Scientific Working Group on DNA Analysis Methods

Guidelines for STR Enhanced Detection Methods

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SWGDAM Guidelines for STR Enhanced Detection Methods

The Scientific Working Group on DNA Analysis Methods, better known by its acronym of SWGDAM, is a group of approximately 50 scientists representing Federal, State, and Local forensic DNA laboratories in the United States and Canada. During meetings, which are held twice a year, Committees discuss topics of interest to the forensic DNA community and often develop documents to provide direction and guidance for the community. This document was approved by the SWGDAM membership and posted for public comment following the July 2014 SWGDAM meeting. The SWGDAM Executive Board reviewed and approved minor revisions to address comments on October 6, 2014.

This document provides guidelines for the use of Enhanced Detection Methods as applied to forensic casework DNA analysis. These recommendations are intended to serve as a guide for laboratories who are engaging in methods that will enhance the recovery from low quality DNA

samples. Because these are guidelines and not minimum standards, in the event of a conflict between the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories (QAS) and these guidelines, the QAS and the QAS Audit Documents have precedence over these guidelines. Additionally, to avoid any such conflict, use of the mandatory term 'shall' has been used when that term is similarly used in the QAS and the use of the term 'shall' is not intended to transform these guidelines into standards. Laboratories are encouraged to review their standard operating procedures and validation protocols in light of these guidelines.

These guidelines are not intended to be applied retroactively. It is anticipated that these guidelines will be updated as needed.

Background

The use of PCR-based analysis methods for detecting DNA recovered from evidentiary items has been routine among forensic laboratories in the U.S. since the mid-1990s. The current short tandem repeat (STR) kits that are validated for forensic usage entail 26 to 32 cycles of PCR amplification with 0.5 to 2 ng of DNA template. Greater detection sensitivity is technologically feasible with modified methods, which include modification(s) to the standard analytical conditions, or other strategies (Gill et al. 2000; Gill 2001). In fact, DNA typing results can be obtained from a single cell with modified PCR amplification conditions (Findlay et al. 1997). Clearly, these modifications would appear to be beneficial for low-quantity and/or low-quality samples, including some "touch" DNA evidence, as well as unidentified human remains from missing person investigations and mass disasters (Wiegand and Kleiber 1997; Alessandrini et al. 2003; Sewell et al. 2008; Kita et al. 2008; Sturk et al. 2009). However, several investigators have demonstrated that some of these modified methods have inherent limitations and the potential for DNA typing inaccuracies (Wickenheiser 2002; van Oorschot et al. 2005; Kloosterman and Kersbergen 2003). The potential for DNA typing inaccuracies, irreproducibility due to stochastic effects, allele drop-in, contamination risks, and interpretational difficulties, are notable concerns that any laboratory considering any Enhanced Detection Methods should contemplate and evaluate.

In 2008, the Scientific Working Group on DNA Analysis Methods (SWGDAM) convened an ad hoc committee, consisting of forensic DNA experts from academic and government laboratories, to assess the limitations of low copy number (LCN) analysis, the accuracy of DNA profiles that are generated with Enhanced Detection Methods, and whether such profiles should be permitted in the National DNA Index System (NDIS). For clarification, in August 2008, the NDIS Board issued the following definition of Low Template or Low Copy DNA analyses:

> "Based upon a laboratory's internal validation, any DNA typing results generated from limited quantity and/or quality DNA template using conditions that have demonstrated increased stochastic effects are defined as Low Copy Number (LCN) or Low Level DNA analyses. The stochastic effects which may be observed in DNA samples subjected to these conditions include allelic drop-in or drop-out, increased stutter and increased intra-locus peak height imbalance. When used to analyze limited quantity and/or quality DNA template below the stochastic thresholds, LCN conditions may include the following: additional amplification cycles, post-amplification purification, reduced reaction volume, injection enhancement by increased voltage or time, and nested PCR."

