39.1 Purpose:

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

39.2 Responsibility:

DNA Unit personnel.

39.3 STR Analysis and Interpretation:

This procedure is a general guideline for the analysis and interpretation of STR profiles when using the GlobalFiler® PCR Amplification kit (GF) and STRmix™ (see WI-34 for GF/STRmix™ workflow overview and WI-35 for GeneMarker overview both found below in the Appendix). 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.

39.4 GlobalFiler® System:

39.4.1 When the GF Kit is employed, the twenty core CODIS STR loci, plus one additional STR locus (SE33), 1 Y-STRs (DYS391), a Y-Indel, and Amelogenin are typed in a single PCR amplification reaction according to the manufacturers and laboratory's protocols.

39.4.2 GlobalFiler® PCR amplification products are separated, detected, and analyzed using an ABI 3500xL Genetic Analyzer, the supplied 3500 Series Data Collection Software 4, and GeneMarker HID (GM-HID) v.2.9.7 software.

39.4.3 These procedures generally follow those outlined in the GeneMarker HID Software User Manual for the version in use.

39.5 GlobalFiler® Analysis:

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

39.5.1.1 Standard analysis parameters: The analytical thresholds for 3500xl data (AT) for forensic unknowns, knowns, ladders, and controls are B-40 RFU, G-55 RFU, Y-35 RFU, R-50 RFU, P-55 RFU.

39.5.1.2 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.

39.5.1.3 Overloaded Data: If too much DNA is added to the amplification reaction or is injected into the 3500xl, 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, including uncommon stutter types and/or amplification artifacts in one or more of the colors. Examine the peak heights in GM-HID software. Peaks greater than 10,000 RFU (30,000 RFU for knowns and database samples) but not off-scale may also lead to artifacts peaks being detected ≥ AT. These samples may need to be reinjected for a shorter time or diluted and re-amplified.

39.5.1.4 Peak identification: Only allele peaks with a height of ≥ AT in GM-HID are called/reported and evaluated using STRmix™. Peaks < AT may be evaluated to address issues such as whether a sample should be re-amped/re-injected but are not used for contributor number or contamination assessment. The identity of peaks called by GM-HID is generally assigned to one of the following categories:

39.5.1.5 Allele Peak: A called allele has a peak height ≥ AT, a fragment size that falls within the range of 60-460 base pairs, has good peak morphology and has the appropriate dye color for the locus. However, not all peaks ≥ AT 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. STRmix™ does not use peaks that do not meet the Laboratory's calling criteria, with the exception of the stutter peaks that are modeled. Analysts may call peaks ≥ AT that are not called by GM-HID on a case-by-case basis. Your technical reviewer must agree with the insertion. If necessary, consult with TL, Assistant TL, or Validation Coordinator. Such peaks are interpreted with caution.

39.5.1.6 Detection of variant alleles: Variant alleles have been identified for many STR loci. These alleles may contain full or partial repeats. In GM-HID, variant alleles may be labeled as Out of Bin (OB) or Off Ladder (OL) alleles. If a particular variant has been observed before by the DESPP CT Division of Scientific Services (CT_DSS), Thermo Fisher, NIST, or in published literature, the sample does not need to be repeated. Change the OB/OL to the proper allele call in GM-HID if needed. However, if the repeat has not been observed, the sample may be re-amplified and/or reinjected to confirm the variant allele as warranted.

39.5.1.7 Stutter peaks (N-1, N-2, N-0.5, N+0.5, and N+1 repeats; N-2 is particularly prevalent if the sample is 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/combined 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-41). Peaks in the stutter position below the Laboratory's stutter thresholds are not listed on the electropherograms or the case report. Note: see DNA SOP-32 for stutter peak types evaluated by STRmix™.

39.5.1.8 Minus A Peaks: GF 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 ≥ 10,000 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.

39.5.1.9 Pull-up Peaks: Pull-up peaks are generally seen when an elevated quantity of 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.

Note: the 3500 Series Data Collection Software 4 has a pull-up correction function applied to the raw data. This function is not necessarily consistent across the entire base-pair range for detecting peaks from amplified DNA; therefore, care must be taken when identifying the percentage of signal for unambiguous pull-up peaks. Using unambiguous pull-up peak(s) in the same locus will most often be optimal. In the case where there are no unambiguous pull-up peaks for the dye channel, consider that the median percentage of pull-up peaks observed during the validation was 0.7%, with 95.1% of the pull-up peaks being < 3% and 99.4% being < 5%.

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 the height of the 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).

39.5.1.10 Pull-down Peaks: Pull-down peaks are generally seen when an elevated quantity of 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.

39.5.1.11 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.

39.5.1.12 Dye Artifacts/Dye Blobs: Broad peaks may result from dissociated dyes or fluorescent particles that are migrating 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 DNA SOP-41).

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

39.5.1.14 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.

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

39.5.1.16 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. The 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.

39.5.1.17 Analysis of known samples amplified with GlobalFiler Express or GlobalFiler

Routine click-offs do not need to be noted on the QR-325 injection worksheet (i.e., pull-up, spikes, dye artifacts, stutter peaks below the CT_DSS allele-specific stutter thresholds/combined stutter thresholds, etc.). Allele changes and sizes of peaks out of ladder ranges, called stutter peaks above the CT_DSS allele-specific stutter thresholds/combined stutter thresholds, possible tri-alleles, non-human artifact peaks, non-routine click-offs, and any low peak height ratios (<60%), should be noted on the appropriate QR-325 injection worksheet. Analysts should be aware that since the GFE_CTDSS_20% knowns panel filters out any peaks < 20% in height of the parent peaks at each locus, there could be a stutter peak present above the allele-specific stutter threshold/combined stutter threshold that does not receive an allele call due to its height being < 20% of the parent peak. The Known PHR Filter Macro is used to assist analysts in identifying any results warranting further review or documentation.

38.5.1.18 Analysis of unknown samples amplified with GlobalFiler

Artifacts, except for any stutter peak, are not labeled in Genemarker. They are removed from the profile, and a comment is unnecessary. This protocol (section 39.5) has detailed definitions of each artifact type and its appearance. Please also refer to DNA WI-35 Genemapper Work Instructions for more information.

For any stutter peak in evidentiary (forensic unknown) profiles, a comment or "label" is added, designating that peak is stutter. This is to aid in the downstream process when making the STRmix project where stutters are left in. Any peak that is to be left in the STRmix project will be labeled in Genemarker. Control samples with stutter do not need a labeled stutter peak as they are not used in a STRmix run.

39.5.2 Control Requirements

39.5.2.1 For evidentiary sample (forensic unknown) amplifications, all amplified controls (RB, NEG, EP1, POS) must give the expected results for each extraction/amplification to pass (exceptions with TL approval). 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. When the positive amplification control does not give the expected results in casework, the TL can approve the passing extraction positive control to serve as the amplification control.

39.5.2.1.1 If 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.

39.5.2.2 If the required control results are not obtained, the amplification set may be re-injected, re-amplified, or re-extracted as warranted.

39.5.2.3 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 of 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.

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

39.5.2.5 The GF profiles for the Kit Positive Control DNA 007 (POS) and the laboratory Extraction Positive Control (EP1) are listed in DNA SOP-41.

39.5.3 Contamination Assessment

39.5.3.1 Potential contamination is assessed by evaluating the evidentiary samples, RB, NEG, and positive controls. Please refer to the workflow in the Appendix below.

39.5.3.2 RB and Negative amp control samples should not have any callable peaks. If more than one callable peak is detected or more than one control is affected, the samples should be first re-prepped and re-injected. If the contamination is still present in the RB, the RB will be reamplified with positive and negative amp controls. If the negative amp control has contamination, the entire set of samples will be reamplified. Rework should be utilized to determine the root cause of the contamination. A Qualtrax workflow will be initiated to document the contamination incident.

Note: The RB may be omitted for re-amplifications if using the same volume (or less) of the template.

39.5.3.3 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. If one or two samples from one analyst is processed with another batch, the cross-comparison should be manually done using just the samples against the rest of the batch.

