

**SAP/EXO1 PCR CLEAN-UP** (chew up excess primers, remove dNTPs)

1. Visualize PCR product to make sure that you have one without sub-bands. A strong amplification product (bright on the gel) will tend to sequence better. Concentration should be at least 20ng/ $\mu$ L as visualized using the MassLadder
2. Add 5  $\mu$ L of each PCR product into a 200 $\mu$ L strip tube:
3. To each sample, add
  - 0.5 U (0.5  $\mu$ L) of shrimp alkaline phosphatase (1 U per  $\mu$ L stock)
  - 5 U (0.5  $\mu$ L) of exonuclease I (10 U per  $\mu$ L stock).Both chemicals are sold by Amersham.

There is nothing magic about this 5  $\mu$ L volume - it provides template for two sequencing reactions if the product is strong. If you need more template than this then scale up.

4. Program a PCR machine to incubate the reactions at 37° C for 30 minutes, kill the enzyme with 80° C for 15 minutes, and then cool the reaction to room temperature, 25° C. This is file #37 on High DNA PCR Machine. The products are now ready for sequencing. Use 1-2  $\mu$ L in a 10  $\mu$ L sequencing reaction, depending on how bright the band was.