By this definition, Low Template or Low Copy DNA analyses involve either amplification or post-amplification efforts to increase detection sensitivity (Westen et al. 2009; Smith and Ballantyne 2007). Pre-amplification strategies such as re-extraction or DNA template concentration are not considered Low Template or Low Copy DNA analyses.

Confusion remains regarding which profiles are permitted in NDIS. In the traditional sense, "LCN Testing" entails increasing the number of amplification cycles. This method is characterized by increased stochastic effects, and if not carefully interpreted, the DNA profiles may be mistyped. However, solely employing an injection enhancement by increasing voltage and/or time has less potential for incorrect DNA profiles.

Therefore, in January 2010, SWGDAM convened a committee to further evaluate and discuss Enhanced Detection Methods. The committee was subsequently named the Enhanced Detection Methods and Interpretation (EDMI) Committee. The committee's focus was to examine the

entire DNA testing process and examine the Enhanced Detection Methods that laboratories have validated and implemented. The techniques may include, but are not limited to, the following:

- Additional PCR amplification cycles greater than the Standard Method
- Post-amplification purification or concentration
- Reduced PCR reaction volume
- Capillary Electrophoresis injection enhancement by increased voltage or time
- Nested PCR
- Increased DNA input during amplification
- Reagent enhancements such as the addition of BSA, $MgCl_2$, or *Taq* Polymerase

This document does not offer an opinion on the viability of any Enhanced Detection Methods, including Low Template or Low Copy DNA Analysis (see Caddy et al. 2008 and Budowle et al. 2009 for more information); however, it does address the suggested validation criteria for laboratories to consider in their individual assessment of an enhanced detection method and provides guidance on data evaluation. DNA results generated using Enhanced Detection Methods must meet certain criteria to increase their confidence to the greatest extent possible.

1. Definitions

For purposes of this document, the following definitions are applicable:

Standard Method is the method routinely employed to generate a complete profile for single-source samples of high quality and quantity. The Standard Method can be applied to all sample types – including mixtures, low-quantity samples, and low-quality samples – wherein data obtained meet the criteria defined through internal validation as reliable. This method is typically not sensitive enough to detect drop-in.

Enhanced Detection Methods are those employed during or subsequent to the PCR amplification step that increases the sensitivity of the Standard Method and are

typically employed with low-quantity and/or low-quality samples. These Enhanced Detection Methods include, but are not limited to, increased amplification cycle number, increased injection time and/or voltage, reduced reaction volume, nested PCR, increasing the amount of *Taq* Polymerase, and post-amplification desalting or concentration. When using Enhanced Detection Methods, the potential for stochastic effects (i.e., elevated stutter, allele drop-out, and intra-locus peak imbalance) may increase. Stochastic effects can be addressed through appropriate interpretation guidelines and relevant thresholds (e.g., an increased injection time may require the adjustment of the stochastic threshold determined from the Standard Method). Therefore, prior to any enhanced detection protocol being implemented, which may include one or more Enhanced Detection Methods, appropriate validations must be performed to address the potential increase in stochastic effects. Appropriate validations shall include assessments of stutter percentages, peak-height ratios, analytical thresholds, stochastic thresholds, locus-to-locus balances, and non-reproducible alleles.

Low Template or Low Copy DNA Analysis is a subset of Enhanced Detection Methods that, in addition to the increased potential for stochastic effects, have an increased potential for non-reproducible alleles.

Points of reiteration for these definitions:

Enhanced Detection Methods are those that are employed during or subsequent to the PCR amplification step that increase the sensitivity of analysis relative to the Standard Method. In order to increase the sensitivity of analysis, a laboratory should already have a validated procedure in place to form a basis for comparison.

Low Template or Low Copy DNA Analysis is a specific type of Enhanced Detection Method. It is understood that there are various methods to increase the sensitivity of analysis; however, Low Template or Low Copy DNA Analysis is a subset of Enhanced

Detection Methods that has an increased potential for non-reproducible alleles. Therefore, replicate analysis should be incorporated into the method.

