STR Interpretation Guidelines

ORIGINAL

1. Definitions

Activity: A point on an electropherogram that is on-ladder, has a maximum height < 75 RFU, and is three times greater than the average background.

Allele: An alternative form of a gene; the allele designation is used to designate a specific size fragment of DNA for a specific locus in STR analysis.

Allelic Dropout: An occurrence where one or more alleles from an individual's DNA profile fails to amplify and is not observed in the profile. Allelic dropout may be detected by severe imbalance of loci where the smaller fragments are observed and the larger fragments are not observed and/or observance of activity as defined above,

Artifact: Erroneous peaks or activity that are byproducts of PCR technology and capillary electrophoresis.

DNA Profile: The genotype obtained from a completed DNA analysis tested at multiple loci.

Injection: When a DNA sample is electrokinetically introduced into a capillary for electrophoretic separation.

Inhibition: The total or partial suppression of the PCR process that would result in partial or no DNA profile being obtained.

Intimate Sample: A sample collected from a person's body or an object collected from a person that has made close physical contact with that person. There is an expectation that the person's DNA profile from whom the sample or object was collected may be observed when analyzing that sample or object. The analyst may use discretion when determining whether or not an item is considered "intimate".

Locus (plural=Loci): The chromosomal location or location of a gene or DNA marker.

Microvariant: An allele that varies by less than the consensus repeat unit and is not defined by a ladder allele. Microvariants are observed "in-between" the ladder alleles for a specific locus.

Mixture: If a DNA profile is observed to have more than two peaks at more than one locus, then there is a high possibility that there is a mixture of two or more

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individual's DNA profiles. **NOTE**: If three peaks are observed at only one locus, then there may not be mixture; the individual contributor may have a tri-allelic pattern at that locus. Both sample and standard should express the tri-allelic pattern in cases of a MATCH.

Off-Ladder Allele: An allele observed outside the region covered by the allelic ladder at a given locus.

Partial DNA Profile: A DNA profile that exhibits probable allelic dropout, degradation, and/or preferential amplification at one or more loci. The analyst may not be able to make conclusions as to the individual(s) that can be potentially included or excluded.

Partial Predominant DNA Profile: A Predominant DNA Profile (see definition below) that exhibits probable allelic dropout, degradation, and/or preferential amplification at one or more loci OR mixture that contains a Predominant DNA Profile (see definition below) at most of the loci.

Peak: A well defined point on an electropherogram that is on-ladder and has a minimum height of ≥75 RFU. Peaks should be considered alleles unless the analyst justifies not doing so. See "Microvariant" and "Off-Ladder Alleles" for exceptions to the "on-ladder" requirement.

Predominant Alleles/Predominance: Given a mixture of two or more contributors at a specific locus, one or two alleles have an RFU value greater than approximately 50% of all other alleles at that locus and a single source may be readily inferred by the observed data.

Predominant DNA Profile: Given a mixture or two or more contributors, one or two alleles at every locus typed has an RFU value greater than 50% of all other alleles and a single source may be readily inferred by the observed data.

Pull-up: A signal from an allele labeled with one dye-set may show up as a peak or Off-Ladder Allele in another dye-set.

Run: Each set of 16 samples that are separated electrophoretically on the Capillary Electrophoresis Unit (ABI 3100 or equivalent).

Shoulder and Tail: A "Shoulder" and "Tail" will be defined as an elongated or raised area to the immediate left and right of a main peak but is not separated from the main peak.

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Spike/electrical Spike: An artifact believed to be caused by a spike in the current within a capillary that causes a sharp increase in signal. This artifact lacks the defined morphology of a peak.

Split peaks: A split peak will be defined as one allele that is represented by two peaks. Lack of full A nucleotide addition may be observed when the amount of input DNA is greater than the recommended protocol. In this case, more time is needed for Taq Polymerase to add the A nucleotide to all molecules. Amplification of too much input DNA will also result in off-scale data (saturation of signal) and will be manifested as split peaks.

Stutter: An artifact of PCR amplification that is one repeat unit less than the corresponding main allele peak.

Tri-allelic Pattern: Three peaks observed at a single locus and is not the result of a mixture. These peaks may or make not be of equal intensity.

Unincorporated Dye: Unincorporated dye (a.k.a. "dye-blobs") can be observed in an electropherogram and are distinct morphologically from a labeled DNA fragment. A Dye-blob does not exhibit the typical sharp, distinct peak that is produced by actual alleles and is observed as a wider, thicker peak and may be lacking the sharply defined slope to the apex of a peak.

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2. DNA Quantitation Interpretation Guidelines for ABI Quantifiler

2.1. Background:

2.1.1. The Quantifiler Human Kit uses a real-time PCR reaction with fluorescent dye chemistry to estimate the quantity of amplifiable DNA in each test sample.

2.1.2. DNA quantitation results are interpreted by checking the analysis settings of the ABI 7000 machine, the slope of the standard curve of the DNA standards, and the R^2 value of the DNA standards.

2.2. Slope:

This value indicates the efficiency of the PCR reaction of the quantification assay. A slope value of -3.32 indicates 100% amplification efficiency. The range of values that is acceptable to deem the assay results as valid is -2.9 to -3.3. If the value falls outside of this range, then one point of the slope may be dropped to account for pipetting variations. If a point is dropped, then both the original and adjusted slopes must be printed and put in case notes.

2.3. R^2 value:

This value measures the closeness of fit between the standard curve line and the Ct values of the DNA standard used in the quantification assay. A value of 1.00 indicates a perfect fit between the standard curve line and the DNA standard data points. In order for the results from a given assay to be valid, the R^2 value must be greater than or equal to 0.98.

2.4. Internal Positive Control:

Each reaction in the Human Quantifiler Kit contains an Internal Positive Control (IPC) to help determine whether a sample is a true negative or whether there is inhibition occurring in the PCR reaction. The normal Ct values for the IPC should range from 20 to 30.

