A.Banta 11/26/02

Dye Terminator Sequencing Protocol

Sequencing Reaction

Introduction

- Keep reagents out of strong light and on ice.
- When setting up these reactions, always be very conscious of their small volumes-it it very important to pipet accurately, vortex a premix if you make one, mix by pipetting up and down when adding an individual reagent to the reaction, and making sure everything is mixed and at the bottom of the tube with no bubbles. One way to accomplish this is to add everything to the bottom of the tube, pipetting up and down as you add each component (but don't go beyond the pipet stop or you will introduce air bubbles)
  - 20 µl Full Reactions are usually good for larger plasmids or sequencing genomic DNA directly
  - 10 µl Half Reactions are usually good for plasmids with a moderate size insert and larger PCR products
  - **WE WILL USE FULL REACTIONS**

Reaction Set-up

	Full Reaction	Half Reaction
Big Dye Terminator Premix	8 µl	4 µl
DNA Template	****see note below**	****see note below**
10X BDT buffer	0	0
Primer	3.2 pmol	1.6 pmol
ddH_2O	(for a total volume of 20µl)	(for a total volume of 10µl)
	20 µl	10 µl

Primers:
we can use either of the primers that we used in the PCR (remember to only use one!). also, note that the primer concentration we used last time was 10 pmol/µl, so we will need to dilute the concentration down to 3.2 pmol/ µl and use one µl in the sequencing reaction

Template:
The amount of DNA necessary will depend on the length of the DNA (molar amount is what really matters). **The range for PCR products is around 10-50 ng** and for typical plasmid DNA it is around 200-500 ng. Using less template than these amounts results in low signal and poor data. Using too much template results in good reads that are shorter than they could be. We will err on having too much template and load between 5 and 11 ul of our PCR product (use the upper end if you have any doubt) or 200 ng of plasmid.

Thermocycler protocol

96.0^0C	2 min	
96.0^0C	10 sec	35 cycles
50.0^0C	10 sec	
60.0^0C	4 min	

4.0^0C	forever

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Sequencing Cleanup using Quiagen DyeEx™ kit

1. Gently vortex the spin column to resuspend the resin.

2. Loosen the cap of the column a quarter turn.

This is necessary to avoid a vacuum inside the spin column.

3. Snap off the bottom closure of the spin column (Figure 1), and place the spin column in a 2 ml collection tube (provided).

4. Centrifuge for 3 min at the calculated speed.

5. Carefully transfer the spin column to a clean centrifuge tube. Slowly apply the sequencing reaction (10–20 µl) to the gel bed (Figure 2).

Notes: • Pipet the sequencing reaction directly onto the center of the slanted gel- bed surface (Figure 2). Do not allow the reaction mixture or the pipet tip to touch the sides of the column. The sample should be pipetted slowly so that the drops are absorbed into the gel and do not flow down the sides of the gel bed. Avoid touching the gel-bed surface with the pipet tip.

• It is not necessary to replace the lid on the column.

6. Centrifuge for 3 min at the calculated speed.

7. Remove the spin column from the microcentrifuge tube.

The eluate contains the purified DNA.

8. Dry the sample in a vacuum centrifuge and proceed according to the instructions provided with the DNA sequencer.