39.5.3.4 The appropriate response for a contamination event (including how the sample(s) are reported) is determined case-by-case. The TL will be apprised of contamination events by the Qualtrax DNA Contamination Tracking and/or by the analyst and TL discussions.

Instances in which a single peak ≥ analytical threshold is observed in a single negative control or reagent blank for each extraction set will not be tracked as a workflow instance.

39.5.3.5 Depending on factors such as the type and extent of contamination and whether the source of the contamination can be identified, the contaminated sample(s) and/or amplification sets may be interpreted with caution or deemed unsuitable for comparison. If any samples are deemed unsuitable for comparison, the TL will review and approve that decision before making a comparison and then a conclusion. (See Alternate Report Template Statements located within the DNA Report Template).

39.5.4 Documentation of controls and standards on DNA QR-325.

The following key should be used as a guideline to determine how controls and standards should be documented on DNA-QR-325. This list is not all-encompassing, and other situations may arise not fully documented here. Any questions on how to document controls and standards will be brought to the Technical Leader. One drop-in peak (peak above AT) in a RB or one peak in a negative amp control does not indicate contamination due to the drop-in and increased sensitivity in the amp kits. However, if two peaks are seen between the RBs and/or the Neg controls for an extraction set, then a Qualtrax DNA contamination workflow must be started. For example, one peak in the negative and one peak in the reagent blank would require a workflow. Another example is one peak in the negative and then a second peak (a different peak than the one at standard injection) is detected on a max injection, which would require a workflow.

Did the ILS perform as expected?

Sizing quality score of less than 88	No
Not all LIZ peaks between 60 and 500 bp are called	No
Score 88 or more, and all peaks between 60 and 500 bp are called	Yes
Broad peaks, but score is 88 or more, and all peaks between 60 and 500 bp are called.	Yes

Did the negative controls perform as expected?

One (1) called peak in Neg, or one (1) called in RB^ (not one peak in both)	Yes#
Called peaks (>1) in Neg or RB*	No
Broad peaks in LIZ, but no called peaks +	Yes

^ No TL initials are needed
* See workflow below in Appendix
+ Still needs reinjection
# Designated in the project as a drop-in

Did the positive controls perform as expected?

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

39.6 Mixed Samples in GF:

39.6.1 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 AT thresholds for GM-HID are not used in determining the number of contributors. (See section 39.7 for detailed guidance on determining the number of contributors to a DNA profile).

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

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

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

39.6.5 Per the "SWGDAM Guidelines on STR Interpretation," a Stochastic Threshold is 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 500 RFU for Standard and Low injection times and 700 RFU for Maximum injection times. Injection time ranges are listed below.

Injection	**3500-1, 2, 3, 5, & 6**	**3500-4**
Low	1.2 kV, 16 sec	1.2 kV, 12 sec
Standard	1.2 kV, 24 sec	1.2 kV, 18 sec
High	1.6 kV, 32 sec	1.2 kV, 24 sec

39.6.6 For the 25-cycle GlobalFiler Express Amplification, the stochastic threshold will be 115 RFU for Low injection and 150 RFU for Max. If samples contain homozygote peaks

below these thresholds, inject the sample longer or re-process the sample, as warranted. The CT-DSS Knowns panel (GFE_CTDSS_20%) will be used for analysis.

Note: Since the GFE_CTDSS_20% knowns panel filters out any peaks < 20% in height of the parent peaks at each locus, it is imperative to look carefully at the baseline of knowns to check for contamination. Using this filter could result in peaks greater than the analytical threshold not receiving an allele call due to their peak height being < 20% of the parent peak at that particular locus. In this instance, contamination is still present and needs to be addressed and documented accordingly.

True alleles of a heterozygous pair may not receive an allele call if the peak height ratio of the pair is < 20%. True tri-alleles may also not receive an allele call if one of the peaks is < 20% of either of the other two peaks. In both instances, analysts would need to manually add the allele call of the smaller peak onto the electropherogram. These instances should be noted on the corresponding QR-325 injection worksheet.

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

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

Single Source Profile Attributes: A single source profile generally has 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, 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 a significant peak height imbalance at one or more loci (see Fig. 1 re: expected heterozygous peak balance vs. peak height. Note: Fig. 1 represents 3130xl data, and the x-axis scale would be considerably different considering 3500 data, but general trends would remain similar.)

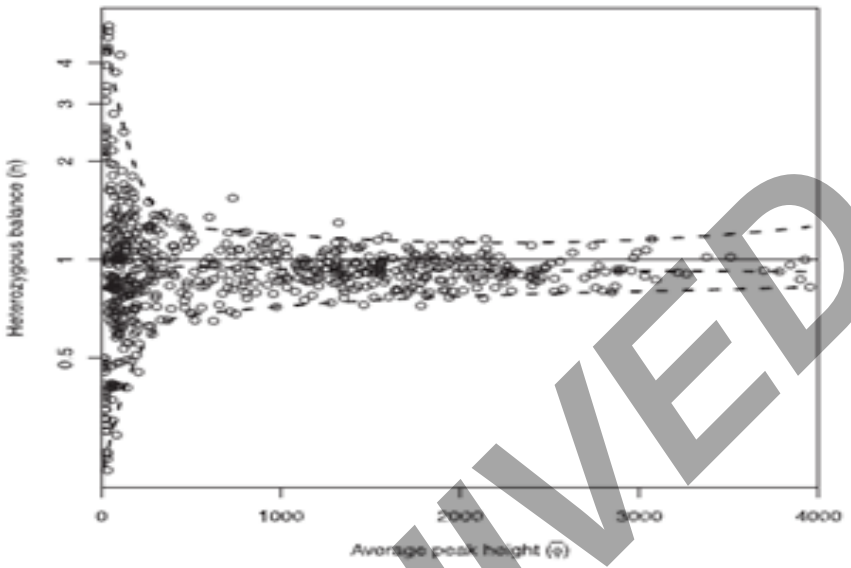


Fig. 1. Heterozygous balance versus average peak height.

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

39.7.1 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 underestimate 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 explain the observed profile reasonably.

A determination of the number of contributors to a profile is generally made by the DNA analyst prior to STRmix™ deconvolution and before 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 and agreed upon by the Technical Reviewer. 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 the 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 judgment to assess the number of contributors and will evaluate if any called peaks should be removed as high stutter where appropriate.

When the number of contributors cannot be adequately assessed or greater than 5 unknown/unconditioned contributors without a clear major contributor being present (see 39.15), the profile is reported as too complex to interpret.

After analysis of the STRmix™ MCMC (Markov Chain Monte Carlo) output, it may be determined that the deconvolution does not conform to scientific expectations and may be re-run under a different number of contributors/iterations. In this event, 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.

39.7.2 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, 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-specific 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). Considering 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 Appendix 1 for an indication of expected heterozygote peak balance for varying template amounts. Note that peak variance (imbalance) increases as the amount of template in the PCR reaction decreases

following a continuous model. With low-template amplifications (≤100 pg), imbalance greater than mean minus 1 SD can provide support for an additional contributor to the profile. However, significant peak imbalance at a locus (e.g., imbalance greater than mean minus 1 SD) is not always a strong predictor itself of an additional contributor, particularly if the imbalance is at 1 or a limited number of loci. Imbalance should be evaluated in the context of the overall profile results to determine the most likely number of contributors to the DNA profile. When peak imbalance is used to infer an additional contributor, the potential impact of additive stutter from adjacent allele(s) must be evaluated.

