Primer Preparation

New primers are designed in consultation with Dr. Edwards, and are ordered from a company called Integrated DNA Technologies (IDT) that specializes in synthesizing nucleic acids. After synthesis, the primers are lyophilized, which means they are sublimated in a freezing solution to yield a dry power. You will first need to suspend the primers to produce a 100 uM stock solution. You will then perform a 1:10 dilution to make a working stock to be used in PCR.

Procedure

1. Quickly spin the tubes from IDT in a microcentrifuge for about 10 sec. This will help pull down any lyophilized bits that may have become dislodged during delivery.
2. Look at the label on the tube containing one of your primers.
3. You should see the amount indicated in nanomoles (nmol), i.e. 38.2 nmol.
4. Multiply the number of nmol by 10 to calculate the amount of TE buffer to add to the tube, i.e. 38.2 nmol x 10 = 382 uL. Adding this volume will produce a final concentration of 100 uM primer. This is your stock primer.
5. Add the calculated amount of TE buffer directly to the IDT primer tube.
6. Repeat steps 2-5 with the other primers, checking the primer amount for each.
7. You now need to make a 'working' 10 uM stock of each primer by performing a 1:10 dilution of the original stock. Add 180 uL of TE buffer to a new tube, then add 20 uL of the original stock primer.
8. Label the working primers with the appropriate name and concentration, along with the date. <u>These are the primers that you will use in your PCR reaction.</u>
9. Put the original and working stocks in the appropriate storage boxes (Stock Primers and Working Primers) in the -20C freezer.