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Research article

Development and usage of a NIST standard reference material for real time PCR quantitation of human DNA☆

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Abstract

National Institute of Standards and Technology SRM 2372 human DNA quantitation standard has been produced to support the need for a human-specific DNA quantitation standard in forensic casework and calibration of new quantitative polymerase chain reaction (qPCR) assays. The conventional DNA concentration has been assigned with one of the U.S. National Reference UV/Visible Spectrophotometers, assuming an absorbance of 1.0 at 260 nm equals 50 ng/μL of double stranded DNA. In addition, an interlaboratory study has been conducted, to verify that the SRM 2372 materials perform well in currently used DNA quantitation assays by the forensic DNA community. Each unit of SRM 2372 consists of three well-characterized DNA extracts. Component A is a single-source human male material derived from blood. Component B is a multiple-source human female material derived from blood. Component C was purchased as a purified unsheared genomic human DNA (Sigma–Aldrich Co., St. Louis, MO) obtained as a lyophilized human genomic extract and has both male and female donors. SRM 2372 is intended to enable the comparison of DNA concentration measurements across time and place. Manufacturers can use SRM 2372 to validate the values assigned to their own reference materials. Individual forensic laboratories can use SRM 2372 to validate DNA quantitation methods and to verify the assigned concentration of in-house or commercial DNA calibration standards.

Keywords: qPCR; SRM; Quantitation; NIST

1. Introduction

Forensic DNA identification laboratories rely on reproducible quantitation of extracted human DNA for downstream analysis. Quantitation inaccuracy leads to too much or too little sample being used in the polymerase chain reaction (PCR) amplification process that may lead to artefacts, wasted time, resources, and potential loss of critical sample.

NIST has conducted several interlaboratory studies to assess the ''state of the art'' in human DNA quantitation methods used by the forensic DNA community and to determine whether or not a standard reference material for DNA quantification would be useful. In NIST Quantitation Study 2004 (QS04), 80 participants provided 287 independent sets of results from 19 different quantification methods [1]. The expected one standard deviation among-laboratory variability for sub-ng/μL DNA concentration samples in QS04 is about a factor of 1.8. (A factor-of-two uncertainty in the amount of template DNA is equivalent to ± a single PCR amplification cycle.) Much of this variability apparently resulted from systematic biases among the 10 quantitative PCR (qPCR) methods used in QS04. Members of the forensic community have also communicated problems with lot-to-lot variability of commercial quantitation calibrators included in commercial human DNA quantitation kits.

The weight of the evidence from QS04 suggests that use of a human DNA standard should help reduce among-laboratory,

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among-calibrant, and among-method quantitation variability. SRM 2372 human DNA quantitation standard was designed to meet this need.

2. Materials and methods

2.1. Material preparation

SRM 2372 contains three components labelled A, B, and C. All components are human genomic DNA. Component A was prepared at NIST from Buffy coat white blood cells from single-source anonymous male. Component B was prepared at NIST from Buffy coat cells from multiple anonymous female donors. Component C was obtained as a commercial lyophilized human genomic extract and has both male and female donors.

The method used to isolate genomic DNA from the white blood cells found in the human Buffy coats is based on a modified ''salting out'' procedure [2]. After the initial extraction, the material was re-extracted to assure purity. In addition, Component A was treated with RNase before re-extraction [3].

2.2. Decadic attenuance measurements

The nominal concentration of a DNA solution is derived from the widely accepted assertion that for a solution of double stranded DNA, an optical density (OD) at 260 nm of 1.0 corresponds to a DNA concentration of 50 μg/mL (50 ng/μL) [3,4]. Optical densities at four additional wavelengths (230 nm, 270 nm, 280 nm and 330 nm) are also traditionally used in the assessment of DNA quality [3]. The SRM 2372 component materials are therefore certified for Decadic Attenuance at 230 nm, 260 nm, 270 nm, 280 nm and 330 nm using UV/vis spectrophotometry. Measurements were performed on the NIST high accuracy reference spectrophotometer. Values at 260 nm along with nominal DNA concentrations for each SRM component are listed in Table 1.

3. Results and discussion

3.1. Interlaboratory testing

A total of 32 laboratories participated in the interlaboratory testing of the materials. Participants ran their qPCR method of choice using the SRM materials. SRM component C was used as the DNA standard to determine the concentration of components A and B. The following eight DNA quantification methods were used in the study: Quantifiler [5], Centre of Forensic Sciences [6], Sybr green ALU [7], Probe-based ALU [8], Picogreen [9], CA-DOJmonoTH01 [10], CA-DOJ-triplexTH01 [10], CA-DOJ-triplexCSF [11]. The results for the majority of the methods fall within a 95% confidence level (data not shown). The net result of the study is that the SRM materials are appropriate for use with different qPCR methods.

Table 1
Decadic attenuance and nominal DNA concentration values for the SRM 2372 ($n = 3$)

Component	260 nm	Error at 260 nm	Nominal [DNA] (ng/μL)
A	1.049	±0.025	52.5
B	1.073	±0.030	53.6
C	1.086	±0.028	54.3

Table 2
DNA standard calibration using SRM 2372

	Commercial standard							
	1		2		3		4	
	[DNA]	S.D.	[DNA]	S.D.	[DNA]	S.D.	[DNA]	S.D.
Dilution								
10×	105	3.2	122	1	126	5.8	256	10.1
50×	105	3.3	122	7.3	145	0.8	272	7.8
100×	99	6.2	113	11.6	138	0.5	270	10.5
200×	100	1.7	137	18.5	137	3.9	311	3.7
Assigned	102		123		136		277	
Stated	200		200		200		260	
Deviation (%)	−49		−38		−32		6	

3.2. Value assignment for DNA standard calibration

An example of DNA standard value assignment is shown in Table 2. DNA concentration values are listed as ng/μL. Four commercial human DNA standards were calibrated using the SRM materials. Samples were evaluated using the Quantifiler qPCR method. Experiments were carried out in duplicate over dilutions ranging from 10× to 200× of the stock materials. Commercial standards 1–3 were found to deviate greater than 30% from the stated concentration supplied with the material.

3.3. Summary

The three SRM components are highly purified human DNA extracts certified for decadic attenuance (absorption), but a nominal DNA concentration can easily be obtained assuming 1 OD = 50 ng/μL of double stranded DNA. The materials are intended to be used as calibrants for standards used in quantitative real time PCR assays. Commercial or in-house DNA standards can be calibrated using the material. The material is not intended for everyday use as a standard. The materials are to be stored at 4 °C and not frozen. SRM 2372 provides an independent means to calibrate the concentration of a DNA standard. DNA standards obtained from different vendors or varying lots can be monitored for concentration accuracy and potential performance variation. Further details about SRM 2372 can be found at http://www.cstl.nist.gov/biotech/strbase/srm2372.htm.

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Conflict of interest

None.

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