

Texas A&M University-Corpus Christi
CHEM4402 Biochemistry II Laboratory
Laboratory 9: DNA Sequencing II

In this week's lab, we will perform the second part of our DNA sequencing experiment. Recall from last time that a cycle sequencing reaction produces a series of fluorescently-labeled fragments that differ in length by a single nucleotide. Using capillary electrophoresis we will separate these fragments according to length. The fluorescent label provides the signal which is detected by the instrument and interpreted as referring to an "A", "T", "G" or "C" (figure 1). Before we can apply our sequencing reaction products to the capillary column, however, we must remove components such as unincorporated dye-labeled nucleotides, DNA polymerase, buffer salts and template DNA. These can clog the capillary and interfere with the signal. We do this by precipitating the labeled DNA fragments with glycogen, and then removing the unincorporated nucleotides and salts by washing with an ethanol solution. Our samples will then be dried down using a vacuum centrifuge. We shall re-dissolve our fragments in sample loading solution, which denatures the double-stranded DNA structure, and then freeze them prior to analysis on the DNA sequencing instrument later in the week. Results will be returned to you next week.

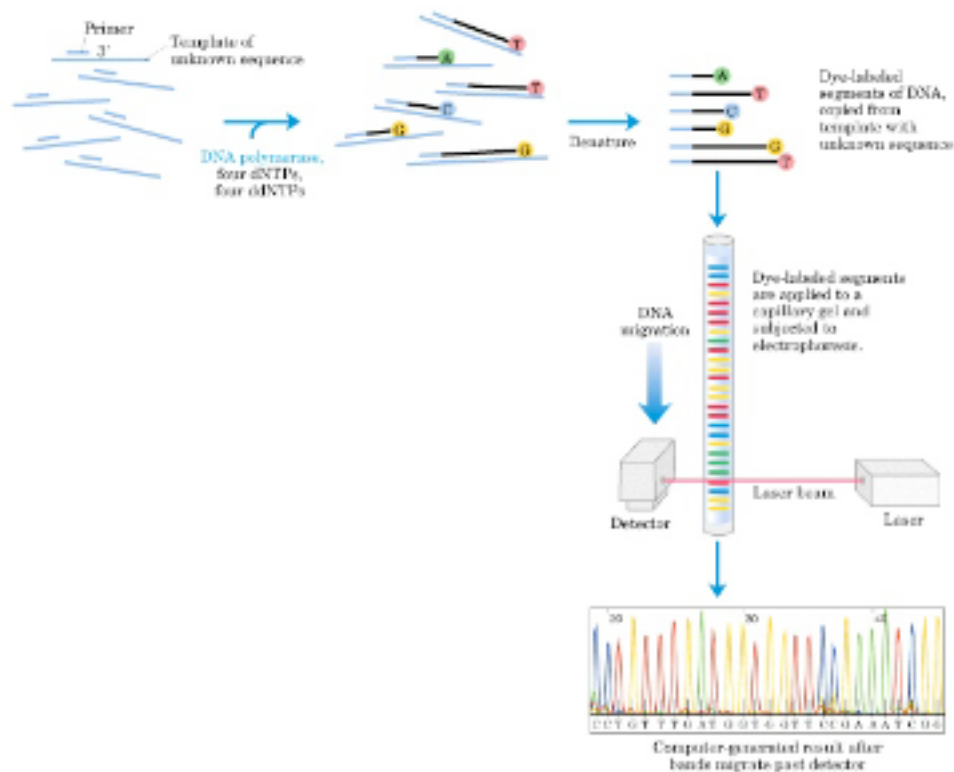


Figure 1. Schematic representation of a DNA cycle sequencing procedure

Materials

95% (v/v) ethanol/dH ₂ O (cold)	Glycogen (20 mg/mL)
70% (v/v) ethanol/dH ₂ O (cold)	Sample Loading Solution (SLS)
1.5 mL sterile microfuge tube	Stop Solution

Procedure

1. Label a 1.5 ml sterile microfuge tube with your lab section (101-106) and group number (assigned by your instructor). **Write down your group number in your laboratory notebook or someplace where you will remember to find it.**
2. Add **4 uL Stop Solution** and **1 uL 20 mg/mL glycogen (instructor)** to your microfuge tube.
3. Transfer your cycle sequencing reaction to the tube and **vortex gently to mix.**
4. Add **60 uL of ice-cold 95% ethanol** and **vortex gently.** Place your sample in the ice bucket. Samples will be centrifuged at **14,000 rpm at 4°C for 15 minutes** (Allegra 21R centrifuge).
5. Use a P-200 pipet to **carefully** remove the supernatant (liquid) from your DNA pellet (small, opaque, white spot near bottom of tube).
6. Add **200 uL of ice-cold 70% ethanol. DO NOT MIX.** Place your sample in the ice bucket. Samples will be centrifuged at **14,000 rpm at 4°C for 15 minutes** (Allegra 21R centrifuge).
7. Carefully remove **ALL** of the supernatant with a P-200 pipet, leaving the pellet at the bottom of the tube.
8. Repeat step 6
9. Repeat step 7
10. When all groups have completed step 9, place sample in vacuum dryer for 40 minutes.
11. After drying, you may no longer be able to see your pellet. Resuspend your sequencing reaction by adding 40 uL of **Sample Loading Solution (SLS)** to the bottom of your tube. Allow samples to sit at room temperature for 10 minutes for full resuspension.
12. Return your sample to your instructor for storage at -20°C. Samples from all laboratory sections will be placed on the genetic analyzer at the end of the week.

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(Due one week after sequencing results are returned)

Reading assignment: Lehninger 8.2-8.3 Nucleic Acid Structure, Nucleic Acid Chemistry,
26.1 DNA-dependent synthesis of DNA, and 27.1 The Genetic Code

1. Make a copy of your sequencing output. Appropriately label with your name, name of product sequenced, lab section and date. (3 pt).
2. Because of the nature of our cloning vector and the sequencing primer binding site, your sequencing results will be from either the “template” or “nontemplate” DNA strands of the GFP gene. Use information from your text to define these terms. (2 pt)

3. If your sequencing output is from the template strand, you will have the “reverse” (3-->5' direction) and “complement” (T for A and G for C) sequence of the “nontemplate” strand. To determine which one you have, look for one of the following nucleotide strings:

CTGGAGTTGTCCCAATTCTT (nontemplate)
AAGAATTGGGACAACCTCCAG (template)

Highlight this string in your results. Is your sample from the *template* or *nontemplate* strand?(2 pt)

4. GFP gets its name from its color, which is produced by a “chromophore” of amino acids that produce light when arranged in a particular fashion. The sequence for this chromophore is TGCTATGGT (nontemplate) or ACCATAGCA (template). Highlight the chromophore sequence in your results. Review the concept of triplet codons and the genetic code in your text. Use the standard genetic code (located inside the back cover of your text) to identify the amino acids in the chromophore and enter below. Remember, if your sequencing output is from the template sequence you must first determine the reverse, complementary sequence before the correct amino acid sequence can be identified. (2 pt)

4. Highlight the Csp45I restriction site (nontemplate: TTCGAA template: TTCGAA) on your sequencing output. The fact that the sequences are the same is not a mistake. Review the concept of *palindromes*, and how they pertain to restriction sites, in your text. (1 pt)

5. Laboratory performance (2 pt).