TECHNICAL NOTE

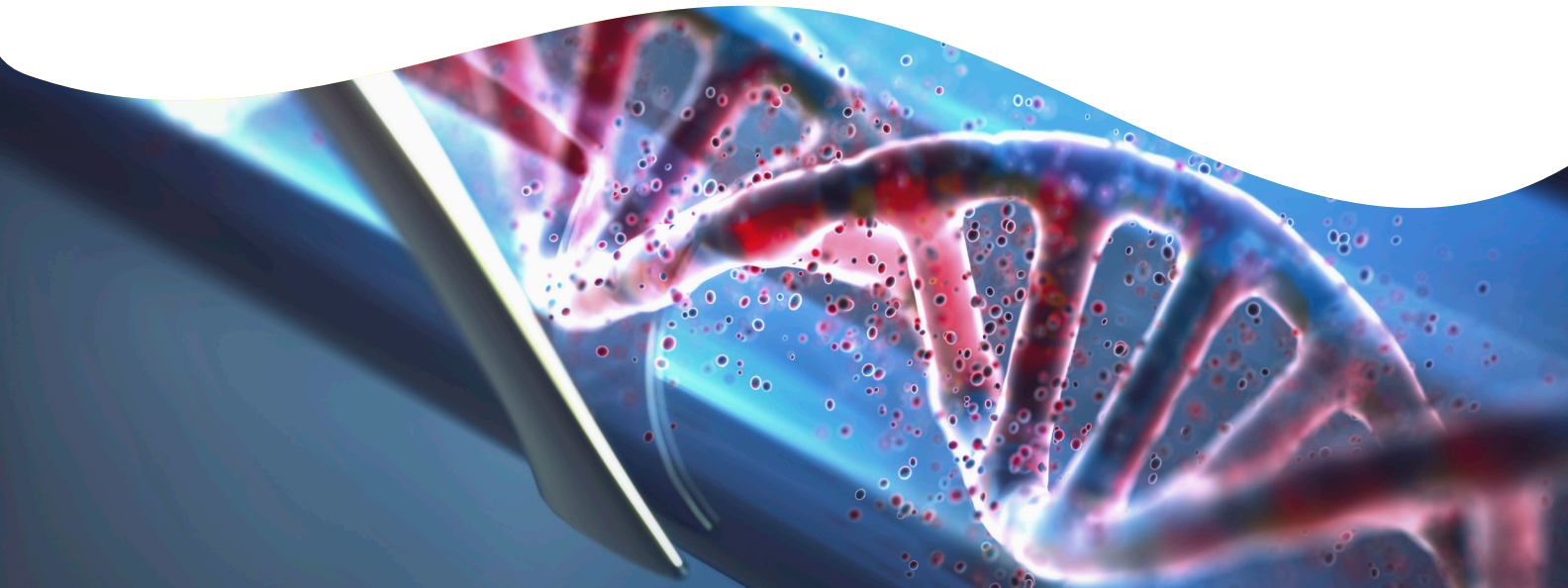
Comparative analysis of DNA quantification methods for gDNA and ctDNA reference standards.

Introduction

Revvity's Mimix™ reference standards are developed to be reliable controls across various sample types and analysis techniques. All our reference standards are generated from cell lines for a closer representation of patient samples, while providing consistency across batches by using robust quality control measures.

In-house, the two most used quality control (QC) techniques for nucleic acid quantification are UV spectrophotometry using the NanoDrop™ (Thermo Fisher Scientific) for gDNA (sample volume=1.5µl), and fluorometry using Qubit™ BR dsDNA assay (Invitrogen) for ctDNA mimetic products (sample volume=2µl). The concentration of the gDNA or ctDNA reference standards are indicated on our Certificates of Analysis (CoA) and on the respective product pages.

We are aware of the variability in concentrations when DNA is quantified using different measurement techniques mentioned above. In this technical note, we investigate how different methods for quantifying our gDNA and ctDNA reference standards can yield varying results.



Methods

A summary of the Nanodrop™ and Qubit™ methods (information collected from the User Guide of the respective kits) is presented in Table 1.

