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dsDNA quantification using Sybr Green I V.1

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protocol .

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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.

James JN Kitson 2021. dsDNA quantification using Sybr Green I. **protocols.io** https://protocols.io/view/dsdna-quantification-using-sybr-green-i-b2f7qbrn James Kitson

The volumes required for n samples in table 2 were unclear, I have updated the table headers to fix this.

Sybr Green 1, dsDNA, quantification

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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 Using NEB lambda DNA (item N3011S) 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*

2 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 samples

3 Aliquot 25 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.*

Part 2 - Make and distribute Sybr Green 1 working solution.

4 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.*
- *If you have previously made your 100X Sybr Green 1 start at this point.*

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

A	B	C
	Volume for one sample	Volume for n + 136 samples
TE	94	
100X Sybr Green 1	1	
Total	**95**	

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

Part 3 - dilute Sybr Green 1 working solution and add DNA.

6 Distribute 95 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.*

7 Using a p10 multichannel pipette, add 5ul of each standard into the first 4 columns of the control plate as shown in figure 1.

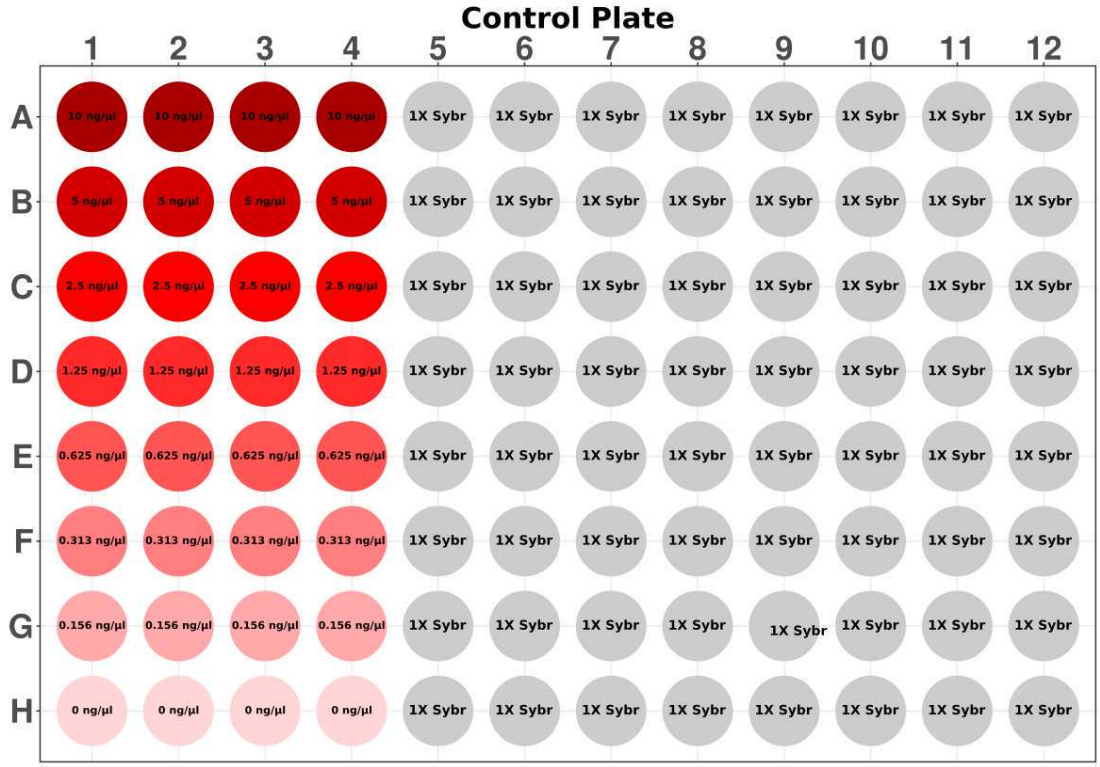

Figure 1: Control plate layout for microplate reader when reading DNA samples.

8 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.*

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

10 Seal with a clear plate seal.

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

Part 4 - load microplate reader and run the assay.

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

13 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.

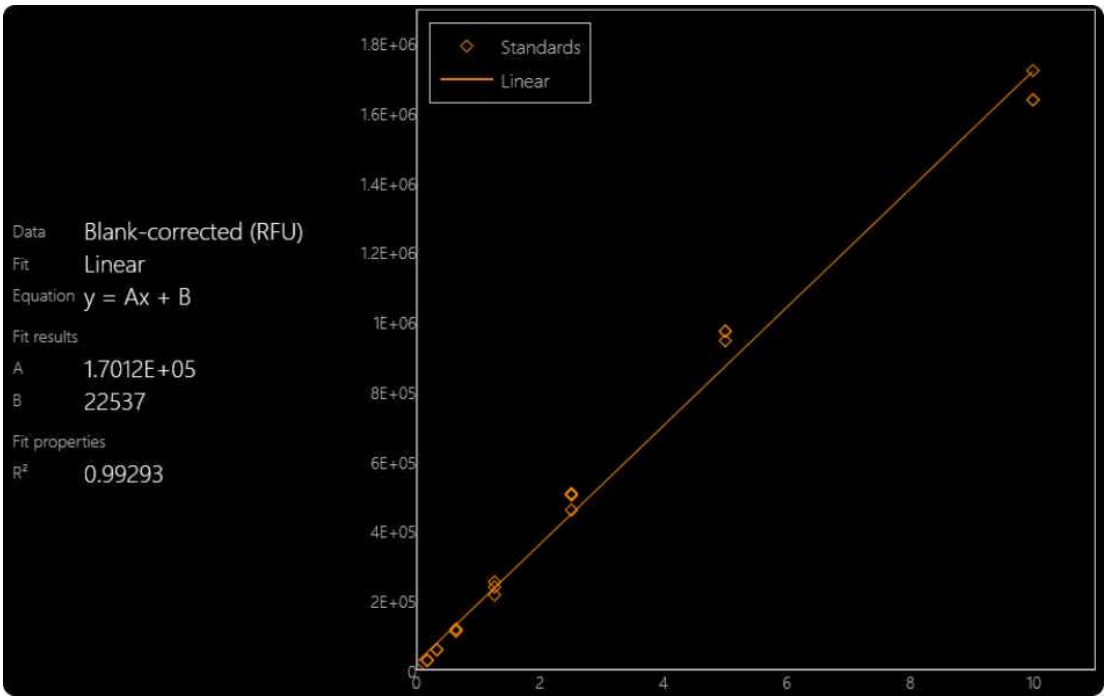

Figure 2: A typical calibration curve from the above protocol