dsDNA quantification with PicoGreen

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Long-term storage (-20)
PicoGreen dye (100 μl each)
20X TE (pH 7.5, 200 mM Tris-HCl, 20 mM EDTA)
λ DNA standard (100 μg/mL) and some 50X diluted (2 μg/mL)

Short-term storage (4C)
One PicoGreen dye (to avoid freeze/thaw)
1X TE in DEPC treated water (50 ml-corning tube)
One λ DNA standard (2 μg/mL)

0. Sample preparation
   a. 100X dilution on soil samples in TE or based on Nanodrop reading for dynamic range of 0-1000 ng/mL
1. Dye dilution
   a. 200X dilution in TE right before the assay (only good for several hours after diluted)
2. Standards preparation (duplicates in 96-well plate)
   a. Sample standards preparation

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>λ DNA (2 μg/mL)</th>
<th>TE (μl)</th>
<th>diluted PicoGreen (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>99</td>
<td>100</td>
</tr>
<tr>
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<td>5</td>
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<tr>
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<td>10</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>200</td>
<td>20</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>500</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

3. Adding samples and standards first (200 μl TE for blanks), then mix with dye (in dark room)
4. Incubate for 2-5 minute in dark before reading in BMG Labtech FLUOstar OPTIMA
   a. Select ‘Test Protocol-PICOGREEN’ for minor adjustment
      i. Specify sample, standards and blank wells by selecting ‘Layout’ tap
   b. Put plate inside of plate reader then click ‘Measure’ icon.
   c. Gain adjustment
      i. Select highest standard well and click ‘Gain Adjustment’ icon at the bottom.
   d. Start measurement

Measure | Plate Out
Moves microplate carrier out of the instrument.

Measure | Plate In
Moves microplate carrier into the instrument.

Measure | Measure
Performs a measurement using a pre-defined test protocol. Before the measurement starts, you can enter plate IDs and perform an automatic gain adjustment.