ASB Standard 078, First Edition
2025

Standard for Training in Forensic Autosomal Short Tandem Repeat (STR) and Y-STR DNA Data Interpretation and Comparison

Standard for Training in Forensic Autosomal Short Tandem Repeat (STR) and Y-STR DNA Data Interpretation and Comparison

ASB Approved Xxxxx 2025

ANSI Approved Xxxxxx 2025

410 North 21st Street
Colorado Springs, CO 80904

Foreword

This standard defines the minimum requirements to be met in a forensic DNA analyst training program for autosomal STR data and Y-STR data interpretation and comparison. The aim is to provide a framework for quality training resulting in consistency within a laboratory and in the forensic DNA community.

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 Biological Data Interpretation and Reporting 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, interpretation, mixture deconvolution, comparison, DNA standard, autosomal STR, Y-STR*

Table of Contents *(to be finalized prior to publication)*

Standard for Training in Forensic Autosomal Short Tandem Repeat (STR) and Y-STR DNA Data Interpretation and Comparison

1 Scope

This standard provides the requirements for forensic DNA laboratory's training program for autosomal and Y-STR data interpretation and comparison. This standard excludes training for DNA sequencing including the derivation of STR profiles from DNA sequencing data.

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
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.

3.2
degradation
The fragmenting, or breakdown, of DNA by chemical or physical means.

3.3
drop-in
Peak(s) in an electropherogram that are not reproducible across multiple independent amplification events.

3.4
drop-out
Failure of an otherwise amplifiable allele to produce a signal above the analytical threshold because the allele was not present or was not present in sufficient quantity in the aliquot that underwent PCR amplification.

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

3.5
inclusion
A conclusion for which an individual cannot be excluded as a potential contributor of DNA obtained from an evidentiary item based on the comparison of known and questioned DNA profiles (or multiple questioned DNA profiles to each other); a statement of inclusion does not confirm that an individual is a source of the DNA.

3.6
inconclusive
A statement provided as the conclusion when testing results are insufficient or lacking in quality and/or quantity, as defined by the laboratory, for comparison purposes; the data are inadequate to draw any meaningful conclusions.

3.7
inhibition
Active interference with or prevention of the synthesis of DNA during the polymerase chain reaction (PCR).

3.8
mixture
DNA typing results originating from two or more individuals.

3.9
mutation
A change in DNA sequence; an alteration or change of an allele at a particular locus resulting in genetic inconsistency between a biological or cellular parent and offspring.

3.10
off-scale STR data
Data produced when the emitted fluorescence from the PCR products being measured saturates the detector; 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.

3.11
peak height ratio
The relative ratio of two peaks at a given locus. Mathematically, the ratio may be calculated in two ways: 1) The shorter peak height (or area) divided by the taller peak height (or area). This is commonly expressed as a percentage, or 2) The peak height (or area) of the longer length allele divided by the peak height (or area) of the shorter length allele.

3.12
preferential amplification
A situation where one allele of a heterozygous pair at a locus is amplified by PCR with greater efficiency than the other allele.

3.13
single source
DNA typing results originating from one individual.

3.14
stochastic threshold
The peak height value in a DNA electrophoretic profile, commonly measured in RFUs, above which it is reasonable to assume that, at a given locus, allelic drop-out of a sister allele in a heterozygous pair has not occurred in a single source DNA sample; due to the possibility of shared alleles in mixed samples, the presence of allele peaks above the stochastic threshold is no guarantee that allele drop-out did not occur in mixed DNA sample profiles.

3.15
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.

3.16
tri-allelic pattern
The detection of three alleles in one individual at a particular STR locus.

3.17
variant allele
A form of an allele due to an insertion or a deletion relative to other commonly seen alleles.

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 forensic autosomal and Y-STR data interpretation and comparison;

b) the laboratory’s applicable validation studies;

c) literature used to support validation;

d) literature used to support the laboratory’s interpretation and comparison protocol;

e) applicable literature as assigned by the trainer;

f) literature on the effects of human factors and cognitive bias in decision-making processes associated with forensic DNA analysis.

4.2.2 The knowledge-based training component of the laboratory's training program shall provide the trainee with basic instruction on the steps for forensic autosomal and Y-STR data interpretation and comparison.

