

dsDNA Quantitation with the Echo 525 Liquid Handler for Miniaturized Reaction Volumes, Reduced Sample Input, and Cost Savings

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Introduction

Precise dsDNA quantitation is a requirement for many workflows in Genomic research. A variety of quantitation kits are commercially available for diagnostics, synthetic biology, and next-generation sequencing applications. Fluorescent-based quantitation kits like AccuBlue® NextGen dsDNA Quantitation Kit and QuantiFluor® ONE dsDNA System dominate the market. The Echo 525 is used to demonstrate the capacity to miniaturize and optimize fluorescent-based quantitation assays. AccuBlue NextGen dsDNA Quantitation Kit has the capacity to cover the lower end of dsDNA quantitation assays (3 ng - 5 pg) and QuantiFluor ONE dsDNA System can cover the higher range of dsDNA quantitation assays (50 ng - 20 pg). The kit manufacturer's recommended volumes are at 200 µL. However, when utilizing the Echo 525, these reaction volumes can be miniaturized up to 10 µL or less. This 20-fold reduction in reaction volume, along with reduced sample input enables testing of up to 20 times more reactions, compared to standard methods. With optimization and miniaturization of kit recommended protocols, standard curves generated are equivalent to that of the regular protocols. The main constraints on DNA quantitation like reagent cost, assay setup time, and required input DNA can be substantially mitigated by miniaturizing the reaction volumes.

The precision and accuracy of the miniaturized reaction volumes for the 2 kits in an Echo 525 Liquid Handler enabled workflow is verified by quantitating libraries of dsDNA samples in a 384-well polypropylene microplate and cross checking the quantitation data against Qubit™ dsDNA (HS) Assay Kit.



Figure 1. (A) Echo 525 Acoustic Liquid Handler. (B) The Echo system transducer rapidly moves between wells on the source plate while the destination plate also moves, allowing rapid transfer from any well to any well for multiple fluid types.

Goal

Our goal is to miniaturize the suggested AccuBlue NextGen dsDNA Quantitation Kit and QuantiFluor ONE dsDNA System protocols without compromising the standard curve quality while improving the quantitation range. With the help of the Echo 525 Liquid Handler, the kit reagents and standard dsDNA serial dilutions are dispensed in miniaturized volumes into microtiter plates. The resulting standard curves have similar R² values as that of the original kit protocols.

Methods and Materials

Initial Miniaturization

Both the kits are fluorescence-based assays and follow similar protocols. Reagents are dispensed in quadruplicates by the Echo 525 Liquid Handler to 384-well black microtiter plates with a range of final volumes, incubated for 5 minutes in the dark and read on PHERAstar fluorometer. Average fluorescence intensity of each reaction, standard deviation and CV were calculated to generate a standard curve. The miniaturized reaction standard curve displays the linearity and R² values comparable to the standard curve values provided by the kit.

Assay Optimization

As shown in Figure 2, each of the reaction volumes in both the kits tested in the initial miniaturization assays displayed a high level of linearity. However, the reactions at 10 µL for AccuBlue NextGen dsDNA Quantitation Kit and 20 µL for QuantiFluor ONE dsDNA System optimized miniaturization and data quality with excellent R² values. For this reason, these volumes were chosen to be the optimal reaction volume for dsDNA quantitation in an Echo 525 incorporated workflow.

