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🌐 DNA quantification in plates (picogreen protocol)

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Protocol status: Working

We use this protocol and it's working

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Troubleshooting

Sample Prep + Standard Curve

1   Allow the Quant-iT PicoGreen reagent to warm to room temperature before opening the vial. PicoGreen is light sensitive; while thawing, wrap in aluminum foil, stick in desk drawer. Note: PicoGreen takes a long time to thaw—**maybe an hour**.

2   **Prepare a 1x working solution of TE** using 20x TE (supplied) and sterile di H_2O.

3   **Prepare a working solution of PicoGreen** by doing a 1:200 dilution (PicoGreen: final volume). Prepare solution in plastic falcon tube wrapped in aluminum foil, as the reagent is light sensitive. Do not use glass as PicoGreen may adsorb to glass.

   You need 48ul of total solution per sample. Make extra for errors.

4   **Prepare standard curve** using Lambda DNA standard (supplied at a concentration of 100ng/ul or 100ug/ml, comes with PicoGreen) and 1x TE in 8 tubes, as below. Load 2ul from each tube into one column (A12-H12). *You will need to increase these volumes if running >2 plates.*

Tube #	Contents		Concentration	Final volume
1	10 ul TE;	10 ul Lambda DNA	50.0 ng/ul	10 ul
2	10 ul TE;	10 ul from tube	125.0 ng/ul	10 ul
3	10 ul TE;	10 ul from tube	212.5 ng/ul	8 ul
4	4 ul TE;	6 ul from tube	37.5 ng/ul	10 ul
5	6 ul TE;	4 ul from tube	35.0 ng/ul	9 ul
6	8 ul TE;	2 ul from tube	32.5 ng/ul	10 ul
7	4 ul TE;	1 ul from tube	51.0 ng/ul	5 ul
8	10 ul TE		0.0 ng/ul	10 ul

5   Pipet **2ul sample into wells in Costar black microplate** (#3915), with the standard curve in wells A1-H1. Use one column of wells for a standard curve in each plate. Pipet your samples into the center of each well. If your DNA is in tubes, this process will take a long time, and you risk dehydration of the samples. You will need to work quickly, and cover or seal plates somehow after finishing one.

6   THE REMAINING STEPS SHOULD DONE BY A PLATE READER- You will need:
   Reservoir and PicoGreen working solution
   P200 multichannel pipet
   As many tips as you have plates/samples
   Waste container
   USB drive
   Samples in Costar plates (stack and use lid for top one)

Aluminum foil covers + piece of aluminum foil
Protocol
Writing utensil
Gloves

Plate Reader

7   Pour PicoGreen solution into reservoir and **pipet 48 ul PicoGreen working solution into each well**. Cover plate with aluminum foil covered Rainin lid. Cover reservoir with aluminum foil. Tip plate slightly and tap sides of plate gently but firmly onto bench to get liquid to cover the bottom of every well. This is difficult, but these wells have a minimum recommended volume of 75ul, and the liquid really should cover the bottom of the well to ensure an accurate reading. You will need to tap harder than you think, but be careful!

8   . **Incubate for 2-5 minutes, keeping foil cover on to protect from light.**

9   Read your samples on the plate reader, be sure to follow its specifications. Below is the settings used for the Willis lab:

Protocol settings for Willis_DNAquant50 protocol

Plate layout: 96 well, read all of wells
Excitation: 485 nm
Emission: 530 nm
Read from top
Endpoint reading
Detect fluorescence
Sensitivity (= Gain): 50

Calculating DNA concentrations

10   Plot your standard curve DNA concentrations in ng/ul (x) and fluorescence scores (y) as a scatter plot.

$$y = mx + b$$

In Excel:
Select a data point. Go to Chart→ Add Trendline. The line should be linear, and check the "display equation on chart" option, and the R^2 to make sure you didn't really mess up the standard curve, although that should be apparent from the line on the chart.

Solve for x to determine the concentration of your samples.