ASB Standard 091, First Edition
~~2024~~2025

Standard for Training in Analysis of Forensic Short Tandem Repeat (STR) DNA Data

Standard for Training in Analysis of Forensic Short Tandem Repeat (STR) DNA Data

ASB Approved Xxxxx ~~2024~~2025

ANSI Approved Xxxxxx ~~2024~~2025

410 North 21st Street
Colorado Springs, CO 80904

Foreword

This standard defines the minimum ~~required elements for~~requirements to be met in a forensic DNA analyst training program ~~on~~for the analysis of STR data. The aim is to provide a framework for quality training resulting in consistency within a laboratory and in the forensic DNA community.

~~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 Human Forensic Biology Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science.~~

The American Academy of Forensic Sciences established the Academy Standards Board (ASB) in 2015 with a vision of safeguarding Justice, Integrity and Fairness through Consensus Based American National Standards. To that end, the ASB develops consensus based forensic standards within a framework accredited by the American National Standards Institute (ANSI), and provides training to support those standards. ASB values integrity, scientific rigor, openness, due process, collaboration, excellence, diversity and inclusion. ASB is dedicated to developing and making freely accessible the highest quality documentary forensic science consensus Standards, Guidelines, Best Practices, and Technical Reports in a wide range of forensic science disciplines as a service to forensic practitioners and the legal system.

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 Human Forensic Biology Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science.

Questions, comments, and suggestions for the improvement of this document can be sent to AAFS-ASB Secretariat, asb@aafs.org or 410 N 21st Street, Colorado Springs, CO 80904.

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

ASB procedures are publicly available, free of cost, at www.aafs.org/academy-standards-board.

Keywords: *training, DNA standard, DNA data, DNA analysis, STR data*

Table of Contents *(to be finalized prior to ~~this document's~~ publication)*

Standard for Training in Analysis of Forensic Short Tandem Repeat (STR) DNA Data

1 Scope

This standard ~~outlines~~ the ~~minimum~~ requirements ~~in~~for a forensic DNA laboratory's training ~~programs~~program for analysis of capillary electrophoresis data including autosomal STRs, X-STRs and Y-STRs.

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*[a].

3 Terms and Definitions

For purposes of this document, the following definitions apply.

3.1
allelic ladder
In STR testing, a standardization tool, consisting of the most commonly observed alleles, used for assigning an allele designation to a peak in an electropherogram at a particular genetic locus.

3.2
analytical threshold
The minimum height requirement at and above which detected peaks on a STR DNA profile electropherogram can be reliably distinguished from instrument background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles.

3.3
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., single or multi-channel voltage spikes or pull-up), or a by-product of primer synthesis (e.g., dye blob) that may be observed on an electropherogram.

3.4
dye blobs
Artifact peaks in capillary electropherograms arising from fluorescent dye molecules coming off their associated PCR primer during primer synthesis (and failing to be purified); generally dye blob peaks are wider than true STR alleles, are lower in signal intensity, and can be characteristically sized in each color channel.

[a] Available from: www.aafs.org/academy-standards-board.

3.5
electropherogram
The graphic representation of the separation of molecules by electrophoresis in which the STR DNA data appear as peaks; the format in which DNA typing results are presented, with the X axis displaying the observed alleles based on fragment size and migration time and the Y axis recording the relative amount of DNA detected based on the relative fluorescent unit (RFU) collected during analysis.

3.6
expert system
A software program or set of software programs that interprets the data generated from a DNA analysis instrument platform in accordance with laboratory defined quality assurance rules and accurately identifies the data that does and does not satisfy such rules.

3.7
genotyping software
A software program or set of software programs that analyzes raw capillary electrophoresis data and, using allelic ladder and size standard information, assigns allele designations to peaks in an electropherogram.

3.8
minus A
–A
A reference to PCR product that does not possess an extra A nucleotide at its 3' end due to incomplete non-template nucleotide addition; sometimes referred to as n-1.

3.9
null allele
The inability to detect an individual's allele during DNA testing. Possible causes include: a variant (mutation) in the template DNA that prevents a PCR primer from binding properly and thus preventing the generation of a DNA ~~productor~~product or the loss of the DNA sequence for the allele from the genome due to a deletion. The detection of a single allele may suggest the individual is homozygous when the individual is actually heterozygous at that locus but the second allele is undetected with the method used.

3.10
off-ladder allele
OL
An STR allele that is of a different length than is found in the allelic ladder for a particular locus.