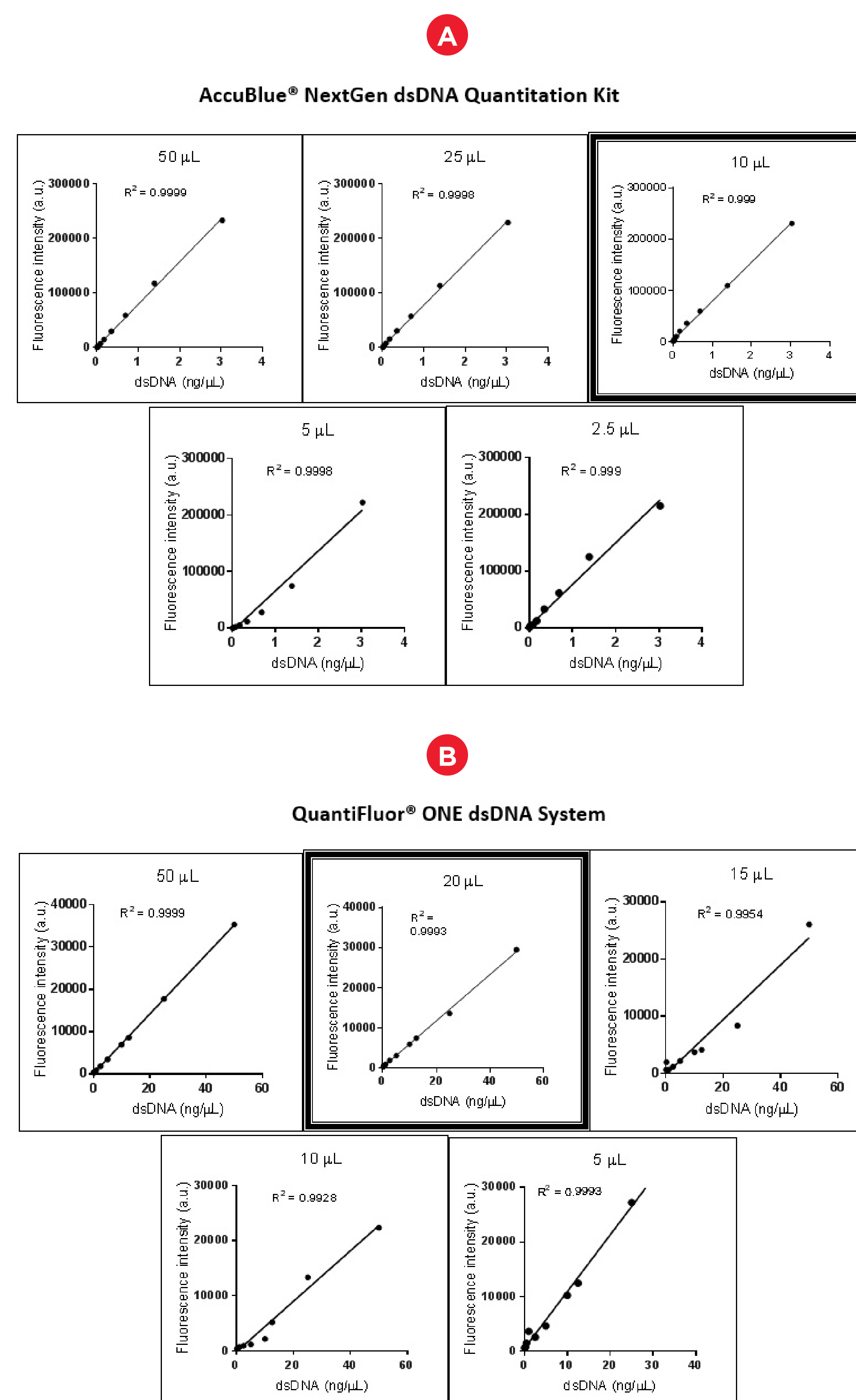


Figure 2. Comparison of multiple standard curves generated via Echo 525 Liquid Handler for (A) AccuBlue NextGen dsDNA Quantitation Kit with reaction volume miniaturized up to 4 fold (50 µL), 8 folds(25 µL), 20 fold (10 µL), 40 fold (5 µL), and 80 fold (2.5 µL), and (B) QuantiFluor ONE dsDNA System with reaction volume miniaturized up to 4 fold (50 µL), 10 fold (20 µL), 13 fold (15 µL), 20 fold (10 µL), and 40 fold (5 µL).

Results

AccuBlue NextGen dsDNA Quantitation Kit

10 µL reaction volume enabled us to extend the linear range beyond the AccuBlue NextGen kit recommended linearity, without compromising the R² value. The extended standard curve enables the quantitation of a broader range of unknown dsDNA samples. The kit recommends 10 ng/µL stock dsDNA to generate the standard curve. This range can be achieved by using two stock dsDNA dilutions, 2 ng/µL and 0.1 ng/µL.

