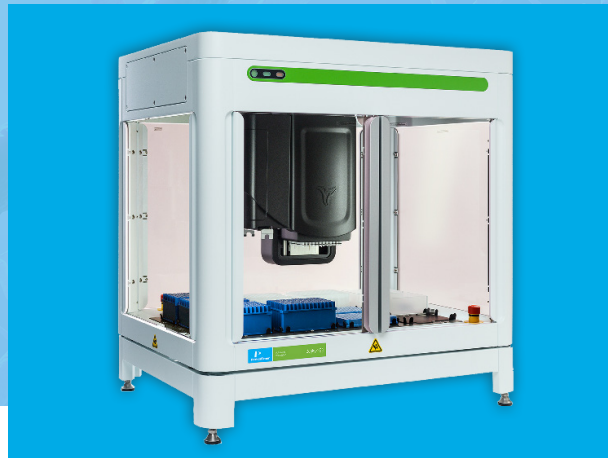
APPLICATION NOTE

NGS Automation

High-throughput Quantification of DNA for NGS Library Prep with the Zephyr® G3 Workstation and the VICTOR Nivo® Plate Reader



Next generation sequencing (NGS) library preparation requires accurate quantification of gDNA input to start the workflow. There are many choices for deciding which solutions to pursue and each can vary in precision, accuracy and throughput. Typically, DNA in NGS workflows is measured using fluorescent based assays on the Thermo Fisher® Scientific Qubit® fluorometer or qPCR on a real-time PCR system. However, these assays are time consuming and require many manual pipetting steps that are can create a bottleneck in a high-throughput lab. Here we obtained consistent, reproducible and high-throughput results using the Zephyr® G3 NGS workstation and the VICTOR Nivo® plate reader to quantify input DNA with reagents from the Thermo Fisher® Scientific Qubit® assay kit.

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INTRODUCTION

Qubit® Reagents for DNA Quantification

This application was developed as an automated 96-well quantification assay for DNA and NGS libraries using the Qubit® BR reagent kit. Typically, Qubit® assays are read on the Qubit® fluorometer. The Qubit® fluorometer is a highly-optimized DNA quantification device based on the fluorescence intensity of dye binding to double-stranded DNA. This method is inconvenient for high throughput quantification, since the samples are read one at a time. In this application we enable plate-based quantification with Qubit® reagents while preserving the convenience and integrity of the tube-based assay on the Qubit® fluorometer.

This application utilizes black 96-well PerkinElmer OptiPlate™ microplates for assay setup via the Zephyr® NGS G3 workstation and subsequent reading on the VICTOR Nivo® plate reader. A 1:200 working solution of Qubit® BR reagent was mixed with BR buffer and 160 µL of the working solution was used per well. Qubit® BR standards and the samples were dispensed at 2 µL into the OptiPlate™ microplate containing the working solution.

Zephyr® G3 NGS Workstation for Plate Setup

The Zephyr® G3 NGS workstation is a benchtop liquid handler designed to automate the construction of NGS libraries. The simplified user-interface and integrated hardware directs users on using the assay on the Zephyr® G3 NGS workstation (Figure 1). The Zephyr® G3 NGS workstation's efficient liquid handling and simultaneous pipetting action is capitalized-on to prepare Qubit® assay plates for 8-96 samples on deck prior to measurement on the VICTOR Nivo® plate reader (Figure 2). Additionally, workbooks packaged with the application are used to guide proper assay preparation (Figure 3) and for tracking automation steps performed by the Zephyr® G3 NGS workstation (Figure 4).

VICTOR Nivo® Plate Reader for DNA Quantitation

The VICTOR Nivo® system is a high-performance filter-based multimode microplate reader equipped with all major detection technologies including fluorescence, absorbance and luminescence detection. It is a compact, light-weight multimode plate reader designed for life science research laboratories requiring a diverse array of applications. The browser-based control software is independent of an operating system, so it can be controlled via a variety of touchscreen devices and operated through a WiFi or network connection.

gDNA for Determining Sample Concentrations

Measurement of a representative DNA sample was performed in this study by using Coriell NA12878 gDNA standard for quantification. NA12878 was selected because this genome has been extensively characterized and is commonly used as a reference genome for validation studies of NGS technologies. Studies include the 1000 Genomes Project and the Illumina Platinum Genomes Project and have developed high-confidence phased variant calls using extensive pedigree information for this family.