Samples containing a low amount of DNA may be processed using Standard Methods and may produce a partial profile; however, this is not considered an Enhanced Detection Method or Low Template or Low Copy DNA Analysis. Conversely, attempting subsequent methods to recover lost loci may fall under the definition of Enhanced Detection Method.

2. General Considerations

2.1 All Enhanced Detection Methods shall be properly validated. The current version of the *SWGDAM Validation Guidelines for Forensic DNA Analysis Methods* remains as the guiding document for a laboratory pursuing the implementation of new methods. However, additional experiments may be required for the developmental or internal validation of Enhanced Detection Methods.

2.2 In order to increase a method's sensitivity, a laboratory should already have a Standard Method in place to form a basis for sensitivity comparison (e.g. Whitaker et al. 2001). If a laboratory does not have enough data from the validation to form a basis for sensitivity comparison, additional studies may be performed to properly make a comparison between the Standard Method and the Enhanced Detection Method.

2.3 During the validation of an Enhanced Detection Method, if a laboratory discovers that the method qualifies as a Low Template or Low Copy DNA Analysis, the laboratory must establish appropriate interpretation and reporting guidelines.

2.4 Where applicable, the current QAS shall be followed.

2.5 Each laboratory should consider under which circumstances the use of an Enhanced Detection Method would be warranted and where in the testing process the Enhanced

Detection Method(s) would be of most benefit. The following should be considered: type of sample, case related facts, single or multiple contributors, amount of starting material, etc.

2.6 During validation, laboratories should be mindful that while Enhanced Detection Methods are usually reserved for low-quantity and/or low-quality samples, an Enhanced Detection Method may also be applied to samples of good quantity and/or quality to recover additional data. For example, in a good quantity/quality DNA mixture where some alleles of the minor component may be below the analytical threshold, a laboratory may choose to increase the capillary electrophoresis injection time to enhance the results.

3. Validation of an Enhanced Detection Method, including Low Template or Low Copy DNA Analysis

During the internal validation of a Standard Method, the laboratory should have followed the *SWGDAM Validation Guidelines for Forensic DNA Analysis Methods* and performed the appropriate studies to evaluate a method to determine its efficacy and reliability for forensic casework. Results from sensitivity studies can be used to determine the dynamic range, ideal target range, limit of detection, limit of quantitation, heterozygote balance (i.e., peak height ratio) and the signal-to-noise ratio associated with the assay. Sensitivity studies can also be used to evaluate excessive random (stochastic) effects generally resulting from low-quantity and/or low-quality samples. Quality assurance parameters and interpretation guidelines are derived from these validation studies. For example, lower template DNA may create extreme heterozygote imbalance. As such, empirical heterozygote peak-height ratio data could be used to determine the appropriate ratio by which two peaks are considered to be a heterozygous genotype.

Some laboratories may choose to further interrogate the lower limit of their detection and employ Enhanced Detection Methods. In that case, a proper comparison of the Standard Method and the Enhanced Detection Method is necessary. This comparison is not solely a

sensitivity comparison, but rather, the precision, accuracy, reproducibility, and repeatability between the two methods must also be compared. If a laboratory does not have enough data from the Standard Method validation to form a basis for comparison, additional studies using the Standard Method may be necessary prior to the validation of the Enhanced Detection Method.

If additional studies using the Standard Method are needed, the laboratory should increase the number of samples run at and below the quantity of DNA that predominantly produces partial profiles. For example, if the Standard Method is validated to produce complete DNA profiles down to 250 pg, then a validation study for an Enhanced Detection Method should be performed at and below 250 pg and compared to the Standard Method validation. In this manner, a laboratory can determine whether or not the new method is, indeed, an Enhanced Detection Method and can use the validation data to establish a new dynamic range specific to the Enhanced Detection Method. However, if the Standard Method Validation does not have sufficient studies in this range, additional studies should be performed at and below 250 pg using the Standard Method to generate relevant data for comparison.

During the validation of any Enhanced Detection Method, at a minimum, the following should be addressed and documented within the studies to determine the limits of the method. All studies should be performed using sample types and templates in which the Enhanced Detection Method would typically be used.