AccuBlue NextGen Standard Curve (10 µL)

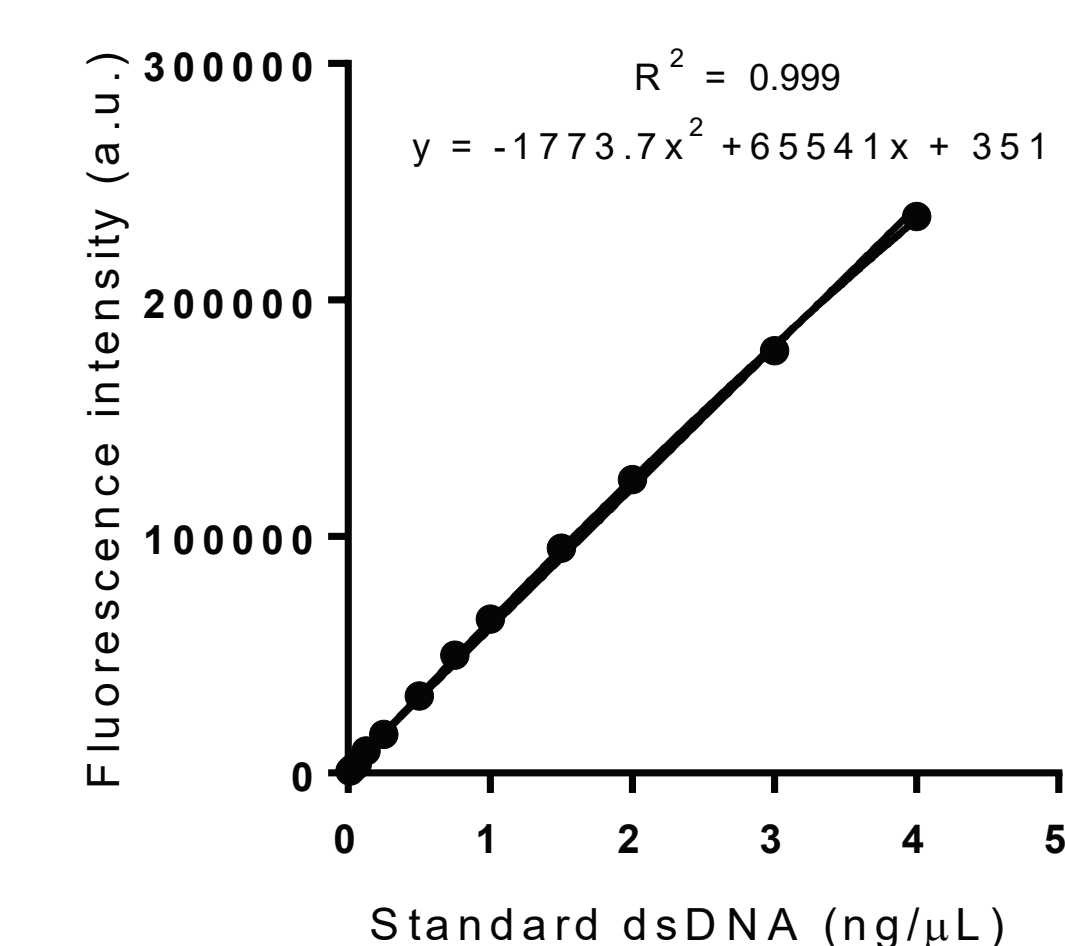


Figure 3. Standard curve created from AccuBlue® NextGen dsDNA Quantitation Kit in a 10 µL reaction volume (4 ng - 5 pg) with R² value of 0.999.

QuantiFluor ONE dsDNA System

A range of 50 ng - 20 pg standard curve can be generated by utilizing 12.5 ng/µL and 1 ng/µL standard stock dilutions in 20 µL reaction volume. This encompasses a wide range for dsDNA quantitation without compromising R² value.