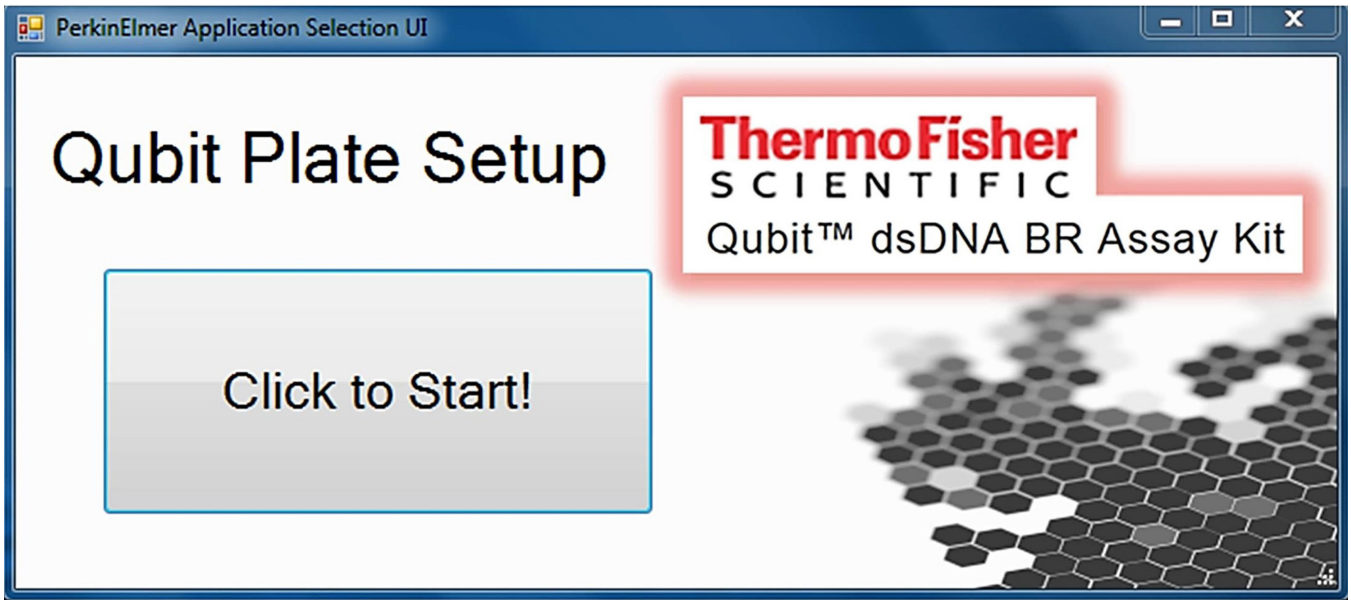

Figure 1. The Zephyr® interface prompts users to start the application before the beginning of assay plate creation by automatically combining samples and working solution into a plate ready for measurement on the VICTOR Nivo® Plate Reader.