4.2.3 The training shall provide instruction on the interpretation and comparison criteria used by the laboratory, how the criteria were determined by the laboratory and any limitations of the laboratory's validation studies (such as mixtures with numbers of contributors above what the laboratory considered during validation).

4.2.4 The training shall address documentation requirements for decisions made during the interpretation and comparison process, to include a basic understanding of the risks of bias and potential for human error.

4.2.5 The training shall include manual interpretation and comparison even when software tools may be used.

4.2.6 The training shall include, at minimum, the following topics, in 4.2.6.1 through 4.2.6.2.

4.2.6.1 Quality control indicators:

a) positive controls;

b) negative controls;

c) internal lane standards;

d) primer peak;

e) allelic ladder.

4.2.6.2 Data suitability for interpretation and/or comparison.

a) Factors in data interpretation:

1) peak height thresholds:

i) analytical threshold,

ii) stochastic threshold;

2) artifacts:

i) stutter (forward and backward),

ii) spike,

iii) pull-up,

iv) other [e.g., (-A), dye blobs];

3) peak height ratios;

4) PCR inhibition;

5) DNA degradation;

6) preferential amplification;

7) data too limited and/or too complex;

8) drop-in/drop-out;

9) other considerations (e.g., mutations, tri-allelic patterns, variant alleles, off-scale STR data, imbalance observed between loci).

b) Requirements for single source data interpretation and comparison.

c) Requirements for mixture data interpretation and comparison.

d) Considerations of mixture interpretation and comparison;

1) mixture assumptions:

i) number of contributors,

ii) allele sharing (for mixtures),

iii) appropriate conditioning/assumption of expected (known) individuals,

iv) estimating the ratio of contributors in mixtures,

v) biological relatives in mixtures;

2) mixture deconvolution:

i) major component(s),

ii) minor component(s),

iii) foreign component(s) (i.e., alleles not belonging to an assumed contributor);

3) probabilistic genotyping (if utilized by the laboratory):

i) scientific principle of probabilistic model,

ii) hypothesis development,

iii) statistical methodology,

iv) limitations of software.

e) Limitations of mixture interpretation and comparison (e.g., if the laboratory is validated to interpret four person mixtures, the trainee needs to be trained on four-person mixture interpretation and comparison and understand why the laboratory does not interpret mixtures with more than four contributors).

f) Comparison of evidentiary data to reference data (as applicable):

1) consistent,

2) inclusion/cannot be excluded,

3) exclusion,

4) inconclusive.

4.3 Practical Training

4.3.1 The practical component of the laboratory's training program shall provide the trainee with practical instruction for the trainee to obtain the skills for the use of the laboratory's forensic autosomal and Y-STR data interpretation and comparison protocol(s).

4.3.2 At a minimum, the practical portion of the training program shall include the observation of a trained analyst performing 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 portion of the training program shall include hands-on exercises representative of the range, type, and complexity of DNA data from routine casework or database samples processed by the laboratory.

4.3.4 The number and quality of samples interpreted by the trainee shall include manual data review and automated data analysis methods, including all validated software programs in use by the laboratory, as applicable. The training shall be appropriate to demonstrate the trainee's ability to follow the laboratory's forensic autosomal and Y-STR data interpretation and comparison protocol(s).

4.4 Competency Component

4.4.1 General

The competency component of the laboratory's training program shall demonstrate knowledge-based and practical competency in the application of forensic autosomal and Y-STR data interpretation and comparison protocol(s) as 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 ANSI/ASB Std 022, *Standard for Forensic 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 forensic autosomal and Y-STR data interpretation and comparison protocol(s). The test(s) shall cover, at a minimum:

a) the theoretical and scientific basis of forensic autosomal and Y-STR data interpretation and comparison;

b) the purpose and function of the controls used in forensic autosomal and Y-STR data interpretation and comparison;

c) the quality control steps pertaining to forensic autosomal and Y-STR data interpretation and comparison;

d) the laboratory's procedures pertaining to forensic autosomal and Y-STR data interpretation and comparison.

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 autosomal and Y-STR data interpretation and comparison protocol(s) for which 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 interpret and compare shall be included in the practical competency test(s). The trainee, at a minimum, shall be able to satisfactorily perform the following:

a) accurately execute forensic autosomal and Y-STR data interpretation and comparison protocol(s);

b) apply the laboratory's interpretation and comparison procedures to a variety of evidentiary casework -or database-type samples, as applicable to the trainee;

c) accurately operate relevant equipment, instrumentation, and software as stated in the laboratory's protocol for forensic autosomal and Y-STR data interpretation and comparison protocol(s);

d) correctly document work performed in accordance with laboratory procedures.