QuantiFluor ONE dsDNA System Standard Curve (20 µL)

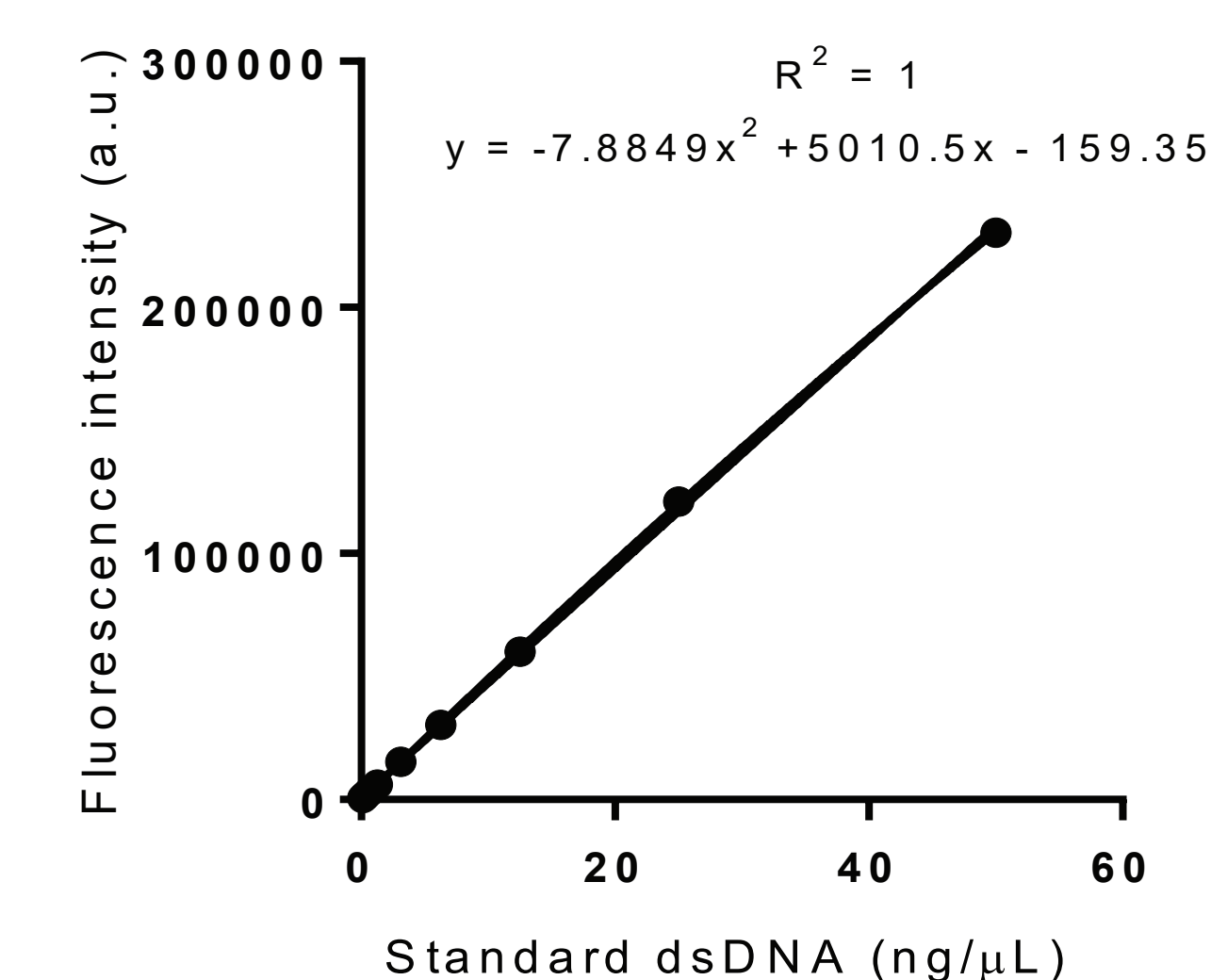


Figure 4. Standard curve created from QuantiFluor ONE dsDNA System in a 20 µL reaction volume (50 ng - 20 pg) with an R² value of 1.

dsDNA Library Quantitation

dsDNA Library sample measured by AccuBlue® Next Gen Quantitation Kit and QuantiFluor® ONE dsDNA System

