Qubit dsDNA assay specificity in the presence of single-stranded DNA

We offer two assays for quantifying double-stranded DNA (dsDNA) using the Invitrogen™ Qubit™ Fluorometer: the Qubit™ dsDNA HS (for High Sensitivity) Assay (Cat. No. Q32851) and the Qubit™ dsDNA BR (for Broad Range) Assay (Cat. No. Q32850). One advantage of these assays is their specificity for dsDNA. This study was carried out to investigate whether Qubit dsDNA quantitation assays accurately determine the concentration of dsDNA when the samples contain short or long single-stranded DNA (ssDNA).

Summary

The Qubit dsDNA HS Assay Kit exhibits high accuracy and precision for pure dsDNA samples in the range of 1–500 ng/mL. Likewise, the Qubit dsDNA BR Assay Kit shows similarly high accuracy and precision for pure dsDNA samples in the range of 0.01–5 µg/mL. This study shows that both Qubit dsDNA assays detected only 2–10% of pure ssDNA for the majority of the range. Similarly, when an equal mass of ssDNA was added to dsDNA, the results generally changed by less than 10% from the results obtained with dsDNA alone.

Experimental method

We tested 2 types of ssDNA, long and short, in both assays. Specifically, we tested an 18-mer oligonucleotide (M13 sequencing primer, sequence 5´ TGTAAAACGACGGCCAGT 3´) and viral ssDNA isolated from M13mp18 phage (7,249 bases, New England Biolabs, Cat. No. N4040S). These were tested against, or in combination with, lambda dsDNA (Invitrogen™ λ DNA, Cat. No. 25250010) using the Qubit dsDNA BR Assay Kit and the Qubit dsDNA HS Assay Kit.

Results

Assaying ssDNA alone

First, we tested each type of DNA (oligo DNA, long ssDNA, and dsDNA) separately in each assay. For both the oligonucleotide and the long ssDNA, the Qubit dsDNA HS and BR Assays detected less than 10% of the actual concentration of ssDNA (calculated according to the manufacturer's reported concentration) in the sample (Figures 1A and 1B). These results show that the Qubit dsDNA assays are highly selective for dsDNA compared to ssDNA.

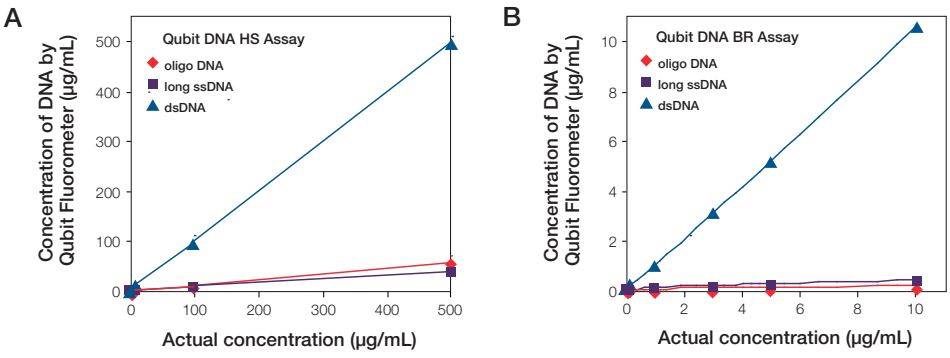

Figure 1. Detection of single-stranded DNA by the Qubit dsDNA HS (A) and BR (B) Assays. Duplicate samples of long ssDNA (squares), oligo DNA (diamonds), or lambda dsDNA (triangles) were added to the Qubit dsDNA HS Assay at concentrations of 0.5 to 500 ng/mL in the assay tube and to the Qubit dsDNA BR Assay at concentrations of 0.01 to 10 µg/mL in the assay tube, according to kit protocols. All stock samples (before dilution in the assay tube) were in 10 mM Tris, 1 mM EDTA (pH 7.5) buffer.

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Assaying dsDNA in the presence of ssDNA with the Qubit dsDNA HS Assay Kit

We also tested dsDNA in the presence of ssDNA in the Qubit dsDNA assays to see if ssDNA would interfere with dsDNA quantitation. For the Qubit dsDNA HS Assay at a 1:1 ratio of oligo DNA concentration to dsDNA concentration, the dsDNA concentration determined by the assay was within 11% of the value without any oligo DNA for all concentrations tested (Figure 2). At a 1:1 ratio of long ssDNA to dsDNA for low concentrations of DNA, the dsDNA concentration determined by the assay was within 8% of the value without any ssDNA (Figure 2A and 2B). However, at higher concentrations of nucleic acid in an assay that contained a 1:1 ratio of long ssDNA to dsDNA, the concentration of dsDNA determined by the assay was lower than the value without ssDNA by 21% (Figure 2C). Therefore, for samples containing at least 400 ng/mL dsDNA where ssDNA contamination is a possibility, we recommend the Qubit dsDNA BR Assay.