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2.5. Interpreting IPC amplification results:

Quantifiler Human (FAM Dye)	IPC (VIC Dye)	Interpretation
No amplification	Amplification	True negative
No amplification	No amplification	Invalid result
Amplification (low Ct and high delta Rn)	No amplification	Disregard IPC result
Amplification (high Ct and low delta Rn)	No amplification	Partial PCR inhibition

Note: The IPC may show inhibition if the Ct value in the sample is greater than the IPC value of the NTC or if the sample reads "Undetermined". In most cases, dilutions should be performed or the samples should be cleaned up using the "Concentration/Clean Up of Extracted DNA" section of the "Organic DNA Extractions" SOP.

2.6. No Template Control (NTC) and Extraction Controls

2.6.1 NTC: For each set of samples analyzed, a "No Template Control" (NTC) must be run to show that there was no contamination during the setup of the assay. Due to the sensitivity of the real-time PCR method, extremely low levels of DNA (levels that do not effect downstream applications such as STR analysis) can be detected. Therefore, if low level DNA with a Ct value of 36 or greater is observed in the NTC, it is still considered to be a negative sample and the results of the assay are still valid. However, if the Ct value of a NTC is less than 36, the NTC sample **may** be contaminated and the entire assay **may** be repeated. However, if the other negative controls show Ct values greater than 36, the analyst may proceed with the amplification of the samples.

2.6.2 Negative Extraction Controls: If the Ct value of a negative extraction control is less than 36, the analyst may either re-extract the set or continue processing the samples, realizing that activity may be observed in those controls in the post amplification product.

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3 PCR STR Interpretation

3.1_ Introduction: The interpretation of results in casework is a matter of professional judgment and expertise. These criteria are based on our validation studies, literature, and over 15 years of forensic DNA casework experience by this laboratory. However, it is not possible to address every situation with a pre-set rule. It is the responsibility of the analyst to use these guidelines in conjunction with their training and experience to provide a solid scientific interpretation of the results.

3.2_ Preliminary Evaluation of Data

3.2.1 General

3.2.1.1 The Peak/Height cut off in the GeneMapper™ ID software will be set at 75 RFU for Casework and 100 RFU for Convicted Offender Testing.

3.2.1.2 "Activity" shall not be designated as alleles for Convicted Offender samples or for determining matches for forensic cases. However, any information obtained from activity may be used for qualitative data interpretation (including, but not limited to, evidence for a mixture within a given sample or exclusions). "Activity" below threshold will be used for exclusion purposes only. The analyst must take great care and take into consideration possible artifacts, high noise, and the general quality of data when making the decision of use data below threshold for this purpose. Section 3.2.1.3 below must also be considered.

3.2.1.3 A general rule is that the Peak/Height to Noise (background) ratio should be 3:1. In other words, the Peak/Height should be at least 3 times greater than the average background for a peak to be called.

3.2.2 Positive Amplification Control: The Positive Amplification Control must have peaks that are in the proper location relative to the allelic markers. If these characteristic peaks are not in their correct position or are not present (too weak to interpret), that particular locus must be considered inconclusive for all samples and must be

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successfully re-injected, re-run, or re-amplified and analyzed before that locus may be used for analysis.

3.2.3 Negative Controls:

3.2.3.1. If any peaks are detected in the amplification negative control or the reagent control samples, then contamination *may* have occurred and the samples may not be interpreted at the locus or loci in question. If possible, the sample(s) associated with the negative controls will be re-analyzed (i.e. re-injected, re-amplified, or re-extracted). If not possible to reanalyze the data because of sample depletion, then the analyst may proceed with great care to interpret results of the samples.

For example, if an analyst's partial profile is observed in a negative control, then justification might exist to use the data from the samples since that profile might not affect the results.

3.2.3.2. If activity is detected in the negative control at a single locus or multiple loci, the analyst should use great care when interpreting the corresponding samples. The controls and/or sample(s) associated with the negative controls may be re-analyzed (i.e. re-injected, re-amplified, or re-extracted).

3.2.3.3. Artifacts observed in the negative samples will not cause those samples to be re-injected. Those artifacts shall be documented in the notes.

3.2.4. Positive Extraction Control: The known bloodstain from MJB is used primarily as an extraction control and the samples may be interpreted if it fails to amplify at all or any loci.

3.2.5. Allelic Ladders: The peaks must be equal to or greater than 75 RFU. If regions of the ladder samples are not present, the specimen peaks shall not be interpreted in these regions. The size standards must be present and correctly called.

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3.2.6. Internal Lane Standards (ILS)/250 bp peak: GeneMapper ID will not size the samples/ladders/controls unless all ILS are present and above threshold. Therefore, the analyst will examine the 250 base pair (bp) ILS peak of each sample. The 250 bp peaks should fall within a size window of < ± 0.5 bp. If a sample or control is >± 0.5 bp, then that sample should be re-run unless the sample or control does not exhibit amplified product (peaks). For example, if the ILS fails for a negative control, that negative control may be used for analysis if no peaks are observed when viewing the raw data. In this instance, the raw data must be printed instead of the electropherogram.

3.2.7. Samples

3.2.7.1. Visually inspect the known and questioned samples. Assess the quality of the peaks including RFU values and if artifacts are present. The peaks must be equal to or greater than 75 RFU for alleles to be called. The size standards within each sample must be present and correctly called.

3.2.7.2. Examine the electropherogram of the mixture. Note any inhibition, allelic dropout, and/or artifacts.

3.2.7.3. If the question sample(s) contain more than two peaks at the same locus, then the results *may* indicate a mixture. NOTE: If three peaks are observed at only one locus, then there may not be a mixture; the individual contributor may have a tri-allelic pattern at that locus. Both sample and standard should express the tri-allelic pattern in cases of a MATCH.

3.2.7.4. Failure of any loci to amplify for a multiplex STR system will not preclude the analysts from reporting those loci that are present, even if only one locus amplifies.

3.2.7.5. Samples that are overblown may need to be

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re-run with a lower amount of amplified product (a dilution) or re-amplified using a lower DNA template, depending on the overall quality of the electropherogram.

