DNA Sanger Sequencing Worksheet Exercise

LB145 S13

>>This **replaces** the Sanger Sequencing Summary Homework on page 79 of the lab manual**<<**
>>DUE on April 15th/16th**<<**
>>Worth **5 points<<**

Let's suppose you have isolated and amplified a short fragment of DNA using Polymerase Chain Reactions. After completing this, you would like to take a closer look at the sequence of nucleotides in the DNA fragment. You use a method called dideoxy Sanger sequencing.

In this worksheet you will mimic the Sanger sequencing method to see how DNA is sequenced.

[Let's Look Into The Future] → Here is what a DNA sequencing lab will tell you…

This fragment of DNA that you have amplified has been sequenced as follows:

```
3' – CGAATATGCGAGTCTGGCAACTCC – 5'   (TEMPLATE STRAND)
     |||||||||||||||||||||||||
5' – GCTTATACGCTCAGACCGTTGAGG – 3'
```

While I've already spoiled the mystery by showing you the sequence of the DNA fragment above, the important thing I want you to learn is **HOW** DNA sequencing is accomplished using the Sanger Method.

Primer:
You have used a primer that has a short nucleotide sequence of: 5' – GCTTA – 3'

Your Task:

- Given this primer, your task is to **write out the set of different DNA fragment that you could synthesize using the dideoxy Sanger Method.**
- To accomplish this, assume that the box on the next page represents the reaction chamber where the dideoxynucleotide reactions occur.
- When you write out a DNA fragment, represent a DNA nucleotide using a normal letter (e.g. A, T, C, or G). To represent a dideoxyribonucleic acid nucleotide, write the letter in the appropriate color to (a) identify it as a *dideoxy*nucleotide and (ii) to show it has a fluorescent tag (as shown in the example on the following page).
- Underline the primer sequence for each fragment.

```
3′ — CGAATATGCGAGTCTGGCAACTCC — 5′   (TEMPLATE STRAND)
     |||||||||||||||||||||||||
5′ — GCTTATACGCTCAGACCGTTGAGG — 3′
```

Reaction Chamber Contains

dNTPs (A, C, T, G)
ddATPs (**A**)
ddCTP (**C**)
ddGTP (**G**)
ddTTP (**T**)

Examples:

5’ – GCTTAT – 3’

5’ – GCTTATA – 3’

5’ – GCTTATAC – 3’

5’ – GCTTATACG – 3’

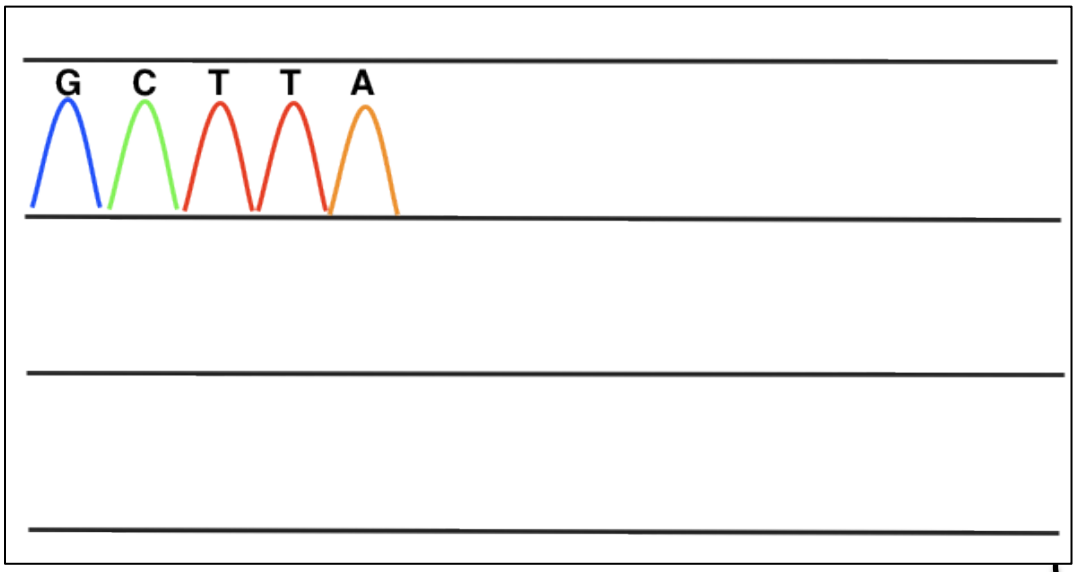
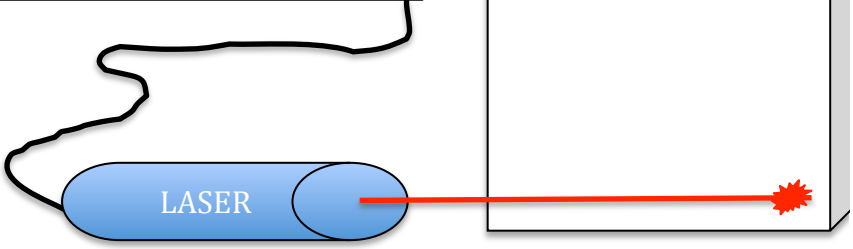
5’ – GCTTATACGC – 3’

etc…

Worksheet Questions:

1. Draw the chemical structures of ribose, deoxyribose and dideoxyribose.

2. Explain what would happen during a PCR elongation step if a dideoxyribonucleotide got incorporated into a growing nucleotide chain. Why would this happen?

3. Based on the DNA sequence you are working with (above), indicate on the gel to the right where you would expect to find each of the fragments you wrote down on the previous page. Assume that electrophoresis has run long enough to separate the fragments, but not so long that any of the fragments have fallen off the edge of the gel. On the gel to the right, w**rite down the nucleotide sequence of each fragment in the place where it would be found after the gel has been run.**

4. As the gel continues to run, the DNA fragments will eventually "fall off" the end. Before they do so, a laser at the bottom of the gel will read each of the fragments as it passes by and generate data on the graph below corresponding to the nucleotides it has identified (based on their fluorescent tags). Complete the graph below using appropriate colors to indicate the nucleotide sequence. Also, indicate which is the 3' end and which is the 5' end of the sequence once you have completed it below.