Cycle sequencing of DNA
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You will need to use a specific amount (25 ng) of your PCR product as a template in your sequencing reaction. This means that you will need to measure the concentration of PCR product in your tubes before you can sequence. A convenient way to confirm the presence of the expected size of PCR product and to estimate the amount of DNA present, is to run the DNA on an agarose gel. By comparing the mobility and intensity of your bands to standards, you can determine the molecular weight of the DNA in your band and how much DNA is there.

Agarose gel analysis of PCR products
1. Prepare a 1.5% agarose gel in 1x TAE buffer. Include 0.1 μg/mL ethidium bromide in the gel.

2. Prepare the samples for loading on the gel as follows:
   4 μL PCR product
   3 μL gel loading buffer (blue)
   8 μL water

3. After mixing the samples well, load each sample onto the 1.5 % gel. Adjacent to your PCR products load one lane with 10 μL (1 μg) of DNA digested with HindIII. Run the gel for about 1 hour.

4. Take a photograph of your gel illuminated by UV light.

5. Determine the molecular weight and the amount of DNA present in each of your PCR product bands. Use this information to determine the concentration of DNA in each of your sample tubes.

NOTE: 1000 ng of DNA digested with HindIII results in the following DNA fragments:

- 23 kbp 500 ng
- 9.4 kbp 200 ng
- 6.6 kbp 150 ng
- 4.4 kbp (don’t use for quantitation)
- 2.3 kbp 50 ng
- 2.0 kbp 50 ng
- 564 bp 10 ng
- 125 bp 2 ng (often this band cannot be seen)
Cycle Sequencing with BigDye terminators
1. Mix the following on ice:
   - 25 ng DNA in water  4.4 µL
   - 2 µM primer  1.6 µL
   - Big Dye RR mix  4.0 µL
   - Water  10.0 µL

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

   ![Cycling Program](image)

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 µL) 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 µL 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° for future use.
RECIPIES FOR BUFFERS ETC.

50x TAE
242 g Tris
    57 g glacial acetic acid
100 ml 0.5 M EDTA pH 8
make up to 1L with water

gel loading buffer
1.25 g Ficoll
12.5 mg bromophenol blue
12.5 mg xylene cyanol
    2 mL 50 x TAE
make up to 10 mL with water