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

## 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 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.

**Keywords:** Training, DNA sequencing, capillary electrophoresis

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# Standard for Training of Forensic DNA Sequencing using Capillary Electrophoresis

## 1 Scope

This standard provides the requirements of a forensic DNA laboratory's training program to ensure proper training in the approved methods of DNA sequencing using capillary electrophoresis (CE) within the trainee's forensic DNA laboratory.

## 2 Normative References

ASB Standard 022 - *Standard for Forensic DNA Analysis Training Programs*<sup>1</sup>

## 3 Terms and Definitions

For the purpose of this document the following definitions apply:

### 3.1

#### **Massively parallel sequencing**

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

### 3.2

#### **Maxam-Gilbert sequencing**

A method of DNA sequencing based on nucleobase-specific partial chemical modification of DNA and subsequent cleavage of the DNA backbone at sites adjacent to the modified nucleotides

### 3.3

#### **Pyrosequencing**

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

### 3.4

#### **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 di-deoxynucleotides by DNA polymerase during in vitro DNA replication.

### 3.5

#### **Sequencing**

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

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## 4 Requirements

### 4.1 Knowledge-based training component

The laboratory's training program shall provide the trainee with an understanding of the fundamental principles of the theory behind DNA sequencing using CE, 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.

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

- a) The laboratory's protocols for DNA sequencing using CE
- b) The laboratory's applicable validation studies
- c) Literature used to support validation
- d) Applicable literature as assigned by the trainer (e.g., see references in Annex A)

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. In-depth understanding of these methods may not be required for successful training.

- a) Principles and limitations of sequencing methods and platforms
  - i. Chain termination (Sanger sequencing)
  - ii. Chemical (Maxam-Gilbert sequencing)
  - iii. High throughput (e.g.; pyrosequencing, massively parallel sequencing)
- b) Quality controls used for DNA sequencing
- c) Evaluation of sequencing results for controls and samples
- d) Laboratory-specific CE instrumentation
  - i. Software
  - ii. Instrument maintenance and calibration
  - iii. Instrument reagents/components
    - a. Capillary length
    - b. Polymer type
    - c. Electrophoresis conditions

- e) Troubleshooting
  - i. Sequencing failure
  - ii. Instrument failure

## **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 DNA sequencing using CE protocols used by the laboratory.

4.2.1 At a minimum, the practical portion of the training program shall include the practical observation of the processes at least once and until clearly understood. These include:

- a) DNA sequencing and CE methods to be utilized by the trainee
- b) Documentation of the process

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

- a) DNA sequencing and CE methods to be utilized by the trainee
- b) Documentation of the process
- c) NOTE: The number and quality of samples processed by the trainee shall be appropriate to demonstrate the ability to follow the laboratory's DNA sequencing and CE 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 in the application of DNA sequencing using CE. The format of the test(s) shall meet Section 4.3 of the ASB Standard 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 DNA sequencing using CE. The test(s) shall cover, at a minimum:

- a) The theoretical and scientific bases of DNA sequencing using CE
- b) The function of the reagents and other components used for DNA sequencing using CE
- c) The quality control steps pertaining to DNA sequencing using CE
- d) The laboratory's analytical procedures pertaining to DNA sequencing using CE methods

### **4.3.2 Practical competency**

The trainee shall successfully complete a practical test covering each of the DNA sequencing using CE 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 DNA sequencing using CE
- b) Apply the laboratory's analytical procedures to a set of samples representing the range of DNA quality and quantity expected to be encountered in the laboratory
- c) Operate relevant 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 Standard 022.

## **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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