Direct Double-Stranded DNA Quantitation from PCR Reactions

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Abstract

For convenience and for PCR products that are challenging to purify with high efficiency (*e.g.*, chemically modified DNAs), it is often desirable to quantitate synthesized DNA directly from a PCR reaction. Here we describe the use of a high-sensitivity Quant-iT™ PicoGreen® dye-based fluorescence assay to quantitate PCR-synthesized, double-stranded, low molecular weight, 5'-modified DNA probes in the presence of single-stranded primers and deoxyribonucleotides.[1]

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Guidelines

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Before start

1. Calibrate your fluorometer as directed by manufacturer's instructions. For example, if using a Thermo Fisher Scientific Qubit 2.0 fluorometer and Quant-iT™ dsDNA High-Sensitivity (HS) Assay Kit, perform a two-point calibration with samples containing 0 ng and 100 ng λ DNA, provided.
2. It is helpful to retain an aliquot (2 µL) of a representative PCR reaction before amplification. Store on ice. Such serves as a blank for subsequent PCR quantitation.

Materials

Qubit™ dsDNA HS Assay Kit Q32851 by Invitrogen - Thermo Fisher

Axygen 0.5 mL PCR tubes, 0.5 mL, thin wall, clear, flat caps PCR-05-C by Corning

Protocol

Prepare Quant-iT™ dsDNA High-Sensitivity Working Reagent [WR1]

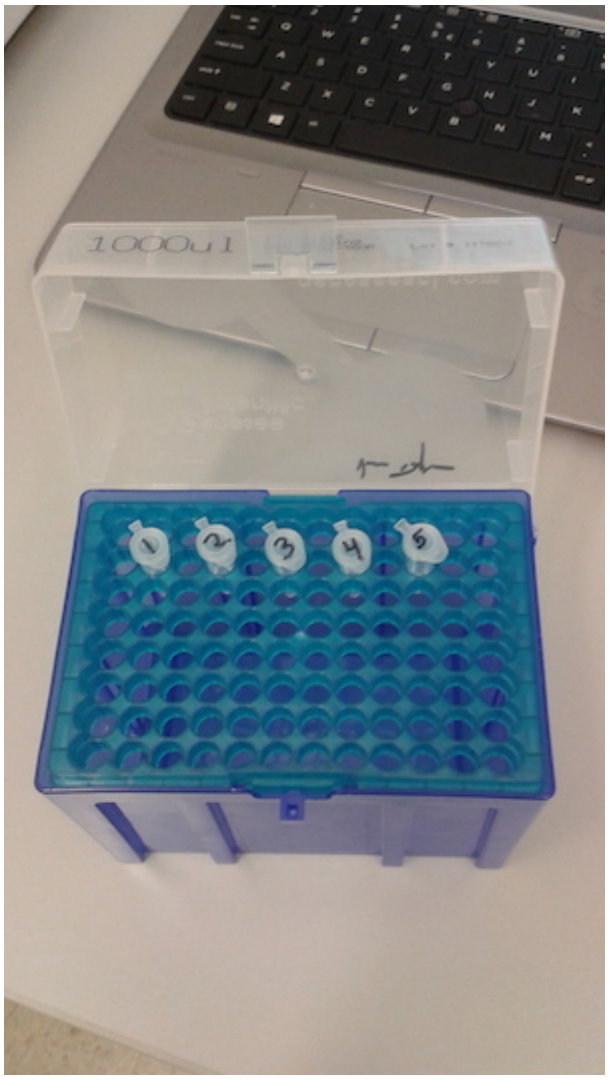
Step 1.

Dilute Quant-iT™ dsDNA HS reagent 1:200 with Quant-iT™ dsDNA buffer to a final volume of 200 µL * 1.1 * (total number of assays to be performed). For example, to perform 10 assays, prepare 1100 µL WR1. Prepare in polypropylene microcentrifuge tube, vortex briefly to mix thoroughly. Store shielded from direct light, room temperature. Use within 3 h preparation. Note: it is possible to substitute water for Quant-iT™ dsDNA buffer in the preparation of WR1. However, measured values for dsDNA will be 33% lower than those obtained with Quant-iT™ dsDNA buffer.

Prepare assay samples

Step 2.

Label high-clarity 0.5 mL polypropylene microcentrifuge tubes on their caps. Axygen thin-wall PCR tubes (PCR-05-C) work well. Include blank if desired. Place tubes in a rack. A used 1000 µL pipet tip rack works well for this purpose.



Prepare assay samples

Step 3.

Aliquot 200 µL WR into each tube. Add 2 µL aliquot from completed PCR reaction. Vortex to mix thoroughly; centrifuge briefly to coalesce. Return tubes to rack. Incubate at room temperature for at least 5 min. Fluorescence values increase slightly with time (≤ 10%) and are stable for at least 1 h.

Read assay samples

Step 4.

Following instructions are for a Qubit™ 2.0 fluorometer. Adjust accordingly for fluorometer being used. Note that the Quant-iT™ dsDNA HS reagent fluorescence excitation maximum is 502 nm (blue) and emission maximum is 523 nm (green).

Read assay samples

Step 5.

Turn on Qubit 2.0 fluorometer. Using touchscreen, “Choose Your Assay: DNA”, “dsDNA High Sensitivity”. “Read New Standards?” If desired “Yes”, otherwise “No”. Insert sample tube, close lid, “Read Next Sample”. Record data by hand or upload upon completion to a USB flash drive.

Read assay samples

Step 6.

Note that the values shown are those for dilution in WR1; units are ng/mL. To determine double-stranded DNA concentration in PCR reaction, multiply values by 100 and convert to ng/µL. For PCR reactions with New England Biolabs *Taq* DNA polymerase and standard *Taq* buffer, synthesizing short 63-bp IRD7-labeled REPSA selection templates, we routinely obtain yields of 36 ng/µL product dsDNA. Blank samples without any added DNA template, whether stored on ice or processed through 20 PCR cycles, routinely show background values of 11.5 ng/µL. Note that the values above will be dependent on primer concentrations, sequence, and lengths of PCR products generated.