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Standard for Training in Forensic Short Tandem Repeat Typing Methods using Amplification, DNA Separation, and Allele Detection

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Foreword

This standard defines the minimum requirements that shall be met in a forensic DNA analyst training program for short tandem repeat typing methods using amplification, DNA separation, and detection. The aim is to provide a framework for quality training that will result in consistency in the forensic DNA community.

This standard was revised, prepared and finalized as a standard by the DNA Consensus Body of the AAFS Standards Board (ASB). The initial draft document was developed by the Biological Methods Subcommittee of the Organization of Scientific Area Committees. All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

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Keywords: Training, PCR, amplification, STR typing, DNA separation, capillary electrophoresis, DNA detection

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Standard for Training in Forensic Short Tandem Repeat Typing Methods using Amplification, DNA Separation, and Allele Detection

1 Scope

This standard provides the requirements of a forensic DNA laboratory's training program in forensic Short Tandem Repeat typing methods using amplification, DNA separation and allele detection.

2 Normative References

ASB Standard 022 - *Standard for Forensic DNA Analysis Training Programs* [1]

3 Terms and Definitions

For purposes of this document, the following definitions apply.

3.1
Allele
One of two or more versions of a genetic sequence at a particular location in the genome.

3.2
Amplification
An increase in the number of copies of a specific DNA fragment; can be *in vivo* or *in vitro*. In forensic DNA testing laboratories, this refers to the use of the PCR technique to produce many more copies of specific genetic loci from samples of known and unknown origin for the purpose of generating DNA profiles for comparison.

3.3
Analytical threshold
1) The minimum height requirement at and above which detected peaks on a STR DNA profile electropherogram can be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles. 2) An acceptable "Relative Fluorescence Units" (RFU) level determined to be appropriate for use in the PCR/STR DNA typing process; a minimum threshold for data comparison is identified by the specific forensic laboratory doing the testing through independent validation studies.

3.4
Artifact
A non-allelic product of the amplification process (e.g., stutter, non-templated nucleotide addition, or other non-specific product), an anomaly of the detection process (e.g., pull-up or spike), or a by-product of primer synthesis (e.g., "dye blob") that may be observed on an electropherogram; some artifacts may complicate the interpretation of DNA profiles when they cannot be distinguished from the actual allele(s) from a particular sample.

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[1] American Academy of Forensic Sciences Standards Board, 4200 Wisconsin Avenue, NW, Suite 106-310, Washington, DC 20016-2143 asb@aafs.org

3.5
Bin
Allele designations corresponding to the window of fragment sizes for each allele, determined by empirical testing.

3.6
Capillary electrophoresis
An electrophoretic technique for separating DNA molecules by their relative size based on migration through a narrow glass capillary tube filled with a liquid polymer.

3.7
Contamination
Exogenous DNA or other biological material in a DNA sample, PCR reaction, or item of evidence; the exogenous DNA or biological material could be present before the sample is collected, or introduced during collection or testing of the sample.

3.8
Electrophoresis
A technique used in laboratories to separate macromolecules based on size. Negatively charged molecules (e.g. proteins, DNA and RNA) migrate towards a positively charged pole through a sieving matrix, which permits a size-dependent separation.

3.9
Inhibitor
As related to the polymerase chain reaction (PCR), any substance that interferes with or prevents the synthesis of DNA during the amplification process.

3.10
Locus (plural loci)
A unique physical location of a gene (or specific sequence of DNA) on a chromosome

3.11
Polymerase chain reaction (PCR)
An enzymatic process by which a specific region of DNA is replicated during repetitive cycles that consist of the following: denaturation of the template; annealing of primers to complementary sequences at an empirically determined temperature; and extension of the bound primers by a DNA polymerase. The goal of the PCR process is to generate many copies (termed products or amplicons) of a specific region of DNA for further analysis.

3.12
Short tandem repeats (STR)
Multiple copies of an identical (or similar) DNA sequence arranged in direct succession where the repeat sequence unit is 2 base pairs (bp) to 6 bp in length; because STRs generally occur in the DNA outside of the constraints (*i.e.,* selective pressure) of genes, the number of repeat units can vary among individuals.

3.13
Spectral calibration
An examination of the contribution of overlap in the emission spectrum of fluorescent dyes used for a specific DNA test on a capillary electrophoresis instrument; permits the color deconvolution

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necessary for multi-color STR typing or sequencing to be performed; a poor spectral calibration may cause artifact peaks or inaccurate peak height determinations.

3.14
Stochastic
1) Chance, or random variation 2) in DNA testing, refers to random sampling error from extracts containing low levels of DNA and/or random variation in selection of alleles amplified at a particular locus.