3.11
off-scale STR data
Data produced when the emitted fluorescence from the PCR products being measured saturates the detector ~~in an electropherogram~~; may result in flat-topped peaks in an electropherogram for STR alleles and pull-up peaks in one or more color channels corresponding to the off-scale peak.

3.12
primer peak
Fluorescence from unincorporated PCR primers present in ~~all~~ amplified DNA product that is detected before the sizing range in an electropherogram.

3.13
pull-up
An artifact that may occur during analysis of fluorescently labeled DNA fragments when signal from one dye color channel produces artificial peaks in another, usually adjacent color, at a similar position on the X axis in an electropherogram; sometimes referred to as bleed-through or matrix/spectral calibration failure.

3.14
size standard
DNA fragments of known length that are analyzed in conjunction with samples during electrophoretic or other instrumental methods.

3.15
spike
An anomalous peak that can occur in capillary electropherograms and may interfere with data interpretation; this instrumental artifact is typically narrow and produces signal in multiple dye channels.

3.16
stochastic effects
Changes in a DNA profile that generally occur when suboptimal or limiting quantities of DNA are tested. This may be due to sampling variation (e.g., pipetting) of the target DNA that goes into the polymerase chain reaction (PCR) and/or random events between primers and target DNA during PCR amplification. The effects may be observed at one or more loci, and include: 1) peak height imbalance of sister alleles in a heterozygous pair; 2) loss of data (referred to as "allele drop out" when one or more alleles are missing at a locus and "locus drop out" when all alleles are missing from a locus); 3) allele drop-in [allelic peak(s) in an electropherogram that are not reproducible]; and 4) elevated stutter peaks (a non-allelic peak in the stutter position exceeding the stutter expectation of the laboratory).

3.17
stutter
An artifact of polymerase chain reaction (PCR) amplification typically observed one or more repeat units smaller or larger than a short tandem repeat (STR) allele in a DNA electrophoretic profile, may result from strand slippage during PCR amplification. A stutter peak is generally of lower relative fluorescence units (RFU) than the allele peak.

3.18
tri-allelic pattern
The detection of three alleles in one individual at a particular STR locus.

3.19
variant allele
A ~~non-standard~~ form of an allele due to a point mutation, an insertion or a deletion relative to other commonly seen alleles in the allelic ladder.

~~NOTE May also be called off-ladder allele or microvariant.~~

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.

The laboratory's training program shall include all requirements applicable to the work conducted by the laboratory and by the individual in training.

4.2 Knowledge-based Training

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

a) the laboratory's protocols for STR data analysis;

b) the laboratory's applicable validation ~~studies, including limitations that affect analysis;~~studiess

c) limitations of the laboratory methods and validation studies that affect analysis;

~~c)~~d) literature ~~that supports the laboratory's~~used to support validation;

~~d)~~e) literature that supports the laboratory's data analysis protocol;

~~e)~~f) manufacturer user guides for applicable software;

~~f)~~g) literature on the effects of cognitive bias in decision-making processes associated with analysis used for forensic STR DNA data;

~~g)~~h) applicable literature as assigned by the trainer.

4.2.2 ~~The~~The knowledge-based training component of the laboratory's training program shall provide the trainee with basic instruction in the analysis of capillary electrophoresis data generated for forensic Short Tandem Repeat (STR) analyses, to include both autosomal and sex-specific STRs. Not all knowledge-based component requirements may be relevant to all types of STR analysis. The training program shall include, at a minimum, the following topics.

a) *Peak Detection and Sizing*

1) genotyping software;

2) analysis method parameters for peak detection including analytical threshold;

3) allelic ladder;

4) size standard and sizing algorithm;

5) controls including primer peak in negative control;

6) raw data including the primer peak;

7) data filters (e.g., smoothing, global cut-off values, stutter filters, –A filters);

8) data resolution including off-scale data.

b) *Artifacts*

1) stutter

i) loci with stutter less than a full repeat unit

ii) the effect of sequence complexity and allele length on stutter,

2) minus A (–A, incomplete adenylation);

3) non-specific amplification products including non-human DNA;

4) electrophoresis data quality issues:

i) raised baseline,

ii) dye blobs,

iii) spikes,

iv) amplification kit artifacts;

c) *Allelic Variations*

1) off-ladder alleles including manual determination of allelic designation;

2) variant alleles (also called off-ladder allele or microvariant);

3) null alleles as a result of deletions or primer binding site mutations;

4) tri-allelic patterns;

5) chromosomal duplications and triplications.