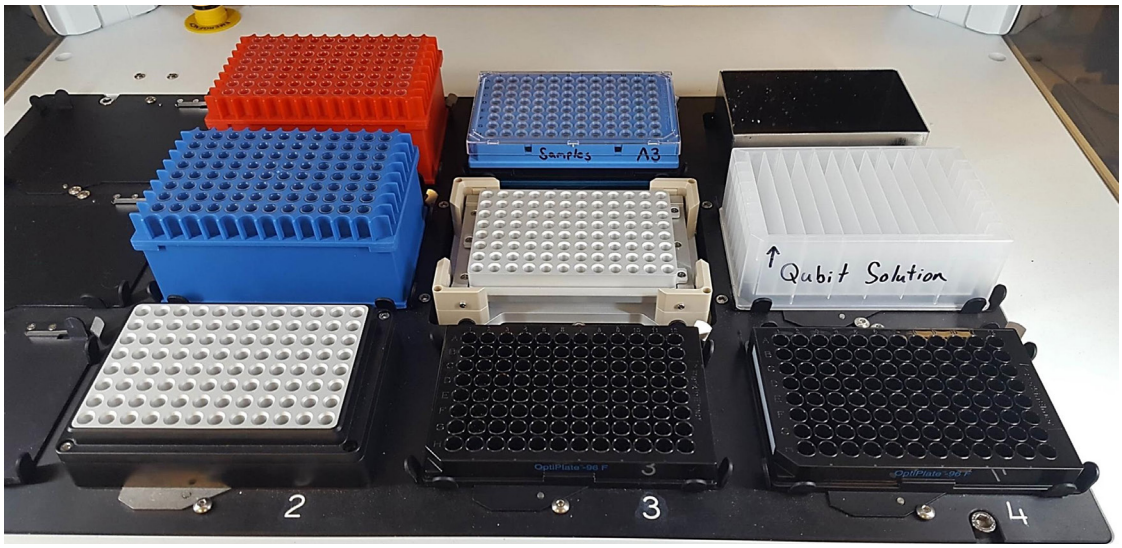

Figure 2. The Zephyr® deck setup required for quantifying 8-96 samples in a 96-well plate of gDNA.

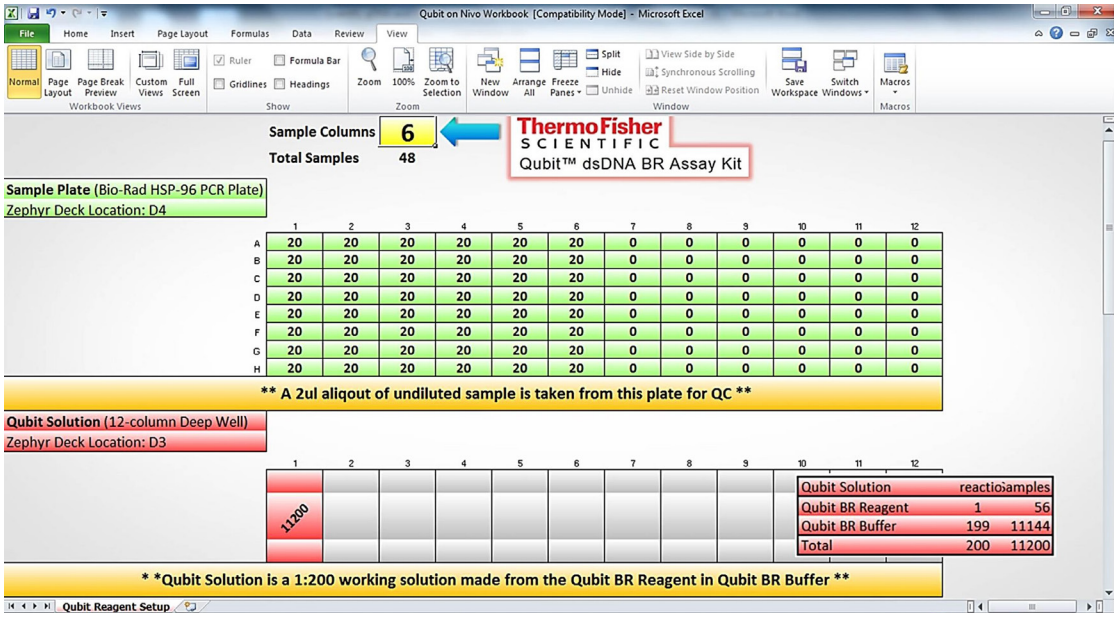

Figure 3. Workbook for guiding the setup of reagents and samples before placing on the Zephyr® deck.