- **Analysis of low-quality/degraded samples.** The laboratory should create degraded samples for experimentation in a controlled manner (e.g. Bender et al. 2004). Because a preparation such as this may be difficult to prepare consistently, an alternative method to preparing samples at controlled levels of degradation may be a titration of known samples to various quantities of template DNA (dilution series). Each sample at each concentration should be amplified multiple times (replicates). The NIST Human DNA Quantitative SRM can be used as an anchor for the quantity of DNA present.

- **Analysis of known samples with genotypes including a wide range of alleles.** A dilution series of these samples should be performed to determine the concentration at which stochastic effects begin to occur for the various combinations of alleles. Each sample at each concentration should be amplified multiple times (replicates).

- **Analysis of known mixtures with genotypes including a wide range of alleles.** Mixed DNA samples that are representative of those that would be subjected to the Enhanced Detection Method should be evaluated. It should be noted that mixtures with a significant difference in the ratio of components should be examined and interpreted carefully since the major component may be enhanced above its optimum level of interpretation. Based on the results, a laboratory can choose to restrict the use of an Enhanced Detection Method to single-source samples; however, interpretation procedures should address the treatment of mixed DNA results.

- **Determination of new analytical and stochastic thresholds.** The laboratory should determine if new analytical and/or stochastic thresholds should be established for the Enhanced Detection Methods. The analytical and/or stochastic thresholds are usually more conservative (higher) than the Standard Method to account for the increased stochastic effects of some Enhanced Detection Methods and the increased potential for non-reproducible alleles of Low Template or Low Copy DNA Analysis.

3.1 The laboratory should determine a method's reliability and reproducibility by comparing the data generated from replicates to each other and to the known profile. Particular attention should be given to incidences of allelic drop-in and allelic drop-out. Next, a comparison should be made between data generated using the Standard Method and Enhanced Detection Method to determine if the sensitivity of the new method has improved and if stochastic effects have increased. Should a laboratory decide to proceed with the use of this Enhanced Detection Method, interpretation procedures should reflect these observations. Where allelic drop-in has been

observed, replicate analysis may be required to establish the reliability of DNA results (see following sections).

3.2 Reagent blank controls ***must be analyzed at the same parameters as test samples and*** should be reviewed for drop-in. The laboratory should run a sufficient number of reagent blank controls to improve the detection of potential drop-in events so they can be appropriately evaluated and understood. Where allelic drop-in has been observed, replicate analysis may be required to establish the reliability of DNA results (see following sections).

3.3 Laboratories may validate and adopt individual Enhanced Detection Methods as well as utilize validated combinations of these methods. Where multiple Enhanced Detection Methods are to be used in combination, a laboratory should understand the effects of combining Enhanced Detection Methods (e.g., improvements in sensitivity and/or causes stochastic effects). Prior to a full validation, each Enhanced Detection Method should first be tested **individually** at the limit of detection to determine how much enhancement each method has on DNA results. Further testing of the **combination** should be done in a step-wise fashion by adding one Enhanced Detection Method at a time. If a peer-reviewed publication exists on the exact combination of the Enhanced Detection Method, it may be used to substitute the testing recommended. This testing does not replace an internal validation since it is not meant to be as extensive and can be as few as five to ten samples. A full internal validation of the Enhanced Detection Method procedure must be conducted in accordance to the QAS. Laboratories should also refer to the *SWGDAM Validation Guidelines for Forensic DNA Analysis Methods*.

For example, if a laboratory would like to develop an enhanced detection protocol that includes reduced reaction volume, increased injection time/voltage, and additional *Taq* polymerase, the laboratory should test each Enhanced Detection Method independently prior to conducting an internal validation. Further testing should incorporate a pair-wise combination of two out of the three Enhanced

Detection Methods, such as reduced reaction volume and increased injection time/voltage. The final testing should be a combination of all three Enhanced Detection Methods. Once it has been determined through this testing that the combination of methods does, indeed, enhance DNA results, then a full internal validation may commence.