d) *Data Outside Optimal Range*

1) the stochastic effects on low template DNA;

i) elevated stutter,

ii) peak height imbalances,

iii) drop-in,

iv) drop-out;

2) off-scale data;

3) pull-up.

e) *Expert Systems, if validated by the laboratory*

1) general understanding of how the system works including expected output;

2) validation and selection of system parameters;

3) data flag indicators (e.g., the "allele number" flag may be indicative of a mixed DNA profile, a tri-allelic pattern, or an elevated stutter peak) or other diagnostic output addressing system performance;

4) understanding of how the expert system parameters were chosen and tested, any filters (or rules) applied to or required of the data, proper evaluation of diagnostic parameters or other system output(s), and the effect of the chosen parameters on data interpretation~~.~~;

5) how to determine if further assessment is required (e.g., are rules established by the laboratory's quality assurance program met) as applicable.

4.3 Practical Training

4.3.1 The practical component of the laboratory's training program shall provide the trainee with ~~sufficient~~practical instruction for the trainee to obtain the skills necessary to analyze forensic STR data according to the laboratory's analysis protocol(s).

4.3.2 At a minimum, the practical portion of the training program shall include the observation of a trained analyst performing ~~the processes at least once or until clearly understood with exercises~~activities representative of the range, type, and complexity of DNA data from routine casework or database samples processed by the laboratory, at least once or until clearly understood.

4.3.3 At a minimum, the practical data analysis exercises shall be representative of the range, type, and complexity of DNA data from routine casework or database samples, and DNA data, technologies, methodologies and platforms in accordance with the trainee's job responsibilities and the extent to which the trainee participates in data analysis.

4.3.4 The practical exercises performed shall be sufficient to demonstrate the trainee's ability to generate, interpret, and compare DNA profiles following the laboratory's protocols for STR data analysis and to produce reliable and accurate results for the types of data that the trainee will be expected to evaluate in casework.

4.4 Competency Component

4.4.1 General

The competency component of the laboratory's training program shall ~~include~~demonstrate knowledge-based and practical competency ~~testing for~~in forensic STR data analysis used by the laboratory. The format of the test(s) and the criteria for passing the competency test(s) shall meet

Section 4.3 of the ANSI/ASB Std 022, *Standard for Forensic Training DNA Analysis Training Programs.*

4.4.2 Knowledge-based Competency

As applicable to the trainee's job responsibilities, the trainee shall successfully complete (as defined by the laboratory's policy) a knowledge-based test covering the critical information obtained during the training on analysis of forensic STR DNA data. The test(s) shall cover, at a minimum, the topics outlined in 4.2 and its subsections.

4.4.3 Practical Competency

The trainee shall successfully complete (as defined by the laboratory's policy) a practical competency test covering each of the forensic STR data analysis protocol(s) for which ~~he or she~~the trainee will be independently authorized to perform. DNA data from samples representative of the range, type, and complexity for which the trainee will be authorized to perform the analysis of forensic STR data shall be included in the practical competency test~~.~~(s).

5 Conformance

In order to demonstrate conformance with this standard, the laboratory shall meet the requirements outlined in ~~section~~Section 5 of ANSI/ASB Std 022 *Standard for Forensic DNA Analysis Training Programs* and all the requirements set forth in this document.

Annex A
(informative)

Bibliography

The following information provides a list of the resources that may assist the DNA technical leader or other appropriate personnel in defining the breadth and scope of the materials to be reviewed by the trainee. This list is not meant to be all inclusive.

1] ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs,* 2019, 1st Ed[b].

2] ANSI/ASB Standard 040, *Standard for Forensic DNA Interpretation and Comparison Protocols*, 2019, 1st Ed[b].

3] Bright, et al. "Investigation into the performance of different models for predicting stutter." *Forensic Science International: Genetics,* 2013, vol. 7, pp. 433–437.

4] Brookes, et al. "Characterizing stutter in forensic STR multiplexes." *Forensic Science International: Genetics,* 2012, vol. 6, pp. 58–63.

5] Butler, J.M., Vallone, P.M., Gettings, K.B., Borsuk, L.A. , Ruitberg, C.M. ,and Reeder, D.J., NIST Short Tandem Repeat DNA Internet Database, National Institute of Standards and Technology, doi:10.18434/T44G6P. SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories[c].

6] SWGDAM Interpretation Guidelines for Y-chromosome STR testing[c].

[b] Available from: www.aafs.org/academy-standards-board

[c] Available from: www.swgdam.org/publications.

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

www.aafs.org/academy-standards-board