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 D1S1656, 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. Amplification results from low-template quantities (particularly less than 100 pg) may exhibit increased stutter and peak variance. Based on the GF/3500xl validation data, it was noted that profiles from low-template contributors may exhibit multiple high stutter peak that can be considerably above the standard stutter thresholds. A similar impact may occur with respect to peak imbalance. Therefore, when these low-template peaks pertaining to stutter or peak imbalance are used to infer an extra contributor, look for data elsewhere in the profile to support this assessment if possible. The effects of the miss-assignment of the number of contributors are typically restricted to false exclusions of true contributors and false inconclusive LRs.
4. DNA QR-342 is used as a tool to calculate possible stutter values not included in the GeneMarker panel (GF_CTDSS). This includes low-template stutter thresholds as well as overlapping/combined stutter. Low-template stutter thresholds are applied to each locus as appropriate based on the estimated quantity of DNA for each contributor to the profile [i.e., (a) when the total quantity amplified is ≤ 100 pg—

apply to all contributors or (b) when total quantity amplified is > 100 pg but the amount of DNA from some contributor(s) is ≤ 100 pg—apply low-template thresholds only to the peaks from the low-template contributors.] Peaks in stutter positions that are below the Laboratory's low-template stutter thresholds (see (a) & (b) above), which have not been removed by GeneMarker are filtered using DNA QR-342 and are not listed on the electropherogram. Low-template stutter thresholds are utilized to aid in determining the number of contributors and to highlight the increased expected stutter variance associated with low-template single source profiles and/or profiles with low-template contributors.

39.8 Highly Degraded/Low Template Samples:

39.8.1 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. Degradation may also result in a particular contributor to a profile being low template at the large amplicons but standard template at the small amplicons as described above. 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 3500xl. A maximum injection may assist in determining the number of contributors during batch processing. In general, maximum should be done for the number of contributor scenarios where the LR is typically more impacted by contributor miss-assignment (i.e., 1 vs. 2 & 2 vs. 3 contributors). Maximum injections can also be done to gather more data from a standard injection. The analyst based on training and experience can make this determination. If more data can be reported, the max injection should be performed.

39.9 Inhibited Samples:

39.9.1 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. If necessary, a Microcon purification can be performed to reduce the amount of inhibitors in a sample.

39.10 **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 the approval of the technical leader. (See DNA SOP-6)

39.10.1 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.

39.10.2 Inconclusive: No conclusion can be drawn from the comparison between the known sample and the evidentiary sample due to uncertainty. There are 3 reasons for inconclusive results:

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

39.10.2.2 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.

39.10.2.3 Profile results only include 1 allele (see 39.10.4.2 below).

39.10.3 Positive Associations

All unknowns within one case will be compared to all knowns in one case except when specifically requested otherwise by agency or SAO.

39.10.3.1 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 in the evidentiary profile at all loci where results are generated (see report templates). An LR ≥ 1,000 is obtained using STRmix.

39.10.3.2 Consistent with Contributor: An individual is consistent with being a contributor to a mixture DNA profile if alleles consistent with the known sample are detected (≥ AT) in the evidentiary profile. (See report templates). When coming to this conclusion, the analyst should keep in mind training and experience with DNA mixtures and the knowledge that STRmix considers the probability of dropout taking into consideration peak height data, the overall quantity of template DNA, information regarding the extent of degradation, inhibition, stochastic effects, and potential masking by stutter or contributor number. An LR ≥ 1,000 is obtained using STRmix.

39.10.4 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:

39.10.4.1 The mixture is too complex. With > 4 unknown (unconditioned) contributors or when the analyst cannot determine the number of contributors with confidence. An exception to this is when a mixture has a clear major contributor in a 5-person mixture. (See 39.15). Another option, with TL approval, allows for multiple NOC scenarios (with STRmix) to be reported if the analyst is undecided based on an insufficient or a complex mixture. This will be approved only on a case-by-case basis on QR-308.

39.10.4.2 Insufficient data is obtained for the questioned DNA Profile. This is 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 cannot be run through STRmix. This includes a low-level contributor(s) associated with a clear major contributor. 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 (data not reported) and remain in the case jacket.

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

39.11 Interpretation of single-source DNA profiles

39.11.1 For single source partial profiles with peaks ≥ AT, report a positive association with potential dropout when the observed allele(s) are consistent with the known source and the LR ≥ 1000.

39.11.2 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.

39.11.3 Note: Report "undetermined" in the "Type" column of the Testing or Conclusions Summary table when the analyst cannot 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 (regardless of X peak above or below the stochastic threshold). Having only an X as a detected result is considered no STR DNA profile and will be reported as no profile detected.

39.11.4 Pseudo-Knowns (such as a water bottle a police officer saw the POI discard) sometimes are sent in as reference samples. They can be reported out as the "DNA profile developed from, (the water bottle)" compared to the evidence item(s). There should be no name used unless a proper known buccal or blood sample is taken.

If the pseudo-known is a mixture, it will not be used as a pseudo-known unless the Technical Leader reviews the profile, and if the TL approves of its use as a pseudo-known, the TL will create a deviation for the specific sample/case. There is the possibility that a clear major can be deduced, but it must be approved through a deviation process.

39.12 Interpretation of DNA mixtures

39.12.1 These general approaches do not account for all possible case mixture scenarios and no two mixtures are the same. Therefore, any scientifically valid approach not described in the SOP can be approved by the Technical Leader.

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.

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

39.12.3 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). Please note that the sample was once one, therefore there is expectation if carry-over peaks are detected.

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

39.12.5 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.

39.12.6 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.

39.12.7 The total number of dropouts (overall and at each locus) that would be required to be still consistent with a contributor conclusion: While allele dropout (below the stochastic threshold) can occur at any locus, extensive validation and case experience have 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.

39.12.8 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.

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

39.13 Statistics – Likelihood ratios

Determining hypotheses for calculating the likelihood ratio (LR)

An LR is calculated using STRmix™ Analysis software for forensic unknown DNA profiles when the DNA profile of the known individual(s) are either consistent with being the source or consistent with being a contributor by a manual comparison. (See DNA SOP-32 STRmix™) 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, an 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 the 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.

39.14 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.

39.14.2 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**. Please refer to the flowchart in the Appendix**.

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

39.14.3.1 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. 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.

The LR threshold to condition ambiguous (where there is uncertainty as to whether the known source is contributing) profiles generated from non-intimate samples is the same as our threshold for reporting positive associations. The known profile in question is first evaluated using STRmix. The known is assumed to the profile and will be used for conditioning 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.

39.14.3.2 If a staff search indicates a putative non-random positive association between a staff member and an evidentiary profile, the staff member may be conditioned in the MCMC on a case-by-case basis 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 impact the LR to the overall profile significantly) 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 Technical Reviewer must agree. If any uncertainty arises, STRmix will be run and handled as above. Any comparison where the reference sample's DNA profile is fully present (i.e., if known is a heterozygote, both alleles must be called) at less than half the loci with results is considered ambiguous, and, therefore must be run through STRmix. Please see the flow chart below.
2) When there are two or more elimination knowns for consideration, it is appropriate to condition the unambiguous known when evaluating the other.
3) Likelihood ratios to ambiguous potentially assumed contributors in 5-person mixtures are calculated using STRmix. If the resulting LR is <1000, the comparison shall be reported as inconclusive or an elimination, based on the likelihood ratio calculated (an LR of 1-999 is inconclusive, while an LR of <1 is an elimination). No probative comparisons will be made to this profile, and reported out as "No additional comparisons to item #XX will be made."
4) Mixtures containing 6-persons, or more are typically considered too complex to determine conditioning. These profiles should be reported as uninterpretable for STRmix, as in 39.10.4.1. A major profile may be used with TL approval on QR-318.

39.14.4 Likelihood Ratio

A conservative 1-sided lower bound HPD (highest posterior density) LR value is reported for GF 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
1-999	Comparison inconclusive, no LR reported
1000 – 100 billion	LR rounded down—use up to two significant figures
>100 billion	Ceiling—report LR is at least 100 billion

39.14.5 Reporting Comparisons using STRmix™ - See DNA SOP 6.

39.15 **5 Person Mixtures with a Clear Major Profile**

39.15.1 During analysis and number of contributor estimation, an analyst can determine that a 5-person mixture has a clear major contributor. For mixtures greater than 5 people, consult with the TL on a case-by-case basis as a deviation may be possible. The following criteria for this must be met at ≥ 10 loci. If any of the following criteria are not met, the locus does not qualify for statistics:

39.15.1.1 All of the alleles of the major contributor are ≥ ST (500 RFU, 700 RFU).

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

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

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

39.15.2 After determining the number of contributors to be 5, the analyst will determine if a clear major profile is present. A macro has been created to assist in this determination. The exported allele table text file will be imported into the GlobalFiler Deconvolution Workbook. If a clear major is present at 10 or more loci, print out DNA QR-318, and keep as a part of the case record. The presence of a clear major profile will be noted on DNA-QR-302 Contributor Estimation Worksheet.

