

ASB Standard 131, First Edition
2020

**Standard for Training in Forensic DNA Sequencing using
Capillary Electrophoresis**



Standard for Training in Forensic DNA Sequencing using Capillary Electrophoresis

ASB Approved Xxxxx 2020

ANSI Approved Xxxxxx 2020



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Foreword

This standard defines the minimum requirements that shall be met in a Forensic DNA Analyst training program for DNA sequencing using capillary electrophoresis (CE) methods. The aim is to provide a framework for quality training that will result in 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 and Wildlife Subcommittees of the Organization of Scientific Area Committees (OSAC) for Forensic Science.

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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, DNA, PCR for sequencing, capillary electrophoresis.*

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Standard for Training in Forensic DNA Sequencing using Capillary Electrophoresis

1 Scope

This standard provides the general requirements for a forensic DNA laboratory's training program in forensic DNA sequencing using capillary electrophoresis. This standard applies to forensic human and wildlife mitochondrial DNA capillary electrophoresis sequencing, and wildlife nuclear DNA capillary electrophoresis sequencing.

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

capillary electrophoresis

CE

An electrophoretic technique for separating DNA molecules by their relative size based on their differential migration through a capillary tube filled with a liquid polymer.

3.2

DNA sequencing

A laboratory technique used to determine the sequence of bases (A, C, G, and T) in a DNA molecule.

3.3

massively parallel sequencing

One of a number of high throughput DNA sequencing techniques. Also referred to as Next Generation Sequencing (NGS).

3.4

pyrosequencing

A method of DNA sequencing which is performed by detecting the nucleotide incorporated by a DNA polymerase.

3.5

Sanger sequencing

A method of DNA sequencing for determining the order of bases in a DNA molecule based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication.

4 Requirements

4.1 General

ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs* provides foundational training requirements for DNA analysts. It shall be used with this document, which outlines further specific requirements.

4.2 Knowledge-based Training

4.2.1 The laboratory's training program shall provide the trainee with an understanding of the fundamental principles behind CE sequencing, the function of the sequencing reagents and CE components, the limitations of sequencing and CE, and the laboratory's own DNA sequencing and CE 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 DNA sequencing using CE;
- 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 methods is intended to provide an educated perspective on current methods.

- a) Principles and limitations of sequencing methods and platforms:
 - 1) sequencing methods;
 - i. chain termination sequencing (Sanger sequencing);
 - ii. pyrosequencing;
 - iii. massively parallel sequencing;
 - 2) reaction components;
 - 3) primer selection.
- b) Quality controls used for DNA sequencing.
- c) Evaluation of sequencing results for controls and samples.
- d) CE instrumentation:

- 1) instrument-specific software;
- 2) instrument maintenance and calibration;
- 3) instrument reagents/components:
 - i. capillary length;
 - ii. polymer type;
 - iii. electrophoresis conditions.
- e) Troubleshooting:
 - 1) sequencing failure;
 - 2) instrument failure.
- 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) Contamination prevention:
 - 1) use of personal protective equipment;
 - 2) decontamination of work surfaces, consumables, and instrumentation [e.g., sodium hypochlorite (bleach), UV light, surfactants];
 - 3) workflow set-up.

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 DNA sequencing using CE 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) DNA sequencing using CE 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) DNA sequencing using CE 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 DNA sequencing using CE 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 DNA sequencing using CE methods used by the laboratory. The format of the test(s) shall meet Section 4.3 of the ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Programs*.

4.4.2 Knowledge-based Competency

The trainee shall successfully complete a knowledge-based test covering the critical information obtained during the training on DNA sequencing using CE methods. The test(s) shall cover, at a minimum, the following.

- a) The theoretical and scientific basis of DNA sequencing using CE methods.
- b) The function of the reagents, instruments, software, and other components used for DNA sequencing using CE methods.
- c) The required quality control steps pertaining to DNA sequencing using CE methods, including the evaluation of controls.
- d) The laboratory's analytical procedures pertaining to DNA sequencing using CE methods.

4.4.3 Practical Competency

The trainee shall successfully complete a practical test covering DNA sequencing using CE protocol(s) for which he or she shall be independently authorized to perform. Samples of known type shall be used. The trainee shall be able to satisfactorily perform the following, as applicable.

- a) Properly and accurately execute the analytical procedures related to DNA sequencing using CE methods without contaminating samples.

- b) Apply the laboratory's analytical procedures to a variety of evidentiary casework or database type samples representing the quality and quantity expected to be encountered in the laboratory.
- 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*.

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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.

- 1] FBI, *Quality Assurance Standards for DNA Databasing Laboratories*, effective September 1, 2011^a.
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- 9] Longo, M.S., M.J. O'Neill, and R.J. O'Neill. "Abundant human DNA contamination identified in nonprimate genome databases." *PLoS One*. Vol. 6:e16410, 2011.
- 10] SWGDAM. *SWGDAM Training Guidelines and References*^e.
- 11] Wilson, M.R., J.A. DiZinno, D. Polanskey, et al. "Validation of mitochondrial DNA sequencing for forensic casework analysis." *International Journal of Legal Medicine*. Vol. 108, 1995, pp. 68-74.

^a Available from <https://www.fbi.gov/file-repository/quality-assurance-standards-for-dna-databasing-laboratories.pdf/view>

^b Available from https://docs.wixstatic.com/ugd/4344b0_809d01b3e9f9451cb9edd9a85f2c2e5b.pdf

^c Available from <https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view>

^d Available from https://docs.wixstatic.com/ugd/4344b0_6782472e073442ec877085584aaffa36.pdf

^e Available from http://media.wix.com/ugd/4344b0_87b2b4a150aa433f9490b7113b1aa4a6.pdf

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