3.2.7.6. It is permissible to combine results from different injections (including dilutions) of the same sample when determining a final DNA profile.

3.3. Artifacts: The PCR process produces artifacts that are known and well characterized. All by-products of PCR and/or capillary electrophoresis will be labeled on the electropherograms as an "artifact" in the case notes.

3.3.1 Stutter

3.3.1.1 The STR results should not be considered to be inconclusive if stutter peaks are present in single source samples. Care must be taken when interpreting samples where mixtures are present in regards to peaks in the stutter position.

3.3.1.2 The GeneMapper™ ID software from ABI contains designated cutoff for peaks in stutter positions and will be used for designating stutter. Based upon analyst discretion, a minor peak in the stutter position that is called by the GeneMapper™ ID software may be disregarded as stutter if the peak in question is 1) not in a mixed sample and 2) when compared to the predominant sister allele, it is close to the percent stutter cutoff for that particular locus. If a mixture is observed, then great care must be used in interpreting weaker peaks in the stutter position.

3.3.2 Pull up: Generally, pull-up can be noted when all the alleles are overlapped using the software and the "pull-up" is observed as a relatively small peak located directly under the larger peak. Analysts should be aware of this phenomenon and use the computer software to aid them in discerning actual alleles from pull-up.

3.3.3 Unincorporated Dye: Analyst should not call "dye-blobs" as an

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actual allele. "Dye-blobs" shall not be considered for interpretation.

3.3.4 Assigning Values to Microvariants and Off Ladder Alleles

3.3.4.1. The GeneMapper™ID determines the base pair sizes of all peaks. The analyst may add the allele call by determining the correct allele size typing in the correct allele designation based on the base pair size.

3.3.4.2. Variant alleles that vary by less than the consensus repeat unit will be designated as an integer of that variation (for example TH01 9.3 allele), as per CODIS recommendations.

3.3.4.3. When the sample contains an off-ladder allele, the analyst must assign the off-ladder allele to the correct locus, if possible. The peak in this situation lies between two loci, so the analyst should first determine if the patterns of the two loci are heterozygous or homozygous. If the pattern is heterozygous at one locus and homozygous at the other, then the off-ladder allele is assigned to the homozygous locus (making it heterozygous). If both loci show homozygous patterns, the analyst will document that the allele was observed between the two loci (a locus will not be designated for that allele).

3.3.4.4. If an allele falls above the largest value or below the smallest value of the allelic ladder for a locus, the allele will be designated as either greater than (>) or less than (<) their respective allelic ladder, as per CODIS recommendations.

3.3.4.5. Microvariants must be documented in the case notes by including either the Sizing Table or the Allele Plots in GeneMapper ID that show the base pair size of the Microvariants and corresponding Ladder alleles. Microvariants do not have to be re-amplified or rerun.

3.3.5. Split peaks: Samples with alleles displayed as split peaks may be called. However, if a potential split peak is observed at a single homozygote locus (and not across the profile), the analyst should

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rerun the sample to ensure it is not actually a heterozygote with a microvariant with a 1 base pair difference between the two alleles.

3.3.6. Shoulder and Tail: Shoulders and tails will not prevent the analyst from assigning the specific peak an allelic value.

3.3.7. n+4 peaks: Analysts should also keep in mind that an artifact peak may appear in the n+4 position. When an n+4 peak is suspected, this should be documented on the allele call sheets or on the electropherograms.

3.4. Comparison of Profiles

3.4.1 The comparison and interpretation of DNA profiles is primarily a qualitative judgment based on careful review by a qualified analyst, utilizing all information pertinent to the tests undertaken.

3.4.2 Matches and non-matches are determined by careful, objective qualitative and quantitative evaluation of the entire profile produced by the various loci tested. It is scientifically acceptable for a match or non-match to be determined for a case when one or more of the loci yield inconclusive results. A match will be based only on loci which yield conclusive results.

3.4.3 Match: DNA profiles are considered to match if their patterns are the same and after taking into consideration the properties of the substrate tested and limitations of the specific techniques used.

3.4.4 Non-Match: Assuming a single source from a forensic sample, two DNA profiles are considered to be a non-match if there is a difference of even one allele after taking into consideration the circumstances of collection and preparation of samples and knowledge of the properties of the substrate tested and limitations of the specific techniques used.

3.4.5 Consistent With/Cannot Be Excluded: In a mixed DNA sample, a person cannot be excluded if the individuals entire profile is present in the mixture or if part of the individuals profile is in the mixture, allelic dropout is present, and the individual cannot be scientifically excluded from the mixture.

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3.4.6 Inconclusive results for an entire sample or for minor alleles in a mixture are usually the result of an insufficient quantity of DNA or complete degradation of DNA present in a sample. Inconclusive results may result from, but are not limited to, the following causes:

3.4.6.1. Insufficient amounts of DNA for that locus in one or more of the samples tested.

3.4.6.2. Degradation of one or more of the peaks in any sample tested.

3.4.6.3. Preferential amplification due to great differences in amounts of DNA present in a sample from multiple contributors.

3.5. Conclusions: Included below is a list of statements that may be used when reporting the results of DNA analysis and when a single source profile is observed. **This is a general list and may not include all possible scenarios. The statements should be used if the scenario fits. However, other statements may have to be used to fit scenarios not included below. Any additional statement should be consistent with the DNA results and conservative and objective in nature.**

3.5.1 Single Contributor

3.5.1.1 A sample may be considered to consist of a single contributor when no more than two alleles are observed at each locus. All loci are to be evaluated in making this decision.

3.5.1.2 If three alleles are observed at one locus, then there may not be a mixture; the individual contributor may have a tri-allelic pattern at that locus.

3.5.1.3 Match (Complete Profile)

If the DNA profile from a known sample matches the DNA profile from a single contributor, then the results

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may be reported as follows:

*"The DNA profile obtained from (Item) **MATCHED** the DNA profile obtained from ____ (Item) and **DID NOT MATCH** the DNA profile obtained from ____ (Item)."*

If the Forensic Unknown profile meets requirements for upload into CODIS, the following statement will be added:

"The DNA profile from Item(s) ____ will be routinely queried against the CODIS (Combined DNA Index System) Database."

