Technical Overview

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Qubit Assay Using the Agilent BioTek Synergy Neo 2 Hybrid Multimode Reader

Introduction

The Qubit dsDNA BR assay is currently used in a large number of laboratories for DNA quantification; however, the kit is designed for a low-throughput, microcentrifuge tube format. Therefore, laboratories looking for increased throughput are adapting the reagent for use in a 96-well microplate assay format. This technical overview describes the materials, methods, and Agilent BioTek Gen5 data analysis software parameters required to establish a standard curve and perform a DNA quantification with the Qubit dsDNA BR assay reagent in a 96-well plate format using an Agilent BioTek Synergy Neo2 hybrid multimode reader. The minimal number of standards required to accurately model the range for the Qubit reagent (0.1 to 1,000 ng/µL) is determined to be four: 0.1, 1, 10, and 1,000 ng/µL.

Experimental

Materials

- Qubit dsDNA BR Assay Kit (part number Q32850) from Molecular Probes (Eugene, OR)
- Purified herring sperm DNA (part number D6898) in TE buffer from Sigma-Aldrich (St. Louis, MO)
- TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0)
- Solid black, 96-well microplates (part number 3915) from Corning (Corning, NY)
- Single- and eight-channel pipettor with 10 to 200 µL range

Assay setup

- The Qubit working solution was prepared by diluting the Qubit BR reagent (200x) 1:200 in Qubit dsDNA BR buffer.
- A 4-, 5-, 6- and 8-point serial log dilution series of standards ranging between 1,000 and 0.1 ng/µL was created from a 2.2 mg/mL stock solution of herring sperm DNA in TE buffer. This is the stated validated range for the Qubit dsDNA Assay Kit.
- The actual concentration of each standard was verified in a Synergy Neo2 through A_{260} read in a 96-well plate with path length correction engaged.
- A multichannel pipettor was used to add 10 µL of DNA standard to the 96-well plate, followed by 190 µL of Qubit working solution (for a final volume of 200 µL) and incubated for five minutes at room temperature, then read on a Synergy Neo2.

Synergy Neo2 setup

- A Corning #3915 black-bottom, 96-well microplate is used for this assay, however the default "96 WELL PLATE" can be selected as the Plate Type.
- Fluorescence intensity is measured using a single top PMT with GFP filter cube (part number 1035108; Ex 485/20 nm, Em 530/25, DM 510).

Gen5 recommended protocol setup

Procedure

1. Select the 96-well plate from the Plate Type dropdown menu.
2. Read method:
   - Detection method: Fluorescence intensity
   - Read type: Endpoint/kinetic
   - Optics type: Filters
3. Read step: Fluorescence intensity
   - Step label: dsDNA Std Crv
   - PMT: Single
   - Filter set 1: 485/528
   - Optics position: Top
   - Gain: 50

Plate layout

See Figure 1.

Data reduction

Transformation	
Type	Blank
Data In	dsDNA Std Crv: 485/20, 528/20
Blank Wells	BLK
New Dataset Name	Blanked dsDNA Std Crv: 485/20, 528/20
Formula	X-BLK
Curve Analysis	
Type	Standard curve
Data In	
Well ID	STD
X-Axis Data	<Concentrations/Dilutions>
Y-Axis Data	Blank dsDNA Std Crv: 485/20, 528/20
Downsize Image	50%
Curve Fit	
Method	4-Parameter nonlinear regression
Axis Presentation	Log (both X and Y)
Y-Axis Data	Use average of replicates
Exploration Factor	1.1
Parameter Constraints	None
Formula	$Y = (A - D)/[1+(X/C)^B] + D$
Data Output Name	[DNA]

