**Restriction Enzyme Lab Honors**

**OBJECTIVES**

Learn how to cut DNA into fragments with restriction enzymes.

Load and separate DNA fragments by gel electrophoresis.

Analyze electrophoresis gels.

# Experiment Outline

In this experiment, plasmid DNA from the bacteriophage lambda (48,502 base pairs in length) will be cut with a variety of restriction enzymes and the resulting restriction fragments will be separated using gel electrophoresis.

Monday -Samples of Lambda (phage) DNA are incubated at 37 degrees C, with either restriction endonuclease enzyme: *BstEII or HindIII*. A third sample will be the negative control in that is will be incubated without any endonuclease as our control.

Tuesday - The DNA samples are then loaded into wells of an agarose gel and electrophoresed, along with loading dyes (see procedure below). An electrical field applied across the gel causes the DNA fragments in the samples to move from their origin (a sample well) through the gel matrix toward the positive electrode (anode). Small DNA fragments migrate faster than larger ones, so restriction fragments of differing sizes separate into distinct bands during electrophoresis. The characteristic number and pattern of bands produced by each restriction enzyme are made visible by staining with a compound that binds to the DNA molecule and flouresencing under UV light.

**PROCEDURES *READ ALL DIRECTIONS CAREFULLY!!!***

# I. Use of the micropipetter

1. Be sure that the volume set on the pipetter is correct.

2. Place **new** tip firmly on micropipetter.

3. Depress plunger to first stop, and hold this position to eliminate any air in tip.

4. Dip tip into solution to be pipetted, and slowly draw fluid into tip by releasing plunger. Always touch pipette tip to side of tube to dislodge any small amount stuck to tip. You now have a sample inside of your pipette tip.

5. To expel sample, touch pipette tip to inside wall of tube into which you want to empty sample. This creates a capillary effect which will help draw fluid out of tip.

6. Slowly depress plunger to first stop and then depress to second stop to blow out last bit of fluid in tip. Do NOT release plunger before removing tip from fluid in tube. Otherwise, it will suck fluid back into tip.

7. When taking a sample, always check for air at the tip. If it is present, put the sample back and begin again.

**We will practice using water first. If you have any questions please ask your teacher.**

**II. Monday - Digest DNA with restriction endonucleases**

**(keep all enzymes on ice!!!!)**

1. You will receive a labeled 1.5ml tube, in which you will perform restriction digestion. This will depend on the enzyme your teacher has given you to work with:

*BstEII* enzyme,

*HindIII* enzyme

The control Lambda DNA uncut with no restriction enzyme

2. Add reagents to each tube in this order: DNA, restriction buffer, and enzymes last (you will have to ask your teacher for them).

**Use a fresh pipette tip for each different reagent.** The amounts are given in microliter (millionths of a liter). You must be very exact or the reaction will not work. **Please let me know if you have any questions!**

 **Your tube should have:**

 **4 µL of the lambda phage plasmid DNA**

 **5 µL of restriction buffer**

 **1 µL of your restriction enzyme**

**For the control, 1 µL water will be added in the place of the restriction enzyme**

3. Cap the tube and pool and mix reagents by placing them in the microcentrifuge and spin for ~20-30 seconds.

4. Incubate all reaction tubes for 30 minutes at 37 degrees C.

# IV. Tuesday - Loading gel with DNA

1. Remove the 4 tubes from the waterbath and pulse spin the tubes in a centrifuge so that all contents go down to the bottom of the tube.

2. Using the pipette with a new tip, add 2 ul loading dye to each reaction tube and tap contents of tube on table top.

3. Use the pipette to load contents of your reaction tube into a separate well in the gel. Set your micropipetter on 12 ul (that should be the total contents in the tube). Make sure to load you gel based on your tube number. There is NO way that the gel can be marked. **Use a clean pipette tip for each different tube.**

 \* Steady pipette over well using 2 hands.

 \* Positioned the pipette tip in the well, and slowly expel the mixture.

# V. Electrophoresis

1. The electrophoresis chamber top is placed on the chamber, the electrodes connected to power supply--anode to anode (red-red) and cathode to cathode (black-black).

2. Power supply is turned on and voltage set. In a few minutes, you should begin to notice the loading dye moving through the gel toward the + pole (anode). We will let it run for about 1 hour.

3. The loading dye will resolve into a band of color. Bromophenol blue migrates through the gel at the same rate as a DNA fragment approximately 300 base pairs long.

4. After 1 hour, the bromophenol band should be nearing the end of the gel. Turn off the power supply and remove the precast gel. We will only run our gel for ~30 minutes.

5. Place the gel on the U.V. box and close the protective cover. We can now view the bands. I will take a picture of your gel and email it to you at the end of class.

**NOTES:**

**You will get a “perfect” gel at the end of class for you to compare you gel to.**

**If we have time while the gel is running we will use a computational tool used in many laboratories to do a virtual restriction. Those instructions will also be handed out in class. If we do not have time students can opt to do this at home, but it will not be required for the lab report.**

# Questions

1. Which restriction enzyme produced the most restriction sites on the lambda DNA?

2. How can you account for differences in band separation and intensity between your gel and the ideal gel?

3. Small restriction fragments of nearly the same length will appear as a single band on this gel, even though it may be run to the very end. Why?

4. The speed of the DNA fragment migration correlates directly with the \_\_\_\_\_ of the fragment.

5. If one DNA strand has the sequence 5’ ATTCCGCTTGCAT 3’ what will the complementary DNA strand look like.

1. 5’ ATTCCGCTTGCAT 3’
2. 5’ ATGCAAGCGGAAT 3’
3. 5’ TAAGGCGAACGTA 3’

6. If a circular piece of DNA has 5 sites for a particular restriction enzyme, into how many fragments will the DNA be cut?

7. If a linear piece of DNA has 5 sites for a particular restriction enzyme, into how many fragments will the DNA be cut?

8. The bond that links adjacent nucleotides (a nitrogenous base, a five carbon sugar called a pentose, and a phosphate group) in DNA is called

1. a phosphate bond
2. an ester bond
3. a phosphodiester bond
4. a polynucleotide bond
5. a hydrogen bond
6. a hydroxide bond

9 . The bond that links nitrogenous bases together (A’s to T’s and G’s to C’s) in DNA is called

a. a phosphate bond

1. an ester bond
2. a phosphodiester bond
3. a polynucleotide bond
4. a hydrogen bond

10. What type of bond does a restriction enzyme break?