**384-Well PicoGreen**

1. **Scope and Applicability:** Quant-iT™ PicoGreen® dsDNA Assay is used for detection and quantitation of double stranded DNA products.
2. **Materials:**
   1. Samples consisting of dsDNA
   2. Quant-iT™ PicoGreen® dsDNA Assay (Thermo Scientific P7589)
      1. Quant-iT™ PicoGreen® dsDNA reagent (Component A)
      2. 20X TE (Component B)
      3. Lambda DNA standard (Component C)
   3. Validated 10 ng/µL standard tubes (Pre-made, 4°C)
   4. 1X TE working stock
   5. Greiner UV plate, black, 384-well (VWR 82051-272)
   6. 1.2 mL Storage Plate (Thermo Scientific AB-1127)
   7. Pipette tips (Rainin GP-L10F, GP-L200F, GP-L1000F)
   8. 25 mL divided reservoirs (VWR 41428-958)
   9. RNase/DNase free water (Ambion AM9932)
   10. 50 mL conical polypropylene Falcon tubes (Corning 352098)
   11. 0.2 mL thin-wall PCR tubes with frosted caps (USA Scientific 1402-8100)
   12. 1.5 mL DNA Lo-bind Eppendorf tubes (Eppendorf 022431021)
   13. 96-well twin.tec® PCR Plate, Full-skirted (Eppendorf 951020401)
3. **Equipment:** 
   1. FilterMax F5 Multi-Mode Microplate Reader with linked PC
   2. Bravo automated liquid handler
   3. Post-AMP Integra
   4. Post-AMP swing bucket centrifuge
   5. Post-AMP hood
   6. Pipettes
   7. Plate-Loc plate sealer
   8. PicoGreen ice bucket (below Post-AMP hood)
   9. 96-well Aluminum Cooler Block (GeneMate R-2027-P)
4. **Safety:**
   1. Nitrile Gloves
   2. Eye protection
   3. Lab coat
   4. Disposable laboratory sleeves

**Warning: Personal Protective Equipment (PPE) should be used always while operating this protocol. If you are unsure what PPE you should be using, see your immediate supervisor.**

1. **Output:** Concentration of PCR products calculated with the standard curve.
2. **Setup:** 
   1. Find the appropriate laminated paperwork for the number of 96-well plates you would like to process.
   2. Label a 50 mL conical falcon tube with “TE” and another 50 mL conical with “PG”
   3. Then, following your paperwork, prepare:
      1. The TE 50 mL Falcon tube with 1X TE at the volume listed as “Sample Aliquot” on the paperwork.
      2. The PG 50 mL Falcon tube with 1X TE with the volume listed under “PG Tube”.
         1. Use a serological pipette for volumes in whole milliliters, then use a P1000 for volumes less than 1 mL.
   4. Remove as many 30 µL aliquots of PicoGreen as needed according to the worksheet.
      1. Place these aliquots in a GeneMate 96-well aluminum block in the dark to thaw (roughly 15 minutes at room temperature).
   5. Fill a designated PicoGreen ice bucket with wet ice and place in the Post-AMP hood.
      1. Place a GeneMate Aluminum Cooler Block on ice to cool.
   6. Take out a standard 10 ng/µL aliquot, spin down, and store in the GeneMate Aluminum Cooler Block.
   7. Retrieve the Proxy aliquots from the 4°C and place them on wet-ice with the standard.
   8. Label the 384-well Greiner plates with numbers to tell them apart.
   9. Label a 96-well twin.tec PCR Plate as “Standards.”
      1. Label a 25 mL divided reservoir as “1X PG” and another as “1X TE”.
   10. Retrieve sample plates to be assayed from the working 4°C and spin down in Post-AMP centrifuge at 1000 RCF for 1 minute.
3. **Methodology/Procedures:**
   1. **Create the TE Plate:**

Following the laminated worksheet, aliquot:

* + 1. Gently pour 1X TE from the “TE” Falcon tube into the labeled 25 mL divided reservoir on the longer (8-channel) side.
       1. Do not use the 1X TE from the “PG” Falcon tube for this step.
    2. Following the worksheet, aliquot into the “TE” 1.2 mL plate the volume specified in the “Plate Aliquots Table” under “1X TE”, “µL / well BRAVO” header.
    3. Seal and briefly (6s) spin down “TE” plate in a Pre-AMP centrifuge.
  1. **Create the Standard Plate:**
     1. Following the laminated worksheet, prepare the 2X standard curve as instructed.
        1. To make standards, take 7 x 0.2 mL tubes and label them according to tube name (Table 1).



* + - 1. On ice, perform serial dilutions using stated volume (Table 1) from parent tubes at each step and 1X TE from the remaining volume in the “TE” reservoir.
      2. Vortex each tube for 15 seconds on maximum speed and spin down between steps. (**Note: Lambda Stock Tube should only be used if there are no 10 ng/µL aliquots available**)
    1. Aliquot 4 µL of each standard into their designated wells of the “Standard” plate (listed in the table) using a multi-channel P10 @ 4 µL.
    2. In wells A12-D12 of the “Standard” plate, aliquot 4 µL of the proxy samples into their matching wells (listed on the cap and sides of each proxy) using a mulit-channel P10 @ 4µL.



