Measuring DNA with SYBR GREEN

Note: This procedure assumes gDNA concentration in the range of 0-10ng/l.
If samples cover a different range of concentrations, the procedure should be modified accordingly. We recommend a two step-dilution for samples with a broad range of concentrations.

1. Create the standards.
   a. You will need to run new standards for each batch of SYBR Green I working solution you use. You will need at least 10L of each standard.
   Use the standards from the Qubit HS DNA kit. The standards have a concentration of 10 ng/L and 0 ng/L. Prepare 12 uL of the standards as outlined in the following table:

<table>
<thead>
<tr>
<th>Standard (ng/L)</th>
<th>Vol. 10 ng/L Standard</th>
<th>Vol. 0 ng/L Standard</th>
<th>Vol. 1 ng/L Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>12 L</td>
<td>0 L</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>9 L</td>
<td>3 L</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6 L</td>
<td>6 L</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>3 L</td>
<td>9 L</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.4 L</td>
<td>21.6 L</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td>6 L</td>
<td>6 L</td>
</tr>
<tr>
<td>0.25</td>
<td></td>
<td>9 L</td>
<td>3 L</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>0 L</td>
<td>12 L</td>
</tr>
</tbody>
</table>

*NOTE: You can make a larger quantity of each standard and store them at 4 C.
***NOTE: It is possible to use the 100 ng/L Standard from the Qubit BR DNA Kit to increase the range of your standards, but be aware on the Tecan Plate Reader using SYBR Green I the fluorescence signal maxes out at around 20 ng/L.

2. Mix 5l of concentrated SYBR Green I with approximately 25mL of TE in a clean reservoir to make a working dye solution.
   a. The same dye solution should be used across all samples and standards.
3. Seal, vortex and centrifuge gDNA (~200rcf for 30s).
4. Add 10 l of gDNA to each well of a fluorometry plate.
   a. Recommended: 96-well pipette or multi-channel pipette.
5. In 8 wells of fluorometry plate, add 10 l of each DNA standard.
   a. Little plate-to-plate variability is seen, as long as the same dye solution is used.
6. Dispense 190 l of dye solution into each well.
7. Incubate the DNA and dye in the dark for 5 minutes, e.g., put in a dark drawer or covered with foil.
8. Read fluorescence on the plate reader using SYBR Green or GFP filters (e.g. Ex:470/40 and Em:520LP, top read). NOTE- This method should be in the Gresham Lab methods folder on the Tecan plate reader computer.
   a. If standard curve is not linear, allow the plate to sit longer and repeat.
   b. If the samples are outside the linear range of standards, repeat with a different dilution of sample.
9. Calculate DNA concentration of each sample using by fitting a linear model to the standards, then calculate the volume of water needed to dilute each sample to the desired concentration (eg. for the Nextera Protocol this will be between 2-2.5 ng/l.
   a. You can use the excel file associated with this page to input your standards and your DNA readings to calculate your concentrations.
10. (Optional) Standardized gDNA libraries can be stored overnight at -20°C.

SYBR_Green_DNA_Conc_Calculation Sheet (Updated 07/14/2017 - Nathan)