Human DNA Quantitation using Alu qPCR Method
Specialized Topics Spring 2008

Supplies needed:
qPCR MasterMix (provided by instructor)
Extracted DNA sample
Extracted Manipulation Blank
Quantitation obtained from Gel electrophoresis

1. Mastermix will be prepared by the instructor. It will contain the following:

<table>
<thead>
<tr>
<th>Component</th>
<th>volume (ul) 1 rxn</th>
<th>volume (ul) 50 rxn</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x SYBR Green JumpStart ReadyMix</td>
<td>12.5</td>
<td>625</td>
</tr>
<tr>
<td>20 pmoles/ul Alu Primer 1</td>
<td>0.4</td>
<td>20</td>
</tr>
<tr>
<td>20 pmoles/ul Alu Primer 2</td>
<td>0.4</td>
<td>20</td>
</tr>
<tr>
<td>250 ug/ml BSA (Sigma number A-9647)</td>
<td>0.8</td>
<td>40</td>
</tr>
<tr>
<td>water</td>
<td>5.9</td>
<td>295</td>
</tr>
<tr>
<td>DNA sample (aim for 1 ng TOTAL)</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td><strong>TOTAL REACTION VOLUME</strong></td>
<td><strong>25</strong></td>
<td></td>
</tr>
</tbody>
</table>

2. Each student will prepare two qPCR reactions.

   Human DNA sample
   Manipulation blank sample

   a. Each student will prepare and label the special tubes required for the Cepheid SmartCycler machine. 20 ul of MasterMix will be taken from the prepared stock and added to the Cepheid tube.

   b. To each tube (of 20 uL) the student will next add their sample (maximum of 5 uL)
DNA dilution for qPCR:

Based on the yield gel quantitation, dilutions will be prepared so that 5 ul of the human DNA sample will give 1 ng of DNA. If no DNA was observed from the yield gel, then 5 ul will be taken from the sample without any dilution and added to the PCR tube.

**EXAMPLE:** Yield gel analysis estimated DNA concentration to be 63 ng DNA/4 ul of sample loaded. DNA concentration is then ~16 ng/ul.

1 ng of DNA sample (16 ng) will be added to 31 ul TE or water to obtain the appropriate concentration needed (0.5 ng/ul). 2 ul of this sample will be used for quantitative PCR. So 2 ul plus 3 additional ul of water will make up the 5 ul sample volume.

Each student will also run a reaction for their manipulation blank. In most cases, no DNA was observed from the yield gel analysis, so 5 ul total will be taken from the undiluted sample for PCR.

3. PCR will be performed. The PCR conditions used will be as follows:

   95°C for 2 minutes (initial denaturation for Hot Start PCR)
   35 cycles of
   95°C C for 15 seconds
   68°C for 15 seconds
   72°C for 15 seconds

4. Following PCR, melt curve analysis will be performed to determine reliability of the reaction.

   72°C for 20 seconds
   Ramp up 1°C at a time, 5 second at each degree

5. Standard curve analysis will be performed for each sample prepared. Standards were prepared by the instructor prior to class. These were made using commercially available DNA (human genomic, Promega) that was purchased and diluted to appropriate concentrations. The starting stock was 184 ng/ul. Understand how all these dilutions were done because they are fair game for any exams, quizzes or homework.
Brief Protocol for tonight’s class

1. Prepare/label 2 tubes.

2. Pipette 20 ul of Mastermix into each.

3. Add human DNA (1 ng) in a volume of 5 ul.

4. Add 5 ul of Manipulation blank sample.

5. Place tubes in PCR machine.

6. Run PCR and melt curve.

7. Determine quantity of DNA.