This document has been accepted by the Academy Standards Board (ASB) for development as an American National Standard (ANS). For information about ASB and their process please refer to asb.aafs.org. This document is being made available at this stage of the process so that the forensic science community and interested stakeholders can be more fully aware of the efforts and work products of the Organization of Scientific Area Committees for Forensic Science (OSAC). The documents were prepared with input from OSAC Legal Resource Committee, Quality Infrastructure Committee, and Human Factors Committees, as well as the relevant Scientific Area Committee. The content of the documents listed below is subject to change during the standards development process within ASB, and may not represent the contents of the final published standard. All stakeholder groups or individuals, are strongly encouraged to submit technical comments on this draft document during the ASB's open comment period. Technical comments will not be accepted if submitted to the OSAC Scientific Area Committee or Subcommittees.

Standard for Training in Forensic DNA Quantification Methods

DRAFT

DRAFT DOCUMENT

Standard for Training in Forensic DNA Quantification Methods

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 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, quantification, quantitative PCR/real time PCR

Table of Contents

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

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

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 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

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

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

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 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 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 quantification

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) Principles of non-PCR based DNA quantification methods

i. Spectrophotometric analysis

ii. Fluorometric methods

iii. Yield gels

iv. Southern blots (e.g., slot blots)

b) Principles of quantitative PCR (qPCR) DNA quantification methods

i. qPCR assays based on fluorescence quenching using specific probes

ii. qPCR assays based on non-specific double-stranded DNA intercalating dye

iii. Digital PCR (dPCR)

iv. End-point PCR assays

v. Standards and standard curves

vi. Cycle threshold (Ct) establishment and its role in the quantification process

c) Characteristics, performance, and information provided by PCR and non-PCR based methods of DNA quantification

i. Sensitivity (limit of detection and reliable concentration range)

ii. Specificity (non-human, higher primate, human, male/female)

iii. Single-copy vs. multi-copy qPCR targets

iv. qPCR target length and consequent performance on degraded DNA

v. qPCR multiplex capabilities

a. Limits of detection

b. Internal positive control for the co-detection of PCR inhibitors

c. Autosomal and Y chromosome co-quantification

d. 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

i. Amount of total DNA or target DNA

ii. Sample purity

iii. Detection of male contributor(s)

iv. Presence of PCR inhibitors

v. DNA degradation levels

vi. Suitability of DNA for downstream DNA typing methods

vii. 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

i. Evaluation of the standard curve (slope, R^2, Y intercept)

ii. DNA concentration in the sample

iii. Presence of PCR inhibitors (if applicable)

iv. Ratio between male and female contributors (if applicable)

v. Level of sample degradation (if applicable)

vi. Determining whether the DNA should be further purified, concentrated, or re-extracted

vii. Determining whether the sample has detectable amounts of DNA

viii. Determining whether it is appropriate to proceed with the amplification step

ix. Determining the amount of DNA to be added to the amplification reaction

x. Method limitations (e.g., non-targeted detection methods, specificity, sensitivity)

f) Instrumentation and reagents

i. DNA quantification instruments and parameters

ii. Software parameters associated with instruments

iii. Maintenance and calibration

iv. Storage and handling of quantification kit reagents

g) Troubleshooting

i. Spectral calibration failure

ii. Standard quantification curve failure

iii. Non-specific detection (e.g., background fluorescence, cellular debris, pigments)

iv. General equipment failure

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 quantification protocols used by the laboratory.

4.2.1 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 quantification methods to be utilized by the trainee

b) Documentation of the process

4.2.2 Practical exercises representative of the range, type, and complexity of routine casework or database samples processed by the laboratory. These shall include

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

a) The theoretical and scientific bases 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

4.3.2 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. The trainee shall be able to satisfactorily perform the following, as applicable:

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) 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.

1) Butler, J. M. (2012) Advanced Topics in Forensic DNA Typing: Methodology. Academic Press, chapter 3, 49-67.

2) Huss, V., et al. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. Systematic and Applied Microbiology 4.2, 184-192.

3) Holden, M. J., et al. (2009). Factors affecting quantification of total DNA by UV spectroscopy and PicoGreen fluorescence. Journal of agricultural and food chemistry, 57.16, 7221-7226.

4) Teare, J. M., et al. (1997). Measurement of nucleic acid concentrations using the DyNA Quant and the GeneQuant. Biotechniques 22.6, 1170-1174.

5) Nicklas, J. A., and Buel, E. (2003). Quantification of DNA in forensic samples. Analytical and Bioanalytical Chemistry, 376, 1160–1167.

6) Ahn, S. J., et al. (1996). PicoGreen quantitation of DNA: Effective evaluation of samples pre- or post-PCR. Nucleic Acids Research, 24, 2623–2625.

7) Budowle, B., et al. (1995). DNA protocols for typing forensic biological evidence: Chemiluminescent detection for human DNA quantitation and restriction fragment length polymorphism (RFLP) analyses and manual typing of polymerase chain reaction (PCR) amplified polymorphisms. Electrophoresis, 16, 1559–1567.

8) Walsh, P. S., et al. (1992). A rapid chemiluminescent method for quantitation of human DNA. Nucleic Acids Research, 20, 5061–5065.

