

NCSBI Forensic Biology Section	DNA SOP	Effective Date: December 15, 2008
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STR Interpretation Guidelines

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.

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

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

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".

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 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.

Peak: A well defined point on an electropherogram that is on-ladder and has a maximum height of ≥ 75 RFU.

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 Profile: Given a mixture of two or more contributors, one or two

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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.

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 may 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.

2.5. Interpreting IPC amplification results:

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<u>Quantifiler Human (FAM Dye)</u>	<u>IPC (VIC Dye)</u>	<u>Interpretation</u>
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 shall 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).

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 shall 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.

3.2.6. Samples

3.2.6.1 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. The 250 bp size

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standard in the samples and controls shall not differ \pm 0.5 base pair from the ladder. If a sample or control differs more than \pm 0.5 base pair from the ladder, then that sample must be re-run.

3.2.6.2 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.

3.2.6.3 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.

3.2.6.4 Samples that are overblown may need to be 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.

3.2.6.5 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

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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 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

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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 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-

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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 individual's entire profile is present in the mixture or if part of the individual's profile is in the mixture, allelic dropout is present, and the individual cannot be scientifically excluded from the mixture.
- 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.
 - 3.4.6.4. Inhibition: Inhibition is total or partial suppression of the PCR process that would result in partial or no DNA profile being obtained.

3.5. Conclusions

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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 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 in nature.

3.5.1.3.1 Match (Complete Profile)

If the DNA profile from a known sample matches the DNA profile from a single contributor, then the results will be reported as follows:

*“The DNA profile obtained from (Item) **MATCHED** the DNA profile obtained from the bloodstain of the , (Item) and **DID NOT MATCH** the DNA profile obtained from the bloodstain of the , (Item).”*

3.5.1.3.2 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:

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*“A partial DNA profile was obtained from (Item). The partial DNA profile **MATCHED** the DNA profile obtained from the bloodstain of the , (Item) and **DID NOT MATCH** the DNA profile obtained from the bloodstain of the , (Item). The match was observed at the following genetic markers: (choose appropriate loci) CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX, vWA, and Amelogenin.”*

Or:

“A partial DNA profile was obtained from (Item). The partial DNA profile was consistent with the DNA profile obtained from the bloodstain of the , (Item). The DNA profile of the , (Item) was not observed in the partial DNA profile. The match was observed at the following genetic markers: (choose appropriate loci) CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX, vWA, and Amelogenin.”

Note: Population Frequency Data should be given in instances where there is an inclusion on a partial profile (if probabitive).

3.5.1.3.3 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:

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*“A partial DNA profile was obtained from (Item). The partial DNA profile obtained from (Item) **ALSO MATCHED** the DNA profile obtained from the bloodstain of the , (Item) and **DID NOT MATCH** the DNA profile obtained from the bloodstain of the , (Item). The match was observed at the following genetic markers: (choose appropriate loci) CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX, vWA, and Amelogenin.”*

Or:

“A partial DNA profile was obtained from (Item. The partial DNA profile was consistent with the DNA profile obtained from the bloodstain of the , (Item). The match was observed at the following genetic markers: (choose appropriate loci) CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX, vWA, and Amelogenin.”

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.”

3.5.1.3.4 Non-Match: If the DNA profile from a known sample does not match the DNA profile from a single contributor, then the

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results will be interpreted as follows:

*“An unknown (choose) DNA profile was obtained from (Item) that **DID NOT MATCH** the DNA profile obtained from the bloodstain of the victim, (Item) or the DNA profile obtained from the bloodstain of the suspect, (Item). This profile will be routinely queried against the CODIS (Combined DNA Index System) Database in search of a match to another forensic case or a convicted offender.”*

Or

*• “A partial (choose) DNA profile was obtained from (Item) at ___ of the 16 genetic markers tested that **DID NOT MATCH** the bloodstain of the victim, (Item) or the DNA profile obtained from the bloodstain of the suspect, (Item). Results were obtained at the following genetic markers: (choose appropriate loci) CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX, vWA, and Amelogenin. This profile will be routinely queried against the CODIS (Combined DNA Index System) Database in search of a match to another forensic case or a convicted offender.”*

3.5.1.3.5 When only the profile of the “owner” of an intimate object is observed, the report shall state:

“No DNA profile different from that of the

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*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 is present, 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.

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 and there are no shared alleles 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 are 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 shall be "No Conclusion can be rendered as to the donor of the minor profile."

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 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:

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

3.5.2.7.2 Mixture: Partial/Incomplete Profile with a major/minor contributor

In cases where an incomplete match or mixture is obtained (i.e. one or more loci cannot be used for comparison purposes), the analyst may specify the number of matching loci and loci that are conclusive.

"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). The match for Item was observed at (choose appropriate loci) CSF1PO, D13S317, D16S539, D18S51,



D19S433, D21S11, D2S1338, D3S1358,
D5S818, D7S820, D8S1179, FGA, TH01,
TPOX, vWA, and Amelogenin.”

