Standard for Training in Forensic DNA Amplification Methods for Capillary Electrophoresis Sequencing

Biological Methods Subcommittee
Biology/DNA Scientific Area Committee
Organization of Scientific Area Committees (OSAC) for Forensic Science

OSAC Proposed Standard

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Foreword

This standard defines the minimum requirements that shall be met in a forensic DNA analyst training program for DNA amplification for capillary electrophoresis (CE) sequencing 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, amplification for sequencing, capillary electrophoresis

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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 amplification for capillary electrophoresis (CE) sequencing used within the trainee's forensic DNA laboratory.

2 Normative References

ASB Standard 022 - *Standard for Forensic DNA Analysis Training Programs*[1]

3 Terms and Definitions

For the purpose of this document the following definitions apply:

3.1
Control region
A presumed non-coding portion of the mitochondrial DNA molecule analyzed through DNA sequencing, which may be used to determine an individual's mitochondrial haplotype or for taxonomic discrimination. The control region often contains hypervariable regions (in humans: HV1 and HV2) that differ in sequence among non-maternally related individuals. The control region encompasses the D-loop region in humans and other species.

3.2
Chloroplast DNA (cpDNA)
A circular DNA molecule, approximately 120-190kbp, which is located in the chloroplasts of plants. The DNA is maternally inherited and contains genes involved in the photosynthetic pathway.

3.3
Degradation
The fragmenting, or breakdown, of DNA or protein (e.g. loss of enzymatic-activity) by chemical, physical, or biological means; a common occurrence when biological samples containing DNA encounter warm moist environments or excessive UV light.

3.4
dNTPs
Abbreviation for deoxynucleotide tri-phosphates; used in PCR as building blocks to construct new DNA strands.

3.5
Hypervariable region
A segment of DNA that often contains polymorphisms and thus is useful for differentiating taxa or unrelated individuals.

3.6
Hypervariable region 1 (HV1)

[1] American Academy of Forensic Sciences Standards Board, 4200 Wisconsin Avenue, NW, Suite 106-310, Washington, DC 20016-2143 asb@aafs.org

A section of the human mtDNA control region spanning nucleotide positions 16024-16365, that often differs among non-maternally related individuals.

3.7
Hypervariable region 2 (HV2)
A section of the human mtDNA control region spanning nucleotide positions 73-340, that often differs among non-maternally related individuals.

3.8
Inhibitor
As related to the polymerase chain reaction (PCR), any substance that interferes with or prevents the synthesis of DNA during the amplification process.

3.9
Mitochondrial DNA (mtDNA)
An often small (~16,500 bp in humans), circular DNA molecule located in eukaryotic mitochondria that is typically maternally inherited; the resistance to degradation and presence of multiple copies of mtDNA in each cell make it useful with samples originating from limited or damaged biological material.

3.10
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.

3.11
PCR product quantification
An analytical procedure to determine the amount of amplicon obtained after DNA amplification.

3.12
Primer
A short polynucleotide chain, usually 18-30 bases long, which targets a specific region of the template DNA and allows a DNA polymerase to initiate synthesis of a complementary strand; two primers are required for amplification of a particular section of DNA. The primers are complementary to opposite strands and are designed to bracket the region of interest for amplification. Polymerization is initiated at the 3' end of the primer and extends in a 5´ to 3´ manner.

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 amplification for CE sequencing, the function of

the amplification reagents and other components used in the method, the limitations of amplification, and the laboratory's own DNA amplification guidelines.

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 amplification for CE sequencing

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 of PCR-based DNA amplification methods for CE sequencing

i. Reaction components

a. dNTPs

b. Primers

c. Buffer

d. Polymerase

e. $MgCl_2$

f. PCR enhancers (if applicable, e.g. bovine serum albumin)

ii. Primer design

iii. Amplification parameters

iv. PCR inhibitors

v. DNA degradation

b) Primer selection for specific target regions of DNA amplification (as applicable to the laboratory)

i. Mitochondrial DNA

a. Control region

b. Portions of control region

c. Whole genome

ii. Chloroplast DNA

a. Coding regions

b. Transgenic spacer regions

iii. Nuclear/genomic DNA

a. Coding regions

b. Non-coding regions

iv. Primer binding site mutations

v. Quality and source of DNA

c) Contamination control and prevention

i. Use of personal protective equipment

ii. Ultraviolet cross-linking of consumables and utensils

iii. Decontamination of work surfaces (e.g. sodium hypochlorite, UV light)

iv. Workflow set-up

d) Quality controls used for DNA amplification

e) Purification of amplification product

f) Post-amplification quantification

i. Evaluation of the controls

ii. Determination of DNA concentration

g) Instrumentation and reagents

i. Thermal cycling instruments and parameters

ii. Software parameters associated with instruments

iii. Maintenance and calibration

iv. Storage of amplification reagents

h) Troubleshooting

i. Amplification and post-amplification quantification failure

ii. Instrument failure and recovery

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 amplification for CE sequencing protocols used by the laboratory.

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

a) DNA amplification for CE sequencing 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 amplification for CE sequencing 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 amplification for CE sequencing 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 testing in the application of amplification of DNA for CE sequencing methods. 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 amplification for CE sequencing methods. The test(s) shall cover, at a minimum:

a) The theoretical and scientific bases of DNA amplification for CE sequencing

b) The function of the reagents and other components used for DNA amplification for CE sequencing

c) The quality control steps pertaining to DNA amplification for CE sequencing

d) The laboratory's analytical procedures pertaining to DNA amplification for CE sequencing methods

4.3.2 Practical competency

The trainee shall successfully complete a practical test covering each of the DNA amplification for CE sequencing 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 amplification for CE sequencing

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

b) Operate relevant equipment and instrumentation used in the laboratory

c) 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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