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# Standard for Training in Forensic DNA Isolation and Purification Methods

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# Foreword

This standard defines the minimum requirements for a Forensic DNA Analyst training program for DNA isolation and purification methods. The aim is to provide a framework for training that will result in quality and 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 Subcommittee 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, nuclear DNA, extraction, isolation, purification.*

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# Standard for Training in Forensic DNA Isolation and Purification Methods

## 1 Scope

This document provides requirements to ensure proper training in the methods of DNA isolation and purification used within the trainee's forensic DNA laboratory.

## 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**
**Chelex®[a] 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**
The unintentional introduction of 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 by chemical, physical, or biological means.

**3.4**
**differential lysis and extraction**
A method of DNA extraction where sperm and non-sperm cells are digested separately based on the different composition of the cell membranes resulting in the generation of a sperm fraction and a 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. Also known as DNA extraction.

[a] This term is used as an example only, and does not constitute an endorsement of this product by the AAFS Standards Board.

**3.6**
**enzyme**
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/membranes, removal of non-DNA materials by washing, and release of DNA from the particles/membranes for analysis.

## 4 Requirements

### 4.1 General

ANSI/ASB Standard 022, *Standard for Forensic DNA Analysis Training Programs* shall be used in conjunction with this document because ANSI/ASB Standard 022 provides the foundational training program requirements upon which additional specific requirements, such as this document, will be based.

### 4.2 Knowledge-based Training

**4.2.1** 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 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 isolation and purification;

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) Composition of DNA within cells, including:

   1) cell and nuclear membrane structure;

   2) structure of DNA and histone packaging of DNA into nucleosomes;

   3) 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 to include the mechanisms of DNA degradation.

c) Cell lysis and separation of DNA from other materials:

   1) function of chemicals, enzymes, and other reagents used in lysis and separation;

   2) impact of pH, salt concentration, heat, molecular weight, and solubility;

   3) use of DNA-preservation treated cards;

   4) limitations of the technology.

d) Methods for DNA isolation and purification used in the laboratory:

   1) organic extraction (phenol:chloroform);

   2) Chelex®[b] extraction;

   3) solid phase-based purification;

   4) differential lysis and extraction;

   5) application of automation and robotic platforms;

   6) other methods not described;

   7) limitations of the above methodologies.

e) Methods based on sample type used in the laboratory:

   1) selection of suitable isolation method for sample type and condition and DNA test to be performed;

   2) pre-extraction cell separation (e.g., cell sorting, laser capture microdissection);

[b] This term is used as an example only and does not constitute an endorsement of this product by the AAFS Standards Board.

3) pre-extraction processing (e.g., soak, grinding, demineralization);

4) post-extraction processing (e.g., filtration, concentration, preservation conditions);

5) direct amplification without extraction;

6) other methods not described;

7) limitations of the above methodologies.

f) DNA Yield:

1) sources of DNA loss during isolation and purification;

2) mechanisms to reduce DNA loss.

g) PCR inhibitors:

1) sources (environmental, chemical);

2) mechanisms of interference with amplification;

3) methods to avoid or reduce effects on amplification.

h) Contamination:

1) sources (environmental, procedural);

2) sample handling strategies and preventative methods;

3) decontamination procedures;

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

i) Quality control in the DNA isolation and purification process to include, reagent blank control(s) and any other extraction controls.

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

k) Troubleshooting, including:

a) forensic DNA isolation and purification errors;

b) general equipment failure.

### 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 successfully performing DNA isolation and purification 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 with 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) 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 hand-on exercises representative of the range, type, and complexity of casework or database samples processed by the laboratory. These include:

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

b) 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 isolation, purification, and documentation protocol(s) and 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 isolation and purification methods. The format of the test(s) shall meet Section 4.3 of 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 isolation and purification methods. The test(s) shall cover, at a minimum:

a) the theoretical and scientific basis 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 and strategy for use;

d) the quality control steps pertaining to DNA isolation and purification, including the evaluation of controls;

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

### 4.4.3 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 to perform. Samples of known type will be used. The trainee, at a minimum, shall be able to satisfactorily perform the following:

a) properly and accurately execute the analytical procedures related to DNA isolation and purification without contaminating the samples;

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) 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[c].

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

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

4] FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories*, effective July 1, 2020[f].

5] SWGDAM. SWGDAM Training Guidelines and References[g].

[c] Available from https://www.fbi.gov/file-repository/quality-assurance-standards-for-dna-databasing-laboratories.pdf/view.
[d] Available from https://docs.wixstatic.com/ugd/4344b0_809d01b3e9f9451cb9edd9a85f2c2e5b.pdf.
[e] Available from https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view.
[f] Available from https://docs.wixstatic.com/ugd/4344b0_6782472e073442ec877085584aaffa36.pdf.
[g] Available from http://media.wix.com/ugd/4344b0_87b2b4a150aa433f9490b7113b1aa4a6.pdf.


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