DNA QUANTIFICATION

A DNA quantification kit can be purchased from Sigma Chemical.

Prepare Assay Solution: Mix 10 μl of Hoescht dye (H33258 stock solution), 10.0 ml of 10X TNE buffer and 90.0 ml of dH2O.

Calibrate the fluorometer: Add 2 ml of Assay solution to the cuvette. Place cuvette was placed in the well, close lid, then press *zero*. Add 2 μl of 100 ng/μl standard DNA to the cuvette and mix with the lid on by tube inversion 10 times. Place the cuvette back into the well, close lid and press *enter* . Press *Calib,* then enter *100* as the standard concentration (100 ng/ml). Wash the cuvette with dH2O. Dry only the outside with a kimwipe. Particles will stick to the inside walls.

Determine DNA concentration:

1) Add 2 ml of Assay solution to the cuvette. Insert the cuvette into the well of the fluorometer. Press *enter* twice, then *Zero*. After "0" displays, remove the cuvette.

2) Add 2 μl of your sample DNA. Place the lid on and invert the cuvette 6 times, mixing well.

3) Place the cuvette in the well, close the lid, press *enter* and read and record the measurement.

4) Wash the cuvette with dH2O, Tap the inverted cuvette on a kimwipe to remove excess water. Repeat Procedures 1-4 for each sample.

5) Each measurement is in ng/μl. A good DNA yield is above 25 ng/μl. You will need approximately 50-100 ng DNA for a PCR reaction.

Example of How DNA Concentration is Calculated:

if fluorometer reads: 100, it means: final concentration = 100 ng/ml

final conc. X volume of assay soltn = original concentration

volume of DNA added

100 ng X 2 ml = 100 ng/μl

1 ml 2 μl

Therefore, the original DNA concentration is in ng/μl.