In the same example, if peer-reviewed publications exist on the individual effects of reduced reaction volume, increased injection time/voltage, and additional *Taq* polymerase, the laboratory may choose to use that data as a substitution for those tests. The laboratory should conduct further testing by incorporating a pair-wise combination of two out of the three Enhanced Detection Methods, such as reduced reaction volume and increased injection time/voltage. The final testing should be a combination of all three Enhanced Detection Methods. Once it has been determined through this testing that the combination of methods does, indeed, enhance DNA results, then a full internal validation may commence.

When Enhanced Detection Methods are validated in combination, laboratories may only utilize the Enhanced Detection Methods individually if an internal validation was conducted to support the use of these methods separately. For example, when a laboratory conducted an internal validation on an enhanced detection protocol that includes reduced reaction volume, increased injection time/voltage, and additional *Taq* polymerase, the laboratory may only utilize a procedure with an increased injection time/voltage individually if an internal validation was independently conducted to support the use of this method.

Laboratories should have validation data to demonstrate that using the Enhanced Detection Method(s) increases the confidence that the alleles called are authentic. It is recommended that DNA profiles that are developed using properly validated Enhanced Detection Methods be eligible for searching at NDIS.

4. Replicate Analysis

Where there is an increased potential for non-reproducible alleles (i.e., Low Template or Low Copy DNA Analysis), additional confidence in determining a profile genotype can be established by replicate analysis. This must be determined by the validation of the Enhanced Detection Method. Where replicate analysis is required, a laboratory shall establish a policy for determining the number of replicates (Benschop et al. 2011) to increase the confidence of the result and whether these replicates must be obtained through multiple amplifications of a single extract or single amplifications of multiple extracts. The use of composite or consensus profiles would also need to be determined by the laboratory and addressed in its protocols (Bright et al. 2012; Benschop et al. 2013).

4.1 If the laboratory has chosen to use Low Template or Low Copy DNA Analysis, it is strongly recommended that the laboratory incorporate **replicate amplification analysis.** This may be accomplished through multiple amplifications of a single extract or a single amplification of multiple extracts from the same source (see Gill et al. 2000).

Laboratories performing Low Template or Low Copy DNA Analysis should have validation data and interpretation protocols that increase the confidence of an allele call. It is recommended that DNA profiles developed from unidentified human remains (UHR) and other presumed single-source samples from missing persons investigations and mass disasters (such as low-quality reference samples) using properly validated Low Template or Low Copy DNA Analysis be eligible for searching at NDIS. At this time, SWGDAM has no recommendations on the NDIS eligibility of DNA profiles developed from other items using properly validated Low Template or Low Copy DNA Analysis.

5. Interpretation of data obtained through Enhanced Detection Methods

The *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories* is the guidance document for the interpretation of autosomal STR results. However, the potential for DNA typing inaccuracies and irreproducibility due to stochastic effects and allele drop-in are notable concerns that any laboratory considering employing an Enhanced Detection Method(s) should contemplate and evaluate. To this extent, the following must be evaluated and, where appropriate, should be incorporated within the laboratory's interpretation guidelines when using Enhanced Detection Methods to enable greater consistency and accuracy among analysts within a laboratory.

- Stochastic Thresholds – The laboratory should carefully evaluate whether or not to incorporate a stochastic threshold to ensure allelic drop-out has not occurred. Additionally, the laboratory should utilize validation data to determine the possibility of loci that may be more susceptible to stochastic effects. In this case, an obligate allele (or otherwise designated) may be reported and used for comparison.

- Replicate Analysis – Where replicate analysis is required, the laboratory must have an interpretation procedure to determine how to generate the final consensus DNA profile.

- Probabilistic Genotyping – This may be useful in the analysis of results obtained from Enhanced Detection Methods (Balding and Buckleton 2009). Probabilistic genotyping approaches (e.g., Mitchell et al. 2012; Taylor et al. 2013) have been published that describe methods of incorporating replicate analyses.