* + 1. Add 4 µL of 1X TE from the “TE” 25 mL reservoir to column 4 of the “Standard” plate to serve as blanks.
       1. Add any additional samples you would like to process to the remaining wells. **Keep track of them using the laminated plate guide located in the PicoGreen drawer.**
  1. **1X TE Addition (19 µL) to the 384-well Plates (BRAVO):**
     1. Set up the Bravo following “384 PG-Bravo Instructions,” or by using the chart on the reverse of the laminated worksheet.
        1. Source plate = “TE” 1.2mL plate
        2. Destination plates = empty 384-well plates labeled 1-4 (or as needed).
     2. Load the BV-008 protocol from the production folder onto the Bravo instrument.
     3. Select the number of quadrants you would like to add TE to and then press the “Run” button.
        1. This will need to be repeated for every 384-well plate.
        2. Change tips between plates as directed by the instrument.
     4. Once TE is added, seal the plates and then bring the plates over to the Post-AMP Hood for sample addition using the Post-Integra.
  2. **Sample Addition (1 µL) to the 384-well Plates (Post-Integra):**
     1. Use fresh racks of Integra tips to keep track of 384-quadrant aliquoting, and retrieve a laminated plate guide from the PicoGreen drawer.
     2. Following the guide, select the appropriate Integra program to run (A1-384, A2-384, B1-384 and B2-384).
        1. These each correspond to 1 µL addition to a particular 384 well quadrant (A1-B2).
     3. Set up the Integra so that your samples are on deck position AB in an aluminum block and the 384-well plate containing 19 µL of TE is on deck position B.
     4. Follow the Integra prompts to begin stamping 1 µL of either sample or standards into each quadrant of the 384 well plate.
        1. Update the guide as you go along to keep track of which plate is in which quadrant.
     5. Seal the plates (source plates) using the plate sealer.
        1. Draw a line across the seal to signify that these are QC plates and not stock plates.
     6. Carefully seal and return the 384-well plates that now have TE plus 1 µL of sample to the Post-AMP hood.
  3. **Prepare 1X PG stock plate:**
     1. Spin down then combine all thawed 30 µL PG aliquots into a 1.5 mL Eppendorf tube.
        1. Vortex for 15 seconds on maximum and spin down.
     2. Following the laminated worksheet, add the listed volume of PicoGreen reagent to the “PG” conical Falcon tube containing TE.
        1. Vortex thoroughly on maximum speed for 30-40 seconds.
     3. Gently pour the PicoGreen reagent from the “PG” conical Falcon tube into the “PG” 25 mL divided reagent reservoir filling the longer 8-channel side.
     4. Aliquot appropriate volume of 1XPG to the “PG” 1.2 mL Thermo storage plate using a P1000 electronic multichannel pipette on the basic dispense option.
        1. This volume is listed on the “Plate Aliquot” table under PG, “µL per well Bravo”.
  4. **Addition of 1XPG to sample plates (BRAVO):**
     1. Take all the 384-well assay plates (containing 19 µL of TE and 1 µL of sample) and “PG” plate to the Bravo.
     2. Follow “384 PG-Bravo instructions” for set up and deck layout. Alternatively, consult the back of your worksheet for a layout guide.
        1. Source plate = “PG” 1.2 mL plate
        2. Destination plates = TE+Sample (20 µL) 384-well plates labeled 1-4 (or as needed).
     3. Load the BV-009 protocol from the production folder on the Bravo.
     4. Select the number of quadrants you would like to add 20 µL of PG reagent into and press “Run.”
     5. Follow the onscreen prompts, changing tips after every quadrant.
     6. Seal the 384-well plate.
     7. Repeat as necessary until the all the plates have reagent added and mixed.
     8. Briefly spin down the plates (6s) in the Post-AMP centrifuge.
        1. Do not leave plates in the centrifuge as temperature affects PG readings.
        2. Plates should be kept at room temperature in the dark (sealed on the benchtop is fine for this).
  5. **Reading Fluorescence Using Filtermax5 Instrument:**
     1. Open the PicoGreen Template on the FilterMax software-“ FM-003\_PicoGreen384\_4\_Plate\_V1.10\_180223”.
  6. Open the drawer and place the first plate into the FilterMax.
  7. Let the plate sit in the dark for ~30s.
  8. Press “Read” to begin reading the plate. Each plate will take roughly 5:40 minutes to complete.
     1. Repeat the above steps for sample plates, incubating each plate inside the FilterMax reader for approximately 30s.
  9. Paste results in appropriate excel file under “Paste\_Raw\_Data” tab.
  10. Copy standard curve, proxy set and sample information then save the data into each excel file that is associated with the worksheet. Save the FilterMax raw data file after each plate to the appropriate directory with filename associated with the worksheet.

1. **Take down:** 
   1. Discard the assay plates in trash bin.
   2. Check that enough PicoGreen 30 µL aliquots are available for the following day. If not, thaw a new PicoGreen dye assay kit. Once the dye is thawed, mix by vortexing 30-40 seconds, spin down and make 30 µL aliquots in 0.2 mL Eppendorf thin-wall PCR tubes. Put the aliquoted tubes in the bag with date in 4°C refrigerator.
   3. Clean the Post-Amp hood and discard the reagents that are not required.
   4. Sign the associated paperwork and place it in “Entry ready” slot of lab file sorter.
   5. Return Proxy samples to the working 4°C.
   6. Discard all other materials and leftover reagent volumes.
   7. Update the RSeq Set Equipment log on PWA.