributors (with (at least) xx of them being male). Assuming xx contributors, given the low likelihood ratio(s) calculated, the results are inconclusive as to whether J. Smith could be a contributor to the DNA profile from item #xx."

31.14.5.3 LR obtained is ≥ 10,000

Single source samples: "The results are consistent with the DNA profile from item #xx originating from a single (male/female) individual. Assuming one individual, the DNA profile from item #xx is at least xx times more likely to occur if it originated from J. Smith than if it originated from an unknown individual."

Mixture samples (with no contributors conditioned/assumed): "The results are consistent with the DNA profile from item #xx being a mixture of xx contributors (with (at least) xx of them being male). Assuming xx contributors, the DNA profile from item #xx is at least xx times more likely to occur if it originated from J. Smith and xx unknown individuals than if it originated from xx unknown contributors."

Mixture samples (with contributors conditioned/assumed): "The results are consistent with the DNA profile from item #xx being a mixture of xx contributors (with (at least) xx of them being male). Assuming xx contributors, where J. Doe is one of the contributors,

the DNA profile from item #xx is at least xx times more likely to occur if it originated from J. Smith , J. Doe, and 1 unknown individual than if it originated from J. Doe and two unknown individuals."

31.14.5.4 For samples that are identical to one another, there is no need to run STRmix again. The same LR may be reported as follows:

"The results from items #xx, #yy, and #zz are identical to the results from item #aa. Therefore, assuming one individual, the DNA profile(s) from item(s) #xx, #yy, and #zz is/are at least xx times more likely to occur if it/they originated from Joe Smith than if it/they originated from an unknown individual."

31.15 **5+ Person Mixtures with a Clear Major Profile**

31.15.1 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:

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

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

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

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

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

31.15.2.1 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.

31.15.2.2 Print DNA-QR-307d Five or More Person Mixture Major deconvolution, and keep a 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.

31.15.3 Qualitative comparisons to known profiles. Known profiles are compared to the clear major profile as described in 31.10.

31.15.3.1 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.

31.15.4 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 31.15.2.1, and click on "LR for Major". DNA-QR-307h, Likelihood Ratio, is printed out for the case jacket. "Min LR/10" will be reported.

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

31.15.5 Reporting Out Complex Mixtures with Clear Majors

31.15.5.1 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.

31.15.5.2 Reports with comparisons to knowns: Both profile and major profile will be included in Testing Summary table. If there is a known consistent to 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.

31.15.5.3 5 person mixture wording: The results are consistent with item #xx being a mixture of xx contributors (with at least xx of them being male).

31.15.5.4 >5 person mixtures: The results are consistent with item #xx being a mixture of at least 5 contributors (with at least xx of them being male).

31.15.5.5 Consistent with Source Wording: "A major (male/female) profile was deduced from item #xx. The results are consistent with J. Doe being the source of the major profile deduced from item #xx. The major profile deduced from item #xx is at least xx times more likely to occur if it originated from J. Doe than if it originated from an unknown individual.

31.15.5.6 Eliminated as Source Wording: "A major (male/female) profile was deduced from item #XX. J. Smith is eliminated as the source of the major profile deduced from item #xx. Due to the complexity of the DNA profile from item #xx, no comparisons will be made as to whether J. Smith could be a minor contributor to the mixture."

31.16 Criminal Parentage Testing:

For criminal parentage testing, standard statistical methods for Fusion 6C results will be used as described below:

31.16.1 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.

31.16.2 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.

31.16.3 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.