39.15.3 Qualitative comparisons to known profiles. Known profiles are compared to the clear major profile as described in 39.10.

39.15.3.1 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.

39.15.4 Statistics for Clear Major Profiles

39.15.4.1 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.

39.15.4.2 Repeat the steps in 39.15.2, when you determined a clear major was present, then click on "LR for Major". DNA-QR-318a, Likelihood Ratio, is printed out for the case jacket. "Min LR/10" will be reported.

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

39.15.5 Reporting Out Complex Mixtures with Clear Majors

39.15.5.1 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.

39.15.5.2 Reports with comparisons to knowns: Both profile and major profile will be included in Conclusions 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 Conclusions Summary table will note "No Comparison" to the full profile.

39.15.5.3 For report wording, see DNA SOP-6.

39.16 Criminal Parentage Testing:

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

39.16.1 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.

Obligate alleles may be searched in CODIS to find the potential unknown maternal or paternal (where applicable) contributor. This is not familial searching.

39.16.2 No statistics are required for non-matches. Due to mutations between generations, an individual must be excluded at more than two loci 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 conclusion and may deviate from the above statement. In the event of apparent mutations, additional testing may be conducted as appropriate.

39.16.3 The RMNE ceiling reported for GlobalFiler criminal parentage testing is 1 in 100 billion.

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

Appendix 1: Expected Heterozygous Peak Balance

Standard injection:

1 ng:

AVE	0.9051	Min	0.6753
SD	0.0803	Max	0.9995
N =	108		

500 pg:

AVE	0.8778	Min	0.6210
SD	0.0825	Max	0.9969
N =	108		

250 pg:

AVE	0.8414	Min	0.6006
SD	0.1061	Max	0.9989
N =	108		

125 pg:

AVE	0.7852	Min	0.3737
SD	0.1471	Max	0.9993
N =	108		

63 pg

AVE	0.6894	Min	0.2584
SD	0.2000	Max	0.9964
N =	108		

31 pg:

AVE	0.6308	Min	0.1871
SD	0.2176	Max	1.0000
N =	100		

8-16 pg:

AVE	0.6155	Min	0.1634
SD	0.2506	Max	1.0000
N =	74		

Max Injections:

4-8 pg:

AVE	0.6018	Min	0.12
SD	0.2378	Max	0.99
N	45		

16 pg:

AVE	0.5939	Min	0.18
SD	0.2174	Max	0.96
N	68		

31 pg:

AVE	0.6029	Min	0.11
SD	0.2090	Max	0.99
N	90		

63 pg:

AVE	0.6922	Min	0.32
SD	0.1660	Max	1.0
N	93		

APPENDIX

WI-34 for GF/STRmix™ workflow Overview for Analyst

1) Bring all data files into GeneMarker and analyze using GF_CTDSS.
2) Save GeneMarker project to file folder that HID files are in (U:\3130 NUCLEAR DNA CASEWORK & QC\To Be Analyzed).
3) Analysts may utilize QR-342 as follows: export text file to your "Analysis Export File" in your "To Be Analyzed folder". Use exported text file to create "Supplemental GlobalFiler Stutter Filter" worksheet, DNA QR-342, to use as a guide for stutter peaks with low template/n-2 stutter contributing. If the supplemental stutter filter is not utilized (e.g., if the profile is single source or if you feel that the filter is not needed), still print out the QR-342 worksheet and note that the batch had no N-2 or low template stutter concerns.
4) Analyze samples and click off artifacts. Please see DNA SOP-39 for further instruction.
5) If a sample needs to be re-injected, or if for another reason you are not reporting out a sample, disable the sample in GeneMarker, noting the reason on QR-325 and in GeneMarker.
6) Re-save GeneMarker file when all click-offs have been made and all appropriate samples have been disabled.
7) Re-export report as a text file to your "Analysis Export File" in your "To Be Analyzed" folder, you can save over the file created in step #3. This text file will be used by the Concordance Checker, Cross-Comparison/Staff Search, Contributor Estimator, Deconvolution workbook, and Allele Table macros.
8) Run the concordance checker (DNA QR-37) if positive controls are present, using the text file created.
9) Print out e-grams from samples and controls. If the analysis is done remotely, printing may be done later.
10) Repeat steps 1 thru 9 if there are multiple injection sheets.
11) Create DNA-QR-302 Contributor Estimation Worksheet for each sample with results. If it's a single source sample, or you feel you don't need the macro to assist you in determining number of contributors, you still need this sheet, but add notes to the comments area as to your determination of number of contributors.
    - Minimum number of male contributors will auto-populate on designated line.

12) Initial and date QR-4A (Batch Paperwork Review Worksheet).

13) Give batch to Technical Reviewer.

Technical Reviewer

1) Review all batch paperwork.
2) Open the analyst's fully analyzed allele table project.
3) Review samples, note any differences to the 1st Analyst. Check that:
    a. Correct analysis template is used.
    b. Analyst's assessment of size standards, ladders and controls are appropriate.
    c. All called peaks appear to be true alleles.
    d. All deletions/edits made are appropriate.
    e. You are in agreement with the number of contributor estimation.
4) Document any corrections on QR-347.
5) Initial and date QR-4A.
6) Give batch to Administrative Reviewer.

Administrative Reviewer

1) Review all batch paperwork.
2) Document any corrections on QR-347.
3) Initial and date QR-4A.
4) Give batch back to Analyst.

Analyst

1) Make changes to project(s), if necessary, according to the technical review. Analytical changes will require updates to allele table GeneMarker project & report text file, STRmix GeneMarker project & report text file and number of contributors worksheet.
2) For each sample project, start a new project by bringing in the same data files into GeneMarker, and analyze them using the GF_CTDSS_STRmix template. Save GeneMarker project with identical name as before, but with "STRmix" added to the end.

OR you can re-analyze your data from the current project by pressing the green play button again. A message will come up asking if you want to re-call the size standard. You want to check the box for "Call Size Again" and click on "Apply to all". Don't forget when you save this file to save it with STRmix added to the end.

3) Disable all samples that you disabled in the previous project.
4) Using your analysis on the e-gram peak tables as a guide, click off all the same peaks you just clicked off with the following exceptions (these exceptions will come up as non-concordances when you run your project comparison, to be noted in the comments section, with the exception of stutter peaks with low template/n-2 stutter contributing, that are below the laboratory stutter threshold):
    a. If you clicked off a stutter peak because pull-up contributed to it, leave this peak in your STRmix project, provided that the peak is still greater than AT, with pull-up subtracted, and the pull-up is not responsible for the majority of the peak height.
    b. If you clicked off a peak as high stutter, leave this peak in your STRmix project.
    c. Same goes if any of these peaks are a combo of different stutters
        i. There might be exceptions to this; for example, if the subtraction of pull-up brings the stutter peak below AT, STRmix should not see this peak, and if the pull-up contribution adds an unusually large amount of height to the stutter peak, it might be better for STRmix not to see such a high peak. Ask for assistance if you are unsure.
        ii. Peaks that you clicked off with n-2 stutter contributing, that are at or below the laboratory's stutter threshold, will remain in the STRmix project. These will not be listed as non-concordances when running the project comparison.
5) Re-save GeneMarker file when all edits have been made and all samples have been disabled.
6) Export report as a text file, to same location as the file created in step 3 on page 1.
7) Complete batch cross comparison and staff search ensuring all completed injections are included.
8) Run DNA-QR-301 Project Comparison Tool using the exported text files for each fully analyzed project and associated STRmix project.
    a. If there are any discrepancies not accounted for in your analysis, you must correct them in the appropriate GeneMarker project. Then re-save the GeneMarker projects AND re-export the text files AND re-run project comparison macro.

b. Keep repeating this until the number of discrepancies can be explained by your analysis.

c. Print out this sheet for the batch paperwork; you only need the final copy in your batch.

