
Procedure for Casework DNA Interpretation

1.0 Purpose – The purpose of this document is to provide guidelines for the interpretation of autosomal DNA results when amplified with Identifiler® Plus.

2.0 Scope – This document applies to casework analysts and trainees in the Forensic Biology Section qualified to perform casework.

3.0 Definitions

- **Allele:** An alternative form of a gene; allele designation is used to represent a specific size fragment of DNA for a specific locus in STR analysis.
- **Allelic Dropout:** Failure to detect an allele within a sample or failure to amplify an allele during PCR.
- **Analytical Threshold (AT):** The minimum height (RFU) requirement at and above which detected peaks can be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles. The threshold for this Laboratory is internally derived by empirical data.
- **Artifact:** Non-allelic byproducts of PCR technology (e.g., stutter, etc), anomalies which occur during capillary electrophoresis (e.g., pull-up, spike, etc), or byproducts of primer synthesis (e.g., dye blob, etc).
- **Combined Probability of Exclusion (CPE):** The probability that a randomly chosen, unrelated person from a given population would be excluded as a potential contributor to the observed DNA mixture.
- **Combined Probability of Inclusion (CPI):** The probability that a randomly chosen, unrelated person from a given population would be included as a potential contributor to the observed DNA mixture.
- **Composite Profile:** A DNA profile generated by combining typing results from different loci obtained from multiple injections of the same amplified sample and/or multiple amplifications of the same DNA extract. When separate extracts from different locations on a given evidentiary item are combined prior to amplification, the resultant DNA profile is not considered a composite profile.
- **Core Loci:** The 13 loci defined by the FBI and required for inclusion within CODIS. The 13 core loci are CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11.
- **Distinguishable Mixture:** A mixture in which relative peak height ratios allow for the determination of a major contributor(s). Separation of contributors (into major and minor components) is based on quantitative peak height information (see Peak Height Ratio).
- **DNA Profile:** The combination of genotypes obtained from DNA analysis testing of multiple loci.
- **Exclusion:** A conclusion reached after comparing the DNA profile of a known sample to the DNA profile of an evidentiary item and the individual in question is not a potential contributor.
- **Full Profile:** A DNA profile that exhibits genotypic information at each locus tested and there is no evidence of allelic dropout, degradation, or preferential amplification.

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- **Inclusion:** A conclusion reached after comparing the DNA profile of a known sample to the DNA profile of an evidentiary item and the DNA profile of the individual in question is a potential contributor.
 - **Indistinguishable Mixture:** A mixture in which the relative peak height ratios do not allow for the determination of a major contributor(s).
 - **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 biological sample from an evidence item that is obtained directly from an individual's body; it is not unexpected to detect that individual's allele(s) in the DNA typing results.
 - **Locus (plural = Loci):** The chromosomal position or location of a gene or DNA marker.
 - **Match:** DNA profiles are considered to match if their patterns are the same after taking into consideration the properties of the substrate tested and limitations of the specific techniques used.
 - **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:** A DNA typing result originating from more than one individual.
 - **Multiple Major Contributors:** The presence of more than one predominant contributor to a mixture profile.
 - **Noise:** signal detected by a data collection instrument.
 - **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 one allele.
 - **Off-Ladder Allele:** An allele observed outside the region covered by the allelic ladder at a given locus.
 - **Off-Scale Data:** The result of excess DNA present in an electrophoresed sample, typically visualized by excessive artifacts as a result of peak heights consistently greater than 7000 RFUs.
 - **Partial DNA Profile:** A DNA profile that does not produce DNA typing results for all loci tested due to DNA degradation, inhibition, or low quantity DNA template.
 - **Partially Predominant Profile:** A Predominant DNA Profile (see definition below) where one or more loci do not exhibit predominance.
 - **Peak Height Ratio (PHR):** The relative ratio of two alleles at a given locus used as an indication of which alleles may be heterozygous pairs.
 - **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 65 % of the remaining alleles at that locus and a single source may be readily inferred by the observed data.
 - **Predominant DNA Profile:** Given a mixture of two or more contributors, one or two alleles at every locus typed has an RFU value greater than 65 % 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 which may show up as a peak or Off-Ladder Allele in another dye-set.
 - **Questioned Sample:** Biological sample recovered from a crime scene or collected from persons or objects associated with a crime.
 - **Random Match Probability:** Refer to the Forensic Biology Section Procedure for Statistical Interpretation.
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- **Reference Sample:** Biological material for which the identity of the donor is established and used for comparison purposes; also referred to as a known standard. These include victim, suspect (subject), elimination and/or witness standards.
- **Run:** Each set of 16 samples that are injected and separated electrophoretically on the Capillary Electrophoresis Unit (ABI 3130XL or equivalent).
- **Shoulder and Tail:** A Shoulder and Tail is 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 an increase 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 is where one allele is represented by two peaks. Lack of full nucleotide A 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 also results in off-scale/overblown data (saturation of signal) and may be manifested as split peaks.
- **Single Source Profile:** A combination of genotypes obtained from STR DNA testing that could originate only from a single individual. 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. If three alleles are observed at one locus, then there may not be a mixture; the individual contributor may have a triallelic pattern at that locus.
- **Stochastic Effects:** The observation of intra-locus peak imbalance and/or allele drop-out resulting from random, disproportionate amplification of alleles in low-quantity template samples.
- **Stochastic Threshold (ST):** The value above which it is reasonable to assume allelic drop-out has not occurred within a single source sample. The threshold for this Laboratory is internally derived through the use of empirical data.
- **Stutter:** An artifact of PCR amplification that is typically one repeat unit less (N-4) or one repeat unit more (N+4) than the corresponding main allele peak resulting from strand slippage during amplification.
- **Triallelic Pattern:** Three peaks observed at a single locus and not the result of a mixture. These peaks may or may not be of equal intensity.
- **Unincorporated Dye:** Unincorporated dye (i.e., dye-blobs) may 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.
- **Uninterpretable Profile:** A DNA typing result which results from an insufficient quantity of DNA, testing of degraded DNA, or preferential amplification. These type profiles provide insufficient data and shall not be used for comparison purposes.

