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.).
- **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.
- **Genotype: Characterization of the alleles present at a genetic locus; the combination of genotypes obtained for multiple loci is referred as a DNA profile**
- **Inconclusive profile/component:** A DNA typing result which stems from an insufficient quantity/quality of DNA, (e.g., degraded DNA, preferential amplification, stochastic effects, and/or number of contributors). This type of profile provides insufficient information to support an inclusion or exclusion and shall not be used for comparison purposes.

- **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).
- **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. (See inclusion definition.)
- **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.
- **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 does not produce DNA typing results for all loci tested due to DNA degradation, inhibition, or low quantity DNA template.
- **Peak Height Ratio (PHR):** The relative ratio of two alleles at a given locus, as determined by dividing the peak height of an allele with a lower relative fluorescence unit (RFU) value by the peak height of an allele with a higher RFU value and then multiplying the value by 100 to express the PHR as a percentage.
- **Predominant DNA Profile/Major Contributor:** An unambiguous single primary source of DNA within a mixture as determined by the application of the PHR.
- **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.
- **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.
- **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.

4.0 Equipment, Materials and Reagents – N/A

5.0 Procedure

5.1 Introduction –The interpretation of results generated from casework samples is a matter of professional judgment and expertise. These guidelines establish minimum criteria for the interpretation and reporting of analytical results which are generally accepted in the scientific community based on years of casework laboratory experience, validation studies, and literature references.

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 and Controls

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 these criteria are not met, refer to the Procedure for GeneMapper ID-X 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).

5.4 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-X 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-X 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 may be interpreted by the Forensic Scientist.

5.4.1.3 Refer to the Procedure for GeneMapper® ID-X 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 **Peak Height Ratio –** Samples shall be examined for balance at each locus. Based upon validation studies, single source samples/single major contributor (predominant profile) should exhibit heterozygote peak height ratios (PHR) greater than or equal to 65 % when both peaks are at/above the stochastic threshold. Low PHR may indicate a null allele, a primer binding site mutation, degradation, or the presence of inhibition.

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 –**Forensic Scientists shall evaluate the profile as a whole to determine if a single unambiguous major contributor can be inferred in the mixture and at which loci statistical calculations can be performed.

An individual's contribution to a mixture is generally proportional to their quantitative representation within the DNA typing results. Accordingly, depending on the relative contribution and assumed number of contributors to a mixture, a single unambiguous major (predominant) contributor might not be inferred.

There exists the potential for aggravated stacking of the X allele at amelogenin in mixtures. As a result, if a male individual is included as a predominant contributor to a DNA mixture based upon STR loci, but the Y allele is assigned as a minor allele, the scientist may draw the conclusion that the male individual cannot be excluded as a predominant contributor to the DNA mixture, despite the Y allele's minor status.

5.9 Evaluation of Samples

5.9.1 General

5.9.1.1 After artifacts have been removed from consideration, the Forensic Scientist shall assess the quality and quantity of data in a sample.

5.9.1.2 Failure of any locus (loci) to amplify shall not preclude the Forensic Scientist from interpreting/reporting those loci that are present.

5.9.1.3 Reasoning for the determination of whether or not a locus is used for statistical interpretation shall be documented. Reasoning may be applied to the entire locus or to a portion of the locus (e.g., minor contributor(s)). Inconclusive loci (or portions) shall not be used for comparison purposes.

5.9.1.4 If no profile above analytical threshold is present, the scientist shall interpret that sample as no DNA profile obtained.

5.9.1.5 The presence of apparent degradation, inhibition, or significant stochastic effects in a profile shall be considered during the interpretation process and may influence the assessments made at each locus. Additionally, the scientist may use additional criteria including quantitative values and/or the totality of the profile to determine if potential stochastic amplification has occurred.

5.9.2 Single Source

5.9.2.1 Generally, a sample is considered to have originated from a single individual if no more than two alleles are present at all loci for which typing results were obtained (although tri-allelic loci may occur).

5.9.2.2 The peak height ratios for all heterozygotes should meet or exceed the empirically determined PHR. It is noted that peak height imbalances may be seen as a result of a primer binding site mutation.

