Protocol For Making 100µM Primer Stocks

Overview of the process
When the primers arrive, punch holes in the Specification Sheets and put them in the appropriate notebook. Primer tubes from IDT* will indicate the OD\textsubscript{260}nmol, and mg along the bottom of the label. It is important to keep these sheets as the OD\textsubscript{260}nmol is very important to this process. When done, you will be able to use this stock for running experiments.

Materials Needed

<table>
<thead>
<tr>
<th>ddH\textsubscript{2}O</th>
<th>Sharpie</th>
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<tbody>
<tr>
<td>Primers</td>
<td>Labeling Paper</td>
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<tr>
<td>Primer sheets</td>
<td>Vortex</td>
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<tr>
<td>Sterile 1.5 Eppendorf Tubes</td>
<td>Microcentrifuge</td>
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</table>

Instructions

1. Open the packets and take out the primer tube.
   a. Remember to hold on to the sheets.
2. Spin down the tubes in a micro centrifuge before opening so that none of the primer falls out.
3. To make a 100µM solution simply add 10 times the amount of water as nmol of your primer.
   a. The label will look like this:
      i. 5.6 OD\textsubscript{260} = 28.18 nmol = 0.18 mg
   b. For example, you will multiply (28.18 x 10 = 281.8)
4. Add 281.8µl of water for this primer. This gives a 100µM stock.
5. Vortex before using to make working solution.
6. Spin down in the micro centrifuge again.
7. To make a working solution, add 12.5 of the forward and 12.5 of the reverse to 975 µl of water for 1 ml of working solution primer in the 1.5ml Eppendorf tubes.
8. This can be stored in the -20°C freezer.
   a. Make many aliquots following step 7 so you don’t have to continually make a new one when you run out.

In order to preserve our primers for longer, aliquot it out and dry it down in the speed vac (with the heat off). To do this, put 12.5 µl of the forward and 12.5 µl of the reverse primer in a 1.5 ml tube. If possible, aliquot out all of the primer in this manner. Place tubes in the speed vac and turn on the vacuum pump and the rotor. It make take several hours for them to dry down. Once dry, store them at -20°C. When ready to use, add 1 ml of water to a tube, vortex and let stand 15 min at room temp before using. This will give a 25 µM working solution.

w. castle Dec 2002