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 Appendix 1 for F6C/STRmix™ work flow overview). However, it is not an exhaustive list of all possible casework scenarios. Where appropriate, other interpretational approaches/methods may be used with approval from the DNA Technical Leader (TL). 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 Autosomal STR:

31.4.1 This procedure is a general guideline for the analysis and interpretation of STR profiles when using the PowerPlex® Fusion 6C System. These procedures generally follow those outlined in the GeneMarker HID (GM-HID) Software v2.9.0 User Manual.

31.4.2 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.

31.4.3 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.

31.4.3.1 Using GM-HID software, analysis of data is performed by the 'Run Wizard'. Open the software and click on the folder icon in the upper left of the menu to open data. When analyzing F6C data, click on the 'Channels' button in the lower left and make sure 6 dyes is selected. Click on 'Change Chemistry' and select 'Promega'. Channel 1 should read 'Blue, FL-6C', channel 2 should read 'Green, JOE-6C', channel 3 should read 'Yellow,

TMR-6C', channel 4 should read 'Red, CXR-6C', channel 5 should read 'Purple, TOM-6C', and channel 6 should read 'Orange, WEN-6C'. Click 'Ok'. Click on the 'Add' button in the upper right and navigate to the location of the data folder you want to import. Double click on the folder to access the .fsa files. Select all or some of the files to import and click 'Open'. The files will be added to the 'Data File List'. The 'Add' button can be clicked again to add more data from other folders. When done adding data, click 'Ok' and the raw data will be added to the project.

31.4.3.2 Click on the green arrow button in the toolbar menu to open the 'Run Wizard' window. Click on 'Select an existing template or create one'. Name the template 'F6C_CT-DSS_29 or F6C_CT-DSS_29_STRmix™' depending on whether the stutter filters are applied. Select the panel with the same name. Set the size standard to ILS500 and the standard color to 'WEN-6C'. The analysis type should be 'HID'. Click on the 'Next' button to display the 'Data Process Options' window.

31.4.3.3 For 'Raw Data Analysis' select 'Auto Range', 'Smooth', 'Superior', 'Spike Removal', and 'Saturation Repair'. For 'Size Call' select 'Local Southern'. For 'Allele Call' deselect 'Auto Range' and set the 'Start' to 60 and the 'End' to 500. Leave the default parameters for 'Peak Detection Threshold'. Click on the 'Next' button to display the 'Additional Settings...' window.

31.4.3.4 Select 'Auto Select Best Ladder' and 'Auto Panel Adjustment'. For the 'Positive Control Template' select 'F6C'. If it is not part of the positive control list, it can be added using the 'Positive Control Template Editor' under the 'Tools' menu. For 'Allele Evaluation' set both 'Peak Scores' to zero. Leave 'Mixture Evaluation' deselected. If this is the first time you are running this 'Run Wizard' template click on the 'Save' button. Click on the 'Ok' button. The raw data will be analyzed and allele calls will be generated. The 'Data processing...' window remains open until you click 'Ok'.

31.4.3.5 To confirm the analysis thresholds for allele peak detection, go to the 'tools' menu and select 'Panel Editor'. In the upper left window of the editor select the panel template (F6C_CT-DSS_29 or F6C_CT-DSS_29_STRmix™) used for the current project (it should be the first one from the top under 'Project Panel'). Once selected the '[Ploidy]' number should be set to '0'. If not, right click on the panel name and select 'edit' and change the ploidy to '0' and click 'Ok'. To check peak detection thresholds and type of stutter filters used, right click on any marker name of the panel at the top center of the window and select 'Edit Marker'. The minimum 'Homozygote', 'Heterozygote', and both 'Inconclusive' intensities should be set to '50' RFU. The 'Max Heterozygote Imbalance (%)' should be 1% for forensic unknown profiles. The 'Min Heterozygote Imbalance (%)' should be 0%. If changes needed to be made, select the 'Apply

Homo/Hetero Settings to All Markers' option. For the stutter filter, 'Use Allele-Specific Values (From Panel)' should be selected. Again, if changes needed to be made, select the 'Apply Stutter Settings to All Markers' option. Click 'Ok'. If changes were applied, click the 'Save Changes' icon in the toolbar menu (**not** the 'Save Changes with Signal Info' icon).