3.5.1.4 Match (Partial Profile of a Sole Sample)

If a sole forensic sample exhibits a partial profile and matches at those loci where data were obtained, then the following statement may be used:

*"A partial DNA profile was obtained from____ (Item). The partial DNA profile **MATCHED** the DNA profile obtained from____ (Item) and **DID NOT MATCH** the DNA profile obtained from____ (Item).*

Or:

*"A partial DNA profile was obtained from (Item). The partial DNA profile was consistent with the DNA profile obtained from ____ (Item) and **DID NOT MATCH** the DNA profile obtained from____ (Item).*

If the Forensic Unknown profile meets requirements for upload into CODIS, the following statement will be added:

"The DNA profile from Item(s) ____ will be routinely queried against the CODIS (Combined DNA Index System) Database."

Note: Population Frequency Data should be given in

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instances where there is an inclusion on a partial profile (if probative).

3.5.1.5 Match (Partial Profile of Additional Samples)

If one or more samples match at all 16 loci and subsequent samples exhibit a partial profile but Match at those loci where data were obtained, then the following statement may be used:

*"A partial DNA profile was obtained from (Item). The partial DNA profile obtained from ____ (Item) **ALSO MATCHED** the DNA profile obtained from ____ (Item) and **DID NOT MATCH** the DNA profile obtained from ____ (Item).*

Or:

"A partial DNA profile was obtained from (Item). The partial DNA profile was consistent with the DNA profile obtained from ____ (Item).

Note: Population Frequency Data may be calculated in this situation if necessary. If Population Frequency Data are not calculated, the following phrase must be added:

"No Population Frequency Data were generated for this Match."

3.5.1.6 Non-Match: If the DNA profile from a known sample does not match the DNA profile from a single contributor, then the results may be interpreted as follows:

*"An unknown DNA profile was obtained from (Item) that **DID NOT MATCH** the DNA profile obtained from ____ (Item) or the DNA profile obtained from ____ (Item). This profile will be routinely queried against the CODIS (Combined DNA Index System) Database."*

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"A partial DNA profile was obtained from (Item) that **DID NOT MATCH** *the ___ (Item) or the DNA profile obtained from ___ (Item). This profile will be routinely queried against the CODIS (Combined DNA Index System) Database."*

3.5.1.7 When only the profile of the "owner" of an intimate object is observed, the report may state:

"No DNA profile different from that of the victim (Item) was obtained from the __ (Item)."

3.5.2 Mixtures

3.5.2.1 A sample may be considered to consist of a mixture of major and minor contributors if there is a distinct contrast in RFU values (approximately > 50%). In cases where there is clearly a major and minor contributor (higher peaks indicating a major or predominant profile and lower peaks indicating a minor profile), a predominant DNA profile may be attributed as coming from a known contributor in the case of a match. However, since heterozygotes have generally been shown to have up to a 50% peak/height imbalance, the analyst must use care in discerning minor and major alleles based on peak height and take into account any "additive effect" that may occur with shared alleles.

3.5.2.2 If a predominant profile will be used for comparison purposes, the minor alleles shall be designated on the Allele Call sheets by placing parenthesis around the minor allele(s). For example, if the profile at vWA is a "15,16,17" and has a minor allele at "16", the call sheet should look similar to the following: "15 (16) 17". All loci must be evaluated in making this determination.

3.5.2.3 If there is evidence of a mixture of only two profiles in the mixture and the predominant profile shares the same alleles at a locus as the minor contributor, then predominance can be called at that locus.

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3.5.2.4 Frequency Calculations

3.5.2.4.1 It is permissible to calculate population frequency data (Random Match Probability) on any predominant profile where a match is observed.

3.5.2.4.2 Furthermore, it is permissible to subtract out the "owner's" DNA profile of an intimate sample when there is no predominant profile and perform Random Match Probability. Donor alleles may only be subtracted out when there are no shared alleles and 1) both donors are heterozygotes or 2) the "owner" is a homozygote and the other donor is a heterozygote.

3.5.2.5 It is acceptable for analysts to define assumptions based on the data observed in the notes. For example, the analyst may preface a mixture statement with "Assuming two contributors,..." or "Assuming multiple contributor,....".

3.5.2.6 In the event that a mixture profile with a major and minor profile (two identifiable contributors) is obtained, the following interpretation guidelines shall be met before declaring a standard as a possible source of the minor contributor:

Two minor alleles are present at one locus in the mixture which is also present in the profile of the standard.

Or

The standards full locus profile is observed in a minimum of 2 loci in the mixture.

If the above criteria fail to be met then the interpretation may be used: "No Conclusion can be rendered as to the donor of the minor profile."

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3.5.2.7 Included below is a list of statements that may be used when reporting the results of DNA analysis and a mixed profile is observed. This is a general list and may not include all possible scenarios. The statements should be used if the scenario fits. However, other statements may have to be used to fit scenarios not included below. Any additional statement should be consistent with the DNA results and conservative in nature.

3.5.2.7.1 Mixture with Predominant Profile - Where multiple contributors are possible (e.g. victim and suspect), all peaks present in the questioned sample can be accounted for by the standards, a clearly predominant profile is observed, the laboratory report may state:

"The DNA profile obtained from ____ (Item) is CONSISTENT WITH A MIXTURE. The predominant profile MATCHED the DNA profile obtained from____ (Item)."

If the Forensic Unknown predominant profile meets requirements for upload into CODIS, the following statement will be added:

"This profile will be routinely queried against the CODIS (Combined DNA Index System) Database."

If a known standard cannot be excluded as a contributor to the mixture, the laboratory report may additionally state:

"The DNA profile obtained from____ (Item #), cannot be excluded *as a contributor to the mixture."*

If a known standard can be excluded as a contributor to the mixture, the laboratory report may additionally state:

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" The DNA profile obtained from_____ (Item #), was excluded as a contributor in the mixture."

