DNA Database Procedure for Autosomal DNA STR Interpretation

1.0 Purpose - To provide guidelines for the interpretation of autosomal DNA results within the DNA Database

2.0 Scope - This document applies to qualified DNA Database Forensic Scientists and DNA Database trainees within the DNA Database Section.

3.0 Definitions

- **3:1 Peak Height to Noise Ratio:** As a general rule, 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.
- **Activity:** A peak less than the analytical threshold (between 100 and 175 RFU, see applicable DNA Database Procedures).
- **Allele:** An alternative form of a gene; allele designation is used to designate a specific size fragment of DNA for a specific locus in STR analysis.
- **Allelic Dropout:** An occurrence where one or more alleles from an individual's DNA profile fail(s) to amplify during PCR and as a result(s) is(are) not detected in the profile. Allelic dropout may be detected by severe imbalance of loci where the smaller fragments are observed and the larger fragments are not observed and/or observance of activity as defined above.
- **Analytical Threshold:** The minimum height (RFU) requirement at and above which detected peaks may be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles.
- **Artifact:** Non-allelic byproducts of PCR technology (i.e., stutter, etc), anomalies which occur during capillary electrophoresis (e.g., pull-up, spike, etc), or byproducts of primer synthesis (i.e., dye blob, etc).
- **DNA Profile:** The combination of genotypes obtained from DNA analysis testing of multiple loci.
- **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.
- **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.
- **Locus (plural=Loci):** The chromosomal location 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. **NOTE**: 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. 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.
- **Noise:** Background 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 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.
- **Off-Ladder Allele:** An allele observed outside the region covered by the allelic ladder at a given locus.
- **Overblown/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 6000 RFUs.

- **Partial DNA Profile:** A DNA profile that does not produce DNA typing results for all loci tested.
- **Peak:** A well defined point on an electropherogram that is on-ladder. See "Microvariant" and "Off-Ladder Alleles" for exceptions to the "on-ladder" requirement.
- **Peak Height/(Signal) to Noise Ratio:** An assessment used to establish an analytical threshold to distinguish allelic peaks (signal) from background/instrument noise.
- **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 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 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 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 only originate 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 tri-allelic 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.
- **Stutter:** An artifact of PCR amplification that is typically one repeat unit less than the corresponding main allele peak resulting from strand slippage during amplification.
- **Tri-allelic 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 - These guidelines are to be used in conjunction with the DNA Database Forensic Scientist's training and experience to provide a solid scientific interpretation of the STR results.

5.2 Interpretation of Samples, Controls, and Allelic Ladders

5.2.1 Examining of the Electropherogram of Samples

Assess the quality of the peaks including RFU values and determine if artifacts are present. (Refer to the DNA Database Section Procedure for GeneMapper ID.)

For Database samples, the profile shall contain the 13 core loci for upload into CODIS.

The use of overblown Database samples shall be at the discretion of the DNA Database Forensic Scientist based upon the training and experience of the DNA Database Forensic Scientist.

It is permissible to combine results from different injections, dilutions and amplifications of the same sample when determining a final DNA profile.

5.2.2 Examination of the Electropherogram of the Negative Controls

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

If activity is detected in the negative control at a single locus or multiple loci, the DNA Database Forensic Scientist shall determine the effects of this activity 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).

Artifact(s) observed in the negative samples does not require the samples to be re-injected. Those artifacts shall be documented in the notes.

5.2.3 Examination of the Electropherogram of the 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 shall be considered inconclusive for all samples and shall be successfully re-injected, re-run, or re-amplified and analyzed before that locus may be used for analysis. If multiple 9947A controls are run, only one must yield a complete profile.

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

Allelic ladder shall be analyzed as specified in the DNA Database Section Procedure for GeneMapper ID.

5.3 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" in the case notes.

5.3.1 Stutter – The GeneMapper® ID software from ABI contains designated cutoff for peaks in stutter positions and shall be used for designating stutter.

5.3.2 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. Scientists shall be aware of this phenomenon and use the computer software to aid in discerning actual alleles from pull-up.

5.3.3 Unincorporated Dye - Scientists shall not call dye-blobs as an actual allele. Dye-blobs shall not be considered for interpretation.

5.3.4 Shoulder and Tail - Shoulders and tails do not prevent the scientist from assigning the specific

peak an allelic value.

5.3.5 n+4 peaks - An artifact peak may appear in the n+4 position. When an n+4 peak is suspected, this shall be documented on the allele call sheets.

5.4 Balance - Samples shall be examined for balance at each locus. For database samples exhibiting heterozygote imbalance (< 50 %), the sample shall be rerun if the imbalance does not allow for proper interpretation by the scientist.

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 DNA DATABASING LABORATORIES." *September 1, 2011.*

DNA Database Section Procedure

Procedure for CODIS

DNA Database Section Procedure for GeneMapper ID

9.0 Records – N/A

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
12/18/2013	1	Original Document