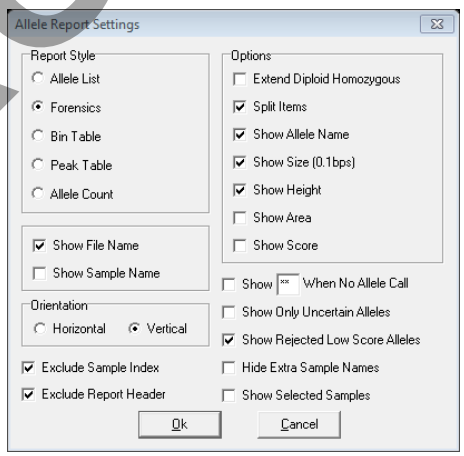
31.4.3.6 Note that even though allele calls have been generated, the project is not saved. To save the project to a network location on the U:\ drive, select 'File' and then 'Save Project' from the toolbar menu. Type in a name for the project and navigate to the network location to save the file to. Click the 'Save' button. For descriptions of all options and tools for GeneMarker, please see the GeneMarker HID User Manual for version 2.9.0.

31.4.3.7 Creating STRmix™ input files from GeneMarker HID

F6C evidentiary profiles that require STRmix™ analysis (SOP32) must be reanalyzed in GeneMarker with the stutter filters turned off as described below:

31.4.3.7.1 After analysis has been repeated with the stutter filters turned off, and all artifact peaks have been clicked off again, click on the "report settings" icon.

31.4.3.7.2 See figure below for the appropriate settings. This will only need to be changed once. Settings will be maintained into subsequent projects.



31.4.3.7.3 Click on the "Save" icon next to the "Report Settings" icon to save report. For "Save as Type:", choose "text file".

31.4.4 **PowerPlex® Fusion 6C Analysis:**

The analysis and interpretation of STR results is discussed below:

31.4.4.1 A GM-HID project is created by importing all of the pertinent data files. Caution: Allele calls are generated without the project being saved. Be sure to save the project file before closing it.

31.4.4.2 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 at loci other than Amelogenin are interpreted with caution with approval from the DNA TL or DNA Lead. With known samples and controls, any peaks > 6000 at loci other than Amelogenin are interpreted with caution. For known samples, including positive controls, the parameters for B, G, Y, R, P may be raised where appropriate.

31.4.4.3 Ensure that all required internal lane size standard peaks are called in all samples. Select the 'Size Calibration' icon in the toolbar menu. Select each sample in the list on the left to view the ILS trace for that sample. Verify that all of the ILS500 standard peaks are present (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, and 500) and the sizing quality score is ≥ 88 (the software flags the sizing quality of the ILS when it is <88). If a sample's internal size standard fails due to a low sizing quality (e.g. pull-up peaks into the size standard), the size standard may be successfully edited to produce a sufficient sizing quality score (see GM-HID User Manual). Alternatively, the sample may be re-injected, re-prepped and re-injected, or re-amplified.

31.4.4.4 Ensure that passing allelic ladders have all allele peaks detected. Ladders are listed in the sample file tree in blue type. Passing ladders will appear in bold blue type. Double-click on the first ladder and select the 'Browse by All Color' icon in the toolbar menu. Verify that the passing ladders have all allele peaks detected. With the 'Set Missed Allele as a Single Line in Ladder' flag enabled (see 31.4.4.5), allele labels for missed ladder peaks will be placed one row above allele labels of peaks that were called and there will be a red vertical line for that allele bin. Failed ladders will not be used to assign alleles to profiles.

31.4.4.5 Before viewing and editing electropherograms (egrams) for the first time, set up your viewing preferences. Select 'View' from the toolbar menu then select 'Preference...'.

31.4.4.6 Under the 'Start up Settings' tab, select 'Wizard' and 'Show Navigator'. The default network path (C:\) for importing/opening raw data and projects and exporting reports can

be modified if desired by checking the appropriate box and selecting the browse button on the right, e.g., U:\3130 NUCLEAR DNA CASEWORK & QC.

31.4.4.7 Under the 'Display Settings' tab 'Allele Label', the 'Decimal Precision' should be '1'. Select 'Mark Off-Marker as 'OL'', 'Mark Off-Bin as 'OB'', and 'Set Missed Allele as a Single Line in Ladder'. Under 'Chart settings', select 'Max # of Open Charts & Max Chart # in Page' (set both to '4') and 'Max Allele Label Layers' (set to '8'). Under 'Peak Label', you may select 'Height' and 'Vertical' and set the 'Position' to 'Allele Label'. For the final egram print for known samples, the peak height label may be deselected.