5.9.2.3 Homozygote alleles will usually appear as peaks with peak heights approximately twice the height of heterozygote alleles.

5.9.2.4 Statistical interpretation (RMP) shall be calculated only as described in the Procedure for Statistical Calculations.

5.9.3 Mixtures

5.9.3.1 General

5.9.3.1.1 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.

5.9.3.1.2 Alleles between the analytical threshold and the stochastic threshold may be used in the assessment of the number of contributors.

5.9.3.1.3 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.

5.9.3.1.4 Allele sharing can exist between multiple contributors which may cause the PHR expectation to vary from the empirically determined value. This may be seen as allele stacking which could create imbalance between heterozygotic peaks of a major contributor. Alternatively, allele(s) attributable to minor contributor(s) may be masked by the major contributor(s). Generally, the more dissimilar the respective contributions of the major and minor contributors, the lesser the potential impact of allele stacking/masking.

5.9.3.1.5 Determination of Number of Contributors in a Mixture

5.9.3.1.5.1 When no more than 4 peaks are present at any locus (above analytical threshold) the profile should be considered a mixture consistent with two contributors.

5.9.3.1.5.2 When 5 or 6 peaks are present at any locus (above analytical threshold) the profile should be considered a mixture consistent with three contributors.

5.9.3.1.5.3 When 7 or more peaks are present at any locus (above analytical threshold) the mixture should be considered a complex mixture (four or more contributors).

5.9.3.2 Mixtures Consistent with Two Contributors

5.9.3.2.1 Indistinguishable – if an unambiguous major contributor cannot be determined from the mixture based on PHR expectations, the profile shall be deemed an indistinguishable mixture. A combined probability of inclusion (CPI) calculation shall be used to describe the rarity of this profile. Only those loci with no indication of dropout and with all alleles above the stochastic threshold may be included in the CPI calculation.

5.9.3.2.2 Distinguishable (major/minor) – If an unambiguous major contributor (predominant) can be determined from the mixture based on PHR expectations, the profile shall be deemed a distinguishable mixture.

5.9.3.2.2.1 An RMP calculation shall be used to describe the rarity of the major contributor profile.

5.9.3.2.2.2 If the minor contributor is present above the stochastic threshold for at least 3 loci, a statistical interpretation may be derived using a CPI calculation.

5.9.3.2.2.3 If the minor contributor is present below the stochastic threshold at a locus, that locus shall be deemed inconclusive with respect to the minor contributor.

5.9.3.3 Mixtures Consistent with Three Contributors

5.9.3.3.1 Due to a larger number of contributors a potential increase in allele sharing and masking may be seen. Discernment of a single major and/or multiple major contributor(s) might not be possible. Additionally, minor contributors may be subject to an increased amount of allelic dropout resulting from an increased amount of data below the stochastic threshold.

5.9.3.3.2 Indistinguishable - if an unambiguous major contributor cannot be determined from the mixture based on PHR expectations, the profile shall be deemed an indistinguishable mixture. A combined probability of inclusion (CPI) calculation shall be used to describe the rarity of this profile. Only those loci with no indication of

dropout and with all alleles above the stochastic threshold may be included in the CPI calculations.

5.9.3.3.3 Multiple major – If there are two major contributors above the stochastic threshold and both meet the PHR expectations when compared against the minor contributor, a restricted CPI calculation may be performed. This calculation shall be performed on the multiple major and may also be performed on the profile as a whole when the minor contributor alleles are above the stochastic threshold and there is no indication of dropout.

5.9.3.3.4 Single major contributor - If an unambiguous single major contributor (predominant) can be determined from the mixture based on PHR expectations, the profile shall be deemed a distinguishable mixture.

5.9.3.3.4.1 An RMP calculation shall be used to describe the rarity of this single major contributor profile.

5.9.3.3.4.2 If the minor contributors are present above the stochastic threshold for at least 3 loci, a statistical interpretation may be derived using a CPI calculation.

5.9.3.3.4.3 If either minor contributor is present below the stochastic threshold at a locus, that locus shall be deemed inconclusive with respect to both minor contributors.

5.9.3.4 Complex Mixtures

5.9.3.4.1 Profiles determined to be complex mixtures are considered to be inconclusive due to complexity related to the quantity of recovered DNA.