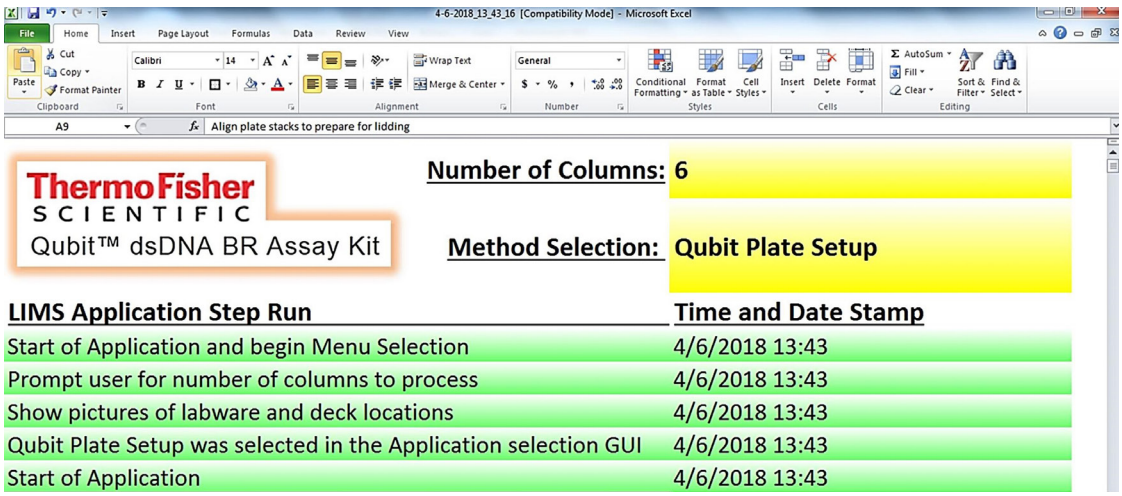

Figure 4. Spreadsheet of steps performed by the Zephyr® G3 NGS workstation that is automatically populated real-time during a run to track processing of samples.

RESULTS

The sample of NA12878 obtained from the Coriell Cell Repository was checked for intact high molecular weight (HMW) gDNA using the Genomic DNA Reagent Kit for the LabChip® GX Touch™ nucleic acid analyzer prior to performing quantification experiments (Figure 5).

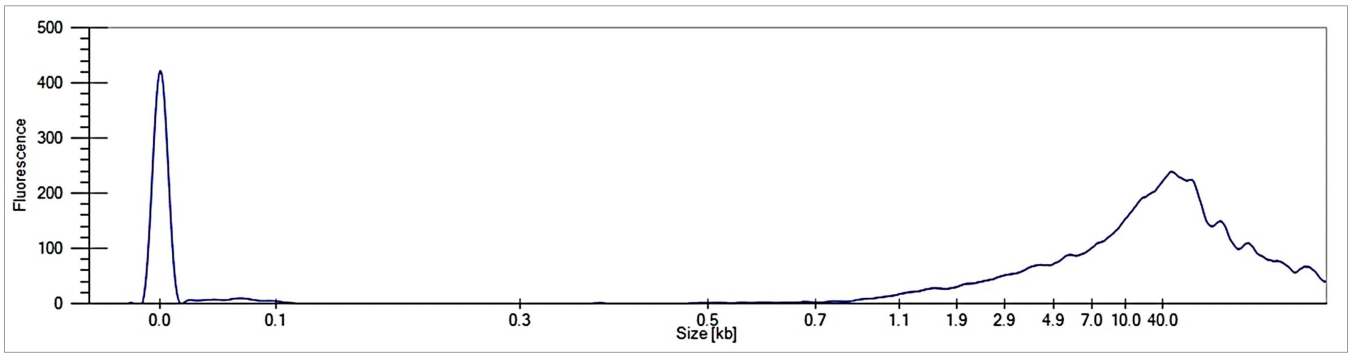

Figure 5. LabChip® GX gDNA assay trace of HMW gDNA from Coriell NA12878. This gDNA sample was tested in this application for validating quantification of gDNA for input into library prep and for adapter-ligated libraries before sequencing.

Fluorescent measurements were performed for three batch replicates of working solution on the VICTOR Nivo® plate reader and analysis was performed on all acquired measurements of data. The relative fluorescence units (RFU) of the standards were used to create a linear regression against concentration (Figure 6).

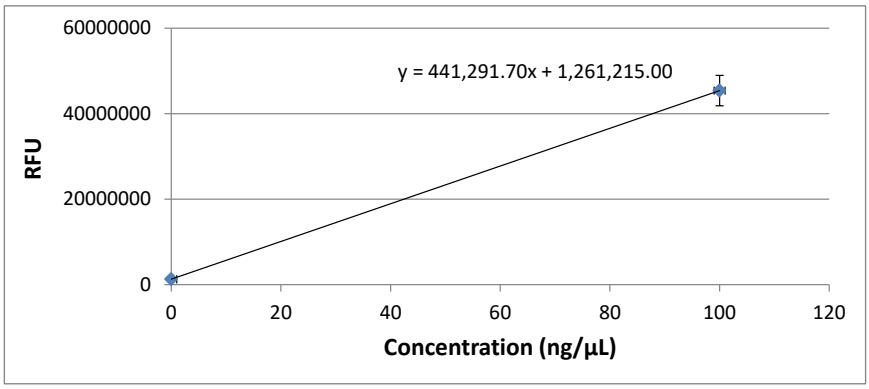

Figure 6. Linear regression analysis of the relative fluorescence units of the Qubit® BR standards measured on the VICTOR Nivo® plate reader using 160 µL of working solution and 2 µL of standards.

