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PicoGreen dsDNA Quantitation Kit (RT-punch tissue): Quantification Protocol using a Linear Standard Curve of quantities ranging from 0 to 1000 pg/uL in a 125pg/uL increment

*** Important:** Read the whole protocol before using any reagent.

Stock Reagents:

1. PicoGreen dsDNA quantitation reagent **(200X)**: 1mL solution in DMSO either in single vial or in 10 vials of 100uL.
2. TE buffer **(20X)**: 25mL of 200mM Tris-HCl; 20mM EDTA, pH 7.5.
3. Lambda DNA standard: 1mL of 100ug/mL (100ng/uL) in TE.

PROTOCOL

1. Calculate the total volume of TE buffer needed for all the reactions with the following formula: (# of samples+standards+1) X 0.5mL = Vol of 1X TE. Then dissolve the 20X TE stock to 1X to that final volume.
2. Dissolve 1uL of Lambda Stock into 199uL of 1X TE in a 1.5mL Eppendorf tube. This tube will be used to make the dilution gradient. **Name it Lambda #2**.
3. Take Lambda #2 and add the following volumes in five different 1.5mL tubes:

----------	Blank	Std 1	Std 2	Std 3	Std 4
Lambda	**X**	7.82uL	15.63uL	31.25uL	62.5uL
TE buffer (1X)	250uL	242.18	234.37uL	218.75uL	187.5uL
Concentration	0 pg/uL	15.64pg/uL	31.25pg/uL	62.5pg/uL	125pg/uL

4. Make 1:100 dilutions of your samples in 1.5 Eppendorf tubes for a final volume of 250uL (2.5uL into 247.5uL 1X TE) and label them: 1:200 for every 1:100 plus the particular name of the sample.
5. Calculate the total volume of PicoGreen needed for all the reactions with the following formula: (# of samples+standards+1) X 0.25mL = Vol of 1X PicoGreen. Then dissolve the 200X PicoGreen stock to 1X to that final volume. Use a 15 or a 50mL conical tube (depending on the volume) and **cover it with aluminum paper to avoid light degradation of the reagent**.

6. Take your standards and your samples and add 250uL of 1X PicoGreen and incubate them for 3 minutes in the dark:

---------- ----------	Blank	Std 1	Std 2	Std 3	Std 4	Sample 1:200
Lambda	**X**	7.82	15.63uL	31.25uL	62.5uL	**X**
TE buffer (1X)	500uL	242.18	234.37uL	218.75uL	187.5uL	**X**
1:100 sample	**X**	**X**	**X**	**X**	**X**	250uL
PicoGreen (1X)	250uL	250uL	250uL	250uL	250uL	250uL
Final Concentration	0 ng/uL	7.82pg/uL	15.625pg/uL	31.25pg/uL	62.5pg/uL	?
Final Volume	0.5 mL	0.5mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL

7. In a 96 well plate make the following arrangement adding 100uL of the final mixture in each well:

	1	2	3	4	5	8	9	10	11	12
A	***Blank***	*Std1*	*Std2*	*Std3*	*Std4*	**1:200**				
B	***Blank***	*Std1*	*Std2*	*Std3*	*Std4*	**1:200**				
C	***Blank***	*Std1*	*Std2*	*Std3*	*Std4*	**1:200**				
D	***Blank***	*Std1*	*Std2*	*Std3*	*Std4*	**1:200**				
E										
F										
G										
H										

8. The empty wells have enough space to run 17 samples of 1:100. **If the number of samples is more than 17, then the remaining samples will have to be measured in a different plate with another set of standards.**
9. Use a spectrofluorometer to read the plate. Make sure to name every sample correctly (Standards, Blanks, and Unknowns) using the software of the machine. Make sure to indicate the theoretical concentration of each standard, and to indicate the dilution ratio of the unknowns also.
10. Use an excitation spectrum of 480-485nm and measure the fluorescence emission intensity on a spectrum of 520 nm.
11. After the reading is done, go through the results and look at the R value of the graph. Values above 0.991 are considered acceptable for low accuracy measurements. For more accurate measurements (Southern Blot, standard curve for real-time PCR, etc) values above 0.998 are required for the conversion.