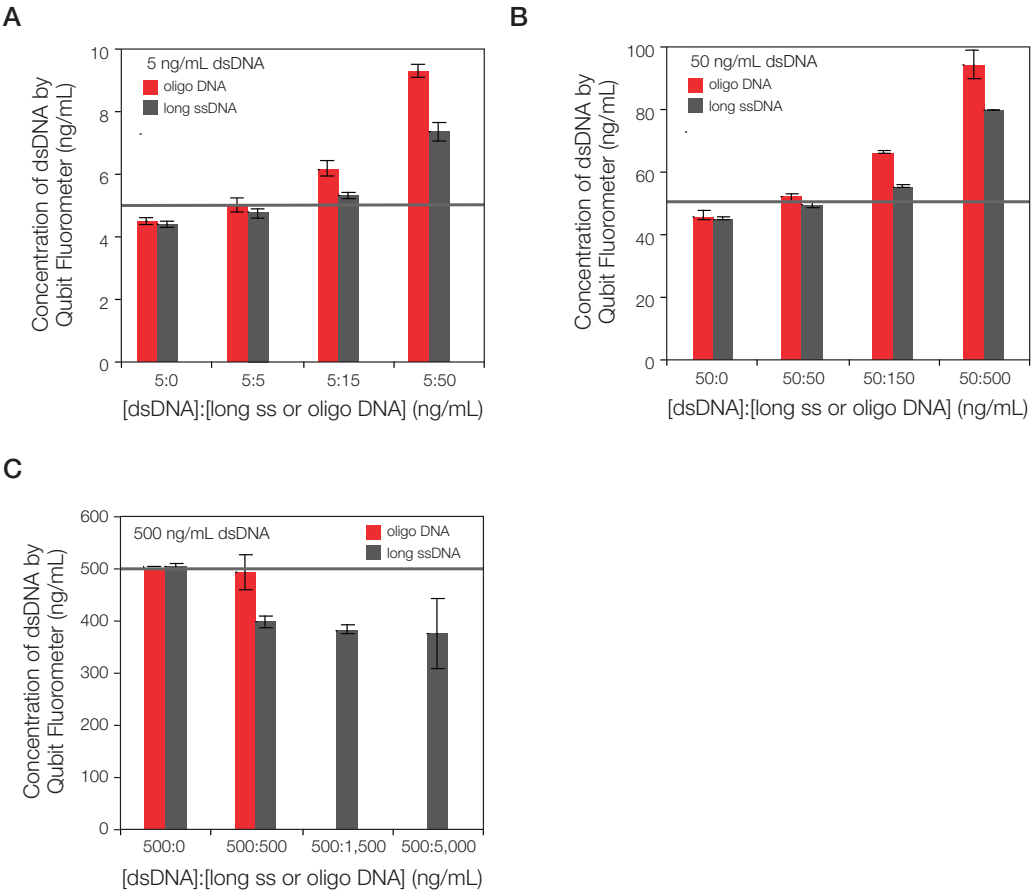

Figure 2. Double-stranded DNA in the presence of oligo DNA or long ssDNA in the Qubit dsDNA HS Assay on the Qubit Fluorometer. Duplicate samples of dsDNA were mixed with 0, 1X, 3X, or 10X concentrations (relative to the dsDNA concentration) of oligo DNA or ssDNA and added to the Qubit dsDNA HS Assay at dsDNA concentrations of 5 ng/mL **(A)**, 50 ng/mL **(B)**, and 500 ng/mL **(C)** in the assay tube. All stock samples (before dilution in the assay tube) were in 10 mM Tris, 1 mM EDTA (pH 7.5) buffer. The horizontal lines indicate actual concentrations of dsDNA.

Assaying dsDNA in the presence of ssDNA with the Qubit dsDNA BR Assay Kit

For the Qubit dsDNA BR Assay, at a 1:1 ratio of oligo DNA concentration to dsDNA concentration, the dsDNA concentration determined by the assay was within 3% of the value without oligo DNA for all concentrations tested. At a 1:1 ratio of long ssDNA to dsDNA, the dsDNA concentration determined by the assay was within 11% of the value without ssDNA for all concentrations tested (Figure 3).