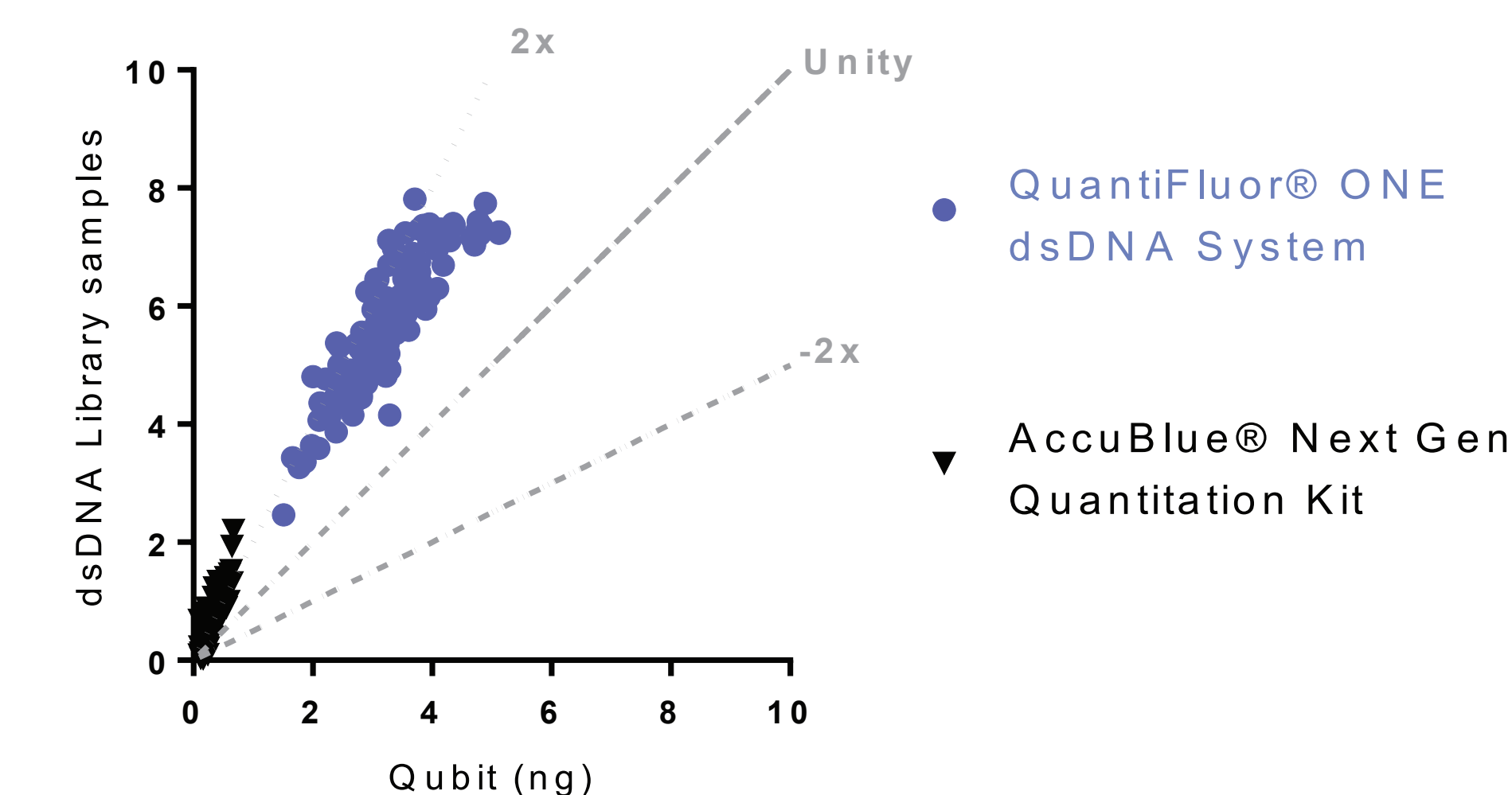


Figure 5. A library of dsDNA was quantitated by AccuBlue NextGen dsDNA Quantitation Kit and QuantiFluor ONE dsDNA System. This data was plotted against the values of the same cDNA libraries quantitated by Qubit™ dsDNA (HS) Assay Kit. The graph displays a comparable correlation between Qubit values and the ones generated from the 2 kits mentioned above.

Summary

- AccuBlue NextGen dsDNA Quantitation Kit and QuantiFluor ONE dsDNA System can be miniaturized up to 20 fold using the Echo 525 Liquid Handler.
- Standard curves generated for both kits in miniaturized reaction volumes display R² values comparable to the kit recommended protocols.
- These kits can be effectively used to quantitate cDNA samples via an Echo 525 Liquid Handler.