9) Complete QR-347. Initial and date QR-4a. Return batch to Technical Reviewer.

Technical Reviewer

1) Review any changes made by 1st analyst and new QRs (batch cross contamination and project comparison tool)
2) Check that the project comparison QR has appropriate number of non-concordances.
3) Add batch name to spreadsheet on U: Drive.
4) Initial and date QR-4A.
5) Return batch to Analyst.

Analyst

1) Scan batch paperwork (not containing sample e-grams & contributor estimator sheets) & save PDF file to U: Drive.
2) Move HID and project files from "To Be Analyzed" to "Completed" folder on U: Drive.
3) Give batch paperwork to Technical Reviewer to review.
4) Each case jacket will contain original e-grams and contributor estimator worksheets and photocopies of QR-4a (Batch Paperwork Review QA Checklist), QR-346 (Male Screen Results Worksheet) and QR-48 (Halt at Quant List), as appropriate.

Technical Reviewer

1) Ensure all pages scanned and PDF file on U: Drive is complete and accurate.
2) Ensure HID folders have been moved to U:\3130 NUCLEAR CASEWORK & QC\Completed.
3) Add initials to batch paperwork spreadsheet.

CASEWORK

Analyst

1) Enter all possible profiles into CODIS.
2) Make comparisons of all knowns to all Q's in case.
    a. Does a sample have 5 contributors?

i. If so, do any of the knowns warrant being assumed and conditioned? If yes, does that bring it down to 4 unconditioned contributors?

1. Yes – can interpret.
2. No – Clear major present – can make comparison to deduced major profile (DNA QR-318). No comparisons will be made to minor components of profile.
3. No – No clear major present – No comparisons will be made to profile.
4. More than 5 person mixtures will not be used for comparison.

b. Samples that are clear eliminations are not run through STRmix, "Eliminate by Analyst" in report macro.

c. Comparisons that are not clear eliminations, are run through STRmix.

i. Reported out comparison based on LR calculated.

ii. The "Sample ID" field shall contain both item # and known # (if applicable) so that there is a unique identifier in the footer of all pages when printed.

d. If reporting a statistic for a comparison, deconvolute in STRmix with appropriate knowns conditioned to profile.

i. Report out comparison based on LR calculated.

ii. If not reporting out a statistic (i.e., intimate sample with no suspect known submitted, single source sample when known expected in sample), no need to run STRmix; enter "assumed" in the report workbook. STRmix may be run if necessary to determine if assuming is appropriate.

e. Does a sample only have one allele at one locus? → inconclusive or eliminated by analyst. If a sample only has X at amelogenin, this is reported as no result.

f. Are you unable to determine the number of contributors with reasonable scientific certainty?

i. Clear major present – Can make comparison to deduced major profile. No comparisons will be made to minor components of profile.

ii. No clear major present – No comparisons will be made to profile.

iii. On a case-to-case basis, with TL approval, multiple contributor scenarios may be reported. For example, the STRmix results of a mixture can be reported if assuming a 2-person and then reported if assuming a 3-person mixture.

g. Is there a problem with the deconvolution discovered Re: 2nd diagnostics that can't be resolved? → inconclusive or eliminated by analyst

3) If a sample is deconvoluted through STRmix:

a. The file will automatically save onto the server, under F:\results.

b. Review the summary report. Ensure that the input file is correct. The letter "S" has previously been added to the beginning of the sample names of the STRmix input file when the project comparison macro was run.

c. Print out pages 1 thru 3 of the STRmix report, or more if "Per Locus Likelihood Ratios" for the three reported populations groups is on subsequent pages.

d. Scrutinize summary reports for any problems with the deconvolution.

i. Fill out DNA-QR-303 for the 2nd diagnostics for each deconvolution. Any assumed contributors will be recorded on DNA-QR-303.

e. Move the file in the F:\results folder to your folder in F:\ results.

f. Create a folder in your folder for this case, named with the case number in format DSS-XX-XXXXXX, and move that folder into this newly created case specific folder.

4) Review case jacket, write report.

5) Give case jacket to TR.

Technical Reviewer

1) Review case jacket, comparisons and report as you normally would. Document any corrections on QR-347.

a. If you disagree with an eliminated comparison, the 1st Analyst will have to run this sample through STRmix.

b. Review the summary report. Ensure that the input file is correct. The letter "S" has previously been added to the beginning of the sample names of the STRmix input file when the project comparison macro was run.

2) If you need to review more of the summary report than is in the case jacket, you can find it on the STRmix server, F:\Results in the Analyst's folder, in the folder specific to this case.

3) Give case jacket to AR. If a separate AR is not required, complete administrative review tasks as well.

Administrative Reviewer

1) Review case jacket and report. Document any corrections on QR-347.
2) Give case jacket back to Analyst.

Analyst

1) Make all appropriate corrections to report and case jacket and document on QR-347.
2) If the TR disagreed with one of your elimination calls, you must run that sample through STRmix, print out appropriate pages and fill out DNA-QR-303.
    a. The report will have to be re-worked:
        i. If it is still an elimination, it is now "elimination by STRmix", and not "elimination by analyst", report wording will be different.
    b. If it is no longer an elimination, report out result based on LR.
3) Once report corrections are complete, save the draft report as both a Word Document and PDF to U: Casereports. Electronically sign the PDF using a secure certificate and save to U:Casereports. If there are STRmix results, move the folder for this case from your folder on the server's F: Drive to F:\results\Completed.
4) Give case jacket to TR for correction check.

Technical Reviewer

1) Ensure all corrections are accurate.
2) If additional deconvolution work needed to be completed, make sure new wording in report is accurate and review all additional paperwork.
3) If there's STRmix data, check to make sure the folder has been moved to F:\results\Completed.
4) Electronically sign the report PDF using a secure certificate and save the final report to U: Casereports.
5) Give completed case jacket to analyst.

CODIS

Analyst

1) If there is a CODIS hit for a sample of yours, you will be notified by the CODIS administrator/alternate or their designee.
2) The CODIS hit might warrant the sample to be deconvoluted in STRmix. If a deconvolution of this sample has already been completed, the case jacket can be brought to the CODIS administrator/alternate or their designee.
3) If a deconvolution of this sample has not yet been completed, deconvolute the sample, making sure to condition any knowns that you assumed when writing the report.
    a. Print out page 1 of the STRmix report, fill out DNA-QR-303, and give the case jacket to your TR to review the additional paperwork. You can check, initial, and date next to 3.D. on DNA-QR-4.

Technical Reviewer

1) Review additional paperwork in case jacket.
2) Check, initial and date next to 3.D. on DNA-QR-4.
3) Give the case jacket to the AR

Analyst

1) When you receive your case jacket back for initials, move your STRmix folder to F:\results\completed. Check, initial, and date that you did so on DNA-QR-4.

OTHER

1) If a known comes in later, that warrants being conditioned, all deconvolutions need to be repeated with a new deconvolution, and a new statistic is calculated.
2) If a known comes in later that is not clearly eliminated regarding a sample that has already been deconvoluted, use the "LR to previously analysis" function of STRmix, and do not repeat the deconvolution.
3) Similarly, if you have a Q in the case, and two knowns are consistent with being a contributor to that sample, only deconvolute the sample once. For the 2nd known, use the "LR to previous analysis" function of STRmix.

WI-35 for GeneMarker

One-Time GeneMarker-HID (GM-HID) Setup

User Management

1. Within the GM-HID software on each analyst's computer, at least two Administrator accounts and one Analyst account are created by going to Help→ User Management. Additional accounts may be created if appropriate.
    a. An Administrator account has full privileges. Only designated staff will have Administrator access. Administrator accounts require a password.
    b. An Analyst account has the default set of privileges for this account type, with the addition of access to "Edit – View Preferences".
    c. Users designated as Administrators still need an Analyst account as well.
2. Log in as an Administrator.
3. Within the User Manager, on the "User Manager" tab, enter an Organization (ex. "CT Forensic Lab"). Check the box for "Run User Protection".
4. On the "Settings" tab, check the box for "Record Data Edit History". Click Ok.

