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

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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 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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the term **'shall'** indicates that a provision is mandatory, and can be audited for compliance

the term **'should'** indicates that a provision is not mandatory, but recommended as good practice.

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

Keywords: *training, DNA, PCR, DNA amplification for sequencing, capillary electrophoresis.*

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

1 Scope

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

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
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.

3.3
degradation
The fragmenting, or breakdown, of DNA by chemical, physical, or biological means.

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
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
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.

3.9
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.

3.10
PCR enhancer
An additive for optimizing the specificity and yield of a PCR reaction.

3.11
PCR inhibitor
Any substance that interferes with or prevents the synthesis of DNA during the amplification process.

3.12
post-amplification quantification
An analytical procedure to determine the amount of amplicon obtained after DNA amplification.

3.13
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 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 DNA amplification for subsequent 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 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 amplification for CE sequencing;

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 historical methods is intended to provide an educated perspective on current methods.

a) Principles of PCR-based DNA amplification methods for CE sequencing.

    1) Reaction components:

        i) dNTPs;

        ii) primers;

        iii) buffer;

        iv) polymerase;

        v) $MgCl_2$;

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

    2) Primer design.

    3) Amplification parameters.

4) PCR inhibitors.

5) DNA degradation.

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

1) Mitochondrial DNA:

i) control region to include hypervariable regions (in humans, HV1 and HV2);

ii) portions of control region;

iii) coding regions;

iv) whole genome.

2) Nuclear/genomic DNA:

i) coding regions;

ii) non-coding regions;

3) Primer binding site mutations.

4) Quality and source of DNA.

c) Contamination:

1) sources (environmental, procedural);

2) sample handling strategies and preventative methods;

3) detection limitations;

4) root cause analysis, corrective action when contamination occurs;

5) procedures for prevention and mitigation of contamination to include;

i. use of personal protective equipment;

ii. ultraviolet cross-linking of consumables and utensils;

iii. decontamination of work surfaces, consumables, and instrumentation [e.g., sodium hypochlorite (bleach), UV light, surfactants];

iv. workflow set-up.

d) Quality control to include positive and negative controls.

e) Storage, preservation, and retention of amplified DNA product according to laboratory policy.

f) Purification of amplification product.

g) Post-amplification quantification.

1) Evaluation of the controls.

2) Estimation of DNA concentration.

h) Instrumentation and reagents, including:

1) thermal cycling instruments and parameters;

2) software parameters associated with instruments;

3) maintenance and calibration;

4) storage of amplification reagents.

i) Troubleshooting, including:

1) thermal cycling errors (e.g., ramping, temperature control);

2) post-amplification quantification failures;

3) general equipment failure and recovery.

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 the DNA amplification for CE sequencing 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 amplification for CE sequencing 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 amplification for CE sequencing 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 amplification for CE sequencing 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 amplification of DNA for CE sequencing 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 Training Programs.*

4.4.2 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 the following, at a minimum.

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

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

c) The required quality control steps pertaining to DNA amplification for CE sequencing, including the evaluation of controls.

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

4.4.3 Practical Competency

The trainee shall successfully complete a practical test covering the DNA amplification for CE sequencing 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 amplification for CE sequencing without contaminating the 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*.

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].

2] FBI, *Quality Assurance Standards for DNA Databasing Laboratories*, effective July 1, 2020[b].

3] FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories (QAS)*, effective September 1, 2011[c].

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