4.0 Equipment, Materials and Reagents – N/A

5.0 Procedure

- 5.1 **Introduction** – The guidelines outlined herein are based upon this Laboratory's validation studies, review of literature, and over 15 years of forensic DNA casework experience. These

guidelines are to be used in conjunction with the Forensic Scientist's training and experience to provide scientific interpretation of the STR results.

5.2 Thresholds

5.2.1 Analytical Threshold - The analytical threshold was established through validation and performance check studies using the Identifiler® Plus PCR Amplification kit. The analytical threshold is set at 50 RFU for all dye channels. Anything present below 50 RFU is considered to be indistinguishable from background noise and shall not be considered for analysis.

5.2.2 Stochastic Threshold – The stochastic threshold is set at 200 RFU. Each instrument has its own specific injection condition in order to maintain the same level of sensitivity across all instruments.

5.3 Interpretation of Allelic Ladders, Controls and Samples

5.3.1 Examination of the Electropherogram(s) of Allelic Ladder(s)

All alleles within the allelic ladder for all loci tested shall be 1) equal to or greater than the analytical threshold and 2) in the correct position in order to use the associated samples and controls. If this criteria is not met, the allelic ladder and associated samples and controls shall be re-injected (refer to the Procedure for GeneMapper ID for Casework and the Procedure for Use of the 3130XL).

5.3.2 Examination of the Electropherogram(s) of the Positive Amplification Control(s)

The positive amplification control must have alleles that are in the proper location relative to the allelic markers. If these expected alleles are not in the correct position or are below the analytical threshold, then that particular locus shall be considered inconclusive for all samples and shall be successfully re-injected. If re-injection is unsuccessful, then the controls and all associated samples shall be re-amplified and analyzed before that locus may be used for analysis (refer to the Procedure for GeneMapper ID for Casework and the Procedure for Use of the 3130XL Genetic Analyzer for Casework).

5.3.3 Examination of the Electropherogram(s) of the Negative Control(s)

If any peaks, not attributable to artifacts, are present above the analytical threshold in the amplification negative control or the reagent controls, the controls shall be reanalyzed (i.e., re-injected or re-amplified.) If further examination is necessary, then the control(s) and associated samples shall be re-extracted. If reanalysis is not possible, then the samples may be interpreted upon consultation with the DNA Technical Leader (TL). The TL shall consider the peak height and number of peaks with respect to the profile. This

consultation shall be documented (refer to Forensic Biology Procedure for Documentation and Review).

5.3.4 Examination of the Electropherogram(s) of the Sample(s)

Assess the quality of the data, including RFU values and determine if artifacts are present.