Analysis Templates

1. Open the software and click on the folder icon in the upper left of the menu to open data. When analyzing GlobalFiler (GF) data, click on the 'Dyes' button in the lower left and make sure 6 dyes is selected. Click on 'Change Chemistry' and select 'AB'. Channel 1 should read 'Blue, 6-FAM', channel 2 should read 'Green, VIC', channel 3 should read 'Yellow, NED', channel 4 should read 'Red, TAZ', channel 5 should read 'Purple, SID', and channel 6 should read 'Orange, LIZ'. 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 .hid 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.

2. Log in as an Administrator (if not already).

3. 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 'GF_CTDSS' or 'GF_CTDSS_STRmix' depending on whether the stutter filters are applied. For known samples amplified by the GlobalFiler Express (GFE) Kit, name the template 'GFE_CTDSS_20%'. Select the panel with the same name. Set the size standard to GS600 and the standard color to 'LIZ'. The GS600 size standard should be edited using the 'Size Template Editor' to disable the following sized peaks of the standard: 20, 40, 514, 520, 540, 560, 580, and 600. After opening the editor and selecting the size standard, change the value in the 'Enabled" column from '1' to

'0' for the sizes listed above. Save the changes. The analysis type should be 'HID'. Click on the 'Next' button to display the 'Data Process Options' window.

a. As a note, the three panels contain the same settings except that the GF_CTDSS_STRmix panel has no thresholds for stutter, and the GFE_CTDSS_20% panel is programmed for diploid results.

4. For 'Raw Data Analysis' select 'Auto Range', 'Smooth', 'Superior', 'Spike Removal', 'Saturation Detection', 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 502. For 'Peak Detection Threshold', leave the default parameters of '50', '32500', and '20' for 'Min Intensity', 'Max Intensity', and 'Percentage >, Global Max', respectively. Click on the 'Next' button to display the 'Additional Settings...' window.

5. Select 'Auto Select Best Ladder' and set 'Allow Match # Variance' to '0', 'Max Average Size Diff' to '0.40' and deselect 'Use Ladder Library'. Select 'Auto Panel Adjustment'. For the 'Positive Control Template' select 'GF'. 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'.

6. 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 each of the three panel templates used for analysis in turn. The '[Ploidy]' number should be set to '0' for the two templates used for evidence and '2' for the template used only for knowns. If not, right click on the panel name and select 'edit' and change the ploidy to the appropriate value 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 are dye specific and should be set to '40' RFU for Blue, '55' RFU for Green, '35' RFU for Yellow, '50' RFU for Red, and '55' RFU for Purple. The 'Max Heterozygote Imbalance (%)' should be 1% for forensic unknown profiles and 21% for knowns. The 'Min Heterozygote Imbalance (%)' should be 0% for forensic unknown profiles and 20% for knowns. The imbalance settings for knowns effectively creates a 20% marker filter in lieu of stutter filters. If changes needed to be made, select the 'Apply Homo/Hetero Settings to All Markers in this dye' option. For the stutter filter, 'Use Allele-Specific Values (From Panel)' should be selected for the GF_CTDSS and GF_CTDSS_STRmix panels and 'Use Marker-Specific Values' for the GFE_CTDSS_20% panel. 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).

Viewing Preferences

1. Select 'View' from the toolbar menu then select 'Preference...'.

2. 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\To Be Analyzed\Analyst Initials.

3. 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 and set to 96, Max Chart # in Page' (set to '4'), and 'Max Allele Label Layers' (set to '8'). Under 'Peak Label', select 'Height' and 'Vertical' and set the 'Position' to 'Allele Label'.

4. Under the 'Forensic' tab, set the Ladder, Positive Control, and Negative Control Identifiers to 'LADDER', 'POS', and 'NEG', respectively. Select 'Show Ladder Samples in Report', Show Control Samples in Report', and 'Mark Deleted/Edited Peaks with Symbols'. When done, click 'Ok'.

Edit Record

1. View any sample in the All Color Browser. Right click on any peak. Select "Edit Comments."

2. Add any necessary comments for edits (i.e., stutter). See SOP 1 Appendix 2 for designated abbreviations. Click Ok.

3. Right click on any peak. Select "Show Columns". Edit this list so that the following columns (and no others) are checked: Size, Height, Marker, Allele, and Allele Comments.

Print Templates

1. Select the 'Print Report' icon in the toolbar menu. Click on 'Select an Existing Template or Create One' and name the template.

2. Under the 'Standard' tab, select 'Normal', 'Selected Samples', 'Electropherogram', '6-FAM', 'VIC', 'NED', 'TAZ', 'SID', 'Hide Bins', 'Peak Table', and 'Start on Separate Page'. Under the 'Advanced' tab, select 'Print Report Header', 'Each Page', 'File Name', 'Print Markers', 'Print Alleles', 'Print Edited Peak Only', 'New Page for Each Sample' and 'Implement Y Axis Settings'. Set the 'Chart Height' to 64. Under the 'Page' tab, select 'Portrait' and set all four Margins to '0.0'. Click 'Save'. Click 'Preview'.

3. If the analyst routinely processes known samples, create a separate print template for known egrams. (Other analysts may add this template as well). Use the same settings as above with

these exceptions: de-select 'Peak Table' on the 'Standard' tab, de-select 'File Name' on the 'Advanced' tab and set the Chart Height to 37-40 depending on whether peak heights will be displayed. Ideally known egrams should be a single page; the Chart Height may be adjusted further.

4. Optionally, analysts may create additional print templates. For example, a template for controls could use the same settings as the known template but have 'Peak Table' selected with the option 'Start on Separate Page'.

Report Settings

1. Click on the "Report Settings" icon.

2. Select the following options: 'Forensics', 'Show File Name', 'Vertical', 'Exclude Sample Index', 'Exclude Report Header', 'Split Items', 'Show Allele Name', 'Show Size', 'Show Height', and 'Show Rejected Low Score Alleles'. De-select all other options. Click Ok.

Analysis in GM-HID

Note that all settings selected remain in effect until changed. If any of the above default settings are changed during the analysis of a project, be sure to change them back again.

Creating a Project

1. Open GM-HID and log in using the appropriate Analyst (not Administrator) account.

2. Import all the pertinent data files as in Step 1 under "Analysis Templates".

3. Select 'File' and then 'Save Project' from the toolbar menu. Save the project to a network folder containing the .hid files analyzed. Type in a name for the project in the format: "Kit-3500#-BatchID-Modifier#-Any Other Details" (ex. "GF-2-MGR050317-2-JES") and navigate to the appropriate location, and click the 'Save' button. Modifier number and other details are optional. For descriptions of all options and tools for GeneMarker, please see the GeneMarker HID User Manual.

4. Click on the green arrow button in the toolbar menu to open the 'Run Wizard' window. Select the template GF_CTDSS, GF_CTDSS_STRmix, or GFE_CTDSS_20% as appropriate to your samples. Click 'Next' on the 'Template Selection' and 'Data Process' screen and 'Ok' on the 'Additional Settings' screen. If this is a re-Analysis of a previous analyzed project, select 'Call Size Again' and click 'Apply to All' when prompted.

5. Note that even though allele calls have been generated, the changes to the project have not been saved. Re-save at any time during analysis.

6. 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 these GS600 standard peaks are present (60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, 460, 480, 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.

7. 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 Step 3 under "Viewing Preferences"), 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.

8. 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.

Editing a Project

1. View each sample in the 'All Color Browser'. (See SOP 39 for details on data analysis.)

2. Select a peak by clicking on it. De-select by clicking a selected peak while holding "Ctrl", or de-select all peaks in a color at once by double-clicking elsewhere in the color.
    a. Note that once a peak has been selected, it remains selected until performing one of the actions to de-select it or closing the All Color Browser.

3. To delete a peak, select it, right-click and select 'Delete'. Multiple peaks can be deleted at once.

4. For any type of stutter peak in evidentiary (forensic unknown) profiles add a comment designating that peak as stutter. Right-click the peak and select 'Edit Comments'. Stutter (st), high stutter (high st), and stutter+pull-up (st+pu) are all examples of the type of comments that should be made. This is to aid in the downstream process when making the STRmix project where stutters are left in. Any peak that is to be left in the STRmix project will be labeled in Genemarker. Controls do not need any artifacts labeled as they do get analyzed with STRmix.