Or:

“A partial DNA profile was obtained from (Item). The partial predominant DNA profile was consistent with the DNA profile obtained from the bloodstain of the , (Item). Results were obtained at the following genetic markers: (choose appropriate loci) CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX, vWA, and Amelogenin.”

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:

“ 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



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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 shall 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 will 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 in search of a match to another forensic case or a convicted offender.”*

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 #).”

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 #).”



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. Random Match Probability: 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. Heterozygote frequencies: $2pq$

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

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

4.2.4. 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).

4.2.5. Random Match Probability shall be used to calculate population frequency statistics in cases of a match using the PopStats program in the CODIS Software.

4.2.6. Random Match Probability 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.

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 (6.5 billion).

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.”

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 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. Determine if there is possible inhibition or allelic drop out.

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. If alleles are not present at one or more locus, then there must be a reason for NOT excluding a standard (e.g. allelic dropout and/or inhibition).
- 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 markers, multiple CPE/CPI calculations may be performed. When performing statistics to determine the probability of an individual 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.

Example: *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 as a contributor. The report wording will be 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 **Allele Dropout:** If there are signal intensities that do not meet the criteria set forth by the laboratory protocols (i.e. activity below RFU cutoff threshold) and there are clearly approved allele calls within the same locus, the entire locus may be excluded from the calculation. Note that only those loci in which an individual is not excluded may be used for statistical calculations.
- 4.4.3.4 **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.
- 4.4.3.5 **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.
- 4.4.3.6 **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.
- 4.4.3.7 **Mixture of Two (or More) Individuals:** If two or more individuals cannot be excluded from a mixture, then multiple CPE calculations may be performed.

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, TH01, 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 wording will be as follows:

“The DNA profile obtained from the gun (Item 1) is CONSISTENT WITH A MIXTURE. The DNA profile obtained from Suspect #1 cannot be excluded as a contributor to this DNA mixture at 10 genetic markers. The DNA profile obtained from Suspect #2 cannot be excluded as a contributor to this DNA mixture at 13 genetic markers.

Suspect #1 cannot be excluded as a contributor to this DNA mixture at 10 genetic markers. 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

(Note: D21, D7, D3, TH01, D16, and D2 were not used in the calculations.)

Suspect #2 cannot be excluded as a contributor to this DNA mixture at 13 genetic markers.



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"

(Note: D3, TPOX, and D5 were not used in the calculations)

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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, 2007	08	Reorganization and revamping of Guidelines for clarification;. Section 1- added definitions; Section 3.2.1.1- Set allele cut off at 75 RFU; Section 4- Addition of CPE. Section 7- Revamp Guidelines for Interpretation.

NCSBI Forensic Biology Section
Quality Assurance Manual- Revision 09
Appendix F – STR Interpretation Guidelines
Effective Date:

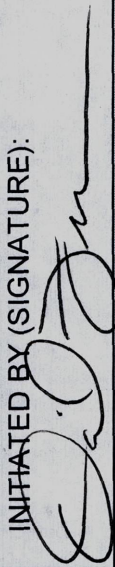


December 15, 2008	09	<p>Section 1- added "Tri-allelic Pattern" in definitions; Section 2.5- added "Note" to address IPC values and additional possible methods that could be used; Section 3.2.1.2- clarified use of information below threshold including ability to use data below threshold for exclusionary 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.</p>
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DOCUMENT APPROVAL REQUEST (DAR) FORM
COMPLETE BOTH SIDES - THIS IS A TWO-SIDED FORM
NOTE: Attach or Reference any necessary supporting documentation

Page 1 of 2

I. INITIATOR INFORMATION	
INITIATED BY (SIGNATURE): 	PRINT INITIATORS NAME: David Freeman
DATE: 12/4/08	
II. SCOPE OF CHANGE	
DOCUMENTATION TO BE CREATED OR CHANGED (Title): STR Interpretation Guidelines	
<input type="checkbox"/> SOP <input checked="" type="checkbox"/> Procedure	
III. REASON FOR CHANGE (Summarize why document was created or changed).	
Changes resulting from annual document review	
IV. DESCRIPTION OF CHANGE (Describe the type of change - i.e., "From...To") - Attach additional pages if necessary.	
See "Revision History" in Document	

V. IMPACT OF CHANGE

OTHER DOCUMENTS AFFECTED?

- NO
 YES (list):

VALIDATION TESTING REQUIRED?

- N/A
 YES (Validation Documentation Reference number)
 NO

Justification for NOT requiring validation testing (attach additional pages if necessary):

SAFETY IMPACT?

- N/A
 No
 Yes (attach additional information)

COMMENTS:

TRAINING REQUIRED? YES NO

To be completed by (date): Prior to use by analyst
 Person responsible for training (name): David Freeman
 Personnel to be trained (function or names): All DNA Analysts
 Type of training: Routing Meeting

VI. APPROVALS

Minimum of two signatures required for all documents. A Quality Assurance/Quality Control representative's signature is needed on all DARs. Enter "NA" for spaces not used.

NAME/TITLE Michael P. Boddy / SAC

DATE 12/8/08

NAME/TITLE Greg Glueck Forensic Biologist II

DATE 12/12/08

NAME/TITLE _____

DATE _____