31.4.4.8 Under the 'Forensic' tab, set the Ladder, Positive Control, and Negative Control Identifiers to 'LADDER', 'POS', and 'NEG', respectively. If 'Mark Deleted/Edited Peaks with Symbols' is selected, then on the egram deleted peaks will be marked with an 'x' and edited peaks will be marked with an 'E'. This setting would not be used for final egram prints, but would be useful for identifying edits made to an electropherogram previously. When done, click 'Ok'.

31.4.4.9 To view and edit electropherograms for the samples, double click the first sample in the sample tree list. Then select the 'Browse by All Color' icon in the toolbar menu. Maximize the egram window. To view electropherograms of other samples, click in the vertical view slider on the far right to toggle up or down one sample at a time. Edit each egram as warranted (see 31.4.6 Analysis of DNA Profiles in GM-HID).

31.4.4.10 To print the electropherogram after the DNA profile editing is complete, close the 'All Color Browser'. Select the 'Set Axis' icon in the toolbar menu and select 'Fixed X'. Set the range from 60 to 480. Click 'Ok'. Select the samples to print by double-clicking their sample name(s) in the sample tree. Select the 'Print Report' icon in the toolbar menu. If printing with this software license for the first time, click on 'Select an Existing Template or Create One' and name the template 'CT-DSS Egram'. Under the 'Standard' tab, select 'Normal', 'Selected Samples', 'Electropherogram', 'FL-6C', 'JOE-6C', 'TMR-6C', 'CXR-6C', 'TOM-6C', and 'Hide Bins'. Under the 'Advanced' tab, select 'Print Report Header', 'Each Page', 'Print Markers', 'Print Alleles', and 'Implement Y Axis Settings'. Set the 'Chart Height' to 65. Click 'Save'. Click 'Preview'. If the electropherogram is not in portrait format, select the 'Page Setup' icon in the Preview toolbar menu and select 'Portrait'. Select the 'Print' icon in the toolbar menu and select the appropriate printer, print range, and number of copies and click 'Ok'.

31.4.5 **GeneMarker HID Analysis-- Control Requirements:**

31.4.5.1 Import the appropriate GM-HID 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. If not, the amplification set may be re-injected, re-amplified, or re-extracted as warranted with input from the TL. 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 an amplification Negative and Positive control. The RB and NEG may be omitted for re-injections of equal or lesser time/sensitivity.

Expected results of controls will be verified and documented on DNA QR-4 which is maintained in the case file. See Fusion 6C Appendices, Appendix 2: Fusion 6C Control Profiles.

31.4.5.2 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. 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, 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, the set of extracted samples may be interpreted with caution with TL approval.

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 as required by CT and Federal laws (see Alternate Report Template Statements located within the DNA Report Template).

Procedures listed here in 31.4.5.2 will be followed for all methodologies, on a case by case basis.

31.4.5.3 Select appropriate Run Wizard template (stutter filter applied, e.g. F6C_CT_29) and proceed with analysis in GMID. Record any editing (including if no edits) of GM-HID results on the appropriate 3130 analysis worksheet (DNA-QR-16b, QR-16c, QR-16d, or QR-16e). Sufficient detail will be noted on the 3130 analysis worksheet comments section so that it is clear to a reviewer. For example: "re-inject at max due to low peaks" or "re-prep sample- ILS failed".

31.4.5.3.1 Please note that DNA profile electropherograms will be printed with allele calls for all allelic peaks ≥ 50 RFU.

31.4.5.4 Control Profiles: Ensure that all control samples performed as expected. The positive controls (POS, EP1) must give the expected results. For known sample processing, only 1 positive control (either POS or EP1) is required. POS/EP1 may be omitted for re-injections.
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.

31.4.6 **Analysis of DNA Profiles in GM-HID:**

31.4.6.1 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 profile. See DNA SOP-6 for standard report conclusions.

31.4.6.2 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 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.

31.4.6.3 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). The identity of peaks called by GM-HID is generally assigned to one of the following categories:

31.4.6.4 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.

31.4.6.6 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 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.

31.4.6.7 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).

31.4.6.8 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.

31.4.6.9 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).

31.4.6.10 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 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.

31.4.6.11 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.

31.4.6.12 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).

31.4.6.13 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.

31.4.6.14 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.

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

31.4.7 **Mixed Samples in F6C:**

31.4.7.1 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 31.4.9 for detailed guidance on determining the number of contributors to a DNA profile).

31.4.7.2 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.