5. Any other type of artifact is deleted; a comment is unnecessary as protocols have detailed definitions of each artifact type and its appearance. Each deletion will be recorded in the casefile as well. DNA SOP-39 "Globalfiler and Globalfiler Express Interpretation" contains these definitions.

6. To confirm a red/yellow flagged peak, right-click the peak and select 'Confirm'.

7. To change an allele call, right-click the peak and select 'Edit Allele" and adjust the label as appropriate.

8. If it is appropriate to insert an allele, right-click the peak and select 'Insert Allele'. Adjust the label as needed.
    a. Zoom in on the peak to insert it at the precise size where the peak is highest.

9. For casework known and database samples, reasons for routine edits do not need to be recorded. Issues such as a change in allele call or insertion of an allele are noted on the 3500xL analysis worksheet.

10. Disable any samples not being reported and note the reason in the Comments section on the 3500xL analysis worksheet. Use sufficient detail to be clear to a reviewer; for example: "re-inject at max due to low peaks" or "re-prep sample- ILS failed."

11. Re-save the project when edits are complete.

Printing Electropherograms

1. After 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 70 to 450. Click 'Ok'.

2. Select the samples to print (all samples in the project that will use the same print template) by double-clicking to checkmark their sample name(s) in the sample tree. Select the 'Print Report' icon in the toolbar menu. Print each set of samples using the appropriate template. See SOP 23 for sample types required to print.
    a. If appropriate for a sample, chart height may be lowered to better fit data on fewer pages or raised for a better view of profile details (see Step 2 under "Print Templates").

3. Should an electropherogram need to be re-printed, be sure to manually note edit changes on the peak table or cross out the edit information for that sample from the original table if printing a new table.

Exporting a Report

1. Click on the "Save" icon next to the "Report Settings" icon.

2. For "Save as Type:", choose "text file". Save in the designated network folder for Analysis Export Files.

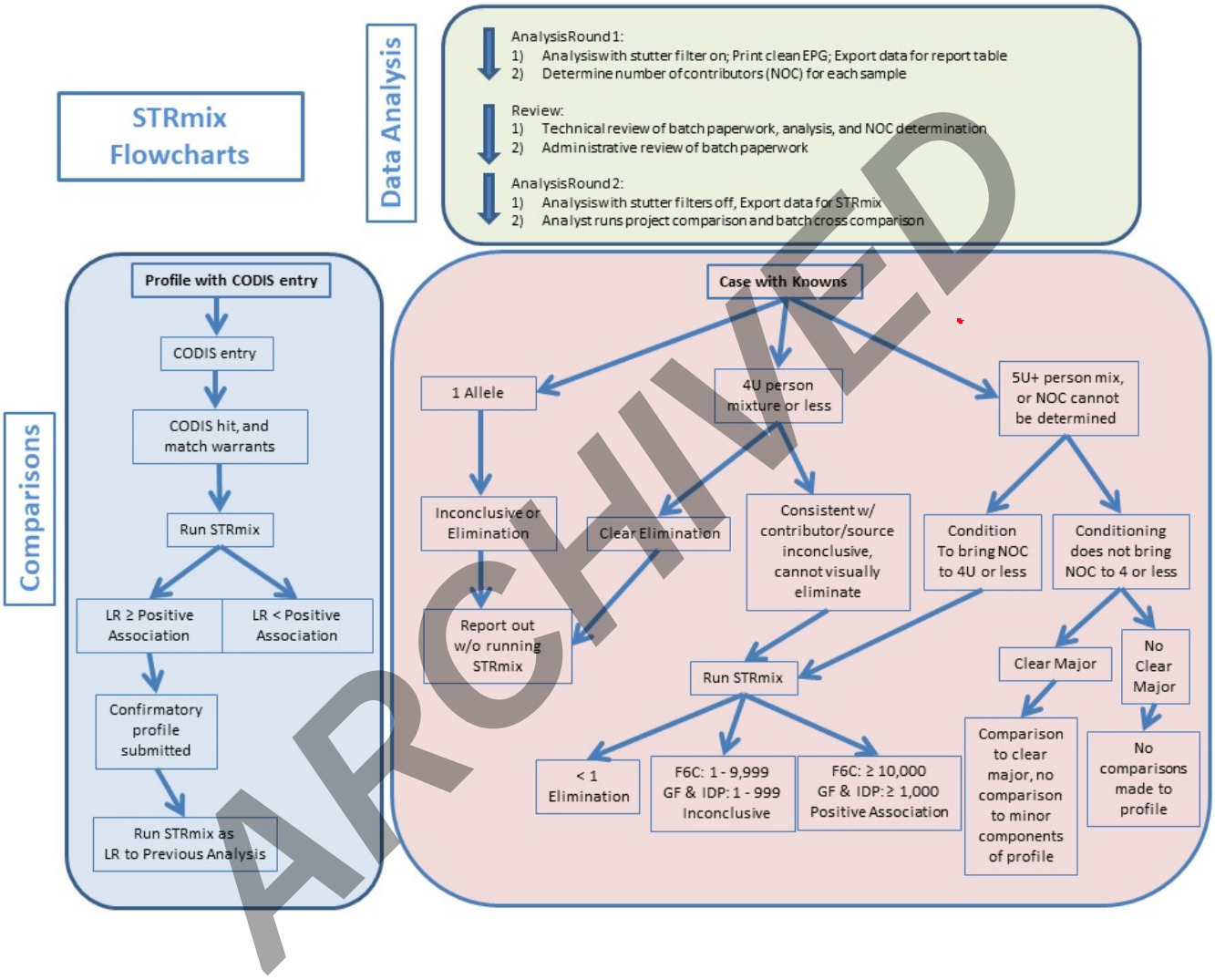
Reviewing a Project

1. Open GM-HID and log in using the appropriate Analyst (not Administrator) account.

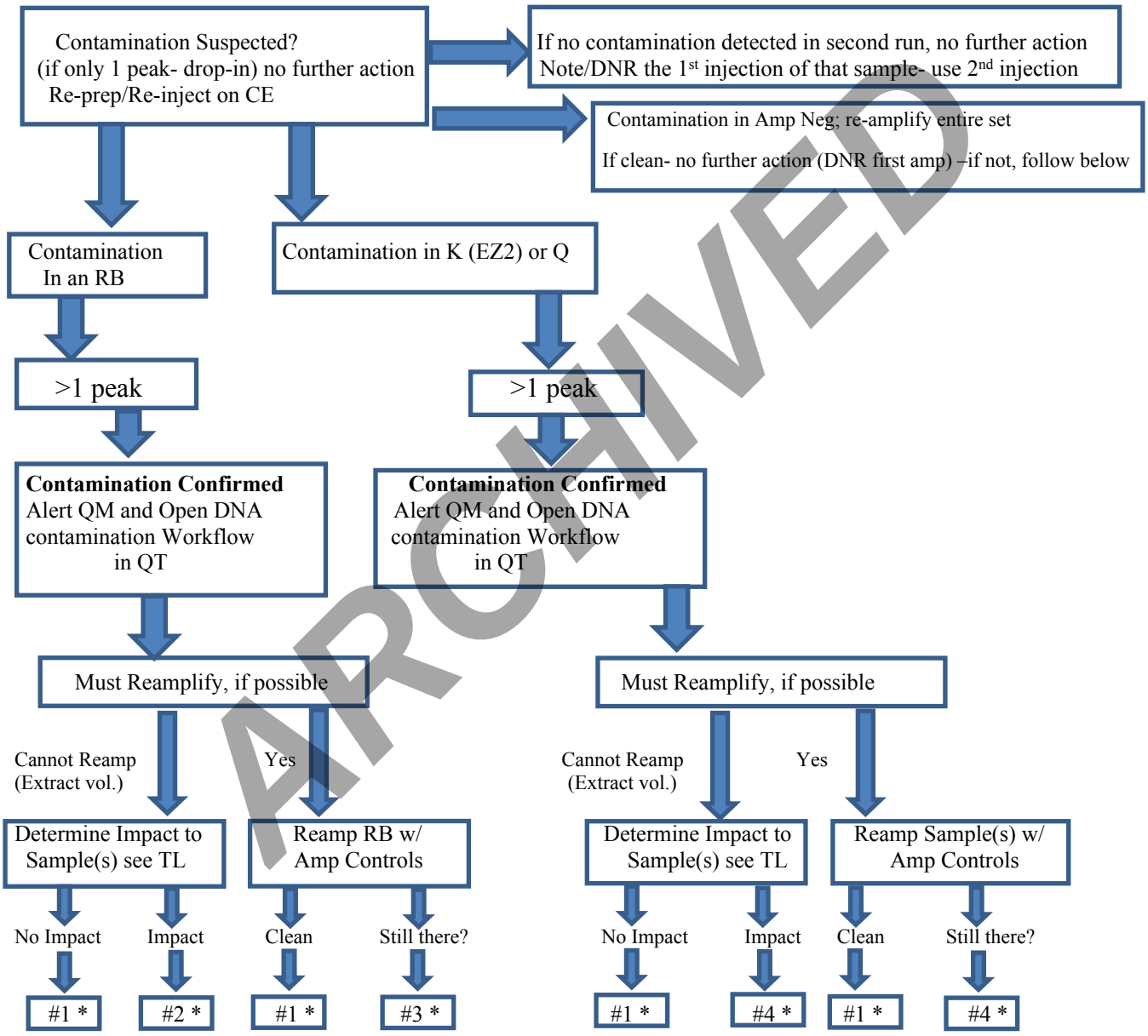
2. See electropherogram headers to check that the correct Analysis Template was used.

