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**Standard for Training in Forensic DNA  
Quantification Methods**



## Standard for Training in Forensic DNA Quantification Methods

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## Foreword

This standard defines the minimum requirements that shall be met in a Forensic DNA Analyst training program for DNA quantification methods. The aim is to provide a framework for quality training that will result in quality and consistency in the forensic DNA community.

This document is part of a series of training documents under 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 '**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, quantification, quantitative PCR/real-time PCR.*

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# Standard for Training in Forensic DNA Quantification Methods

## 1 Scope

This standard provides the requirements for a forensic DNA laboratory's training program in DNA quantification.

## 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

#### **DNA quantification**

A process by which the DNA concentration in a sample is determined.

### 3.2

#### **cycle threshold**

Cycle number (in quantitative PCR) at which the fluorescence generated within a reaction exceeds a defined threshold; this value is converted to a DNA concentration for each sample tested using a standard curve developed from DNA samples of known concentrations.

### 3.3

#### **degradation**

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

### 3.4

#### **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.

### 3.5

#### **Quantitative PCR**

##### **qPCR**

A means for quantifying the amount of nucleic acid present in a sample using PCR.

## 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 various DNA quantification methods, the function of the reagents and other components used in each method, the information generated by each assay, the limitations of each method, and the laboratory's own DNA quantification 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 quantification;
- 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 and limitations of non-PCR based DNA quantification methods:
  1. spectrophotometric analysis;
  2. fluorometric methods;
  3. yield gels;
  4. slot blots.
- b) Principles and limitations of quantitative PCR (qPCR) DNA quantification methods:
  1. PCR assays based on fluorescence quenching using specific probes;
  2. PCR assays based on a reduction of fluorescent signal during amplification;
  3. qPCR assays based on non-specific double-stranded DNA intercalating dye;
  4. end-point PCR assays;

5. standards and standard curves;
  6. cycle threshold ( $C_T$ ) establishment and its role in the quantification process;
  7. the use of melting temperature ( $T_m$ ) to assess specificity of the reaction.
- c) Characteristics, performance, limitations, and information provided by PCR and non-PCR based methods of DNA quantification:
1. sensitivity (limit of detection and reliable concentration range);
  2. specificity (non-human, higher primate, human, male/female);
  3. single-copy vs. multi-copy qPCR targets;
  4. qPCR target length and performance on degraded DNA;
  5. qPCR multiplex capabilities;
    - i. limits of detection;
    - ii. internal positive control for the co-detection of PCR inhibitors;
    - iii. autosomal and Y chromosome co-quantification;
    - iv. detection and characterization of degradation based on targeting fragments of different lengths in a multiplex reaction.
- d) Characteristics of results of different methods of DNA quantification:
1. amount of total DNA or target DNA;
  2. sample purity;
  3. detection of male contributor(s);
  4. presence of PCR inhibitors;
  5. DNA degradation levels;
  6. suitability of DNA for typing methods;
  7. impact of the source of the DNA standard (e.g., cell line, single source genomic DNA, pooled genomic DNA) on the quantification estimate.
- e) Interpretation of results:
1. evaluation of the standard curve (slope,  $R^2$ , Y-intercept);
  2. DNA concentration of the sample;
  3. presence of PCR inhibitors (if applicable);

4. ratio between male and female contributors (if applicable);
  5. level of sample degradation (if applicable);
  6. evaluation of the melting temperature ( $T_m$ ) (if applicable);
  7. determining whether the sample has detectable amounts of DNA;
  8. determining whether the DNA should be further purified, further concentrated, or the sample should be re-extracted;
  9. determining whether it is appropriate to proceed with the amplification step;
  10. determining the amount of DNA to be added to the amplification reaction;
  11. method limitations (e.g., non-targeted detection methods, specificity, sensitivity).
- f) Instrumentation and reagents:
1. DNA quantification instruments and parameters;
  2. software parameters associated with instruments;
  3. maintenance and calibration;
  4. quality control, storage and handling of quantification kit reagents.
- g) Troubleshooting:
1. spectral calibration failure;
  2. standard quantification curve failure
  3. non-specific detection (e.g., background fluorescence, cellular debris, pigments);
  4. 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 quantification 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 quantification 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 casework or database samples processed by the laboratory. These include:

- a) DNA quantification 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 quantification 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 DNA quantification. 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 quantification methods. The test(s) shall cover, at a minimum:

- a) theoretical and scientific basis of DNA quantification;
- 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 quantification;
- e) the laboratory's analytical procedures pertaining to DNA quantification methods.

##### **4.4.3 Practical Competency**

The trainee shall successfully complete a practical test covering each of the DNA quantification protocol(s) for which he or she will be independently authorized to perform. At a minimum the trainee shall be able to satisfactorily perform the following:

- a) properly and accurately execute the analytical procedures related to DNA quantification;
- 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<sup>a</sup>.
- 2] FBI, *Quality Assurance Standards for DNA Databasing Laboratories*, effective July 1, 2020<sup>b</sup>.
- 3] FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories (QAS)*, effective September 1, 2011<sup>c</sup>.
- 4] FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories*, effective July 1, 2020<sup>d</sup>.
- 5] SWGDAM. *SWGDAM Training Guidelines and References*<sup>e</sup>.

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<sup>a</sup> Available at <https://www.fbi.gov/file-repository/quality-assurance-standards-for-dna-databasing-laboratories.pdf/view>.

<sup>b</sup> Available at [https://docs.wixstatic.com/ugd/4344b0\\_809d01b3e9f9451cb9edd9a85f2c2e5b.pdf](https://docs.wixstatic.com/ugd/4344b0_809d01b3e9f9451cb9edd9a85f2c2e5b.pdf).

<sup>c</sup> Available at <https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view>.

<sup>d</sup> Available at [https://docs.wixstatic.com/ugd/4344b0\\_6782472e073442ec877085584aaffa36.pdf](https://docs.wixstatic.com/ugd/4344b0_6782472e073442ec877085584aaffa36.pdf).

<sup>e</sup> Available at [http://media.wix.com/ugd/4344b0\\_87b2b4a150aa433f9490b7113b1aa4a6.pdf](http://media.wix.com/ugd/4344b0_87b2b4a150aa433f9490b7113b1aa4a6.pdf).



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