

Name _____

Pre-lab 1–DNA Analysis/Gene Expression Part 2

1. Sequence specific endonucleases cut DNA at _____ while micrococcal nuclease cuts DNA _____.
2. DNA fragments will “run” toward the _____ electrode during gel electrophoresis because the _____ groups make it a negatively charged molecule.
3. The statement that DNA fragment size is inversely proportional to mobility means: (Circle the single best response)
 - A. that fragments above a certain size are too large to migrate through the agarose gel.
 - B. that polar biomolecules are best suited for this technique.
 - C. that small fragments will migrate further than large fragments over a given time because they are able to weave through the agarose more easily.
 - D. none of the above are true
4. True or False: The buffer added to the gel box during electrophoresis contains TBE to aid in conduction of electrical current.
5. All *E. coli* cells that were able to grow and survive on the petri dishes with antibiotic added must be producing the _____ protein, which means the cells have taken up the plasmids and expressed the resistance gene.
6. True or False: Ethidium bromide is used to make the DNA visible when exposed to ultraviolet light.

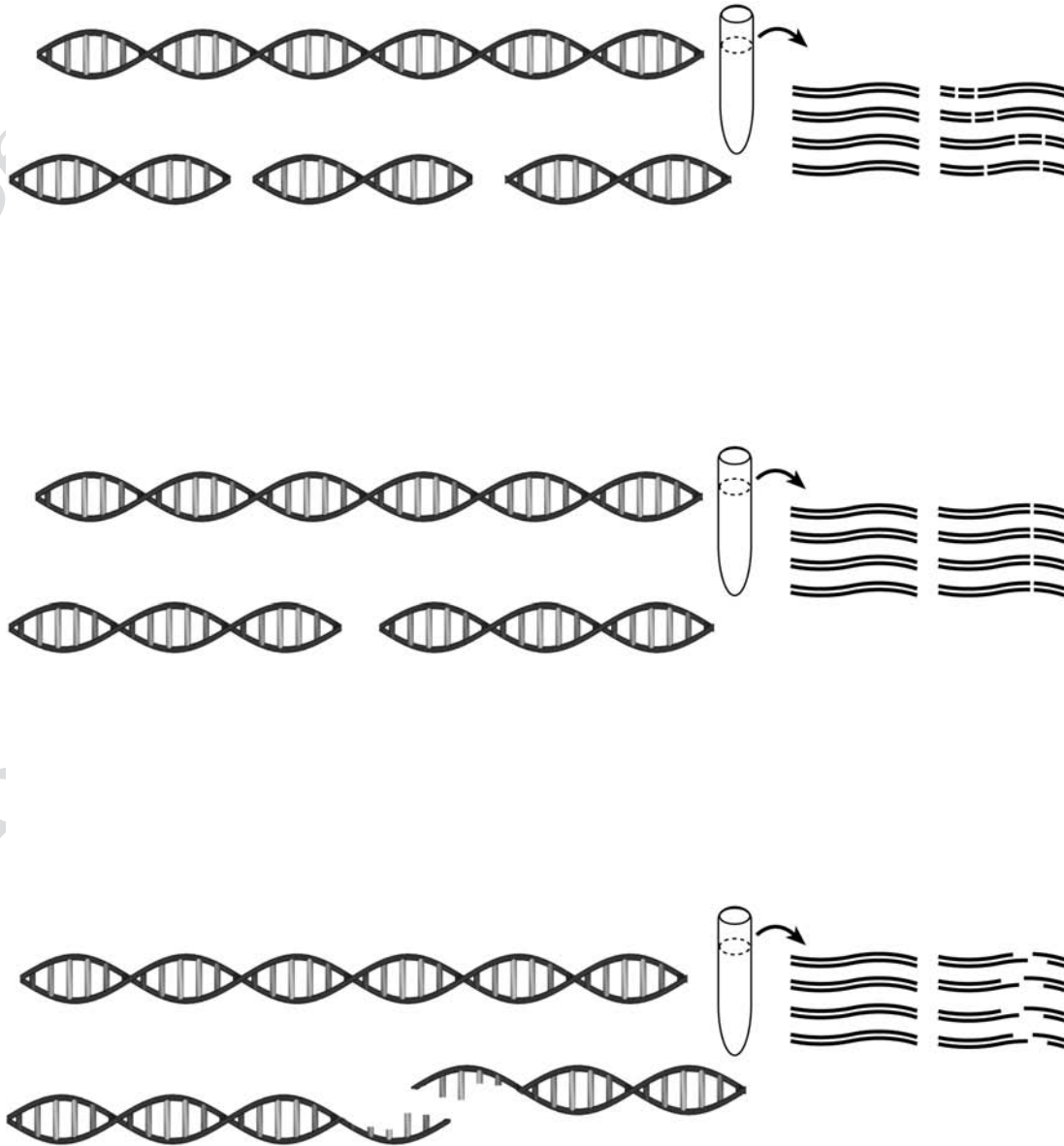


Figure 1.1 Cutting DNA with Restriction Enzymes.

