dsDNA Quantification

(Quantifluor™ Dye Systems; Promega # E2670)

1. Prepare standard curve:

Standard	Volume of Lambda dsDNA Standard	Volume of 1X TE Buffer (µL)	Final Concentration of dsDNA (ng/µL)
Blank	0	1000	0.0
A	20 µL DNA Std + 980 µL 1X TE	0	2.0
B	500 µL of Std A	500	1.0
C	500 µL of Std B	500	0.5
D	400 µL of Std C	600	0.2
E	500 µL of Std D	500	0.1
F	200 µL of Std E	800	0.02

2. In a regular 96-well plate, prepare a 1:100 dilution of each unknown sample (2.5 µL sample + 250 µL AE Buffer.
3. In a black 96-well plate, transfer 100 µL of each standard and diluted sample *in duplicate* (use multi-channel pipette and Tip One filter tips to triturate 4x and transfer diluted samples to plate).
4. Prepare Quantifluor™ dsDNA Dye working solution: (10 µL 200X dye + 1,990 µL 1X TE) and add 100 µL per well (use multi-channel pipette and Tip One filter tips).
5. Incubate for approximately 5 min at RT, protected from light.
6. Measure fluorescence using the GMB Protocol on the BioTek SynergyMx 96-well plate fluorimeter. (Ex. 504; Em. 531).
    a. Log onto computer.
    b. Open Gen5 1.09 software.
    c. Create New Expt
    d. Select GMB_Quantifluor protocol
    e. Maximize Protocol on left side of screen and adjust plate layout if necessary.
    f. Under Plate at the top, select “Read”.
    g. Click the Read button.
    h. Save file in GMB folder.
    i. Insert plate according to instructions on screen.
    j. Click OK.
    k. After the machine finishes reading the plate, and data appears on the screen, click the “Statistics” Tab. For “Data”, choose Conc x Dil.
    l. Save as an excel spreadsheet by clicking on the excel icon button. (R:GMI\GMB\Data\QUANTIFLUOR)
    m. Log off and close drawer (little black button).