5.10 Comparison of Profiles

5.10.1 When interpreting DNA typing results, the analyst must interpret the results from evidentiary items prior to comparison of any known samples, other than those of assumed contributors (e.g., victim standard on vaginal swabs). This includes whether each locus meets the analytical threshold or stochastic threshold, is single-source, mixed (distinguishable or indistinguishable), meets PHR expectations, and/or has potential allelic dropout.

5.10.2 Those loci which will be used in statistical calculations shall be determined prior to comparison to known reference samples.

5.10.3 The victim's known profile may be used during the interpretation of intimate samples in order to determine the obligate alleles of the putative perpetrator for CODIS entry purposes.

5.10.4 Results

5.10.4.1 Inclusion – A known individual's profile is included in a single source or mixed sample if the genotype is present at all loci at which DNA typing results are deemed interpretable with no unexplained differences. The loss of an allele due to preferential amplification, stochastic effects, mutation, or other factors must be considered and does not necessarily indicate an exclusion.

5.10.4.2 Exclusion – A known individual's profile could not have contributed to or is not a part of the questioned profile from a single source or mixed sample if the genotype is missing at any loci at which the DNA typing results are deemed complete.

5.10.4.3 Inconclusive – Inconclusive results which are not suitable for comparison may be narrowed to the following:

5.10.4.3.1 The profile has limited data available due to the possibility of allelic dropout, degradation, preferential amplification, and/or the potential for the masking of minor alleles by the major profile or in stutter positions in the mixture. For example, a two person mixture with results at two loci with no data above the stochastic threshold.

5.10.4.3.2 The profile is too complex due to the total number of possible contributors present, the possibility of allele sharing between multiple contributors, and/or the possibility of allelic dropout of lower level contributors. For example, a mixture of at least four contributors with no single unambiguous major contributor.

Note: The inconclusive conclusion will be applied to the profile as a whole or minor contributor(s) and not on an individual basis when comparing different known contributors. Such data is not suitable for inclusion or exclusion due to insufficient information.

5.10.5 Incidences of employee, vendor or batch case matches/associations shall be immediately conveyed to the DNA TL. Any incidences of the unintentional introduction of exongenous DNA into a control shall also be immediately conveyed to the DNA TL.

5.10.6 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.

5.10.7 DNA profiles for questioned items 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.) after consultation with the DNA Technical Leader.

5.11 Documentation of Interpretation

Analysts shall document on the allele call tables any assumptions used (e.g., use of victim for intimate samples, number of contributors), determination of predominant (major) profiles (to include listing the specific loci used), the non-use of loci for interpretation and reason (e.g., imbalance, stochastic effects), and list loci which may be used to perform statistical interpretation. Additionally, any other notes, remarks, and observations used to make an interpretation and/or conclusion regarding unknown samples shall be documented on the allele call tables.

6.0 Limitations – These guidelines are not meant to cover all situations, and shall not be applied retroactively to analysis performed under previous versions of this procedure without the documented authorization of the DNA Technical Leader. Interpretation of low level samples should be approached with caution due to the limits in sensitivity of the STR typing testing procedure. Interpretation of low level results must also factor in the potential loss of data due to the inability to detect all genotypes present in a sample in an effort to ensure a reliable result is obtained.

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 – N/A

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
01/24/2014	6	5.11 – clarified wording
04/18/2014	7	3.0 – updated definitions; 5.3.1 – removed re-injection requirement; 5.4.1.2 – removed automatic inconclusive determination; 5.6 – clarified balance wording; 5.8 – added wording to clarify explanation; 5.10.3 – added requirement for controls; 5.10.7 – split into additional section for clarity; 5.11 – added section about documentation of interpretation
12/28/2015	8	3.0 – removed definition for off-scale (in GMID-X procedure), non-match, and uninterpretable profile, added genotype and inconclusive profile/component; 5.1 – updated wording; 5.3 - removed Samples and 5.3.4; 5.6 – clarified PHR wording; 5.8 – clarified predominant as unambiguous; 5.9 - added section on sample evaluation; 5.10 – removed original opinion statement and added comparison wording based on 5.9; 6.0 – updated limitations; removed attachment