9) Greenspoon, S. A., et al. (2006). Automated PCR setup for forensic casework samples using the Normalization Wizard and PCR Setup robotic methods. Forensic Science International, 164, 240–248.

10) Hayn, S., et al. (2004). Evaluation of an automated liquid hybridization method for DNA quantitation. Journal of Forensic Sciences, 49, 87–91.

11) Allen, R. W., & Fuller, V. M. (2006). Quantitation of human genomic DNA through amplification of the amelogenin locus. Journal of Forensic Sciences, 51, 76–81.

12) Fox, J. C., et al. (2003). Development, characterization, and validation of a sensitive primate-specific quantification assay for forensic analysis. BioTechniques, 34, 314–322.

13) Zipper, H., et al. (2004). Investigations on DNA intercalation and surface binding by SYBR Green I: Its structure determination and methodological implications. Nucleic Acids Research, 32, e103.

14) Higuchi, R., et al. (1992). Simultaneous amplification and detection of specific DNA sequences. Biotechnology, 10, 413–417.

15) Higuchi, R., et al. (1993). Kinetic PCR analysis: Real-time monitoring of DNA amplification reactions. Biotechnology, 11, 1026–1030.

16) Nicklas, J. A., & Buel, E. (2003). Development of an Alu-based, real-time PCR method for quantitation of human DNA in forensic samples. Journal of Forensic Sciences, 48, 936–944.

17) Nicklas, J. A., & Buel, E. (2005). An Alu-based, MGB Eclipse real-time PCR method for quantitation of human DNA in forensic samples. Journal of Forensic Sciences, 50, 1081–1090.

18) Alonso, A., et al. (2004). Real-time PCR designs to estimate nuclear and mitochondrial DNA copy number in forensic and ancient DNA studies. Forensic Science International, 139, 141–149.

19) Kontanis, E. J., et al. "Evaluation of Real-Time PCR Amplification Efficiencies to Detect PCR Inhibitors." Journal of forensic sciences 51.4 (2006): 795-804.

20) Andreasson, H., et al. (2002). Real-time DNA quantification of nuclear and mitochondrial DNA in forensic analysis. BioTechniques, 33, 402–411.

21) Swango, K. L., et al. (2007). Developmental validation of a multiplex qPCR assay for assessing the quantity and quality of nuclear DNA in forensic samples. Forensic Science International, 170, 35–45.

22) Hudlow, W. R., et al. (2008). A quadruplex real-time qPCR assay for the simultaneous assessment of total human DNA, human male DNA, DNA degradation and the presence of PCR inhibitors in forensic samples: A diagnostic tool for STR typing. Forensic Science International: Genetics, 2, 108–125.

23) Nicklas, J. A., & Buel, E. (2006). Simultaneous determination of total human and male DNA using a duplex realtime PCR assay. Journal of Forensic Sciences, 51, 1005–1015.

24) Green, R. L., et al. (2005). Developmental validation of the Quantifiler real-time PCR kits for the quantification of human nuclear DNA samples. Journal of Forensic Sciences, 50, 809–825.

25) Barbisin, M., et al. (2009). Development validation of the Quantifiler Duo DNA quantification kit for simultaneous quantification of total human and human male DNA and detection of PCR inhibitors in biological samples. Journal of Forensic Sciences, 54, 305–319.

26) Frégeau, C. J., (2015). The Qiagen Investigator® Quantiplex HYres as an alternative kit for DNA quantification. Forensic Science International: Genetics, 16, 148-162.

27) Holt, A., et al. (2016). Developmental validation of the Quantifiler® HP and Trio Kits for human DNA quantification in forensic samples. Forensic Science International: Genetics, 21, 145–157.

28) Kanthaswamy S, Premasuthan A, Ng J, Satkoski J, Goyal V. Quantitative real-time PCR (qPCR) assay for human-dog-cat species identification and nuclear DNA quantification. Forensic Sci Int Genet. 2012;6(2):290-5. Epub 2011/07/19. doi: 10.1016/j.fsigen.2011.06.005. PubMed PMID: 21764401.

29) Tobe SS, Linacre AMT. A technique for the quantification of human and non-human. mammalian mitochondrial DNA copy number in forensic and other mixtures. Forensic Sci Int-Gen. 2008;2(4):249-56. doi: 10.1016/j.fsigen.2008.03.002. PubMed PMID: WOS:000261500500001.

30) Wozney KM, Wilson PJ. Real-time PCR detection and quantification of elephantid DNA: Species identification for highly processed samples associated with the ivory trade. Forensic Science International. 2012;219(1-3):106-12. doi: 10.1016/j.forsciint.2011.12.006. PubMed PMID: WOS:000304626800022.

31) Evans, J. J., Wictum, E. J., Penedo, M. C. T., & Kanthaswamy, S. (2007). Real-time polymerase chain reaction quantification of canine DNA. Journal of Forensic Sciences, 52(1), 93-96.

32) EM Jesmok, JM Hopkins and DR Foran 2016 Next-Generation Sequencing of the Bacterial 16S rRNA Gene for Forensic Soil Comparison: A Feasibility Study. Journal of Forensic Sciences 61, 607–617.

33) Lindquist CD, Evans JJ, Wictum EJ. 2011. Developmental validation of feline, bovine, equine, and cervid quantitative PCR assays. J Forensic Sci. 56(S1):S29–S35.