Procedure to Quantitate DNA with the Mini-fluorometer

1. Turn the machine on 30 minutes before using.

2. Make 100 ml of 1X TNE (10 ml 10x TNE plus 90 ml H_2O.)

3. Add 10 ul of dye to the TNE (0.1 ug/ml.)

4. Clean the cuvette by squirting distilled H_2O in it a few times.

5. Pipet 2 ml of the dye solution into the cuvette.

6. Turn the scale knob fully clockwise.

7. Adjust the reading to 000 with the zero contrast knob after the cuvette is in the machine.

8. Now make a standard curve.

   **The Standard Curve**

   a. Pipet 2 ml of the dye solution into the cuvette. Add 2 ul of the 100 ug/ml standard. Cover the cuvette opening with parafilm and mix by inverting the cuvette several times. Place the cuvette in the fluorometer and adjust the scale knob to 100.

   b. Remove the cuvette, empty it, and rinse it with distilled H_2O. Refill it with 2 ml of the dye mix. Add 1 ul of the 100 ug/ml and take a reading. Record this.

   c. Repeat step two with 250 ug/ml and 500 ug/ml.

9. To quantitate your DNA sample by adding 2 ul of the sample into the cuvette that has 2 ml of 1X TNE, then place the cuvette in the mini-fluorometer to obtain the reading (in ng/ul).

10. Rinse the cuvette with deionized water and repeat step 9 for the next sample.
NOTES

1. The readings are in ng/ml but since our DNA sample was 2ul of the 2ml total sample; therefore, we interpret the readings as ng/ul.

2. Use 1 ul of lambda DNA as a check.

10X TNE Buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris</td>
<td>12.1 gm</td>
</tr>
<tr>
<td>EDTA-Na₂</td>
<td>3.1 gm</td>
</tr>
<tr>
<td>NaCl</td>
<td>58.4 gm</td>
</tr>
</tbody>
</table>

10X TNE Buffer contains 12.1 gm Tris, 3.1 gm EDTA-Na₂, and 58.4 gm NaCl.

pH to 7.4 with conc. HCl, then make to 1 liter with H₂O.