If the questioned sample(s) contains more than two alleles at the same locus, then the samples *may* indicate a mixture. NOTE: If three alleles are observed at only one locus, then there may not be a mixture; the individual contributor may have a triallelic pattern at that locus. Both the questioned sample and the known sample shall express the triallelic pattern.

Failure of any locus (loci) to amplify for a multiplex STR system shall not preclude the Forensic Scientist from reporting those loci that are present.

Samples that are off-scale shall be re-amplified using a lower DNA template and shall not be used for comparison purposes (refer to the Procedure for GeneMapper® ID for Casework).

5.4 Artifacts – The PCR process produces artifacts that are known and well characterized. All by-products of PCR and/or capillary electrophoresis shall be labeled on electropherograms as artifact (refer to the Procedure for GeneMapper® ID for Casework).

5.4.1 Stutter

5.4.1.1 The STR results shall not be considered inconclusive if stutter peaks are present in single source samples.

5.4.1.2 The GeneMapper® ID software contains stutter percentages for the loci used in the Identifiler® Plus amplification kit and applies them to the data. 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 not in a mixed sample. In mixed samples with major/minor components, minor peaks in stutter position that are indistinguishable from stutter shall not be removed as artifacts and the minor component at that locus shall be deemed inconclusive.

5.4.1.3 Refer to the Procedure for GeneMapper® ID for Casework for locus-specific stutter percentages.

5.4.2 Pull up/Incomplete Spectral Separation – 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. Forensic Scientists shall be aware of this

phenomenon and use the computer software to aid in discerning actual alleles from pull-up.

- 5.4.3 Unincorporated Dye** – Forensic Scientists shall not call dye-blobs as an actual allele. Dye-blobs shall not be considered for interpretation.
- 5.4.4 Shoulder and Tail** – Shoulders and tails do not prevent the Forensic Scientist from assigning the specific peak an allelic value.
- 5.4.5 N+4 Peaks** – An artifact peak may appear in the n+4 position. Due to the rarity of N+4 peaks, caution shall be observed when designating these peaks as artifacts.
- 5.5 Amelogenin Results** – Under rare circumstances a male individual may not display the Y chromosome of this test; therefore, scientists shall not interpret an X as originating from a female. If Y is present in a single source unknown or predominant unknown profile, male shall be used as a qualifier for that unknown profile.
- 5.6 Balance** – Samples shall be examined for balance at each locus. Based upon validation studies, single source samples should exhibit heterozygote peak height ratios (PHR) greater than or equal to 65 %. The sample shall be re-injected, re-amplified, or re-extracted if the overall imbalance of the sample does not allow for interpretation by the Forensic Scientist. Intra-locus PHRs are calculated for a given locus by dividing the peak height of an allele with a lower RFU value by the peak height of an allele with a higher RFU value, and then multiplying this value by 100 to express the PHR as a percentage. PHR expectations are only applicable to alleles that meet or exceed the stochastic threshold.
- 5.7 Composite Profiles** – It is permissible to combine results from different injections, dilutions and amplifications of the same sample when determining a final DNA profile. In order to call predominance at a locus, all results shall show the same predominance. When separate extracts from different locations on a given evidentiary item are combined prior to amplification, the resultant DNA profile is NOT considered a composite profile. Unless there is a reasonable expectation of sample(s) originating from a common source (e.g., duplicate vaginal swabs or a bone), allelic data from separate extractions from different locations on a given evidentiary item should not be combined into a composite profile.
- 5.8 Predominant Profiles** – In order to establish a predominant profile (single or multiple major), at least 75 % of the loci that have data present above the stochastic threshold (not including Amelogenin) shall exhibit predominance based upon peak height ratios.
- 5.9 Number of Contributors to a DNA Profile**
- 5.9.1** Generally, a sample is considered to have originated from a single individual if one or two alleles are present at all loci for which typing results were obtained (although tri-allelic loci may occur) and the peak height ratios for all heterozygotes are within the empirically determined values. It is noted that peak height imbalances may be seen in the

typing results from, for example, a primer binding site variant that results in attenuated amplification of one allele of a heterozygous pair.

- 5.9.2** Generally, a sample is considered to have originated from more than one individual if three or more alleles are present at one or more loci (excepting tri-allelic loci) and/or the peak height ratios between a single pair of allelic peaks for one or more loci are below the empirically determined heterozygous peak height ratio expectation. Generally the minimum number of contributors to a mixed sample can be determined based on the locus that exhibits the greatest number of allelic peaks. As an example, if no more than five alleles are detected per locus, then the DNA typing results are consistent with having arisen from at least three individuals.
- 5.9.3** Alleles between the analytical threshold and the stochastic threshold may be used in the assessment of the number of contributors.
- 5.9.4** An estimation of the minimum number of contributors to a mixture should not be construed as designation of an absolute number of individuals that must have contributed to a mixed specimen. Rather, this estimation is provided to describe the fewest number of individuals who must have contributed to a mixture.