LABORATORY

DNA Analysis Using Restriction Enzymes and Gel Electrophoresis; Gene Expression Part 2

INTRODUCTION

A. Restriction Endonucleases

Endonucleases are enzymes that cut between nucleotides of DNA at internal positions as opposed to exonucleases which digest DNA from the ends. They were first discovered in the 1960's when it was observed that the bacterium, *Escherichia coli*, possessed an enzyme system that could recognize and destroy invading bacteriophage DNA by cutting it into fragments. This action "restricted" the growth of the phage. For this reason these enzymes are more commonly called restriction endonucleases or just restriction enzymes. There are now several hundred restriction endonucleases that have been isolated from many prokaryotic organisms. Restriction enzymes are named according to the organism from which they are isolated. For example:

Restriction enzyme, EcoRI: E = genus *Escherichia*
 co = species *coli*
 R = strain RY13
 I = first endonuclease isolated from *E. coli* strain RY13

There are two kinds of restriction endonucleases:

1. Sequence specific restriction endonucleases. These enzymes recognize specific DNA base pair sequences and cut at these sites. Some cut cleanly through the double stranded DNA creating blunt ends and some cut each strand off center causing "sticky ends."

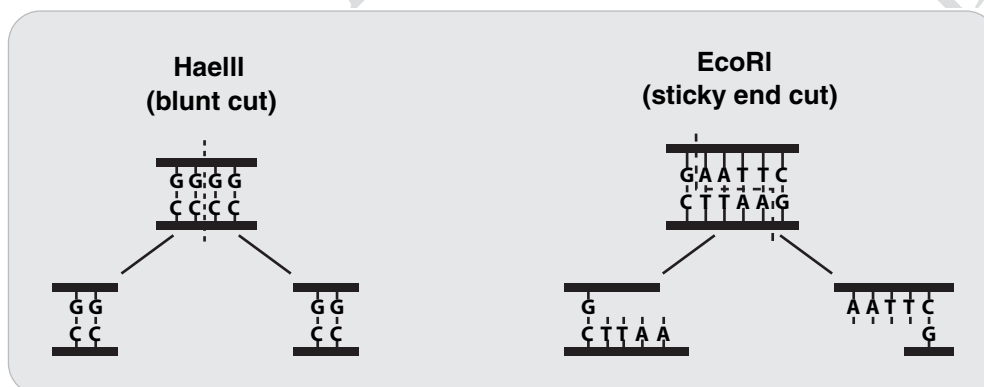


Figure 1.2 Sequence Specific Endonuclease Cuts.

The fragments generated by these enzymes can then be used in a variety of methods such as gel electrophoreses, DNA sequencing, gene analysis, cloning and DNA fingerprinting. These methods are used heavily in research, forensics, gene therapy, and determining parentage/lineage.

2. Non-sequence specific restriction endonucleases. These enzymes are not specific to any base pair sequence. Micrococcal nuclease is an example of a non-sequence specific endonuclease. Micrococcal nuclease cuts DNA randomly.

B. Gel Electrophoresis

Electrophoresis is one of the more commonly used research techniques in molecular biology. The root words electro- and -phoresis literally mean “to carry with electricity,” but the process is more commonly defined as the movement of charged particles (ions) under the influence of an electric field. The principles of electrophoresis are quite simple: charged molecules placed in an electric field will move to the oppositely charged electrode. Since biomolecules such as DNA, RNA and proteins have charges, they can be analyzed using this technique.

A supporting gel medium, such as agarose, is used to hold and separate biomolecules. They form a jello-like dense substance consisting of a network of cross-linked molecules through which the charged molecules must travel. This gel matrix will differentially sort molecules by size because it acts as a sieve to impede mobility. Smaller molecules will move farther from the point of origin than larger molecules in a given time, making molecular mobility inversely proportional to molecular size. All like-sized molecules will form into bands on the gel.

Since biomolecules in the gel are usually colorless, they must be stained with dyes. Ethidium bromide (EtBr) is commonly used in research as a DNA dye. It is a fluorescent dye that intercalates (stacks) between the nucleotides of the DNA helix and makes the DNA bands visible only when exposed to UV light. **Caution** must be taken when using ethidium bromide since it is a known mutagen. **Gloves MUST be worn.** Other dyes, such as methylene blue, can be used but low concentration DNA bands sometimes cannot be detected and staining/destaining takes several hours.

