B60 An Assessment of the NovaQUANT™ Human Nuclear and Mitochondrial DNA Quantitative Polymerase Chain Reaction (qPCR) Assay Using the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2372a

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Learning Overview: The goal of this presentation is to inform attendees of an evaluation of the NovaQUANT™ Human Mitochondrial to Nuclear DNA Ratio Kit utilizing the NIST SRM 2372a.[1,2]

Impact on the Forensic Science Community: This presentation will impact the forensic science community by discussing qualities of an accurate and robust real-time qPCR assay and how the SRM 2372a can be used to verify the concentration of DNA standards used in absolute qPCR assays. In turn, downstream analysis failures can be minimized, particularly when valuable casework samples are limited.

In forensic DNA casework, a highly accurate real-time qPCR assay is recommended per the Scientific Working Group on DNA Analysis Methods (SWGDAM) to determine whether the DNA is of sufficient quantity and robust quality to move forward with downstream Short Tandem Repeat (STR) or sequencing analyses. One fundamental issue with absolute qPCR is that the quantifiable concentration of the commercial assay standards can vary depending on origin (i.e., whether from a cell line or a human subject, supplier, lot number, shipping method, etc.). In 2018, NIST released a human DNA standard reference material for evaluating qPCR quantification standards, SRM 2372a, which contains three well-characterized human genomic DNA samples, including (A) a single male$_1$ donor, (B) a single female$_1$ donor, and (C) a 1/3 male$_2$/female$_2$ donor, each with certification data for nDNA and as well as for mitochondrial DNA/nuclear DNA (mtDNA/nDNA) ratios.[2]

The SRM 2372a was used to evaluate the commercial qPCR assay NovaQUANT™, which amplifies four targets, each in separate singleplex SYBR® Green qPCR assays: two mtDNA gene targets ((ND6 (96bp) and (ND1(153bp)) and two nDNA targets ((BECN1(129bp) and (NEB (116 bp)).[1] NovaQUANT™ was performed using the absolute standard curve method utilizing osteosarcoma cell line 143B DNA.[1] The data was evaluated using the Minimum Information for Publication of Quantitative Real-Time Experiments (MIQE) guidelines.[3] While the data for all four targets for qPCR efficiency (perfect doubling of product with each cycle) and correlation coefficients (r^2) values fell within the MIQE guidelines, the quantification accuracy, which was affected by sensitivity and specificity, was less than optimal. The results showed that all three SRM 2372a components (i.e., A, B, and C) quantified higher than their expected NIST values.[2] A second analysis, not reliant on the 143B DNA standard, was conducted using the NovaQUANT™ Relative Copy Number Method.[1] Those results showed that SRM 2372a quantified much more accurately compared to the absolute quantification method. Therefore, the inaccuracies associated with absolute quantification may be due to quantitative imprecision and/or qualitative issues of the cell line 143B DNA standard itself.

In conclusion, the availability of a commercial qPCR assay like NovaQUANT™ that can quantify mtDNA and nDNA simultaneously is a valuable tool for forensic DNA analysts, potentially saving time and reducing cost. However, when utilizing an absolute qPCR method with a standard, such as 143B cell line DNA, the standard should be verified using a well-characterized DNA standard reference material like the NIST SRM 2372a to ensure high qPCR accuracy and success.

Reference(s):

1. NovaQUANT™ Human Mitochondrial to Nuclear DNA Ratio Kit. EMD Millipore Corporation 2011.
2. Romsos, E. et al. *Certification of Standard Reference Material 2372a; Human DNA Quantitation Standard.* 2018.
3. Bustin, S.A. et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical chemistry*, v. 55, n. 4, p. 611-622, 2009. ISSN 0009-9147.

NovaQUANT™ qPCR, NIST SRM 2372a, Absolute Quantification

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