6. Workflow Criteria

A laboratory must establish criteria on when to allow the use of an Enhanced Detection Method. These criteria can be based on the quantity of DNA template present, the relative fluorescent units (RFU) seen during a Standard Method run, the completeness of a profile, or

for a specific sample type (e.g., skeletal samples, touched samples, etc.). Workflow criteria would likely be different for samples presumed to be from a single source as opposed to samples presumed to contain mixtures of small quantities of DNA, none of which individually may be present in sufficient quantity to produce a high quality DNA profile. As an example, skeletal samples might have different criteria for Low Template or Low Copy DNA Analyses than a touched sample.

7. Quality Practices

With the increased detection sensitivity using any Enhanced Detection Methods, extra care must be taken to prevent the contamination of samples. While the prevention of contamination is important, the laboratory must also have interpretation guidelines that address appropriate actions should contamination occur.

7.1 Reagents and Consumables – A laboratory should perform quality control checks of critical reagents and implement quality measures on consumables such as tubes, pipette tips, and filters in which Enhanced Detection Methods are performed. The quality control procedures must be performed using the Enhanced Detection Method to ensure that any prevalent contamination would be detected.

7.2 Controls – Reagent blanks shall be treated the same as the evidence samples. A laboratory must have a procedure to evaluate the performance of controls.

7.3 Laboratory cleanliness – A laboratory performing any Enhanced Detection Method should be cleaned using techniques similar to those used by mitochondrial DNA laboratories. For example, using 10% bleach to clean all surfaces prior to performing examinations and exposing tubes and lab equipment to ultraviolet (UV) light are two techniques which are effective in preventing or reducing contamination.

7.4 Personnel DNA Database – A DNA database containing the DNA profiles of individuals who have access to laboratory space and/or may come into contact with

an item of evidence prior to or during processing should be maintained. This database is an essential part of the quality assurance program of a laboratory, especially one employing any Enhanced Detection Methods, and should be searched in order to assure that no evidence DNA profile was contaminated by someone during or after the investigation. Access to this database shall be limited to those tasked with addressing contamination. A laboratory should consult its legal representative when establishing a personnel/staff DNA database to ensure the database is developed in accordance with applicable federal, state and local laws and regulations. The individuals included in this DNA database should include past and present personnel of the laboratory and others who have access to the space (such as members of housekeeping staff and evidence intake staff). To the extent permitted by federal, state or local law/regulations, the personnel DNA database should also include equipment vendors, members of first-response teams, various visitors to the laboratory, and known exogenous DNA profiles from consumables.

7.5 Personal Protective Equipment (PPE) – PPE is designed to protect employees from serious workplace injuries or illnesses resulting from contact with chemicals, reagents, or biological hazards. PPE includes a variety of devices and garments such as goggles, gloves, lab coats, etc. Proper PPE must be worn during sample processing, and required PPE may vary from location to location depending on the hazards of the area. While PPE is designed to protect employees, it can also prevent the transfer of DNA from employees to work surfaces or evidence. For example, in areas of the laboratory where Low Template or Low Copy DNA Analysis takes place, the addition of wearing booties and/or bouffant caps can prevent the transfer of DNA from employees.

8. Additional Quality Practices for laboratories using Low Template or Low Copy DNA Analysis

For laboratories using Low Template or Low Copy DNA Analysis, additional quality practices should be considered to ensure the continuous reliability of results.

8.1 Proficiency Testing – Proficiency testing using Low Template or Low Copy DNA Analysis should be considered. Unless proficiency tests specific for Low Template or Low Copy DNA Analysis are available from approved test vendors, laboratories using Low Template or Low Copy DNA Analysis should dilute proficiency test samples to at least the target amount deemed appropriate for Low Template or Low Copy DNA Analyses. All proficiency testing standards within the QAS shall be followed at all times.

