**Pico-Green Assay:**

Note: Pico Green is light sensitive. Keep covered.

1. Make a working stock of 1x TE (20x TE lives in the fridge - small plastic vial, white cap, on the left side, second shelf).

*Dilute to make fresh 1x TE: See the 1xTE calculator spreadsheet in the drive*

Use a 15 or 50 mL conical (shelves above PCR machine)

1. Prepare the standards: label a set of PCR strip tubes A-H.
2. Add 100 µL 1x TE buffer to B-H.
3. Add 200 µL of 20 ng/µL lambda DNA (in common reagents box) into A.

For easy reference:

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Tube | A | B | C | D | E | F | G | H |
| 1x TE (µL) |  | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 20ng/µLLamda (µL) | 200 | 0 |  |  |  |  |  |  |

1. Use a serial dilution to complete the curve by taking 100 µL from A, mixing it into B via back-pipetting, taking 100 µL from B, mixing it into C, and so on. **STOP** before H, and discard the excess 100 µL. Do not continue the dilution through H because **H is your blank**. The strip tube should have 100 µL volume per tube.

The concentrations of your standards should now be as follows:

|  |  |
| --- | --- |
| **Well** | **[λ] in strip tube (ng/µL)** |
| A | 20 |
| B | 10 |
| C | 5 |
| D | 2.5 |
| E | 1.25 |
| F | 0.625 |
| G | 0.3125 |
| H | 0 |

1. Because diluting the standards is tricky, you should run one test with just the standards first, this way you don’t waste reagents to later find out your standard curve is not usable.
2. Create a master mix just for your standards, combining the Pico Green reagent (in enzyme box in -20°C freezer) and 1x TE as follows:
   * 1. First thaw the pico green reagent. PUT FOIL OVER IT as it is light sensitive.
     2. It can take a long time to thaw and sometimes remains somewhat crystalline. It’s fine to briefly heat it at 37 °C in the heat block to liquify it
     3. Vortex once thawed
     4. Wrap a conical vial in foil for your master mix
     5. Add 845.75 µL of 1X TE and 4.25 µL of Pico Green reagent
     6. Vortex and keep in the dark (put in drawer while you set up)
     7. Leave pico reagent in drawer as you’ll need it again shortly

1. Grab a NEW black plate from the shelf above the plate reader. These are expensive so try to run as many samples as possible to maximize their use. Ask your lab mates if they need to assay any samples and combine samples onto one plate after the standard curve is verified to be usable. You should continue using this plate for your sample measurements.
2. Add 100 µL of 1x TE buffer to 8 wells (A1-H1)
3. Add 2 µL of each standard to each well. Make sure there aren’t bubbles or splashes in the wells
4. Add 98 µL of the master mix (7.v) to each well, again ensuring there are no bubbles or splashes in the wells.
5. Incubate for 5 minutes IN THE DARK (place in box, or drawer)
6. In the meantime set up the plate reader
   1. Turn on computer and open plate reader software
   2. Open the pico green assay file (Excitation should be at 480 nm and emission at 520 nm).
   3. Go to plate template and designate A1-G1 as standards with the concentrations listed above, with a 1/2 dilution factor starting with the concentration in A1 and then H1 as a blank. (Temperature should read ~26 C).
7. Place the plate in the plate reader, lining up well A1 with the corner of the plate reader labeled A1 and press run
8. Use the standard curve equation to calculate your standard curve accuracy. Use the google sheet in Pico Assay folder on lab drive
9. If your R2 was > 0.98 then you can proceed to quantify your samples. Otherwise make a new set of standards and try again (you will need to make more 1xTE if your standards are off)
10. Create a master mix just for your standards and samples, combining the Pico Green reagent (in enzyme box in freezer) and 1x TE as follows:
    * 1. Wrap a conical vial in foil for your master mix (you can use the same conical as before)
      2. Add 99.5\*(#of samples+0.5) µL of 1X TE and 0.5\*(#of samples+0.5) µL of Pico Green reagent
      3. Vortex and keep in the dark (put in drawer while you set up)
11. Add 100 µL of 1x TE buffer to wells
    1. If running a lot of samples, it may be easiest to put the buffer in a reagent reservoir and use a multichannel to pipette into each well
12. Add 2 µL of each standard to wells A2-H2. Then add 2 ul of your samples to each respective well. Make sure there aren’t bubbles or splashes in the wells
13. Add 98 µL of the master mix to the wells, again making sure there are not bubbles or splashes.
    1. If running a lot of samples, it may be easiest to put the mastermix in a reagent reservoir and use a multichannel to pipette into each well. If you do so, be sure to cover the top with foil when you aren’t drawing from it.
14. Incubate for 5 minutes IN THE DARK (place in box, or drawer)
15. Measure with plate reader and use google sheet to get your concentrations :)
    1. Double check your standard curve to make sure the R2 value is greater than 0.98.