To check the accuracy of the plate-based assay, the concentrations determined by manual and automated preparations were compared to the concentrations determined by conventional tube-based assay measured on the Qubit® fluorometer by using pre-prepared standards from the Quant-iT™ assay kit (Figure 7).

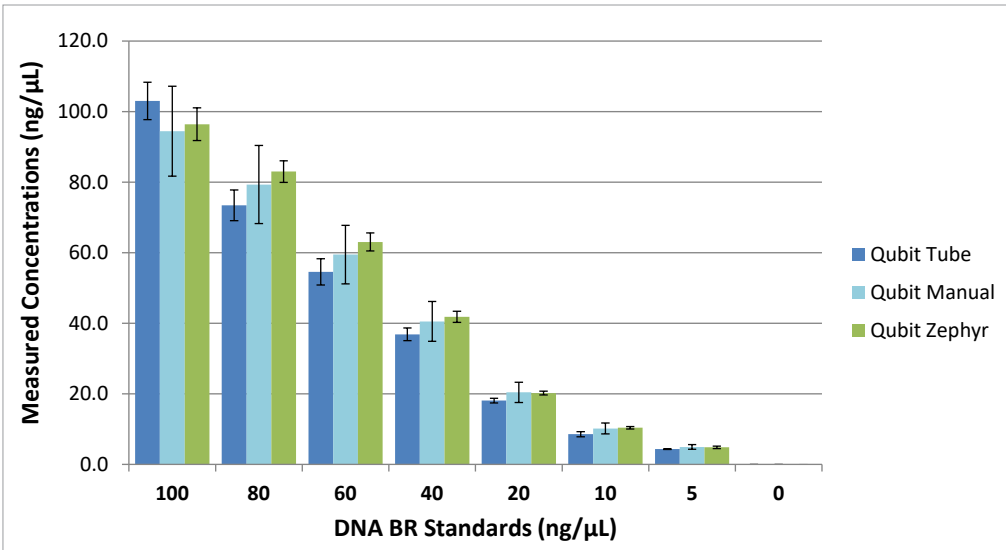

Figure 7. The measured concentrations (ng/µL) of standards obtained from the Quant-iT™ BR assay kit measured on the Qubit® 2.0 fluorometer compared to the results measured on the VICTOR Nivo® plate reader.

To check the accuracy and determine the low end of the dynamic range, pre-prepared standards from the Quant-iT™ HS assay kit were tested (Figure 8). The results were also compared to the concentrations determined by the tube-based Qubit® BR assay.

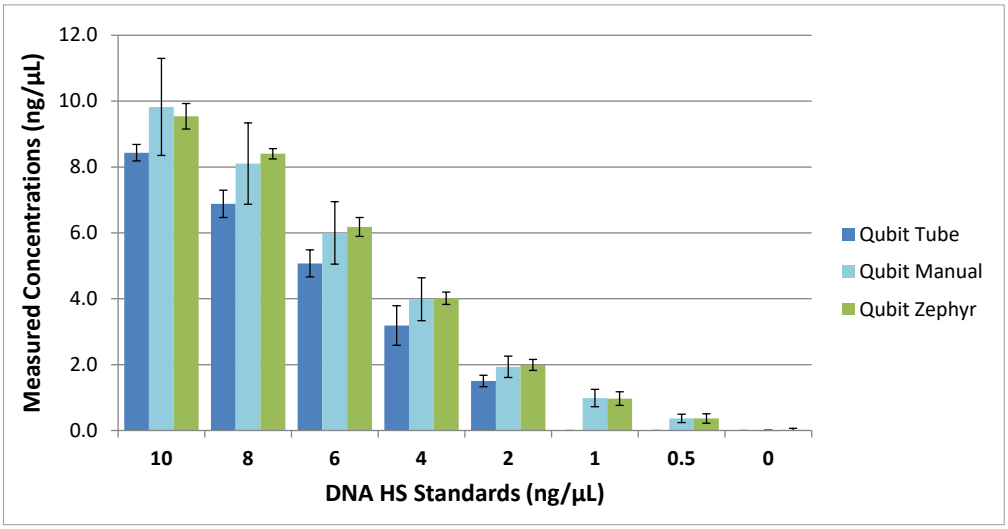

Figure 8. The measured concentrations (ng/µL) of standards obtained from the Quant-iT™ HS assay kit measured on the Qubit® 2.0 Fluorometer compared to the results measured on the VICTOR Nivo™ plate reader.