Table 1. A comparison of Qubit (Invitrogen, Qubit™ dsDNA HS Assay Kit: Q32851, Q32854. Qubit™ dsDNA BR Assay Kit: Q32850, Q32853) and Nanodrop (Thermo Scientific NanoDrop 8000).

Properties	Qubit (Fluorometry)	Nanodrop (Spectrophotometry)
Sample requirement	1µl to 10µl	0.5µl-2µl
Principle	Fluorochrome chemistry	Spectroscopic analysis
Quantification Range	BR 4-2,000ng HS 0.1-120ng	10ng-10µg
Sample processing	2-5 minutes	None
Accuracy	99%	~90%
Sample preparation	Required	Not required
Qualitative analysis (purity)	Not possible	Possible and highly accurate
Advantages	Accurately quantifies low dsDNA concentration, distinguishes between DNA, RNA, and free nucleotides	Measures qualty and quantity in a single assay in small sample volume
Disadvantages	Separate kits are required to measure dsDNA/ssDNA/RNA; sample preparation required prior to measurement	Measures free ssDNA and small dsDNA fragments affecting the resuts accuracy

A total of 20 catalog gDNA and 10 catalog ctDNA products were used for the study to represent a variety of reference material and cell lines. The concentration of these gDNA reference standards were quantified using fluorometry (Qubit dsDNA BR assay) and spectrophotometry (Nanodrop) approaches, while the ctDNA reference standards were quantified with the most used fluorometry assays (Qubit dsDNA HS and Qubit dsDNA BR assays).

Results

Comparison of gDNA samples by Nanodrop and Qubit dsDNA-BR assay

A total of 20 gDNA reference standards at 50 ng/µl (as reported on the CoA) were quantified using Nanodrop™ spectrophotometer and Qubit™ fluorometer BR dsDNA assay using an input volume of 2µl. All the gDNA samples measured by Nanodrop™ fell within the acceptance criteria (48.0-52.0 ng/µl, Figure 1). However, all the gDNA samples measured by Qubit™ BR assay showed significantly lower DNA concentrations when compared to Nanodrop™ - Qubit BR assay (mean = 45.16, standard deviation (SD) ±3.658), Nanodrop readings (mean = 50.27, standard deviation (SD) ±0.778.

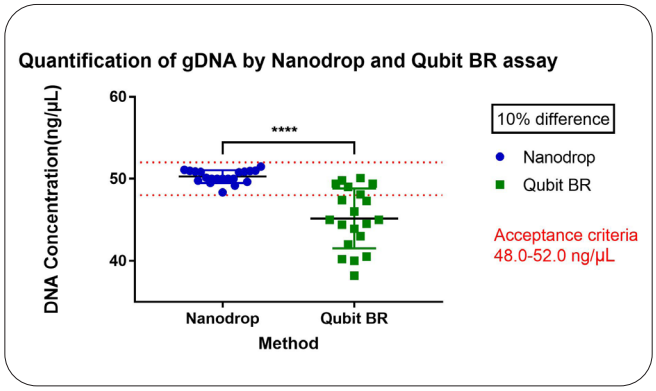


Figure 1: Quantification of gDNA concentration as measured by Nanodrop™ and Qubit™ BR assay. DNA concentration was measured in 20 reference standards. The Nanodrop™ (blue circles) values are significantly higher than Qubit™ (green squares) ($p<0.05$) representing a difference of 10%.

Comparison of ctDNA mimetic samples by Qubit™ BR and Qubit™ dsDNA HS assays

To investigate the variability associated with using Qubit™ BR and Qubit™ HS dsDNA assay, two input volumes, 2µl and 5µl, (in triplicates) were used in the assay for each of the 10 ctDNA products at 20 ng/µl (measured by Qubit™ as reported on the CoA). Comparison between sample input volume of 2µl and 5µl measured with Qubit™ BR assay showed no significant difference ($p>0.05$). Similar results were obtained when 2µl and 5µl of sample were used for Qubit™ HS assay. However, when 2µl input sample was measured by Qubit™ BR and HS, the variability between the assays was observed to be statistically significant. Finally, a significant difference was observed in the results for 5µl input volume sample measured by Qubit™ HS and BR. Generally, the Qubit™ HS ended up with slightly higher values for most of the samples (2µl BR: mean = 17.93, standard deviation (SD) ±0.746, 5µl BR: mean = 18.29, standard deviation (SD) ±1.026, 2µl HS: mean = 18.93, standard deviation (SD) ±0.998, 5µl HS: mean = 20.18, standard deviation (SD) ±2.000).

