

## Cycle sequencing of DNA

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Very often, molecular biologists will isolate a cDNA clone for a gene of interest. The sequence of the cDNA then needs to be determined to find out what protein is encoded. In this laboratory exercise you will have four cDNA clones that you need to sequence. Each of these cDNAs has already been placed into the multiple cloning site of the pBluescript vector (see Figure 1). Over the next two weeks, you will determine the sequence of the cDNA insert in each of these plasmids.

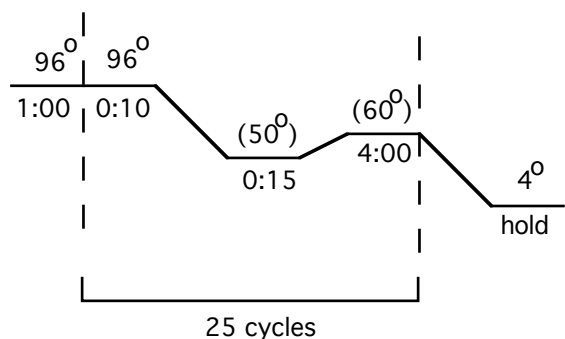
You will be given four (already purified) plasmid preparations at a concentration of 50 ng/ $\mu$ L. These will serve as your sequencing templates.

### Cycle Sequencing with BigDye terminators

1. The components needed for a sequencing reaction are shown below. Keep everything on ice while making up the reactions. Since you need to carry out four sequencing reactions it will be a good idea to make a master sufficient for five reactions. Then you can combine 6.5  $\mu$ L of the master mix with 4.5  $\mu$ L of each plasmid DNA sample.

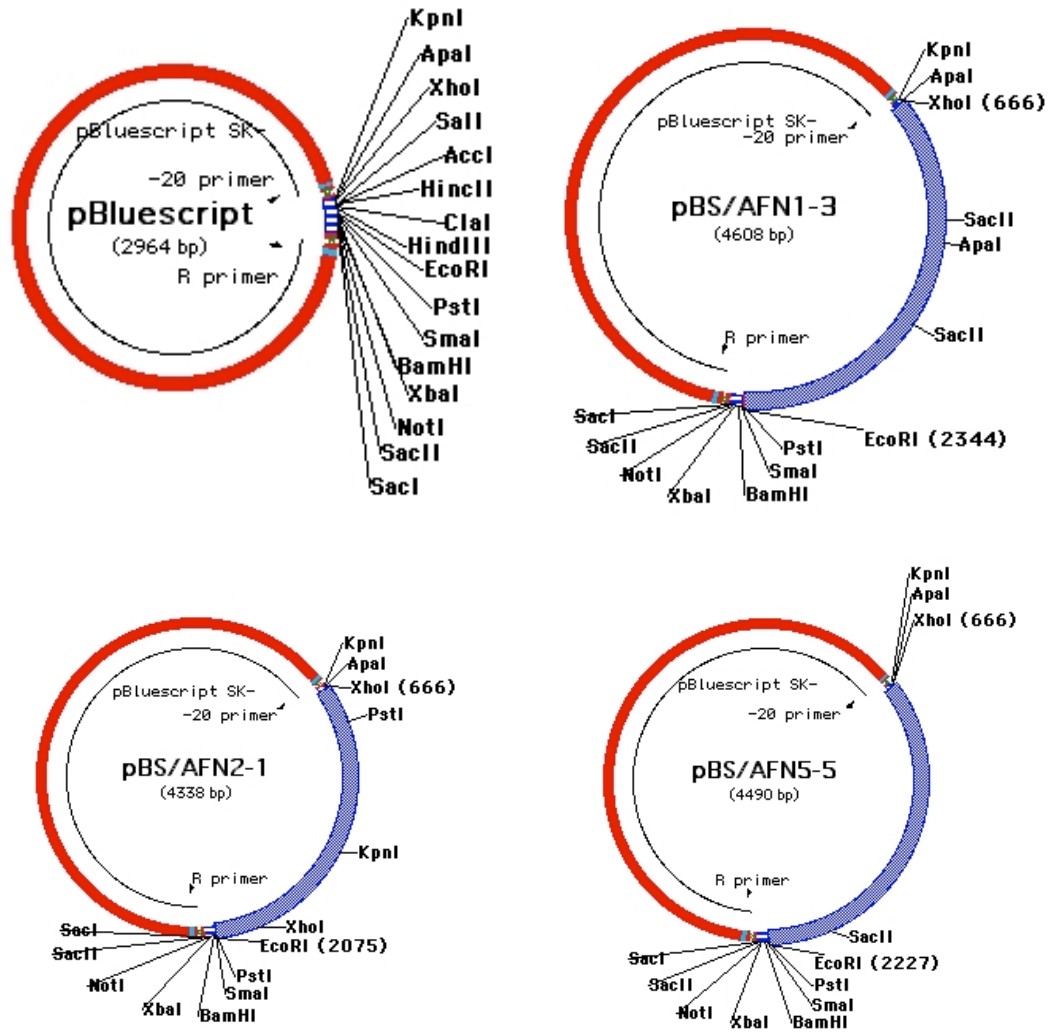
(225 ng) DNA + water	4.5 $\mu$ L	<u>master mix (5x)</u>
2 $\mu$ M primer	1.5 $\mu$ L	7.5 $\mu$ L
5x Buffer	3.0 $\mu$ L	15.0 $\mu$ L
Big Dye mix	<u>1.0 <math>\mu</math>L</u>	<u>5.0 <math>\mu</math>L</u>
	10.0 $\mu$ L	32.5 $\mu$ L

2. Place tubes in the thermal cycler and run the following program.



### **Purification of sequencing reaction products**

- 1.** Obtain a dry CENTRI-SEP column and tap the gel down to the bottom. Remove the cap and add 0.8 mL water to the dry gel. Put the cap on and shake the gel to fully hydrate it. Slowly loosen the top cap and allow the column to sit for 15 minutes at RT.
- 2.** Tap or shake the column as necessary to obtain a good bed of gel that is free of air bubbles. Let the column stand up for a couple of minutes to settle.
- 3.** Remove the column end stopper and the cap from the column. Avoid introducing any bubbles into the column as you do this. Allow the excess fluid (about 200  $\mu$ L) to drip out into a 2 mL collection tube. Discard this fluid.
- 4.** Spin the column and collection tube at 3,000 rpm for 2 minutes in a microcentrifuge. Discard the excess fluid.
- 5.** Immediately transfer 10  $\mu$ L of completed sequencing reaction onto the top of the gel. Spin the column with a clean 1.5 mL collection tube at 3,000 rpm for 2 minutes in a microcentrifuge. Collect the purified sample from the collection tube.
- 6.** Dry the sample in a vacuum centrifuge. Store the dried samples at  $-20^{\circ}$  for future use.



**Figure 1.** Plasmid vector pBluescript with and without cDNA inserts.