4 Requirements

4.1 Knowledge-based training

The laboratory's training program shall provide the trainee with an understanding of the fundamental principles of the theory behind PCR amplification, DNA separation, and allele detection methods, the function of the reagents and other components used in each method, the limitations of each method, and the laboratory's own STR typing guidelines

4.1.1. At a minimum, the knowledge-based portion of the training program shall require review of the following:

a) The laboratory's protocols for PCR amplification, DNA separation, and allele detection

b) The laboratory's applicable validation studies

c) Literature used to support validation of the methods

d) Applicable literature as assigned by the trainer

4.1.2 At a minimum, the knowledge-based portion of the training program shall cover the following topics:

NOTE: Knowledge of historical methods is intended to provide an educated perspective on current methods. In-depth understanding of these methods may not be required for successful training.

a) STRs in forensic DNA analysis

i. History

ii. Structure and nomenclature

iii. Methods of analysis

iv. STR typing systems (e.g., commercially produced kits)

v. Core STR loci (e.g., CODIS)

b) Polymerase chain reaction

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i. History

ii. Biochemical principles

a. Hot-start PCR

b. Multiplex PCR

iii. Function of reagents

iv. Specificity, fidelity, and optimization

v. Limitations

vi. PCR inhibitors

vii. Stochastic events

viii. Amplification artifacts

ix. Contamination and quality control

c) DNA separation

i. Theory of electrophoresis

ii. Capillary electrophoresis advantages and disadvantages

iii. Function of reagents

iv. Electrokinetic injection

v. DNA sieving

vi. Sample preparation

vii. Electrophoresis artifacts

viii. Contamination and quality control

d) DNA detection

i. History of DNA detection methods

ii. Fluorescent dye detection

a. Excitation

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b. Emission

iii. Dye-labeling of PCR primers

iv. Computer software programs for DNA detection

v. Multicomponent analysis/spectral calibration

vi. Analytical threshold

vii. Fragment sizing and allele calling

viii. Virtual bins

e) Instrumentation and reagents

i. Thermal cycling instruments and parameters

ii. DNA separation and detection instruments and parameters

iii. Software parameters associated with instruments

iv. Maintenance and calibration

v. Storage of STR typing kit and DNA separation reagents

f) Troubleshooting

i. Thermal cycling errors (e.g., ramping, temperature control)

ii. DNA detection errors (e.g., spectral calibration failure, resolution failure)

iii. General equipment failure

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4.2 Practical training

The laboratory's training program shall provide the trainee with sufficient practical instruction for the trainee to obtain the skills for performing the STR PCR amplification, DNA separation, and allele detection protocols used by the laboratory.

4.2.1 At a minimum, the practical portion of the training program shall include exercises representative of the range, type, and complexity of routine casework or database samples processed by the laboratory. These include:

a) STR PCR amplification, DNA separation, and allele detection methods to be utilized by the trainee

b) Documentation of the process

4.2.2 Practical exercises representative of the range, type, and complexity of routine casework or database samples processed by the laboratory. These shall include

a) STR PCR amplification, DNA separation, and allele detection methods to be utilized by the trainee

b) Documentation of the process

c) The number and quality of samples processed by the trainee shall be appropriate to demonstrate the ability to follow the laboratory's STR PCR amplification, DNA separation, and allele detection protocol(s) and to produce reliable and accurate results.

4.3 Competency testing

The laboratory's training program shall include knowledge-based and practical competency testing in the application of STR PCR amplification, DNA separation, and allele detection protocols used by the laboratory. The format of the test(s) shall meet section 4.3 of ASB 022.

4.3.1 Knowledge-based competency

The trainee shall successfully complete a knowledge-based test covering the critical information obtained during the training of STR typing methods using PCR amplification, DNA separation, and allele detection methods. The test(s) shall cover, at a minimum:

a) The theoretical and scientific bases of STR PCR amplification, DNA separation, and detection

b) The function of the reagents and other components used in each method

c) The proper application of each method

d) The quality control steps pertaining to PCR amplification, DNA separation, and allele detection

e) The laboratory's analytical procedures pertaining to PCR amplification, DNA separation, and detection

4.3.2 Practical competency

The trainee shall successfully complete a practical test covering each of the PCR amplification, DNA separation and detection protocol(s) for which he or she will be independently authorized. The trainee shall be able to satisfactorily perform the following, as applicable:

a) Properly and accurately execute the analytical procedures related to PCR amplification, DNA separation and detection

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b) Apply the laboratory's analytical procedures to a variety of evidentiary casework or database samples

c) Operate equipment and instrumentation used in the laboratory

d) Document work performed in accordance with laboratory procedures

5 Conformance

In order to demonstrate conformance with this standard, the laboratory shall meet Section 5 of the ASB 022.

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Annex A
(informative)

Bibliography

The following information provides a list of the literature resources that may assist the DNA technical leader in defining the breadth and scope of the materials to be reviewed by the trainee. This list is not meant to be all inclusive. The laboratory shall develop a list tailored to its specific needs. Updated references shall be added to the laboratory's list as new methods or technologies are incorporated into the laboratory's protocols.

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