Qubit Quantification of DNA

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1 Introduction

The protocol uses a Qubit Fluorometer (Invitrogen) to quantify DNA, e.g. from environmental DNA extractions or purfied PCR products. The Qubit kit is stored at 4°C (Quant-iT dsDNA Assay Kit, High Sensitivity, 1000 assays. Invitrogen product no. Q33120).

2 Protocol

2.1 Prepare working solution

Prepare a master mix of working solution by diluting Florescent Dye (Quant-iT dsDNA HS reagent) to Quant-iT dsDNA HS buffer at a final ratio of (1:199): add 1 µl of dye into every 198 µl of Qubit buffer. Ensure that all solutions are fully liquid before mixing; the Florescent Dye may crystallize during storage at 4°C.

2.2 Calibrate Qubit

Before quantifying DNA, the Qubit machine should be calibrated using standard solutions. However, if the machine has been recently calibrated, select "use last calibration" when prompted on screen.

2.3 Prepare DNA samples

Prepare sample solution by adding 1-10 µl of each DNA sample to working solution (190-199 µl, respectively). Each sample should be prepared in a separate 0.3 ml Qubit tube (Qubit assay tubes, Invitrogen product no. Q32856), where the final volume of the prepared sample solution is 200 µl (DNA sample mixed with working solution).

2.4 Measure Sample Concentration

Measure the DNA concentration of each sample, as follows:

- On the Qubit screen prompt, chose "Quant-it dsDNA, HS" (high sensitivity DNA quantification)
- Choose "use last calibration"
- Insert sample into slot, close plastic cover, and press "GO" button
- Select "calculate the sample concentration" and then choose the input sample volume used in Step 2.3
- If the Qubit cannot quantify DNA ("sample concentration too low"), prepare a new sample solution by increasing the amount of DNA diluted into the working solution (Step 2.3, for example, use 5 µl instead of 1 µl of your DNA sample). Re-measure new sample solution.