

## DNA SEQUENCING

Ex Application: PCR products identified as possibly being products of the candidate gene primers can be sequenced for comparison to candidate gene sequence.

### Procedure:

For each template to be sequenced, a PCR/Sequencing rxn must be set up for each primer (f & r) of the pair. **1 template = 2 rxn set-ups**

### 1X:

1  $\lambda$  Big Dye reagent (pol,dNTPs\*,etc.) Add to premix last  
1.5  $\lambda$  5X Sequencing Buffer  
0.5  $\lambda$  DMSO  
0.5  $\lambda$  Primer  
4  $\lambda$  Template DNA  
2.5  $\lambda$  dH<sub>2</sub>O  
10  $\lambda$

1. Set-up above reaction in 96 deep-well plate.
2. Run PCR program Stand files: **CYCSTAND** (3hrs.)
3. Clean PCR products with Sephadex G50 columns.  
**Collect Clean PCR Products in specialized 96 well plate for Sequencer!**  
(Check first to see if someone already has a plate started.)
4. Wrap plate with foil. Date and write how many samples ready. Put in -20°.