A likelihood ratio (LR) is calculated for DNA profiles (single source and mixtures) as described in section 31.8.

31.4.7.3 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.

31.4.7.4 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.

31.4.7.5 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". The below table lists the applicable stochastic thresholds for CT DSS based on 3130xl intruments and injection times.

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

	Injections Time Ranges				
Stochastic Threshold	**3130-1**	**3130-2**	**3130-3**	**3130-4**	**3130-6**
150 RFU	5 sec	5 sec	5 sec	5 sec	5 sec
300 RFU	35 sec	22 sec	20 sec	22 sec	16 sec

31.4.8 **Deconvolution F6C profiles (see MCMC & STRmix SOP 32 for detailed guidance)**

31.4.9 **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.

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™ SOP32).

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 Figure 1 re: expected HPB vs peak height).

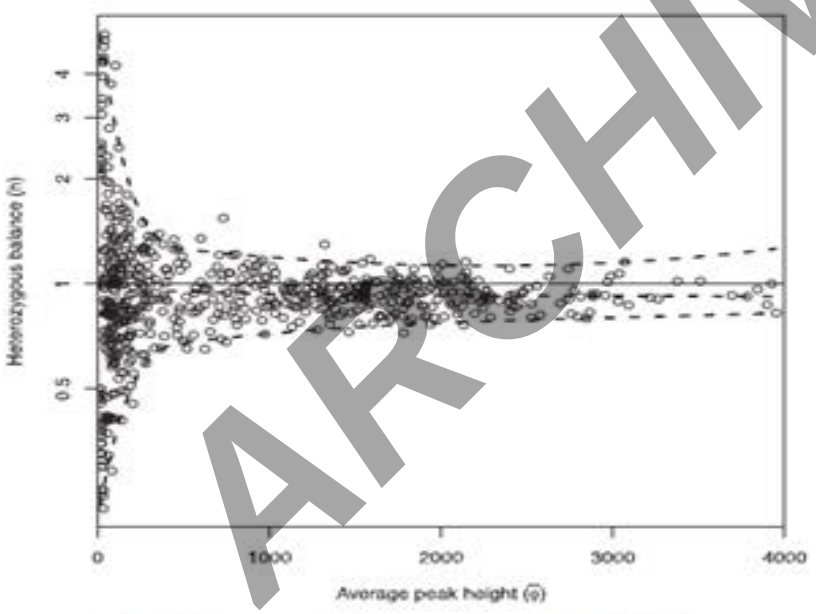


Fig. 1. Heterozygous balance versus average peak height.

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.

A determination of the number of contributors to a profile must be made by the DNA analyst prior to STRmix™ deconvolution and prior to the comparison of the profile to any reference samples in STRmix™. 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.

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 take into account sub-analytical threshold (AT) peaks (≥ 25 RFU) and high stutter as appropriate.

In circumstances when the number of contributors cannot be adequately assessed, no clear major contributor is present, or when there are greater than 4 unknown/unconditioned contributors (see SOP 32), the profile is reported as too complex to interpret. Report language:

"Due to the complexity of the DNA profile for item #xx, it is unsuitable for STRmix™ interpretation."

After analysis of the STRmix™ MCMC output and prior to comparison with the reference profiles in STRmix™, 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, the analyst's assumptions are documented on the STRmix™ output and the electronic file of both deconvolutions is retained in the casework folder.

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.

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. Peaks below the AT, but not in artifact positions, are useful to identify the presence of low level/minor additional contributors. When sub AT peaks are used to infer an extra contributor there should be data elsewhere in the profile to support this. Sub AT peaks are unlikely to significantly affect the interpretation of the other contributors.
4. The effects of the miss-assignment of the number of contributors are typically restricted to false exclusions of true contributors and false inconclusive LRs.

31.5 Highly Degraded/Low Template Samples:

31.5.1 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 amplications 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. However, profiles with any data > 4000 RFU are interpreted with caution.

31.6 Inhibited Samples:

31.6.1 Inhibited samples may exhibit allele, locus, or complete profile dropout, unbalanced heterozygous alleles, and interlocus 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**.7** **Qualitative Interpretation of STR Results:**

31.7.1 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)

31.7.1.1 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.

31.7.1.2 Inconclusive: No conclusion can be drawn from the comparison between the known sample and the evidentiary sample due to uncertainty. There are 4 reasons for inconclusive results.

