ANSI/ASB Standard 115, First Edition
2020

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

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

ASB Approved September 2019

ANSI Approved August 2020

Academy Standards Board
410 North 21st Street
Colorado Springs, CO 80904

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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 training that will result in quality and consistency in the forensic DNA community.

This document is part of a series of training documents under ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs*.

This document was revised, prepared, and finalized as a standard by the DNA Consensus Body of the AAFS Standards Board. The draft of this standard was developed by the Biological Methods Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science.

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>
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All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

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

The following reference is indispensable for the application of the standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs*

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. In forensic DNA testing laboratories, this refers to the use of the PCR technique to produce many more copies of fragments at 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) A "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 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.

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
The unintentional introduction of 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 and charge. Negatively charged molecules (e.g. 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
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 necessary for multi-color STR typing or sequencing to be performed; a poor spectral calibration may cause artifact peaks or inaccurate peak height determinations.

3.13
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.

3.14
virtual bins
Virtual bins are alleles that are not present in the allelic ladder, but have been previously reported or discovered during developmental validation of a particular chemistry.
(GeneMapper® [a] ID-X Software and Bin Overlap User Bulletin[b],)

4 Requirements

4.1 General

ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs* shall be used in conjunction with this document because ANSI/ASB Standard 022 provides the foundational training program requirements upon which additional specific requirements, such as this document, will be based.

4.2 Knowledge-based Training

4.2.1 The laboratory's training program shall provide the trainee with an understanding of the fundamental principles of the theory behind PCR amplification, DNA product 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 protocols.

4.2.2 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 and the test methods in the laboratory;

d) applicable literature as assigned by the trainer.

4.2.3 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.

a) STRs in forensic DNA analysis including:

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[a] This term is used as an example only and does not constitute an endorsement of this product by the AAFS Standards Board.
[b] https://assets.thermofisher.com/TFS-Assets/LSG/manuals/100029546_GeneMapperIDX_BinOverlap_UB.pdf

1. history of development and use;
2. structure and nomenclature;
3. methods of analysis;
4. STR typing systems (e.g., commercially produced kits);
5. core STR loci (e.g., CODIS);
6. limitations of the technology.

b) Polymerase chain reaction, including:

1. history of development and use;
2. biochemical principles;
3. hot-start PCR;
4. multiplex PCR;
5. function of reagents;
6. specificity, fidelity, and optimization;
7. limitations of the technology;
8. PCR inhibitors;
9. stochastic effects;
10. amplification artifacts;
11. contamination and quality control.

c) DNA separation, including:

1. theory of electrophoresis;
2. capillary electrophoresis advantages and disadvantages;
3. function of reagents;
4. electrokinetic injection;
5. DNA sieving;
6. sample preparation;
7. electrophoresis artifacts;

8. contamination and quality control;
9. limitations of the technology.

d) DNA detection, including:

1. history of DNA detection methods;
2. fluorescent dye detection;
   i. excitation,
   ii. emission;
3. dye-labeling of PCR primers;
4. computer software programs for DNA detection;
5. multicomponent analysis/spectral calibration/spatial calibration;
6. analytical threshold;
7. fragment sizing and allele calling;
8. bins (including virtual bins);
9. limitations of the technology.

e) Instrumentation and reagents, including:

1. thermal cycling instruments and parameters;
2. DNA separation and detection instruments and parameters;
3. software parameters associated with instruments;
4. maintenance and calibration;
5. storage of STR typing kit and DNA separation reagents.

f) Contamination:

1. sources (environmental, procedural);
2. sample handling strategies and preventative methods;
3. decontamination procedures;
4. detection limitations;
5. root cause analysis, corrective action when contamination occurs.

g) Quality control in the amplification, DNA separation and allele detection process to include appropriate controls.

h) Storage, preservation, and retention of amplified DNA product according to laboratory policy.

i) Troubleshooting, including:

1. thermal cycling errors (e.g., ramping, temperature control);

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

3. general equipment failure.

4.3 Practical Training

4.3.1 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.

4.3.2 At a minimum, the practical portion of the training program shall include the observation of the process at least once or until clearly understood, and 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) the use of appropriate controls;

c) proper documentation of the process.

4.3.3 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) use and evaluation of controls and expected results;

c) proper documentation of the process;

d) 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.

4.4 Competency Testing

4.4.1 General

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 ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs*.

4.4.2 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 basis of STR PCR amplification, DNA separation, and detection;

b) the function of the reagents, instruments, software and other components used in each method;

c) the proper application of each method and strategy for use;

d) the required quality control steps pertaining to PCR amplification, DNA separation, and allele detection, including the evaluation of controls;

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

4.4.3 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 to perform. Samples of known type will be used. 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 without contaminating the samples;

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

c) operate relevant equipment, instrumentation, and software used in the laboratory;

d) correctly 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 ANSI/ASB Standard, 022 *Standard for Forensic DNA Analysis Training Programs*.

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.

1] FBI, *Quality Assurance Standards for DNA Databasing Laboratories,* effective September 1, 2011[c].

2] FBI, *Quality Assurance Standards for DNA Databasing Laboratories*, effective July 1, 2020[d].

3] FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories (QAS),* effective September 1, 2011[e].

4] FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories*, effective July 1, 2020[f].

5] SWGDAM. *SWGDAM Training Guidelines and References*[g].

[c] Available at https://www.fbi.gov/file-repository/quality-assurance-standards-for-dna-databasing-laboratories.pdf/view.

[d] Available at https://docs.wixstatic.com/ugd/4344b0_809d01b3e9f9451cb9edd9a85f2c2e5b.pdf.

[e] Available at https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view.

[f] Available at https://docs.wixstatic.com/ugd/4344b0_6782472e073442ec877085584aaffa36.pdf.

[g] Available at http://media.wix.com/ugd/4344b0_87b2b4a150aa433f9490b7113b1aa4a6.pdf.

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