3. When checking edits, match edited peaks onscreen (marked with an "X" or "E" symbol) to edits listed in the printed peak tables using size by holding crosshairs above the edited peak.

4. To confirm that reasons for not reporting samples, as listed in Comments on the 3500xL analysis worksheet, are appropriate, re-enable disabled samples. (Do not save the project).


STRmix Flowcharts
Data Analysis
Analysis Round 1:
1) Analysis with stutter filter on; Print clean EPG; Export data for report table
2) Determine number of contributors (NOC) for each sample
Review:
1) Technical review of batch paperwork, analysis, and NOC determination
2) Administrative review of batch paperwork
Analysis Round 2:
1) Analysis with stutter filters off, Export data for STRmix
2) Analyst runs project comparison and batch cross comparison
Comparisons
Profile with CODIS entry
CODIS entry
CODIS hit, and match warrants
Run STRmix
LR ≥ Positive Association
LR < Positive Association
Confirmatory profile submitted
Run STRmix as LR to Previous Analysis
Case with Knowns
1 Allele
4U person mixture or less
5U+ person mix, or NOC cannot be determined
Inconclusive or Elimination
Clear Elimination
Consistent w/ contributor/source inconclusive, cannot visually eliminate
Condition To bring NOC to 4U or less
Conditioning does not bring NOC to 4 or less
Report out w/o running STRmix
Run STRmix
Clear Major
No Clear Major
< 1 Elimination
F6C: 1 - 9,999 GF & IDP: 1 - 999 Inconclusive
F6C: ≥ 10,000 GF & IDP: ≥ 1,000 Positive Association
Comparison to clear major, no comparison to minor components of profile
No comparisons made to profile

Contamination Workflow



***See below for Numbered Outcomes**

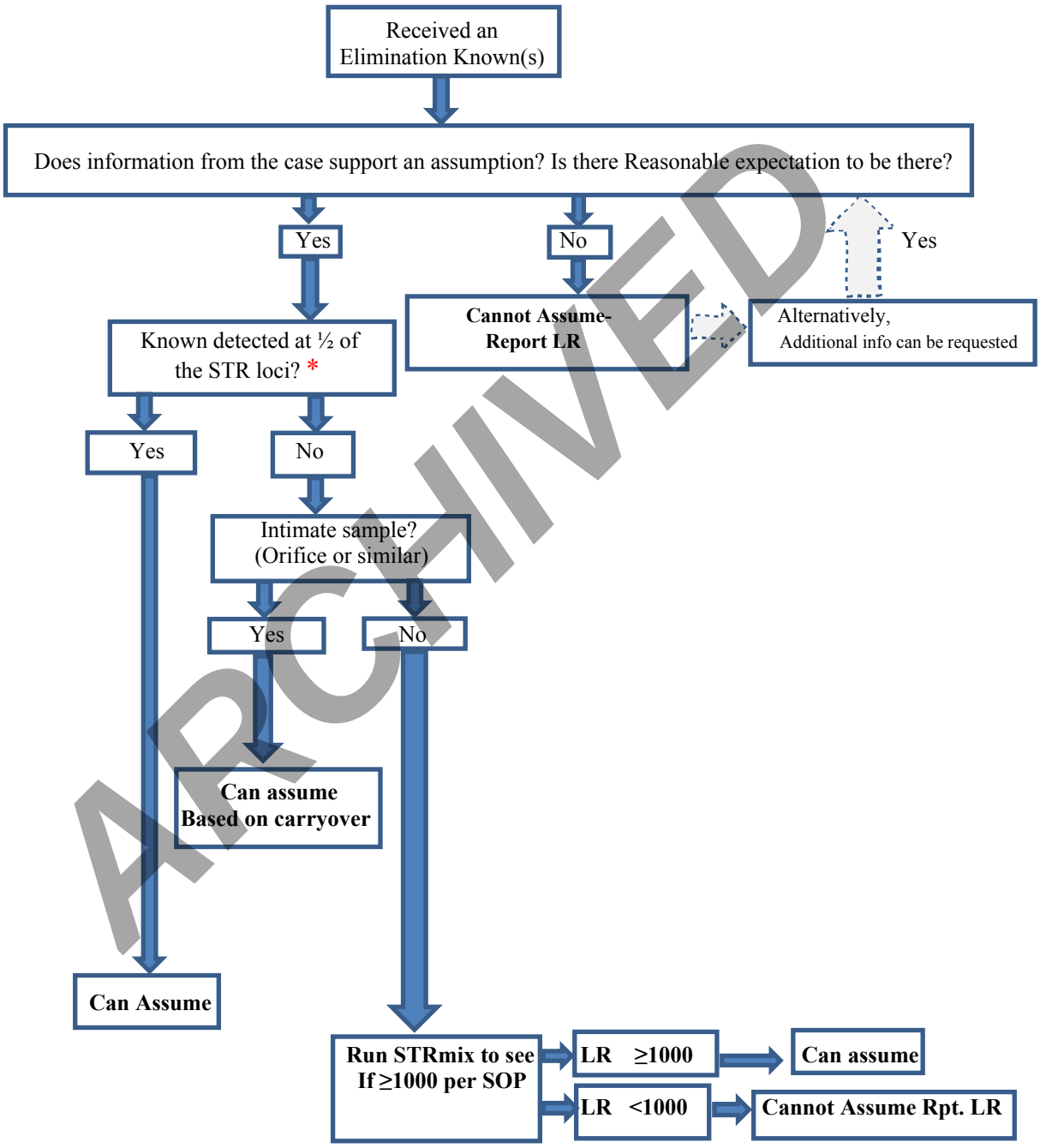
#1. No Additional Work. May report as normal.

#2. Samples impacted will not be used for any comparison. Report out contamination event. Discuss with TL.

#3. Decide if the contamination impacts samples. If no, use #1. If yes, use #2.

#4. If it is an identified source (such as a staff contamination), see if you could assume and then condition in STRmix. If it is an unknown source of contamination, consider consumables, work area, instrument, etc., and discuss with TL. Anything impacted will not be used for comparison purposes. Report out contamination event(s).

Workflow for Elimination Knowns vs. Assuming/Conditioning.



***If you have a 4–5-person mixture: needs to be more loci and definitive due to coincidental matches. Use caution. See TL for any questions.**