31.7.1.2.1 Mixture too complex: With > 4 unknown contributors, report the following statement: "Due to the complexity of the DNA profile for item #xx, it is unsuitable for STRmix™ interpretation. (see exception for > 4 unknown contributors in 31.7.1.4).

31.7.1.2.2 The LR is greater than 1 but less than 10,000, report the following statement: "…given the low LRs calculated, the results are inconclusive..."

31.7.1.2.3 Insufficient for comparison (IFC): "Insufficient amplification products were detected from item #xx for comparison to the known DNA profile of J. Smith." The 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™.

31.7.1.2.4 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."

31.7.1.3 Positive Associations

A. Cannot be eliminated (CBE). LR ≥ 10,000 using STRmix. 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.

B. Inclusion. LR ≥ 10,000 using STRmix. An individual is included as a potential source of (or a contributor to) a 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).

31.7.1.4 With a mixture of > 4 unknown contributors (no conditioning possible) where there is a clear major contributor at all loci, the major profile may be deconvoluted with TL approval. The LR (1/RMP) is calculated for the deconvoluted major genotype as described in 31.8 without utilizing STRmix™ software.

31.7.2 **Interpretation of single source DNA profiles** (see section 31.4.9 for detailed guidance on determining the number of contributors to a DNA profile).

31.7.2.1 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.

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.

31.7.2.2 The use of "undetermined" in the Report Conclusions Summary table (in the **Type** column): applies to the detection of male vs female DNA. To be used when the analyst is unable to determine whether a profile is male or female.

31.7.3 **Interpretation of DNA mixtures**

31.7.3.1 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.

31.7.3.2 **Factors taken into consideration when interpreting mixtures:**

31.7.3.2.1 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.

31.7.3.2.2 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).

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

31.7.3.2.4 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. If not, this may not optimally fit the CBE conclusion (note: this may not apply to degraded profiles).

31.7.3.2.5 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.

31.7.3.2.6 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.

31.7.3.2.7 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.

31.7.3.2.8 If the analyst and reviewers feel that the above rules do not adequately apply to a specific mixture comparison, alternate conclusions may be used with TL approval.

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

31.8 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 by a manual comparison. (see STRmix™ SOP32) LRs are calculated according to the following equation:

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

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 where the data is explained by an inclusion of the person of interest. 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 (ss, 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.

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.

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

Description of profiles: Use the standard terminology regarding single source samples and mixtures per current SOPs.

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.

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

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

Reporting Comparisons using STRmix™

1. Where LR is less than 1

The following is the standard reporting statement used when the LR is less than 1, i.e. the likelihood of obtaining the results is greater for H_2 than H_1.

Example 1: LR<1

This is reported as an exclusion of the individual considered in H_1.

"The results are consistent with the DNA profile from item #xx originating from a single individual. The results do not support the hypothesis that this DNA profile originated from J. Smith. Based on the assumptions listed above, J. Smith is excluded from being the source* of the DNA profile from item #xx." (*or contributor to if a mixture)

2. Example 2: LR > 1 but less 10,000. The comparison is inconclusive:

"The results are consistent with the DNA profile from item #xx originating from a single individual. Based on the assumption listed above and given the low LRs calculated, the results are inconclusive regarding whether J. Smith could be the source* of the DNA profile from item #xx." (*or contributor to if a mixture)

3. Example 3: LR ≥ 10,000

A. For a single source profile where the reference sample is included, report the following statement:

"The results are consistent with the DNA profile from item #xx originating from a single individual. Based on the assumption listed above, the DNA profile obtained from item #xx is at least 1 million times more likely to occur if it originated from J. Smith (item #xx) than if it originated from an unknown individual."

B. For a mixture profile (with no contributors conditioned/assumed) where the reference sample is included, report the following statement:

"The results demonstrate that the DNA profile from item #xx is a mixture. Assuming 3 contributors, The DNA profile obtained from item #xx is at least 1 million times more likely to occur if it originated from J. Smith (item xx) and 2 unknown individuals than if it originated from 3 unknown contributors."

C. For a mixture profile (with contributors conditioned) where the reference sample is included, report the following statement:

"The results demonstrate that the DNA profile from item #xx is a mixture. Assuming 3 contributors to the profile where J. Doe is one of the contributors, the DNA profile obtained from item #xx is at least 1 million times more likely to occur if it originated from J. Smith (item #xx) and J. Doe (item #yy) and 1 unknown individual than if it originated from J. Doe and two unknown contributors."

F6C/STRmix Work Flow