3.5.2.7.2 Mixture with either a Partial Predominant Profile or an incomplete profile

In cases where a mixture is obtained with either a partial predominant profile or an incomplete profile due to allelic dropout (one or more loci cannot be used for comparison purposes) and there is a major/minor contributor, the laboratory report may state:

"The partial DNA profile obtained from ____ (Item) is indicative of originating from more than one contributor and is CONSISTENT WITH A MIXTURE. The predominant profile MATCHED the DNA profile obtained from____ (Item). The weaker profile is CONSISTENT WITH the DNA profile obtained from ____ (Item).

Or:

"A partial DNA profile was obtained from ____ (Item). The partial predominant DNA profile was consistent with the DNA profile obtained from ____ (Item).

If the Forensic Unknown predominant profile meets requirements for upload into CODIS, the following statement will be added:

"This profile will be routinely queried against the CODIS (Combined DNA Index System) Database."

If a known standard cannot be excluded as a contributor to the mixture, the laboratory report may additionally state:

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"The DNA profile obtained from ____ (Item #), cannot be excluded *as a contributor to the mixture."*

If a known standard can be excluded as a contributor to the mixture, the laboratory report may additionally state:

" The DNA profile obtained from ____ (Item #) , was excluded as a contributor to the mixture."

Note: Population Frequency Data may be calculated in this situation.

3.5.3 Mixture with no major/minor contributor

Combined Probability of Exclusion (CPE) Frequency Calculations will be performed on these samples. See Section 4.4 for CPE guidelines.

3.5.4 Mixture of Victim and Foreign Profile

In the event that a profile is obtained that is consistent with a mixture of the victim and a foreign profile and the victim profile is not the predominant profile, the alleles that are solely from the victim should not be entered into CODIS. Alleles that are shared should be entered into CODIS and alleles that are solely from the foreign profile should be entered into CODIS as obligate alleles. **NOTE: The criteria for NDIS samples (4x4 rule) must be met before samples can be uploaded.** This may be reported as:

"The DNA profile obtained from ____ (Item) is ***CONSISTENT WITH A MIXTURE*** *of the victim and one unknown contributor. This profile will be routinely queried against the CODIS (Combined DNA Index System) Database. "*

3.5.5 Inconclusive Results

In the case where an analyst does not have sufficient data to reach a conclusion, then the report should state:

"No conclusive DNA profile was obtained from _____(Item #)."

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3.5.6 No DNA Results

In the case where no profile is obtained from an item, then the report should state:

"No DNA profile was obtained from _____, (Item #)."

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4 Statistical Interpretation

4.1. This laboratory will utilize the North Carolina Databases which have been reviewed by Dr. Bruce Weir. Databases for the North Carolina Caucasian, Black, and Lumbee Indian populations were generated by this laboratory. The North Carolina Hispanic database was developed by the Charlotte/Mecklenburg Police Department Crime Laboratory. These data have been entered into PopStats. Analysts will use the PopStats program provided with the CODIS software to calculate population frequency information.

4.2. Statistics for a Match

4.2.1. Random Match Probability (RMP): The formulas used in the calculation of the frequency of a DNA profile will be in accordance with those published in the NRC II guidelines and in PopStats.

4.2.1.1. Heterozygote frequencies: $2pq$

4.2.1.2. Homozygote frequencies: $p^2 + p(1-p)\,\theta$, where $\theta = 0.01$

4.2.1.3. Multi-locus frequencies: the product rule will be used

4.2.1.4. Minimum allele frequency: $5/2N$ ($2N$= # alleles in the population database at that locus)

The minimum allele frequency will be used for any allele which is seen less than 5 times in the population frequency database (to include variant and off-ladder alleles).

4.2.1.5. RMP will be used to calculate population frequency statistics in cases of a probative match using the PopStats program in the CODIS Software.

4.2.1.6. RMP may be used to calculate population frequency statistics in cases of a match generated by a partial profile or a mixed profile with a predominant profile or a partial predominant profile. In these situations, only the loci that are used to call the Match may be used for population frequency data.

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4.2.2. Match Statement

4.2.2.1. The Match Statement when the RMP for all population subgroups exceeds 1 in 1 Trillion (greater than the world's population) may be as follows:

"This laboratory maintains population databases for the NC White, Black, Lumbee Indian and Hispanic populations and has access to other population databases which can be used as appropriate.

*"The DNA profile obtained from ____ (Item) **MATCHED** the DNA profile obtained from ____ (Item). The probability of randomly selecting an unrelated individual with a DNA profile that matches the DNA profile obtained from the ___ is 1 in greater than 1 Trillion (which is more than the World's population) in the NC Caucasian, Black, Hispanic, and Lumbee Indian populations".*

4.2.2.2. When the RMP for some of populations exceed 1 in 1 Trillion (greater than the world's population) and one or more populations do not, the Match Statement may be as follows:

"This laboratory maintains population databases for the NC White, Black, Lumbee Indian and Hispanic populations and has access to other population databases which can be used as appropriate.

*"The DNA profile obtained from ____ (Item) **MATCHED** the DNA profile obtained from ____ (Item). The probability of randomly selecting an unrelated individual with a DNA profile that matches the DNA profile obtained from the ____ is approximately 1 in _____ in the NC Black population, 1 in _____ in the NC Hispanic population, 1 in _____ in the NC Lumbee Indian and 1 in greater than 1 Trillion (which is more than the World's population) in the NC Caucasian population."*

4.2.2.3. When the RMP for all the population subgroups do not exceed 1 in 1 Trillion (more than the World's population), the Match Statement may be as follows:

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"This laboratory maintains population databases for the NC White, Black, Lumbee Indian and Hispanic populations and has access to other population databases which can be used as appropriate.
*"The DNA profile obtained from ____ (Item) **MATCHED** the DNA profile obtained from ____ (Item). The probability of randomly selecting an unrelated individual with a DNA profile that matches the DNA profile obtained from the ____ is approximately 1 in _____ in the NC Black population, 1 in _____ in the NC Hispanic population, 1 in _____ in the NC Lumbee Indian and 1 in ____ in the NC Caucasian population."*

4.3. Identity Statement

Analysts in the Forensic Biology Section may provide opinion testimony as to the uniqueness of a DNA profile in cases when population frequency calculations for all population groups exceed the current estimated population of the world.