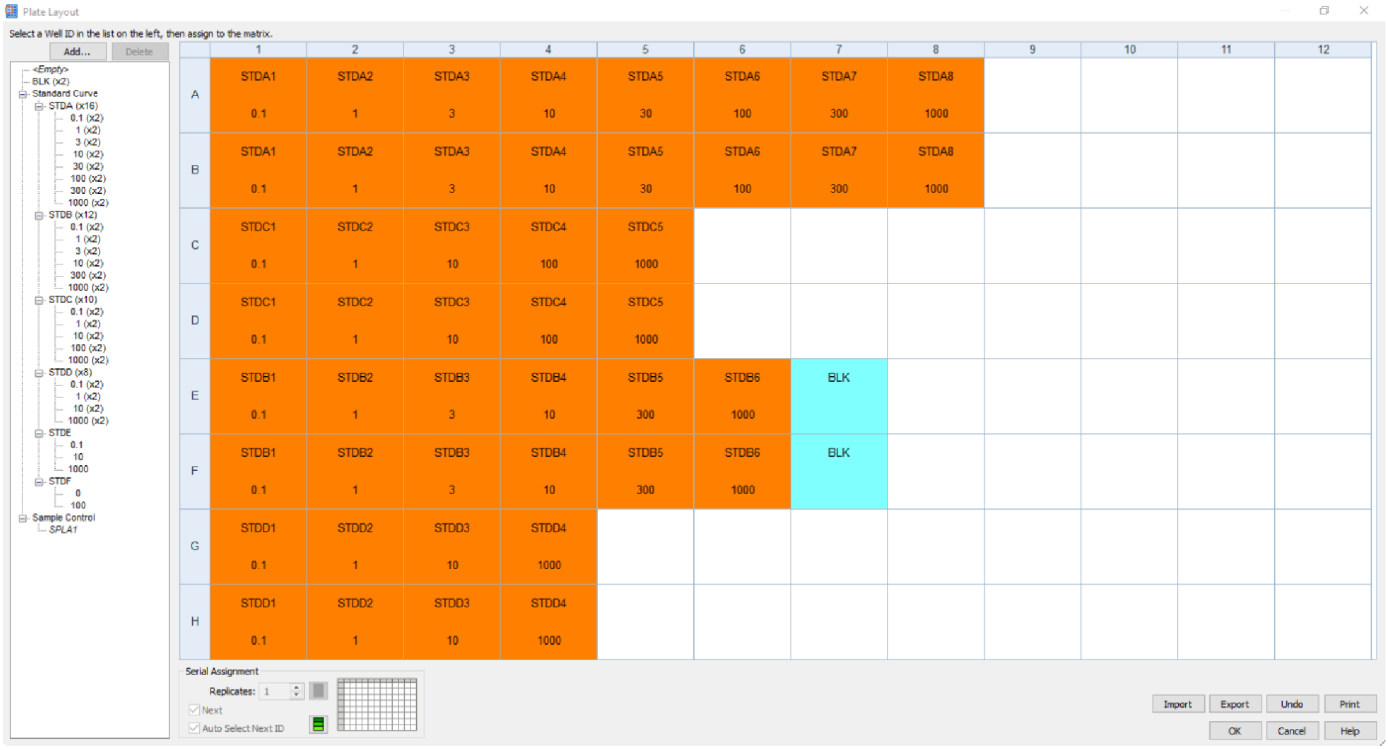

Figure 1. Plate Layout window.

Results and discussion

The composite data were plotted in Agilent BioTek Gen5 for standard curves with four (Figure 2A) and eight (Figure 2B) standards. The plot shows excellent correlation between standard curves fit using a four-parameter nonlinear regression analysis generated with as little as four standard concentrations; 0.1, 1.0, 10, and 1,000 ng/µL dsDNA.

Conclusion

The use of fluorescent reagents can increase the specificity and sensitivity of nucleic acid quantification. The use of Qubit dsDNA BR reagent in conjunction with the Agilent BioTek Synergy Neo2 hybrid multimode reader allows dsDNA quantitation with as few as four DNA standards in a 96-well microplate format. The curve fit produced with four standards is comparable to that of standard curves produced with as many as eight standards, allowing significant reagent savings.

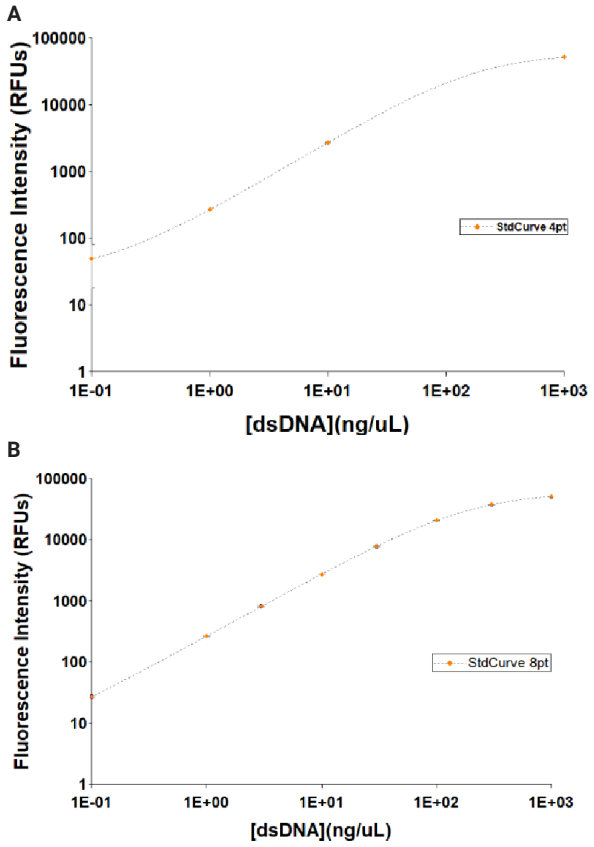

Figure 2. Standard curves using four (A) and eight (B) standards.

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