

**PicoGreen Protocol**  
**May 31, 2018**

**Preparation of the Standard Curve**

1. Prepare standard A by diluting lambda DNA standard to 10ng/uL (high sensitivity) or 50ng/uL (broad range) in 1xTE. If high DNA concentration is expected, use the broad range dilutions; if low concentration is expected, use the high sensitivity curves.
2. Prepare remaining standards (B-G) by serially diluting standard A 1:1 with 1xTE. Mix each dilution by thoroughly vortexing and a quick spin down.
3. Add a blank made from 1xTE as standard H.

<b>Standard</b>	<b>Concentration (ng/uL) High Sensitivity</b>	<b>Concentration (ng/uL) Broad Range</b>	<b>Volume</b>	<b>Total DNA (ng) High Sensitivity</b>	<b>Total DNA (ng) Broad Range</b>
A	10	50	5	50	250
B	5	25	5	25	125
C	2.5	12.5	5	12.5	62.5
D	1.25	6.25	5	6.25	31.25
E	0.625	3.125	5	3.125	15.625
F	0.3125	1.5625	5	1.5625	7.8125
G	0.156	0.78125	5	0.78125	1.953125
H	Blank	0	5	0	0

4. We typically use the broad range standards and make 400uL of each standard so that we can pull from a stock (rather than making new standards each time).
5. Example serial dilutions...

A → 40uL Lambda (500 ng/uL) + 360uL 1xTE → 400uL of A (50 ng/uL)  
B → 200uL of A (50 ng/uL) + 200uL 1xTE → 400uL of B (25 ng/uL)  
C → 200uL of B (25 ng/uL) + 200uL 1xTE → 400uL of C (12.5 ng/uL)  
D → 200uL of C (12.5 ng/uL) + 200uL 1xTE → 400uL of D (6.25 ng/uL)  
E → 200uL of D (6.25 ng/uL) + 200uL 1xTE → 400uL of E (3.125 ng/uL)  
F → 200uL of E (3.125 ng/uL) + 200uL 1xTE → 400uL of F (1.5625 ng/uL)  
G → 200uL of F (1.5625 ng/uL) + 200uL 1xTE → 400uL of G (0.78125 ng/uL)  
H → 200uL 1xTE

**Preparation of the Unknown Samples**

1. If high concentrations are expected, dilute DNA in 1xTE. Otherwise, use stock DNA as is.

**Preparation of PicoGreen Master Mix**

1. Prepare Master Mix as described below.

Reagent	Per Sample (uL)	Per 96 Well Plate (uL)
1xTE	94.5	9450
200x PicoGreen	0.5	50
TOTAL	95	9500

### Preparation of Costar 96-well Assay Plate (black)

1. Add 5uL of each standard (A-H) in duplicate to a microplate.
2. Add 5uL of each sample to its own well in the microplate.
3. Add 95uL Master Mix to each Standard, Blank, and Sample.
4. Incubate for approximately 5min (no more than 10min) at room temperature in the dark (to avoid photobleaching).
5. Use the following settings to read the Costar black solid bottom plate (3915) on the BioTek Synergy MX Plate Reader (in the Genomics Core Facility):

Variable	Setting
Step Label	Default
Excitation	485
Emission	528
Optics Position	Top
Gain	Auto
Read Speed	Normal
Read Height	8.0mm

6. This information should be stored in the linda\_picogreen protocol saved in the Gen5.20 program on the Genomics Core Facility computer next to the BioTek.
7. Save results as an excel spreadsheet.

### Interpreting Results

1. Use the Pico\_Results\_Blank.xls template to interpret results.
2. Open the Data sheet.
3. Add RFU values from each standard to cells B3->B10 and H3->H10.
4. Select the standard curve with the higher  $r^2$  value and input the slope and baseline to cells E31 and E32.
5. Ensure that the value for STOCK supplied is correct in cell J31.
6. Add RFU values to cells B37->B132.
7. Paste plate map in upper right corner.
8. Paste all raw data in the Raw\_Data sheet.
9. The Results sheet should automatically populate.