

## SEQUENCING REACTIONS

- Write out samples to sequence along with primer to use for each template. The reactions will be done in the order the samples are written out. Organize templates with primers in a rack.

For each reaction add DNA template + H<sub>2</sub>O for a total volume of 8 microliters. Plasmid DNA should be no more than 1 to 3 micrograms. Generally 5-8 µl Qiagen miniprep DNA is good in a reaction. Once the amount of template needed is figured out go ahead and add the water needed first to each 9600 PCR reaction tube. Then add desired template to each tube.

Add 4 microliters of 3.2 pmol sequencing primer or 1 µl 20 µM concentration. For 3.2 pmol, dilute 4 microliters of 20 micromolar concentration primer with 96 microliters of water. To figure out micromolar concentration of primer follow formula : A<sub>260</sub> spec reading X 100 X 100 (spec reading on 1:100 dilution of resuspended primer) divided by Mer or length of primer.

$$\frac{A_{260} \times 100 \times 100}{\text{Mer}}$$

Mer

Finally, add 8 microliters of Perkin Elmer Dye terminator mix (catalogue # 402079; 100rxns; \$489) – this is changing. Keep cold and return to -20 degrees promptly. Also, when adding the 8 microliters of terminator mix, pipette up and down to mix well with template and primer.

The total volume of the reaction is 20 microliters.

The 9600 PCR machine is already set up with a sequencing reaction program. It is called method 82. Just go to Edit via Option and

select 52.  
follows:

Enter all the way through. Conditions should be as

25 cycles

94 degrees 4 minute hold for hot start (put samples in when machine reaches 94 degrees.

Cycle program: 96 degrees 10 seconds; 50 degrees 5 seconds, 60 degrees minutes.

4

15 degree cooling hold.

Reactions may be set up overnight.

## IV. ETHANOL PRECIPITATIONS

- For each reation prepare a 1.5 ml microcentrifuge tube by adding the following:

2.0 microliters 3M Sodium acetate pH 5.3 or pH 4.6 ok too.  
48.0 microliters absolute ethanol.

Add 50 microliters of above mix to another tube for each reaction set up in the PCR machine. Make sure to number each tube.

Add entire 20 microliters of sequencing reactions to each 50 microliters of the ethanol/sodium acetate mix. Vortex and put on ice for 10 minutes.

so Centrifuge in cold centrifuge for 15 minutes. Make sure tubes are placed that location of pellet is known. Often pellet will not be visible.

Carefully aspirate ethanol solution from each tube. Remove as much as possible without disturbing the pellet.

Wash with 1ml 80% Ethanol and centrifuge 2 minutes. Carefully remove wash. At this point sometimes pellet falls to bottom of tube. Remember that sometimes the pellet is not visible. Remove as much ethanol as possible. If there is just a little left at bottom of tube, that's ok.

Put in speed vac for 10-15 minutes until dry (without heat).