Analysts are to advise the prosecutor in advance of their testimony that they will provide opinion testimony as to identity, and provide the prosecutor with some form of the following question to ask:

"Based on your professional knowledge, careful reading of the pertinent scientific literature, and years of experience with forensic DNA testing - have you developed an opinion, satisfactory to yourself as to whether or not the stain on State's Exhibit # ____ (description of the State's Exhibit item) could have originated from (the defendant or victim) _____?"

Analysts are to use great care with the wording of their opinion on the uniqueness of the DNA profile and are to paraphrase the following statement as close as possible (to fit the case scenario):

"It is my opinion that it is scientifically unreasonable to expect that the DNA profile derived from the (semen, blood, saliva) stain detected on State's Exhibit # _____ (description of the State's Exhibit item) could have originated from anyone other than _____ _____, unless this individual has an identical sibling."

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4.4. CPE/CPI Interpretation Guidelines

4.4.1 General

4.4.1.1 If the data demonstrate that no more than one individual contributed to a DNA profile, the Random Match Probability calculation for a single source sample should be used for statistical purposes. The interpretation of genotypes present in a mixture is more complicated when the observed peaks from the contributors is approximately equal (based on allele intensities) and therefore a major contributor cannot be determined, or when a contributor's alleles are masked by stutter products or other alleles in the mixture (DAB 2000). It is not always possible to determine all of the alleles present in a mixture, especially with a partial profile from a degraded DNA sample or when allele dropout may have occurred. As a result, the Combined Probability of Exclusion (CPE) may be used as a tool to give weight to the evidence.

4.4.1.2 CPE is used to conservatively interpret complex DNA mixtures (Butler 2005). The Combined Probability of Exclusion (CPE) provides an estimate of the portion of the population that has a genotype composed of at least one allele not observed in the DNA mixture profile (DAB 2000) or the probability that a given DNA type would be excluded as a contributor to a mixed sample.

4.4.1.3 Each mixture will present unique interpretation issues. Not every mixture situation can be covered by a preset rule and therefore, not every situation will fit into a particular subset of these guidelines. **It is the responsibility of the analyst to use these guidelines in conjunction with their training and experience to provide a solid scientific interpretation of the results.**

4.4.2 Procedure

4.4.2.1 Before a statistical calculation is applied, the DNA

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profile will be evaluated to determine whether an individual (e.g. suspect or victim) is included or excluded as a possible contributor to the mixture. Only if the individual cannot be excluded will a CPE calculation be performed. The possibility of allelic dropout must be considered in determining whether an individual may be excluded or included in the mixed profile.

4.4.2.2 Basic Steps

4.4.2.2.1 Examine the electropherogram of the mixture. Note any inhibition, allelic dropout, and/or artifacts.

4.4.2.2.2 Compare the DNA profile obtained from the standards with the mixture. When determining the contributors to a DNA mixture profile, each locus must be considered independent of the DNA Standards from suspects and victims.

4.4.2.2.3 Determine if the standard(s) can be excluded.

4.4.2.2.4 If the standard(s) cannot be excluded, perform the CPE calculation using the NCSBI CPE Mixture Program.

4.4.2.3 All alleles detected at a particular locus must be included in the CPE calculation unless there is a compelling reason to exclude the alleles.

4.4.2.4 Intimate sample alleles may not be subtracted from a CPE calculation.

4.4.2.5 Frequencies for the Amelogenin locus will not be computed.

4.4.2.6 If the DNA profiles from two (or more) individuals cannot be excluded from the profile and the DNA from the individuals is not present at all genetic

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markers, multiple CPE/CPI calculations may be performed. When performing statistics to determine the probability of a person being included in a mixture, only the genetic markers where that individual CANNOT be excluded shall be used in the CPE calculations.

4.4.2.7 CPE on minor components: If a DNA profile from an evidentiary sample is a mixture of contributors whose genotypes can be separated based on allele intensities and a major profile discerned, the report must state that a major profile is evident. In this case, a statistic for the major DNA profile may be calculated using Random Match Probabilities. When a Major DNA profile and an obvious DNA minor profile are present, a CPE statistic may be reported for the minor DNA profile.

Note: Obvious shall be defined as three or more alleles or an imbalance between allele intensities at a locus.

4.4.3 Report Statements and Examples

4.4.3.1 Basic Statement: A statement(s) regarding inclusion or exclusion of individuals must be included in the report. The statement may read as follows:

"The DNA profile obtained from the vaginal swabs (Item 1-2) is CONSISTENT WITH A MIXTURE. The DNA profile obtained from the suspect, John Smith (Item 2) cannot be excluded as a contributor to the DNA. In addition, the DNA profile obtained from the victim, Sue Jones (Item 1A) cannot be excluded as a contributor to the DNA mixture."

4.4.3.2 CPE on minor components

Example: A DNA profile is obtained at 16 genetic markers from a knife handle. The victim is the major DNA profile at all 16 genetic markers. There is an obvious DNA mixture at D8 (8, 10, 11) and THO1 (6, (8)) from which Suspect 1 cannot be excluded as a contributor. Suspect 2 is excluded

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as a contributor. The report may be worded as follows:

" The DNA profile obtained from ___ (Item _) is CONSISTENT WITH A MIXTURE. The predominant DNA profile MATCHED the DNA profile obtained from ___. The DNA profile obtained from _____ cannot be excluded as a contributor to the minor profile.

