Genetic Sequencing Simulation C. Kohn, Waterford WI

Group Names (F&L):   
  
Hour Date: Why late? Score: + ✓ - If your project was late, describe why

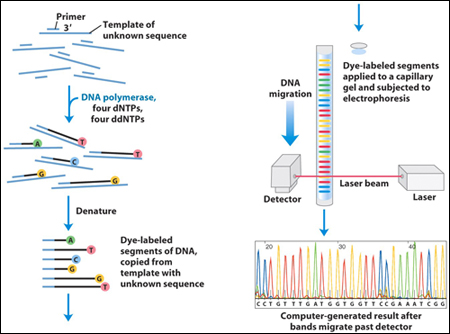
**Objective**: in this lab you will be replicating some of the steps in which scientists read DNA using the Sanger Method. Because we do not have access to the same equipment that a geneticist would use, we will replicate these steps using slips of paper instead of actual DNA. While our lab will not be an exact replication of the Sanger Method, it will help you to better understand how this process works.

**To complete this lab, complete the following steps:**

1. Begin by reading the Pre-Lab Summary answering the Pre-Lab Questions.
2. Obtain an envelope of paper “DNA fragments” from your instructor.
3. Sort the fragments from longest to shortest (in the same way a gel would separate the DNA fragments).
4. Convert the colors in the fragments into the bases each color represents.
5. Record the DNA sequence from shortest fragment to longest in the space provided in this packet.
6. Complete the post-lab questions and submit to your instructor.

# Pre-Lab Summary.

The **Sanger Method** is the technique used to read a **genome** of a species base by base. To read an animal’s DNA, a scientist must isolate a sample of white blood cells and extract their DNA. The DNA must be broken into smaller chunks using a restriction enzyme. The DNA chunks are inserted into bacteria which will reproduce the snippets of DNA millions of times.

The amplified DNA is removed from the bacteria and **denatured** (heated so that it unravels and becomes single-stranded). **Primers** are added, which tell the **polymerase** where to begin the process of replicating the DNA. **Nucleotides** are also added. Polymerase enzyme will add the bases to the single-stranded DNA to make it double-stranded once again.

Some of the nucleotides that have been added are modified so that they stop the transcription by polymerase at random points. These bases are also color coded; when excited by a laser, these special nucleotides (called **ddNTP’s**) will flash a color indicating the last base added.

Each copy of the chunk of DNA will be a different length depending on where the ddNTP stopped the polymerase for that particular copy of the DNA. When the millions of differently-sized copies of this chunk of DNA are pulled through an electrified gel, they will line up from shortest to longest. As each chunk moves through the gel past a laser, the chunk will flash a color that indicates that base added (the ddNTP). This sequence of colors will be sensed and recorded by a computer. The sequence of colors will correspond to the bases in the DNA, providing a scientist with the letters that make up the genes in the animal’s genome.

# Background.

Imagine you are a team of scientists trying to create a transgenic species of cattle that produce insulin in their milk. This would allow people with diabetes to avoid taking insulin injections with a needle and syringe. Your team has already spliced the gene for human insulin into the cow’s genome, but your team needs to confirm that your work was successful.

To test this, you have extracted the DNA of one of your cows. You have used a restriction enzyme to cut out the portion of the cow’s DNA where milk proteins are found and have amplified this section of the DNA using bacteria. You now have a purified and amplified form of the DNA for milk proteins and now must determine if the gene for insulin is found in this section of DNA. If the gene for human insulin can be found in this chunk of DNA, your work to create a transgenic cow was successful.

# Pre-Lab Questions.

1. In this lab, you will separate the fragments of DNA by size with your hands. In the Sanger Method, how would scientists actually separate the DNA on the basis of longest to shortest?   
     
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2. The Sanger Method requires many, many copies of the same piece of DNA. How are these copies of DNA made?  
     
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3. Once the copies of DNA are made, the DNA must be removed from the bacteria and denatured. What does this mean?   
     
   \_
4. After the DNA has been denatured, a scientist must add a primer, polymerase, and nucleotides. Describe the role of each of the following below:  
     
   Primer: \_   
     
   Polymerase: \_   
     
   Nucleotides: \_
5. Some of the nucleotides are ddNTP’s, or “fake” nucleotides. These nucleotides stop the transcription process and give a color to each copy of the chunk of DNA. Explain why this is necessary below:   
     
   \_   
     
   \_

# Post-Lab Questions.

1. What was the DNA sequence you worked with? Record it below (remember to start with the shortest strand and work up to the longest strand):   
     
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2. If the gene for human insulin[[1]](#footnote-1) is G-G-T-A-C-G-T-T-C-A-C-T-A-G, was this gene successfully added to your transgenic cows? (*NOTE: depending on how you arranged your DNA fragments, the sequence might be backwards*).  
     
   \_ Explain how you know:   
     
   \_
3. Name two ways this simulation is similar to how DNA is actually read:  
     
   \_   
     
   \_   
     
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4. Name two ways in which this simulation is different from how DNA is actually read:   
     
   \_   
     
   \_   
     
   \_
5. How would this be different if this test were done using…  
     
   Pyrosequencing:\_   
     
   \_   
   *(includes 454-Roche and Illumina Bridge)*Ion-Torrent:\_   
     
      
   *(i.e. hydrogen ion sensing sequencing)*  
   Nanopore:

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G

A

T

C

C-A-T-A-T-G-G-T-A-C-G-T-T-C-A-C-T-A-G-T-A

1. *Note: this is not actually the gene for human insulin. The gene for insulin is much larger and has a different sequence of bases. For the sake of time and ease, a shortened version of this gene has been created to make it easier for you.* [↑](#footnote-ref-1)