Pico Green DNA Quantitation

Reagents:

1x TE — Procedure calls for "assay buffer." Use water if diluted samples will be needed for PCR
Prepare from 20X concentrate (stored in -20)
Make ~1mL for each sample you are going to analyze, including duplicates and standards.

Picogreen — Prepare 0.5 mL for each sample to be analyzed, including duplicates and standards.
Mix from 200X stock stored in -20. Make dilutions in plastic as Picogreen can sorb to glass. Cover in foil and store in a cool place. Use within 2-3 hours of preparing

2 μg/mL DNA
Dilute from 50 x stock of λ DNA stored in -20. Amount varies based on standard curve. The table below is for a standard curve set up in a well plate.

DNA ng/mL	2 μg/mL DNA λ μL	TE μL	Picogreen μL
0	0	100	100
10	1	99	100
50	5	95	100
100	10	90	100
200	20	80	100
500	50	50	100

Sample Prep:

For soil samples prepare a 0.01 dilution of extracted DNA (5 μL in 495 μL water). Add 100 μL of diluted sample to each well and add 100 μL of picogreen dye.

Fluorometer Settings:

Turn on the fluorometer and the computer and allow the fluorometer to warm up for 15 minutes before using.

Under “Instrument” and “Read” the settings should be:

Fluorescence
Excitation 480 Slit Width 15
Emission 520 Slit Width 20
Integrate time 20 seconds

The local contact for the fluorometer is John Shannon jds1c@virginia.edu