5 Conformance

In order to demonstrate conformance with this standard, the laboratory shall meet the requirements outlined in 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 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.

1] ANSI/ASB Standard 018, *Standard for Validation of Probabilistic Genotyping Systems*, 1st Ed., 2020. [b]

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

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

4] Buckleton, J., et al. "Towards Understanding the effect of uncertainty in the number of contributors to DNA stains." *Forensic Science International: Genetics,* vol. 1, 2007, pp. 20–28.

5] Buckleton, J., M. Krawczak, and B. Weir. "The interpretation of lineage markers in forensic DNA testing." *Forensic Science International: Genetics*, vol. 5, 2011, pp. 78–83.

6] Budowle, B., et al. "Mixture Interpretation: Defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework." *Journal of Forensic Sciences*, vol. 54(4), 2009, pp. 810–821.

7] Budowle, B., J. Ge, X.G. Aranda, J.V. Planz, A.J. Eisenberg, and R. Chakraborty. "Texas Population Substructure and Its Impact on Estimating the Rarity of Y-STR Haplotypes from DNA Evidence." *Journal of Forensic Sciences*, vol. 54, 2009, pp. 1016–1021.

8] Butler, J. *Advanced Topics in Forensic DNA Typing: Interpretation,* Elsevier Academic Press, 2015.

9] Dror, I., et al. "Subjectivity and Bias in Forensic DNA Mixture Interpretation." *Science and Justice*, vol. 51(4), 2011, pp. 204–208.

10] Haned, H., et al. "Estimating Number of Contributors to Forensic DNA Mixtures." *Journal of Forensic Sciences*, vol. 56 (1), 2011, pp. 23–28.

11] Ge, J., B. Budowle, J.V. Planz, A.J. Eisenberg, J. Ballantyne, and R. Chakraborty. "US forensic Y-chromosome short tandem repeats database." *Legal Medicine*, vol. 12, 2010a, pp. 289–295.

12] Gill, P., et al. "Commentary on Budowle, B., Onorato, A.J., Callaghan, T.F., Della Manna, A., Gross, A.M., Guerrieri, R.A., Luttman, J.C., McClure, D.L. Mixture Interpretation: Defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework." *Journal of Forensic Sciences*, vol. 55(1), 2010, pp. 265–268.

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

13] Gill, P., et al. "An Investigation of the rigor of interpretation rules for STR derived from less than 100 pg of DNA." *Forensic Science International,* vol. 112, 2000, pp. 17–40.

14] Gusmão, L., J.M. Butler, Á. Carracedo, P. Gill, M. Kayser, W.R. Mayr, N. Morling, M. Prinz, L. Roewer, C. Tyler-Smith, et al. "DNA Commission of the International Society of Forensic Genetics (ISFG): An update of the recommendations on the use of Y-STRs in forensic analysis." *Forensic Science International*, vol. 157, 2006, pp. 187–197.

15] National Research Council. *The Evaluation of Forensic DNA Evidence*, National Research Council, 1996, Chapters 1–6.

16] Perez, et al. "Estimating the number of contributors to two, three and four person mixtures containing high template and low template amounts." *Croatian Medical Journal*, vol. 52(3), 2011, pp. 314–326.

17] SWGDAM. SWGDAM Interpretation Guidelines for Autosomal and Y-STR Typing for Forensic DNA Testing Laboratories[c].

18] Taylor, M., E. Romsos, K. Ballantyne, D. Moore Boswell, and T. Busey. (2024), *Forensic DNA Interpretation and Human Factors: Improving the Practice Through a Systems Approach*. NIST Interagency/Internal Report (NISTIR), National Institute of Standards and Technology, Gaithersburg, MD.[d]

[c] Available from: http://media.wix.com/ugd/4344b0_da25419ba2dd4363bc4e5e8fe7025882.pdf
[d] Available from: https://www.nist.gov/publications/forensic-dna-interpretation-and-human-factors-improving-practice-through-systems

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

www.aafs.org/academy-standards-board