5.10 Comparison of Profiles

- 5.10.1** The comparison and interpretation of DNA profiles by a qualified Forensic Scientist is a qualitative judgment based on review of all information pertinent to the tests performed. Questioned samples shall be interpreted (i.e., designating alleles/loci for use in statistics, assessing predominance) prior to comparison of known reference samples.
- 5.10.2** Matches and non-matches shall be 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 shall be based only on those loci which yield conclusive results.
- 5.10.3** Incidences of employee, vendor or batch case matches/associations shall be immediately conveyed to the DNA TL.
- 5.10.4** With unknown profiles in a case, comparisons shall be made only between single source unknowns and predominant unknown profiles. Comparisons between unidentified single source or predominant profiles to mixtures is not permitted.
- 5.10.5** For intimate samples, the known profile from an assumed contributor may be used to establish the obligate alleles for a putative perpetrator (refer to the Procedure for CODIS).
- 5.10.6** For the interpretation and comparison of profiles, refer to the flow chart (see attachment). For performing statistical calculations, refer to the Procedure for Statistical

Interpretations. For reporting of results and conclusions, refer to the Procedure for Casework Report Writing.

5.10.7 The following DNA profiles generated under prior technology (e.g., Identifiler, Quantifiler, etc.) may be compared to standards generated using new technology (e.g., Identifiler Plus, Quantifiler Duo, etc.): single source DNA profile(s), predominant DNA profile(s), and CODIS eligible sample(s). Statistical calculations may be performed for these. For limited profile(s) and minor profiles/alleles in a mixture (which do not exceed more than four alleles at any locus and have a minimum of three loci for comparison), comparisons may be performed to submitted standards analyzed using new technology. These interpretations shall be only for exclusionary purposes or to report that no conclusion can be rendered.

5.11 Opinion Statement

Forensic Scientists in the Forensic Biology Section may provide opinion testimony regarding the DNA profile in cases when population frequency calculations for all population groups exceed the current estimated population of the world (refer to Forensic Biology Procedure for Casework Report Writing Section 5.14.1).

6.0 Limitations – N/A

7.0 Safety – N/A

8.0 References

Butler, J.M. *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers*. 2nd ed. Burlington, MA: Elsevier Academic Press, 2005.

Federal Bureau of Investigation. "QUALITY ASSURANCE KNOWN SAMPLES FOR FORENSIC DNA TESTING LABORATORIES." *Forensic Science Communications*, October 2008, Volume 10, Number 4.

Forensic Biology Section Procedure for Statistical Interpretations

Forensic Biology Section Procedure for GeneMapper® ID for Casework

Forensic Biology Section Procedure for Casework Report Writing

Forensic Biology Section Procedure for CODIS

Forensic Biology Section Procedure for Use of the 3130XL for Casework

State v. Ragland, __N.C. App__, __ S.E.2d__, April 16, 2013.

