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Department of Genomic Medicine

Cancer Immune Monitoring and Analysis Center

Division of Cancer Medicine

The University of Texas MD Anderson Cancer Center

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Director:

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SOP PicoGreen – Detection of dsDNA on the BioTek Synergy™ Microplate Reader

To Prepare the Reagents:

- Remove reagents from the fridge and allow to warm to room temperature.
  - The dye reagent is light-sensitive, so keep the tube in the reagent bag before and after use.
- Dilute 20X TE Buffer to 1X (50µl + 950µl H_2O) – make sure to check for aliquots in the fridge (they're good for 1 week)
  - You will need 6.5mL for the standards and an additional 1mL per sample.
  - Remember to consider the amount of 1X TE to be used to prepare the dye (see below).
- For the dye, add 5µl stock dye concentrate + 995µl 1X TE
  - You will need 1mL of dye reagent for each standard and sample (6 + x samples).

To Prepare the Standards (one set per run):

- Using the stock provided in the PicoGreen kit (100µg/ml), prepare a 2µg/mL standard by adding 30µl of the stock DNA (100µg/ml) and 1.47mL 1X TE.
- The four standards and one reference sample can be prepared (see table below):

Standard No. (ng/ml)	Amount of 2µg/mL DNA Stock (ul)	Volume of 1X low TE (ul)	Volume of Dye Reagent (ul)
STD1 (1000)	1000	0	1000
STD2 (100)	100	900	1000
STD3 (10)	10	990	1000
STD4 (1)	1	999	1000
STD5 (-)	0	1000	1000

To Prepare the Samples:

- Prepare a 1:1000 dilution of each sample by adding 1µl of stock DNA sample and 999µl of 1X TE.
- Add 1mL Dye Reagent to each dilution.
- Cover the standards and samples with a foil lid and turn off the bench overhead light – let the samples incubate for 2-5 minutes at room temperature.

To Prepare the Microplate for Analysis:

- Aliquot 200ul of each standard and sample in the microplate wells – place according to the sample layout specified on the Synergy instrument.

Operation of the BioTek Synergy™ Microplate Reader:

- Open the Gen5 software, click Instrument Control, and select Control Lamp to warm the fluorescent bulb.
    - Make sure to turn the bulb off when not in use – it will automatically shut off after 4 hours

Running the DNA Samples

- Select File -> New Task -> Experiments -> Create using an existing protocol -> dsDNA Quantitation using PicoGreen.
- Select Plate Layout, right-click and select Empty Layout.
- Add the standards, blanks, and samples according to the plate layout.
- To run the plate, select the green play button at the top. Follow the prompts as directed.

Data Analysis & Export

- Click on the Graphs tab and verify that the R^2 value is > 0.95.
- Click on the Statistics tab and select Concentration from the Data drop-down menu.
- Right-click, select QuickExport, and choose the location and save the file.

*Make sure to keep a key of the samples included in the plate layout as the sample IDs cannot be changed in the software.

Genomics Laboratory

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[Signatures] and [dates] 7/9/18