The estimates of the combined probability of inclusion (i.e. the chance of selecting an unrelated individual at random that would be expected to be included) for the observed DNA mixture profile is approximately:

N.C. Caucasian: 1 in 209
N.C. Black: 1 in 1,070
N.C. Hispanic: 1 in 235
N.C. Lumbee Indian: 1 in 394"

4.4.3.3 Predominant Profile with Minor Alleles of Interest:

Example: A predominant profile with 5 minor alleles at 4 loci is obtained and the suspect cannot be excluded at the minor alleles. CPE may be performed on the mixture, but only at the 4 loci.

4.4.3.4 Intimate Samples with No Predominant Profile: If a mixed profile is obtained from an Intimate Sample with no Predominant profile, CPE must be calculated on the entire sample. Alleles from the "owner" of an Intimate Sample cannot be pulled out of Mixture.

4.4.3.5 Complex Mixtures: If a complex mixture with multiple contributors is observed and individuals cannot be excluded, then CPE may be performed on entire profile. However, if a single individual is being considered, then CPE calculations should be performed on loci where that individual cannot be excluded.

4.4.3.6 Mixture of Two (or More) Individuals: If two or more individuals cannot be excluded from a mixture, then multiple CPE calculations may be performed.

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Example: The following profile is obtained from a swabbing from the handle of a gun used to rob a bank. The following profile was observed:

Item	D8	D21	D7	CSF	D3	TH01	D13	D16	D2	D19	vWA	TPOX	D18	Am	D5	FGA
Gun	(13) 14 (15)	29	8 12	(10) 11 (12)	14 15	6 (7)	11 12 13	9	17 23 24	13 14 15	(14) 16 (17)	10, 11	12 (15) 17	XY	10 (11) (12)	(22) (23) 24
Susp 1	13 14	29 30	Inc	10 12	14 17	6 9.3	11 12	12 13	19 23	13 14	14 17	10, 11	12 17	XY	10 12	24
Susp. 2	14 15	29	8 12	11	15 16	6 7	12 13	9	17 24	14 15	16	9, 11	15 17	XY	9, 11	22, 23

Allele dropout was noted D21, D7, D3, THO1, D16, D2, TPOX, and D5. Suspect # 1 cannot be excluded as a contributor to the DNA mixture at 10 genetic markers. Suspect # 2 cannot be excluded as a contributor to the DNA mixture at 13 genetic markers. The report may be worded as follows:

"The DNA profile obtained from the gun (Item 1) is CONSISTENT WITH A MIXTURE.

Suspect #1 cannot be excluded as a contributor to this DNA mixture. The estimates of the combined probability of inclusion (i.e. the chance of selecting an unrelated individual at random that would be expected to be included) for the observed DNA mixture profile is approximately:

N.C. Caucasian: 1 in 5,340
N.C. Black: 1 in 7,990
N.C. Hispanic: 1 in 11,210
N.C. Lumbee Indian: 1 in 4,100

Suspect #2 cannot be excluded as a contributor to this DNA mixture.

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The estimates of the combined probability of inclusion (i.e. the chance of selecting an unrelated individual at random that would be expected to be included) for the observed DNA mixture profile is approximately:

N.C. Caucasian: 1 in 15.3 Million
N.C. Black: 1 in 1.96 Million
N.C. Hispanic: 1 in 15.8 Million
N.C. Lumbee Indian: 1 in 8.97 Million"

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Revision History		
Effective Date	Revision Number	Reason
Unknown	00	Original Document
June 21, 2001	01	Procedure Update
November, 2002	02	Procedure Update
June 13, 2003	03	Addition of Mixture Statistics Calculation
November 17, 2003	04	1) Addition of DNA Quantitation Interpretation Guidelines 2) Update to Reporting Guidelines (Section 2.5)
May 21, 2004	05	1) Addition of DNA Interpretation Guidelines for ABI Capillary System.
December 23, 2004	06	Addition of ABI 7000 Interpretation Guidelines
December 22, 2005	07	1.3 Allow for one point of slope to be deleted for pipetting inaccuracies 1.7- Specify acceptance criteria for low level DNA detection during Quantitation; 2.2.1- Lower absolute minimum cut off from 75 rfu to 50 rfu for the ABI Capillary Electrophoresis System; 2.2.2- Specify Positive amp. Control must be included in each 3100 run; 2.2.3.5- Added new Section dealing with activity in negative controls; 2.2.6.4- specify re-injection of appropriate negative controls; 2.4.1.3- Change wording to allow analysts to disregard alleles in the stutter position in certain circumstances; Reorganized Section 2.6; Addition of Section 2.6.4 "Incomplete Profiles"; delete FMBIO/gel references; Minor wording changes for clarification;
January 30,	08	Reorganization and revamping of Guidelines for clarification;. Section 1- added definitions;

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2007		Section 3.2.1.1- Set allele cut off at 75 RFU; Section 4- Addition of CPE. Section 7- Revamp Guidelines for Interpretation.
December 15, 2008	09	Section 1- added "Tri-allelic Pattern" in definitions; Section 2.5- added "Note" to address IPC values and additional possible methods that could be used; Section 3.2.1.2- clarified use of information below threshold including ability to use data below threshold for exclusionary purposes. Throughout Document- General rewording for clarification with no substantive changes, removal of reference to calling male/female profiles based on Amelogenin; Section 2.3.6.1- Clarify procedure for sample handling if the 250 bp size standard falls outside the required ± 0.5 base pair from the ladder. Section 3.3- All artifacts shall be labeled as "artifact"; Section 3.5.6.2- set standard for calling the minor contributor in a mixed sample. Section 3.3.4.5- Clarifies handling of Microvariants.
February 25, 2009	10	Section 1- Added "DNA Profile", "Locus", "Partial DNA Profile", Partial Predominant DNA Profile" definitions and clarified definition for "Peak"; Section 3.2.3.1- changed "shall" to "may" and clarified when samples may be used when negative controls are positive; Section 3.2.6.1- loosened requirements for re-running samples that do not contain peaks; Section 3.5.1- Removed loci from reporting statements, added CODIS search statements for Forensic Unknowns that meet upload requirements; removed statement referring to the number of genetic markers where results were obtained for partial profiles; Section 3.5.2.2- Clarified when minor allele must be labeled; Section 4.2.1.5- clarified use of RMP for "probative Matches"; Section 4.2.2: Added guidelines for reporting RMP for match statements, changed the reporting guidelines for population statistics from a likelihood statement to the RMP statement; Section 4.4.2.2.1- Added requirement for documenting inhibition, dropout, and artifacts on electropherograms of mixtures; 4.4.2.2.3- Removed second sentence; 4.4.3- Removed "unrelated" from report statements for CPI Calculations.
October 12, 2009	11	Minor wording changes for clarity throughout document ; Section 1-Definitions: Added "Allelic Dropout" and changed "DNA Profile"; Addition of Section 3.2.6, updated and clarified requirements for ILS; moved requirements of denoting artifacts, etc from 4.4.2.2.1 to 3.2.7; 3.5.2, minor wording changes for clarity;

