# Pico Green DNA Quantification

*Delaney Nolin & Rae Santora*

August 2019

Materials

- 1X TE
    - May already be aliquoted or may need to be made fresh from 20X stock
- Pico green 1:200
    - Should be in 10 µL aliquots in the 4°C fridge. Keep on ice and in the dark.
- 100 ng/µL $\lambda$ DNA standard
    - Should be in the 4°C fridge. Keep on ice.
    - Thermo ref: P7589, Lot 1639100
- Optically pure 96 well plates & caps
    - Thermo AB-0866 (caps)
    - Thermo AB-0600-L (plates)

Notes

- Turn the Stratagene qPCR machine on 20 min before running to allow the lamp to warm up (you must both turn on the machine and open the MxPro software in the computer for the lamp to begin warming up)
- Make sure to use 96 well plates that are optically pure or the assay may be inaccurate. Do not label the lids!
- Using the multi-channel pipette can really save you time, especially if you have a lot of samples!
- If you think you have a very low concentration of DNA you can add > 1 µL of DNA to each well
- Make sure than if you add more/less than 1 µL of DNA that you **multiply your results by the dilution factor!!** (e.g. if you use 5 µL, divide your final concentrations by 5).

Procedure

1. Turn on the computer
2. Prepare 1X TE if necessary, using PCR water (0.02 µm filtered, autoclaves MilliQ water) and the 20X TE stock solution
3. **Make the standard curve.** Prepare the standards as described in the table below in 0.5 mL Lo-bind tubes.

    NOTE: The "upper limit" of this assay is ~10 ng/µL, as it gets nonlinear above this threshold, so if you think your sample has more DNA than this, you will need to dilute it.

Table 1: standard curve dilution table.

| **Vol of previous tube ( uL)** | **Vol of TE to add (uL)** | **Final Concentration (ng/uL)** |
|---|---|---|
| 1 ( of 100 ng/uL STOCK SOLUTION) | 9 | 10 |
| 5 | 5 | 5 |
| 2 | 8 | 1 |
| 1 | 9 | 0.1 |
| 0 | 10 | 0 - BLANK |

4. Add standard curve (from table above) to plate by adding **1 µL of standard dilution** to each well first and **then** adding **14 µL of TE** to each well.

a. We recommend adding everything vertically so you can place lids on the plate as you finish each column.

b. By adding the DNA first, you can see the dot of liquid on the tube to make sure you have added it properly

Table 2: example 96 well plate layout.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 10 | SAMPLE | SAMPLE | SAMPLE | SAMPLE | SAMPLE | | | | | |
| 5 | 5 | SAMPLE | SAMPLE | SAMPLE | SAMPLE | SAMPLE | | | | | |
| 1 | 1 | SAMPLE | SAMPLE | SAMPLE | SAMPLE | SAMPLE | | | | | |
| 0.1 | 0.1 | SAMPLE | SAMPLE | SAMPLE | SAMPLE | SAMPLE | | | | | |
| blank | blank | SAMPLE | SAMPLE | SAMPLE | SAMPLE | SAMPLE | | | | | |
| | | SAMPLE | SAMPLE | SAMPLE | SAMPLE | SAMPLE | | | | | |
| | | SAMPLE | SAMPLE | SAMPLE | SAMPLE | SAMPLE | | | | | |
| | | BLANK | BLANK | BLANK | BLANK | BLANK | | | | | |

5. Add **1 µL of sample DNA** of unknown concentration to each designated well first, then add 14 µL of TE.
6. Prepare the **1:200 dilution of the stock Pico** and keep in the dark (we will often tape over the outside of the tube to keep it in the dark while on ice)
    a. Sample 1:200 Pico calculation for 40 samples:
        Final Volume: need 15 µL/well with sample or standard → 40 samples + 10 standards = 50 (16) = 800 µL (to allow for pipetting error)
        Stock Pico: $\frac{1}{200} = \frac{x}{800}$ 200x = 800  x = 4 µL Pico
        1X TE: 800 µL total - 4µL Pico = 796 µL 1X TE
7. Add 15 µL of 1:200 Pico to each well.
    a. Add carefully but quickly, as the Pico should be exposed to light for as little time as possible.
8. Vortex plate + spin down using "salad spinner" or centrifuge.
9. When finished, incubate the plate in the dark for 5 minutes.
10. Set up the computer while the plate is incubating.
    a. Select "Quantitative Plate Read"
    b. Set up each of your standards by entering their concentration, the unit (nanograms), and the dye (SYBR)
    c. Select all wells that have your sample DNA and label them as "unknown" with SYBR
11. Place the plate into the plate reader.
    a. Lift the heated metal lid of the 96-well sample holder inside the Stratagene and place the plate inside the sample holder (not on top).
12. Click "Run" and select "post-read"
13. After the run is complete, check the $R^2$ value of the standard curve. It should be ~0.99.
14. Check the concentrations of your samples in the text report:
    a. You can so some manipulations in the software if your $R^2$ value is a little low and visual inspection of the standard curve graph suggests it may be just one point that is throwing off the linearity.
    b. Your samples should all fall within the standard curve values (0-10 ng/uL). If some are larger, or ALL are extremely low (between 0-0.1), you may want to dilute the samples or use more than one uL and re-run the assay.
15. Export both the text report and the standard curve values to Excel workbooks by right clicking.