8.2 Monitoring of Analytical Procedures – The laboratory should check its Low Template or Low Copy DNA Analyses procedures, either annually or whenever substantial changes are made, against an appropriate and available NIST standard reference material (SRM) or standard traceable to a NIST standard. To the extent possible, laboratories using Low Template or Low Copy DNA Analysis should dilute samples to at least the target amount deemed appropriate for Low Template or Low Copy DNA Analyses.

Glossary

Analytical threshold: The minimum height 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. [From *SWGDAM Interpretation Guidelines for Autosomal STR Typing (2010)*]

Additional amplification cycles: One or more amplification cycles beyond what the laboratory had validated in the Standard Method of its STR kit (not necessarily that above manufacturer's recommendations).

Chemical Enhancements: Addition of a variety of chemicals/reagents to improve the efficacy of the PCR reaction. This includes, but is not limited to, adding more BSA, $MgCl_2$,

polymerase, or using commercial products developed to enhance the PCR and/or STR process beyond the Standard Method.

Composite/Consensus Profiles: 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. [From *SWGDAM Interpretation Guidelines for Autosomal STR Typing (2010)*]

Contamination: The unintentional introduction of exogenous DNA into a DNA sample or PCR reaction. [From *SWGDAM Validation Guidelines for DNA Analysis Methods (2012)*]

Drop-in: Non-reproducible allele(s) that show up in the profile or control that does not originate from the principal DNA donor(s). Typically, drop-in events are not detected using Standard Methods.

Drop-out: Failure to detect an allele within a sample or failure to amplify an allele during PCR. [From *SWGDAM Interpretation Guidelines for Autosomal STR Typing (2010)*, under "Allelic dropout"]

Enhanced Detection Method: See Section 1 of this document.

Heterozygous balance: Intra-locus balance of two sister alleles measured as a function of relative florescence units (RFU). Heterozygous balance is determined by the laboratory during its initial validation. Related to: Peak Height Ratio (PHR).

Increased injection time: An increase in time of the electrokinetic injection above that which the laboratory had validated for the Standard Method.

Increased injection voltage: An increase in voltage of the electrokinetic injection above that which the laboratory had validated for the Standard Method.

Low Template or Low Copy DNA Analysis: See Section 1 of this document.

Low Copy Number DNA Profile: A consensus DNA profile generated and interpreted from Low Template or Low Copy DNA Analysis.

Nested PCR: PCR amplification with two pairs of PCR primers used in two successive PCR runs. The first pair of primers amplify the targeted alleles, but may not produce enough PCR product for detection. The second pair of primers (nested primers) consist of sequences internal to the first set of primers and bind to the PCR product to generate sufficient signal for analysis.

Non-reproducible alleles: These include preferential stutter, allele drop-in, and authentic alleles at levels that may not be detected in replicate analysis.

Partial Profile: A DNA profile for which complete typing results are not obtained at all tested loci due to probable allelic dropout, degradation of DNA, and/or preferential amplification.

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 this value by 100 to express the PHR as a percentage; used as an indication of which alleles may be heterozygous pairs and also in mixture deconvolution. [From *SWGDAM Interpretation Guidelines for Autosomal STR Typing (2010)*]

Post-amplification purification: The purification or "cleaning up" of PCR product or amplicon to reduce or eliminate amplification reaction components from competing for

electrokinetic injection. This could be achieved by methods such as, but not limited to, desalting or filtration.

Standard Method: See Section 1 of this document.

Stochastic effect: The observation of intra-locus peak imbalance and/or allele drop-out resulting from random, disproportionate amplification of alleles in low-quantity template samples. [From *SWGDAM Interpretation Guidelines for Autosomal STR Typing (2010)*]
Stochastic threshold: The peak height value above which it is reasonable to assume that, at a given locus, allelic drop-out of a sister allele has not occurred.

Stutter: The tendency of PCR product to become displaced typically by one repeat unit compared to the template DNA during the reaction. This results in DNA fragments that are one repeat unit shorter or longer than the desired fragment size.

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October 6, 2014	SWGDAM Executive Board's review and approval of minor revisions following public comment period; approval to post final version at www.swgdam.org.