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DOCUMENT APPROVAL REQUEST (DAR) FORM

NOTE: Attach or Reference any necessary support documentation

I. INITIATOR INFORMATION		
INITIATED BY (SIGNATURE):	PRINT INITIATORS NAME: David Freeman	DATE: October 5, 2009
II. SCOPE OF CHANGE		
DOCUMENTATION TO BE CREATED OR CHANGED (Title): **STR Interpretation Guidelines (Rev 11)**		
X SOP ☐ Procedure		
III. REASON FOR CHANGE (Summarize why document was created or changed).		
Annual Review		
IV. DESCRIPTION OF CHANGE (Describe the type of change - i.e., "From...To") - Attach additional pages if necessary.		
See Document Revision History		

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V. IMPACT OF CHANGE

OTHER DOCUMENTS AFFECTED?	VALIDATION TESTING REQUIRED?	SAFETY IMPACT?
X NO ☐ YES(list):	☐ N/A ☐ YES (Validation Documentation Reference number) X NO Justification for NOT requiring validation testing (attach additional pages if necessary): Training for a new job position.	☐ N/A X No ☐ Yes (attach additional information)
COMMENTS:	TRAINING REQUIRED? X YES ☐ NO To be completed by (date): Person responsible for training (name): Personnel to be trained (function or names): Type of training: X Routing ☐ Meeting	

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VI. APPROVALS **Minimum of two signatures required for all documents. The Technical Leader's signature is needed on all DARs (if not the initiator). Enter "NA" for spaces not used.**

NAME/TITLE		DATE
Michael J. Le Boyd	SAC	10/5/09
Amanda Wilson	ASAC	10/5/09
— NA —		— NA —

FORENSIC BIOLOGY TRAINING RECORD

I. TRAINING INFORMATION	
TRAINING TITLE: STR Interpretation Guidelines Training	
TYPE OF TRAINING: X Routing Meeting	
DOCUMENT TITLE: STR Interpretation Guidelines	
DOCUMENT #/REVISION LEVEL: 11	
OTHER REFERENCE MATERIALS: none	
DATE TRAINING COMPLETED:	
II. TRAINER'S INFORMATION	
TRAINER'S NAME	D. Freeman
III. PERSONNEL TRAINED	
EMPLOYEE NAME (Print Name)	EMPLOYEE SIGNATURE
GINA AUTRY	
JESSICA BADGER	
TIM BAIZE	
SUSAN BARKER (ASAC)	
LINDA BLACKMON (CONTRACT)	NA
CAROLYN BUSH (CONTRACT)	NA
Laura Clements	
SARA CLAY	NA
CORTNEY COWAN	
MACKENZIE DEHAAN	
JENNY ELWELL (ASAC)	
ERIN ERMISH	
TABITHA EURE	
CHRISTIANA FISCHER	
AMANDA FOX	

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II. TRAINER'S INFORMATION	
TRAINER'S NAME	David Freeman
III. PERSONNEL TRAINED	
EMPLOYEE NAME (Print Name)	EMPLOYEE SIGNATURE
DAVID FREEMAN (ASAC)	
MICHELLE HANNON	
REGINA HEDGEPETH	
STEPHEN HENDERSON	
SHARON HINTON	
RUSSELL HOLLEY	
KRISTIN HUGHES	
SHELLY JACKSON	
SARAH JOHNSON	
ZACH KALLENBACH	
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CHRIS PARKER (ASAC)	
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DATE TRAINING COMPLETED:	
II. TRAINER'S INFORMATION	
TRAINER'S NAME	David Freeman
III. PERSONNEL TRAINED	
EMPLOYEE NAME (Print Name)	EMPLOYEE SIGNATURE
LINDA RICHARDS (SEC)	- NA -
TONYA RUSH	Tonya Rush
ELAINE STALEY	Elaine M. Staley
KAREN STANCIL	- NA -
KEISHA STEWART	—
AMANDA THOMPSON(ASAC)	Amanda Thompson
MARTHA TRAUGOTT	N/A
JODY WEST	Jody West
RACHEL WINN	Rachel Winn
KAREN WINNINGHAM	

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DOCUMENT REVIEW RECORD (DRR)

Document Review Form		
Document Reviewed (Include Revision #): STR Interpretation Guidelines Rev 08		
Unit Affected Section ☐ Body Fluid ID ☐ Database ☐ DNA ☒		
Reviewer (Print Name and Initial)	Date of Review	
M.K. Budzynski	MKB	4/9/08
Chris Parker	AMP	4/3/08
~~Amanda Fex~~		
Kristin Meyer	KMM	4.23.08
David Freeman		7/16/08
Suzi Barth	S Barth	1/18/08
Amanda Thompson	AMT	2/14/08
Jenny Ewell	JE	3/24/08
To Be Completed by Technical Leader After Review		
Revision Required?	☐ NO	☒ YES
New Revision Number:	09	
Training Required?	☐ NO	☒ YES
Type of Training:	☐ Routing ☒ Meeting	☐ Training Program
If "Yes", Who is Responsible for Training:	DNA Tech Leader	

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