dsDNA quantification using Sybr Green I V.2

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This is a simple protocol that uses Sybr Green 1 and a microplate reader to quantify dsDNA concentrations in unknown samples. This is best suited for situations where a high number of samples need to be quantified (e.g. normalisation of samples before pooling for Illumina sequencing). For smaller sample numbers (e.g. < 50 samples) it will be more efficient to simply use a qubit or nanodrop.

You need to pick an appropriate standard concentration range for the type of samples you will be testing. For genomic DNA it is probably better to have a lower concentration range (0-10 ng/µl) whereas for PCR products, standards will probably need to be much more concentrated (0-50 or maybe even 100ng/µl).

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10,000X Sybr Green 1
TE buffer
Black flat bottomed microtitre plates
Plate reader

Part 1 - Make a dilution series of a Lambda DNA standard:

1 **Select which standards we need to make and use based on the strength of the samples being tested.**
Step 1 includes a Step case.
Genomic DNA
PCR products

step case

Genomic DNA

The following steps outline an appropriate dilution series for weaker DNA solutions.

2 Using NEB lambda DNA (item N3011S) make a **10 ng/µl** dsDNA standard in TE (20ul of 500ng/µl lambda DNA + 980 µl of TE).
- *TE =10 mM Tris HCl, 1 mM EDTA, pH 8*

3 Serially dilute this as below:

A	B	C	D
	lambda DNA (µL)	TE (µL)	lambda DNA (ng/µl)
1	1000 (from 10 ng/µl stock)	0	10
2	500 (from dilution 1)	500	5
3	500 (from dilution 2)	500	2.5
4	500 (from dilution 3)	500	1.25
5	500 (from dilution 4)	500	0.625
6	500 (from dilution 5)	500	0.313
7	500 (from dilution 6)	500	0.156
8	0 (from dilution 7)	500	0

Table 1: Dilution series for control DNA samples

4 Aliquot 20 µl of each of these into PCR strips and store at -20 °c until needed. Thaw one for each run on the plate reader.
- *If you have previously made your dilution series start at this point.*

5 Make a 100x Sybr Green 1 solution (5ul Sybr Green 1+ 495 µl of TE).
- *This can be pipetted out into 50 - 100 µl aliquots and stored at -20 °c. Once thawed, do not reuse.*
- *96 µl of 100x Sybr Green 1 is required per plate of samples.*

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- *If you have previously made your 100X Sybr Green 1 start at this point.*

6 Make a solution of 1x Syber Green 1 as below:

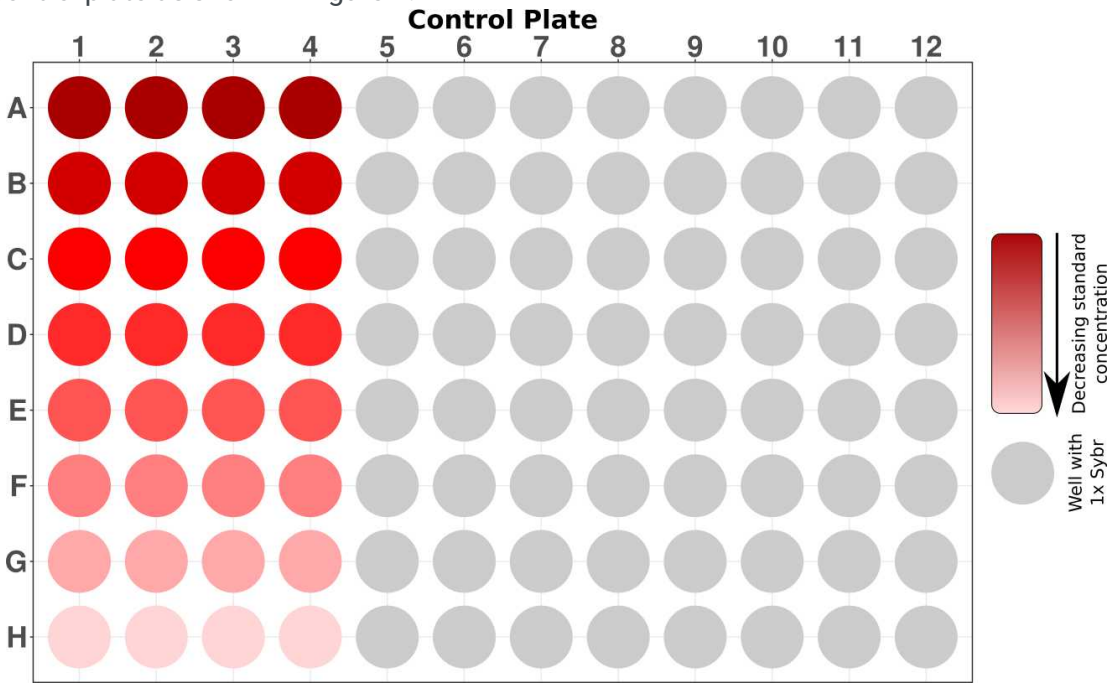
A	B	C
	Volume for one sample	Volume for n + 136 samples
TE	97	
100X Sybr Green 1	1	
Total	**98**	

