PicoGreen Assay

***PicoGreen binds to DNA so handle accordingly: wear appropriate PPE.**
***Protect PicoGreen stocks and solutions from light.**

Step 1: Calculate # of samples and prepare 1X TE:

#____ of samples + 6 Standards = ______

Amount of 1xTE to prepare:
110µL (for picogreen solution) x ______ + 140 µL (for standard dilutions) = _______
Use 20xTE stock (provided) to prepare appropriate amount of 1xTE from above calculation (or use your own, 1X TE= 10 mM Tris 8, 1 mM EDTA).
Remove 140 µL for use of making Standards in next step.

Step 2: Prepare Standards:

Use 2µL of stock λ-DNA of 100 ng/µL (provided) + 18µL of 1X TE = 20µL of 10 ng/µL working solution, this is standard #1. Make five serial dilutions using 2 ul working solution into 18 ul TE to make the following standards:

#2) $\frac{(2\ \mu L)(10 ng/\mu L)}{(20 \mu L)} = 1\ ng/\mu L$

#3) $\frac{(2 \mu L)(1 ng/\mu L)}{(20 \mu L)} = 0.1\ ng/\mu L$

#4) $\frac{(2 \mu L)(0.1 ng/\mu L)}{(20 \mu L)} = 0.01\ ng/\mu L$

#5) $\frac{(2 \mu L)(0.01 ng/\mu L)}{(20 \mu L)} = 0.001\ ng/\mu L$

Put 10 uL of the original 10 ng/ul λ-DNA standard into a designated well on your plate (typically a 96 well full skirted black plate from MJ Research), and continue by adding 10 ul of dilutions 2-5 into the next series of wells. Put 10 ul TE with no DNA into the next well. Now you should have 6 standard wells containing 10, 1, 0.1, 0.01, 0.001, and 0 ng/ul of λ-DNA (make note of where each was put).

Step 3: Prepare PicoGreen Solution:

Dilute the 200X PicoGreen stock to 1X PicoGreen with 1X TE prepared above (excluding amount removed for Standards step).

Put 100µL of 1xPicoGreen in each well containing 10µL of Standard with pipetting up and down to mix.
Continue to add 100 µL of 1xPicoGreen to each well that will be used to measure your samples.
Add 10 µL of each sample to wells that have 100µL of 1X PicoGreen and pump up and down to mix.

Notes: Your standard curve should be linear to the 0.01 ng/ul sample, then it will bottom out, and 0.001 ng/ul will probably be the same as 0. Readings can be done right away, but they don't have to be: the dye:DNA mixes are stable for at least 24 hrs at 4°. It may also be possible to do readings in a 384 well format, talk to us if you're interested in trying that out.