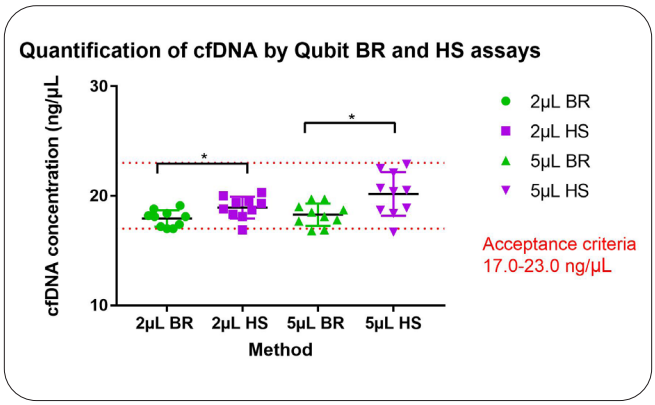


Figure 2: Quantification of ctDNA concentration as measured by Qubit™ BR and Qubit™ HS assays. 10 ctDNA reference standards were measured in two sample volumes (2µl and 5µl). No significant difference was observed between the two sample volumes when measured by the same assay (BR assay- green circles and triangles; HS assay -purple squares and inverted triangles). However, when the same sample volumes were measured by HS and BR assay, there was a statistically significant difference in the results ($p<0.05$; green circles vs purple squares; green triangles vs purple inverted triangles).

Discussion

Nanodrop measurements showed higher readings for gDNA samples

In this study, we have demonstrated that Nanodrop™ and Qubit™ assays may show different results while measuring the same samples, like other observations reported in the literature (Simbolo et al, 2013; Hussling et al, 2018). Generally, concentrations measured with Nanodrop™ are significantly higher than those measured with Qubit™ BR ($p<0.05$ or 10% difference). While we use Nanodrop™ to define our gDNA product specifications, other quantification methods such as Qubit™ might be more suitable for specific downstream applications (like NGS, Microarray etc.). It is therefore suggested to keep the differences in mind while planning your experiments. The expected differences between these two technologies has been documented in the following technical note from Thermo Fisher Scientific: https://assets.fishersci.com/TFS-Assets/LSG/Technical-Notes/fluorescence-UV-quantitation-comparison-tech-note.pdf.

ctDNA samples measured with Qubit BR assay showed less variability

We have illustrated here that the sample volume is not a parameter significantly affecting ctDNA quantifications while using the Qubit™ BR or HS assay. The concentration measurements fell within the acceptance range for both 2µl and 5µl sample volumes. However, the difference in results between BR and HS assays was found to be statistically significant, with higher variability within the samples, when using Qubit™ HS assay at a higher sample volume (5µl).

In summary, we highlight the differences in outcomes of the two measurement techniques. This information will enable the end users to make informed decisions on choosing the right instruments and/or assays for accurate gDNA and ctDNA quantification. Mimix™ reference standards are subject to strict quality control procedures and are assessed using reproducible methods. Data analysis, records, and processes are in accordance with ISO 9001:2016 and ISO 13485:2015 standards.

References

1. William W. Wilfinger, Karol Mackey, and Piotr Chomczynski, Effect of pH and Ionic Strength on the Spectrophotometric Assessment of Nucleic Acid Purity: BioTechniques 22:474-481

2. Simbolo M, Gottardi M, Corbo V, Fassan M et al (2013) DNA quantification workflow for NGS of histopathological samples. Journal.pone.0062692

3. Hussling C, Kampmann M, Mogensen H, Borsting C, Morling N (2018) Quantification of massively parallel sequencing libraries-a comparative study of eight methods. Scientific report 8, 1110

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