NA12878 dilutions of unknown concentrations were measured to confirm results obtained from the plate-based assays prepared on the Zephyr® G3 NGS workstation are comparable to the results obtained from the tube-based Qubit® fluorometer (Figure 9).

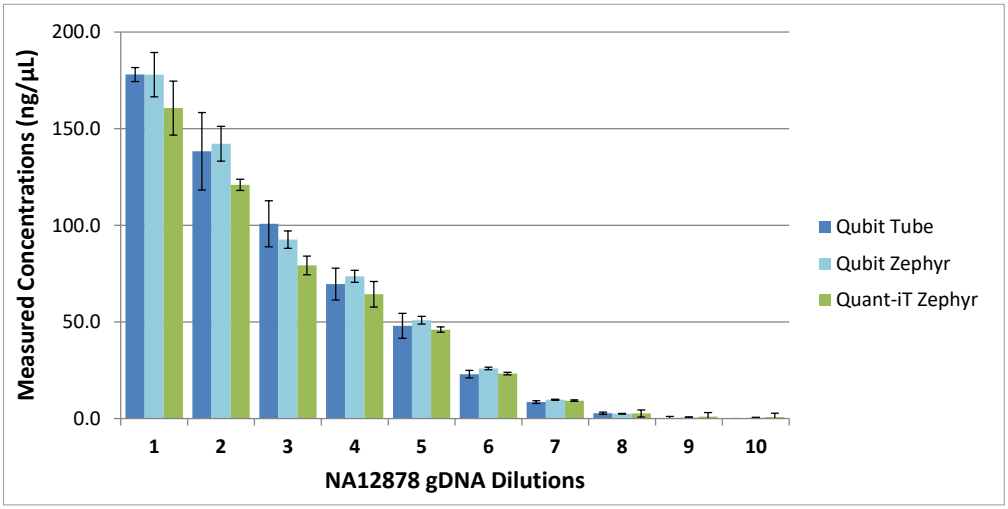

Figure 9. The concentrations (ng/µL) of NA12878 gDNA measured in a tube on the Qubit® 2.0 fluorometer compared to the results measured in a plate on the VICTOR Nivo® plate reader for both Qubit® BR reagents and Quant-iT™ BR reagents.

SUMMARY

This application shows that the Zephyr® G3 NGS workstation can be combined with Qubit® reagents and the VICTOR Nivo® plate reader to provide a high throughput solution for quantifying gDNA and NGS libraries consistent with the tube-based method on the Qubit® Fluorometer. The tube-based Qubit® assay is time consuming and cumbersome for quantifying a large number of samples. Using the Zephyr® G3 NGS workstation, assay plates take approximately 13 minutes to prepare 96 genomic DNA samples for analysis with an additional 5 minutes of hands-on time. The quantification on the VICTOR Nivo® plate reader takes less than 2 minutes to read the entire 96-well plate. Using the workflow presented here, 96 genomic DNA samples can be quantified in only 20 minutes.

Interestingly, the dynamic range of the assay developed in this application can cover typical library inputs amounts (1-200 ng total mass) and adapter-ligated libraries in the range of 0.5 ng/µl – 100 ng/µl, therefore, a higher sensitivity assay with a lower dynamic range may not be needed for certain NGS workflows. If needed, it is possible to expand the high end of the dynamic range by increasing the standard/sample volume used in the assay and the low end of the range by utilizing Qubit® HS reagents.

We also show that this application is compatible with the Quant-iT™ BR reagent kit containing additional standards (Figure 9). Comparisons between the Quant-iT™ dsDNA assay and the Qubit® dsDNA assay have been thoroughly examined. The accuracy of the Quant-iT™ dsDNA assay depends on how well the curve was generated by the user on the day of the assay using the standards provided in the kit. It is important to be aware of the total amount of DNA standard used compared to the amount of sample used in the measurement for the most accurate quantification. It is recommended for assays in the low range to use smaller volumes of the standards; for example, 2 µL volumes for a standard curve ranging from 0–200 ng.

It is important to note that using Qubit® reagents may not be as accurate as qPCR for quantifying adapter-ligated libraries because qPCR is specific to the Illumina® indexes and only measures sequencable DNA. The Qubit® application developed can be adopted for sequencing normalization because quantifying library pools can be fine-tuned for optimal cluster density and instrument reproducibility based on the recommended nM range of loading the flow cell.

REFERENCES

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