Standard for Training in Forensic DNA Isolation and Purification Methods

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

OSAC Proposed Standard

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Prepared by
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This document has not been published by an SDO. Its contents are subject to change during the standards development process. All interested groups or individuals are strongly encouraged to submit comments on this proposed document during the open comment period administered by the American Academy of Forensic Science Standards Board (www.asbstandardsboard.org).

Foreword

This standard defines the minimum requirements that shall be met in a Forensic DNA Analyst training program for DNA isolation and purification 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, nuclear DNA, mitochondrial DNA, extraction, isolation, purification

Table of Contents

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1 Scope

This document provides requirements to ensure proper training in the approved methods of DNA isolation and purification 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 purposes of this document, the following definitions apply.

3.1

Chelex extraction

A method of DNA extraction involving Chelex resin; since one step of the method requires boiling, the extracted DNA is single-stranded.

3.2

Contamination

Exogenous DNA or other biological material in a DNA sample, PCR reaction, or item of evidence; the exogenous DNA or biological material could be present before the sample is collected, or introduced during collection or testing of the sample.

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

Differential lysis and extraction

A method of DNA extraction where sperm and non-sperm cells can be digested separately based on the different composition of the cell membranes. The goal of the procedure is to separate a sample that would otherwise generate a mixed DNA profile into two separate DNA profiles by enriching for the DNA from the male sperm donor (for example, collected in a sexual assault case) in the sperm fraction and enriching for the DNA from the donor of the non-sperm cell fraction.

3.5

DNA isolation and purification

The process of releasing DNA molecules by cell lysis, attempting to remove the non-nucleic acid components of the sample, and recovering purified DNA.

3.6

Enzyme

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

A catalytic protein that can speed up a specific chemical reaction without being changed or consumed in the process.

3.7
Organic extraction
A method of isolating DNA from cells involving phenol and other organic chemicals.

3.8
Nucleases
Enzymes that degrade (break down) nucleic acids.

3.9
PCR inhibitor
Any substance that interferes with or prevents the synthesis of DNA during the amplification process.

3.10

Solid-phase based purification

A method of isolating DNA from cells involving selective absorption to small silica or other particles in an acidic buffer with a high salt concentration, removal of non-DNA materials by washing, and release of DNA from the particles in an alkaline buffer with a low salt concentration.

4 Requirements

4.1 Knowledge-based training

The laboratory's training program shall provide the trainee with an understanding of the fundamental principles of the theory behind the various isolation methods, the function of the reagents and other components used in each method, the limitations of each method, and the laboratory's own DNA isolation and purification 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 isolation and purification

b) The laboratory's applicable validation studies

c) Literature used to support validation

d) Applicable literature as assigned by the trainer

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

a) Composition of DNA within cells, including
    i. Membrane structure
    ii. Histone packaging of DNA into nucleosomes
    iii. Nucleases and other enzymes that can act on DNA in the cell
b) Impact of exposure to heat, humidity, mechanical breakage, and chemicals on DNA stability
    i. Mechanisms of degradation
    ii. Characteristics of degradation
c) Cell lysis and separation of DNA from other materials
    i. Function of chemicals, enzymes, and other reagents used in lysis and separation
    ii. Impact of pH, salt concentration, heat, molecular weight, and solubility
d) Methods for DNA isolation and purification as appropriate for the laboratory
    i. Organic extraction (phenol:chloroform)
    ii. Chelex extraction
    iii. DNA-preservation treated card purification
    iv. Solid phase-based purification
    v. Differential lysis and extraction
    vi. Application of automation and robotic platforms
e) Methods based on sample type as appropriate for the laboratory
    i. Selection of suitable isolation method for sample type and condition
    ii. Pre-extraction cell separation (e.g., cell sorting, laser capture microdissection)
    iii. Pre-extraction processing (e.g., soak, grinding, demineralization)
    iv. Post-extraction processing (e.g., filtration, concentration, preservation conditions)
    v. Non-extraction direct amplification approaches
f) DNA Yield
    i. Sources of DNA loss

ii. Mechanisms to reduce DNA loss

g) PCR inhibitors

i. Sources (environmental, chemical)

ii. Mechanisms of interference with amplification

iii. Methods to avoid or reduce effects on amplification

h) Contamination

i. Sources (environmental, procedural)

ii. Sample handling strategies

iii. Decontamination procedures

i) Quality control in the DNA isolation and purification process

j) Storage, preservation, and disposal of extracted DNA according to laboratory policy

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 DNA isolation and purification 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 or until clearly understood. These include:

a) DNA isolation and purification 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 or database samples processed by the laboratory. These include:

a) DNA isolation and purification methods to be utilized by the trainee

b) Documentation of the process

c) The number and quality of samples processed by the trainee shall be appropriate to demonstrate the ability to follow the laboratory's DNA isolation and purification protocol(s) and to produce reliable and accurate results.

4.3 Competency

The laboratory's training program shall include knowledge-based and practical competency testing in the application of DNA isolation and purification methods. The format of the test(s) shall meet Section 4.3 of ASB 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 isolation and purification methods. The test(s) shall cover, at a minimum:

a) The theoretical and scientific bases of DNA isolation and purification methods

b) The function of the reagents and other components used in each method

c) The proper application of each method

d) The quality control steps pertaining to DNA isolation and purification

e) The laboratory's analytical procedures pertaining to DNA isolation and purification methods

4.3 2 Practical competency

The trainee shall successfully complete a practical test covering each of the DNA isolation and purification methods 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 isolation and purification

b) Apply the laboratory's analytical procedures to a variety of evidentiary casework- or database-type samples

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 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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