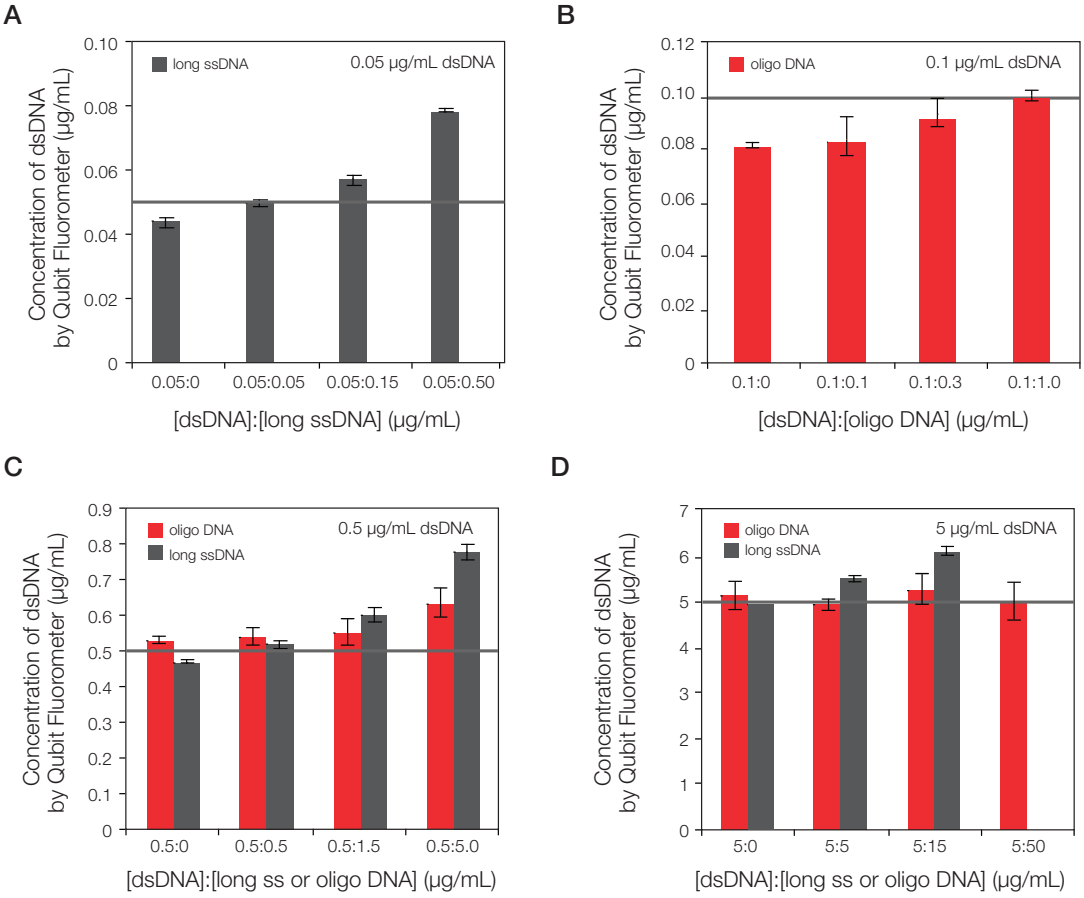

Figure 3. Double-stranded DNA in the presence of oligo or long ssDNA in the Qubit dsDNA BR Assay on the Qubit Fluorometer. Duplicate samples of dsDNA were mixed with 0, 1X, 3X, or 10X concentrations (relative to the dsDNA concentration) of oligo DNA or long ssDNA and added to the Qubit dsDNA BR Assay at dsDNA concentrations of 0.05 µg/mL **(A)**, 0.1 µg/mL **(B)**, 0.5 µg/mL **(C)**, and 5 µg/mL **(D)** in the assay tube. All stock samples (before dilution in the assay tube) were in 10 mM Tris, 1 mM EDTA (pH 7.5) buffer. The horizontal lines indicate actual concentrations of dsDNA.

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Conclusion

The concentration detected for a mixture of equal masses of dsDNA and ssDNA was generally within 10% of the concentration determined for dsDNA alone. For example, a mixture of 0.50 µg/mL dsDNA and 0.50 µg/mL long ssDNA was measured as 0.52 µg/mL dsDNA using the Qubit dsDNA BR Assay. However, at the high end of the dsDNA concentration range for the Qubit dsDNA HS Assay, with equal masses of double-stranded and long single-stranded DNA, the concentration determined by the assay was only 80% of the actual dsDNA concentration. For this reason, for concentrations of dsDNA above 400 ng/mL (in the assay tube), when long ssDNA contamination is suspected, we recommend using the Qubit dsDNA BR Assay. When concentrations of oligo or long ssDNA exceeded the concentration of dsDNA by three-fold or more, the concentration of dsDNA determined by either assay was in most cases more than 10% higher than the actual dsDNA concentration. Table 1 summarizes the results.

Table 1. Summary of ssDNA contamination results using the Qubit dsDNA HS and BR Assays.

Amount of ssDNA tolerated in the assay*		
	Qubit dsDNA HS Assay	**Qubit dsDNA BR Assay**
18-mer oligo ssDNA	1:1 ssDNA:dsDNA, across the full assay concentration range	1:1 ssDNA:dsDNA, across the full assay concentration range
M13mp18 phage (long) ssDNA	1:1 ssDNA:dsDNA, up to 400 ng/mL†	1:1 ssDNA:dsDNA, across the full assay concentration range

* Level of ssDNA contamination tolerated by the assay with <11% perturbation of results.

† For dsDNA concentrations above 400 ng/mL in the assay tube, where long ssDNA contamination is suspected, the Qubit dsDNA HS Assay is not recommended. We recommend using the Qubit dsDNA BR Assay.

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