Table 2: Illustrative table for calculating the dilution to make enough 1x Sybr Green 1 for n samples

7 Distribute 98 µl of Sybr Green 1 working solution into the first 4 columns of a Nunc black microtiter plate and 100 µl into the last 8 columns.

- *This is best done using a multichannel electronic repeater or 96 well pipette if you are testing a lot of samples.*

8 Using a p10 multichannel pipette, add 2ul of each standard into the first 4 columns of the control plate as shown in figure 1.



General control plate layout

Pipetting accuracy is key here. Accurate standards will greatly improve the accuracy of the samples. You can increase the number of standard replicates if you wish.

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9 Distribute 98 µl of sybr green 1 working solution into n wells of a nunc black microtiter plate.
- *Use multiple plates for more than 96 samples.*

10 Add 2 µl of sample dsDNA into each of the sample wells.

11 Seal with a clear plate seal.

12 Incubate at room temperature in the dark for 10 minutes. **00:10:00**

13 Run samples on your plate reader according to manufacturers instructions with an excitation wavelength of 485 nM and an emission wavelength of 535 nM.

14 Check that your calibration curve has a very high R^2 value (greater than 0.95) and multiply your measured concentrations by 2.5 to get a final concentration.

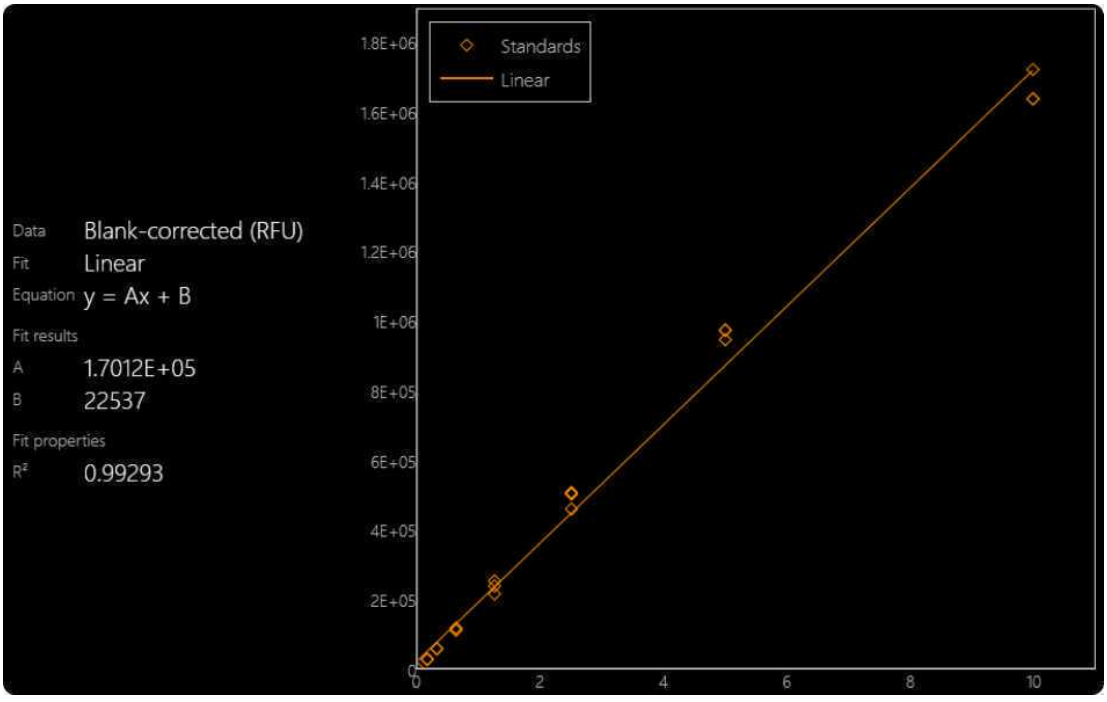

Figure 2: A typical calibration curve from the above protocol

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