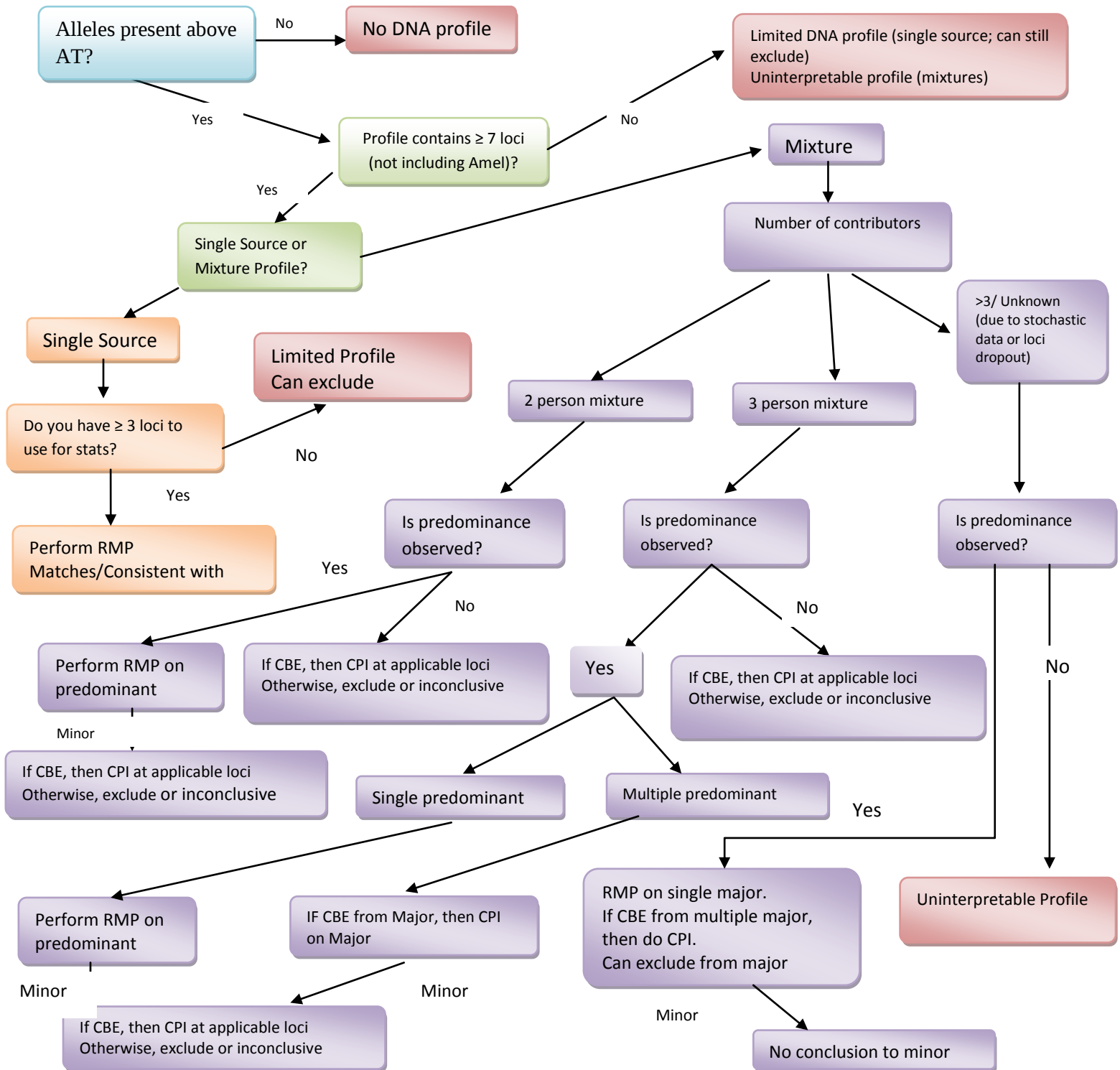
9.0 Records – N/A

10.0 Attachments

- Flow chart

Revision History		
Effective Date	Version Number	Reason
01/03/2013	1	Original Document
02/01/2013	2	5.9.7 - Added requirement for comparison of evidence worked prior to 01/03/2013
03/08/2013	3	Definitions – reworded allelic drop-out, and non-match, clarified CPE, CPI, Locus, and Peak Height Ratio; 5.3.2 – clarified requirements for re-amplification of samples; 5.3.3 – clarified wording; 5.6 – changed PHR requirements to PHR expectations; Added new 5.9 section for number of contributors to a DNA profile; Attachment – clarified flow chart; grammar
09/25/2013	4	Header – added titles of issued by; 3.0 – added subject to definition of reference sample; 5.1 – clarified wording; 5.8 – changed 10 loci to 75 %; 5.10.5 – clarified wording to assumed contributor; 5.10.7 – clarified when profiles generated under prior technology can be used for comparison; 5.11 – changed header from identity to opinion, addressed testimony statement, added citation for court case (8.0)
12/18/2013	5	5.3.3. – clarified wording for evaluation of controls; 5.3.4 – removed reference to database; attachment – updated flow chart; 5.8 – changed “analytical” to “stochastic”; edited 5.11

Attachment



AT = 50 rfu ST = 200 rfu Predominance ≥ 75% of loci (not including Amel)
PHR = 65% RMP: Heterozygotes ≥ AT, Homozygotes ≥ ST
RMP & CPI: must have ≥ 3 loci to use (Amel, D2 and D19 do not apply)
Partial profiles follow the same rules

If a standard cannot be excluded but no CPI can be performed (due to stochastic effects), then no conclusion can be rendered for that standard