Date: **Initials:**

This document contains supply checklists and the protocol for the QuantiFluor dsDNA System using the GloMax Fluorometer to measure DNA quantity (concentration) prior to sample submission for Genotyping-by-Sequencing (GBS).
* All solutions, standards and unknowns should be at room temperature prior to pipetting.

Supplies Checklist:

- Gloves
- Pipettes (list sizes)
    - Multi-channel
    -
- Pipette tips
- 96-well plate
- 1.5 ml microcentrifuge tubes
- Falcon tubes
- Dish thingy - does this have a specific name?
- USB-drive!

Reagents Checklist:

- Promega QuantiFluor dsDNA System kit (small yellow box in freezer)
    - 20X TE Buffer
    - QuantiFluor dsDNA dye
    - Lambda dsDNA
- Nuclease-free water
- Experimental (“unknown”) DNA samples

1. Prepare 1X TE Buffer:

- Dilute 20X TE buffer 20-fold (1:20) with nuclease-free water. When doing a full plate, prepare enough diluted buffer for both serial dilutions and to create the working solution for 100 samples.
    - ** Remember: 1, 000 ul = 1 ml

20X TE buffer	**1000 ul + 30 ul**
Nuclease-free water	**19, 000 ul + 570 ul**
1X TE buffer	**20, 000 ul + 600 ul**

2. Prepare Working Solution:

- Dilute the QuantiFluor dsDNA dye 1:400 in 1X TE buffer to make the working solution. For a full plate, you need enough working solution for 200 ul in each of the 96 wells (19, 200 ul).
    - The working solution is stable for at least 2 hours at 25 C.

QuantiFluor dsDNA dye	**50 ul**
1X TE buffer	**19, 950 ul**
Working solution	**20, 000 ul**

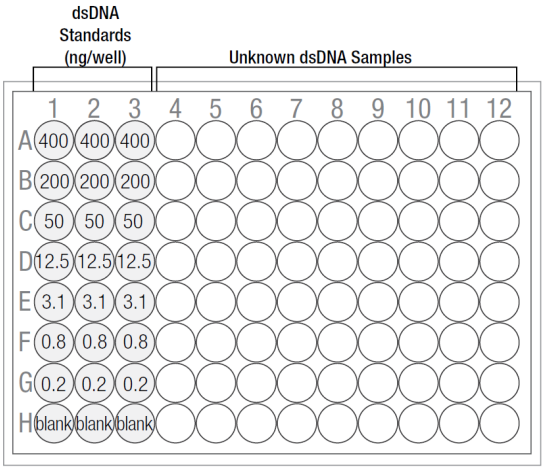
3. Prepare a Standard Curve:

- Label seven in 1.5 ml tubes for each standard as follows:
    - 200, 50, 12.5, 3.1, 0.78, 0.2, and 0.05.
- Prepare dsDNA standards by mixing the Lambda DNA Standard (100 ng/ul) and serially diluting as shown in table below.
    - Shake DNA to mix since the concentration gradient. Do not vortex since DNA is fragile.
    - Take care not to introduce air bubbles. If this happens, spin them down.
    - Vortex well after each subsample - common error affecting standard curve.

Preparing recommended dsDNA standard curve samples:			
Standard	**Volume of dsDNA Standard**	**Volume of 1X TE Buffer (ul)**	**Final dsDNA Concentration (ng/ul)**
A	20 ul	80	20
B	25 ul of Standard A	75	5.0
C	25 ul of Standard B	75	1.25
D	25 ul of Standard C	75	0.31
E	25 ul of Standard D	75	0.078
F	25 ul of Standard E	75	0.02
G	25 ul of Standard F	75	0.005

TIP: Run GloMax to measure fluorescence of ONLY standards and blanks to make sure dilutions worked. Then load/run experimental ("unknown") samples. This can prevent frustration of doing a full plate only to find the dilutions were done incorrectly. Be aware that fluorescence will wane because of longer procedural time.

4. Load Standards and Samples:



- Dispense 10 ul of the prepared dsDNA standards in columns 1 - 3, rows A - G (figure at right).
    - Keep plate covered to prevent evaporation of samples.
- For the blank, pipet 10 ul of 1X TE Buffer into row H.
- Pipet 2 ul of the "unknown" dsDNA samples to wells in columns 4 - 12, rows A - H.
    - There is room for 72 samples.
    - Record original sample label info in table below.
    - Use the 2.5 ul pipette and tips.
- Pipet 200 ul of the working solution into each well.

5. Measure Sample Fluorescence Using the GloMax:

- Turn on the machine (switch is on the back) and press button to open door.
- Place the plate in machine. ** Remove clear plate lid!
- Press the button to close door - do not manually close it.
- Select the preloaded protocol: QuantiFluor ONE dsDNA System.
    - Drag the "Incubate" icon into the protocol and set for 5 minutes at room temperature (~ 20 C).
    - Drag the "Shake" icon into the protocol to mix the plate thoroughly.
    - Recommended to do two loops and readings.
    - Run the protocol
- Export the results to a USB-drive, adding "Jade" to the beginning of the file name.
- Calculate the dsDNA concentration by copying and pasting the raw fluorescence data into the analysis Excel workbook.

72 "Unknown" dsDNA sample ID table:									
	4	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A									
B									
C									
D									
E									
F									
G									
H									