Quantification of DNA
by Robyn Tanny November 2014, adapted from protocol by Josh Bloom

NOTE: I never got this assay to work reproducibly with the Synergy 4. The higher standards never read correctly. I have done tests altering the parameters and the amount of Qubit reagent added - nothing seems to help the issue. The Kruglyak lab uses a Synergy 2 and that might be the difference in whether this works.

1. For this quantification, we use Quant-IT DNA High Sensitivity (HS) reagents (cat. # Q32854, Life Technologies) and a Synergy 4 Microplate reader (Biotek). Each 96-well plate is split into two quantification plates. The first three columns are the dilution curve of the standards, and the last six columns are the DNA samples from the 96-well plate.

Amount of 100 ng/µL Qubit stock (µL)	Amount of TE buffer (µL)	Final Concentration (ng/µL)
10	190	5
20	180	10
40	160	20
80	120	40
120	80	60
160	40	80

2. Make up standards in an eight-tube PCR strip by the dilution table below using the 100 ng/µl stock provided by the Quant-IT DNA Broad Range (BR) reagents (cat. # Q32850, Life Technologies). You will need enough to have triplicate of the dilution curve in each 96-well quantification plate. You can save the remaining standards at 4°C.

3. Make up enough Qubit working solution for the quantification. For each sample, use 1 µl of sample and 199 µL of working solution. Each 96-well plate to be quantified requires 16 mL (enough for 80 samples) of working solution (mix 80 µL of Qubit dsDNA HS reagent with 15.92 mL of Qubit dsDNA HS buffer). Cover the working solution tube to keep it out of the light.

4. Using a 12-channel pipette with the tips removed where there are blanks, pipette 199 µL of working solution into each well. The table below can be used as a guide. (S means sample; E means empty)

	1	2	3	4	5	6	7	8	9	10	11	12
A	**0**	**0**	**0**	**E**	**S**	**S**	**S**	**S**	**S**	**S**	**E**	**E**
B	**5**	**5**	**5**	**E**	**S**	**S**	**S**	**S**	**S**	**S**	**E**	**E**
C	**10**	**10**	**10**	**E**	**S**	**S**	**S**	**S**	**S**	**S**	**E**	**E**
D	**20**	**20**	**20**	**E**	**S**	**S**	**S**	**S**	**S**	**S**	**E**	**E**
E	**40**	**40**	**40**	**E**	**S**	**S**	**S**	**S**	**S**	**S**	**E**	**E**
F	**60**	**60**	**60**	**E**	**S**	**S**	**S**	**S**	**S**	**S**	**E**	**E**
G	**80**	**80**	**80**	**E**	**S**	**S**	**S**	**S**	**S**	**S**	**E**	**E**
H	**100**	**100**	**100**	**E**	**S**	**S**	**S**	**S**	**S**	**S**	**E**	**E**

5. Using an 8-channel pipette, pipette 1 µL of each standard into the first three columns of the plate. Double check that each tip takes in 1 µL of the sample.

6. Mix the DNA with the multi plate vortexer at 1200 rpm for 10 seconds.

7. Using an 8-channel pipette, add 1 µL of each DNA sample into the sample wells. Double check that each tip takes in 1 µL of the sample.

    a. Open the Gen5 software used by the Synergy 4 Microplate Reader. Create a new Protocol and use the following parameters for the Read Step:
        i. Detection Method: Fluorescence
        ii. Read Type: Endpoint

iii. Read Speed: Normal
iv. Light Source: Tungsten
v. Excitation: 485/20
vi. Emission: 528/20
vii. Optics Position: Top 50%
viii. Sensitivity: 35
ix. Top Vertical Probe Offset: 7.00 mm

8. Once you have created the protocol, start a new experiment and use this protocol to run it. Make sure to indicate your standards values in the plate setup. When the plate has finished reading, you can have the software output the concentrations based on the fluorescence readings of your standards and samples.