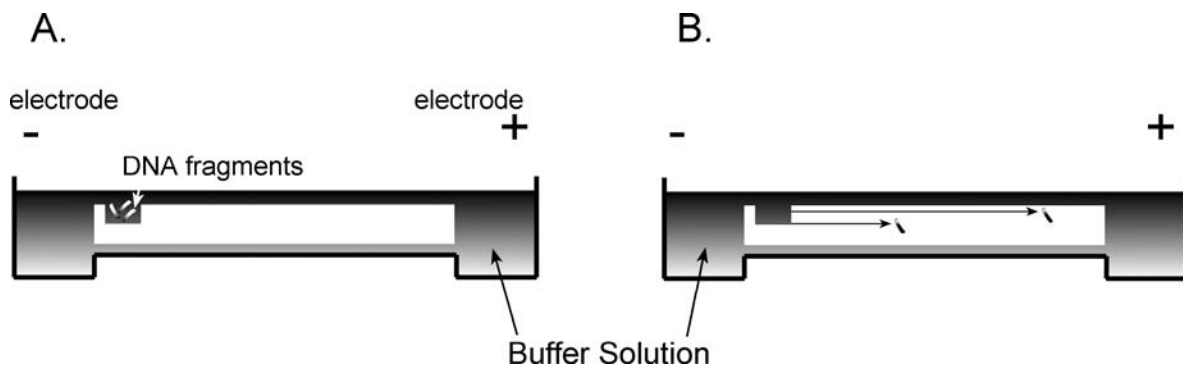


Figure 1.3 Movement of Biomolecules in a Gel.

A loading dye, also called a tracking dye, is put into the samples in order to monitor biomolecular migration during electrophoresis. When the dye is 1-2 cm from the bottom of the gel, the current can be cut off.

PART A: GEL ELECTROPHORESIS

Background

In this lab session, gel electrophoresis will be used to separate fragments of DNA cut with restriction endonucleases. Students will work in groups of 4-5. Each group will analyze an uncut onion DNA sample, an onion DNA sample cut with micrococcal nuclease, two unknown plasmid samples, and a DNA size standard (has bands of known size).

Materials

liquid agarose(1%, no EtBr)
gel bed
color coded DNA samples
well comb
3-cycle semi-log paper

gel box
micro pipettes
GLOVES
power supply box
Ethidium bromide staining solution
IX TBE buffer (No EtBr)

UV gel illuminator
gel camera
practice gels
practice loading dye

PROCEDURE

A. Cast agarose gels

1. Pour 40 mL melted 1% agarose with into gel bed as directed.
2. Place well comb into hot agarose.
3. Let gel cool approximately 15 minutes to set. Gel will look cloudy when set.
4. Gently remove comb from gel.

5. Once the experimental gels have set, each group will load one gel as follows:

<u>Lane</u>	<u>Sample</u>	<u>Amount</u>
1	uncut onion DNA	10 μ l
2	onion DNA cut with micrococcal nuclease	10 μ l
3	RE cut plasmid #1: _____	10 μ l
4	RE cut plasmid #2: _____	10 μ l
5	DNA standard: _____	10 μ l

Each sample contains a loading dye of bromophenol blue with sucrose, which allows one to visualize the movement of the DNA. Bromophenol blue migrates at about the same rate as a 300 base pair molecule. The density of sucrose helps keep DNA in the wells.

6. Put gel bed into gel box with wells oriented nearest the negative electrode.

B. Add buffer to gel box

TBE (Tris Borate EDTA) buffer without ethidium bromide is added to the gel box to aid in conducting electrical current and to stain DNA. **Pour buffer into gel box very slowly and carefully so as not to wash the DNA samples out of the wells.**

C. Electrophoresis

1. Attach lid to gel box and plug electrodes into power supply. The electrode nearest the wells should be plugged into the negative outlet.
2. Turn current on. Set voltage at about 100-125 volts. Increased voltage will increase the rate at which the DNA travels. At 100 volts, the gel should be ready to observe in 1 to 1 1/2 hours.
3. Turn off current when the loading dye is about 2-3 cm from the end of the gel.

D. Staining DNA bands in the gel

1. Gently remove gel from gel box and place into plastic dish containing ethidium bromide staining solution.
2. The gel will be ready for viewing using UV light after 20 minutes.

CAUTION: Students who handle gels must wear gloves. Ethidium bromide is a known mutagen.

E. Band observation

1. Gently remove gel bed from staining dish and place gel on the UV transilluminator. The transilluminator is an instrument used to illuminate ethidium bromide stained gels for viewing and photographing.

PART B: FRAGMENT SIZE DETERMINATION

Background

While the agarose gel is being run, students will practice graphing a standard curve and determining fragment sizes from a photo of a gel. Fragment mobility is inversely proportional to size. More specifically, the distance moved is inversely related to the log of the molecular weight. The standard contains fragments of known sizes. Plotting the distance traveled by the bands of the standard against the known fragment sizes of the standard gives a standard curve. Semilog paper is used (your instructor will demonstrate). Since fragment size is expressed as the log of the number of base pairs they contain, this transforms the data to a straight line instead of a curve that would be produced on linear graph paper. From this standard plot, unknown fragment sizes can be determined.

Materials

ruler
computer gel
semilog graph paper
photographs of experimental gels

PROCEDURE

1. Measure the migration distance in mm for each band produced by the DNA size standard on the computer-generated gel. Measure from the bottom of the well to the bottom of the band and record each distance in Table 1.1. Band sizes for the DNA standard are given.

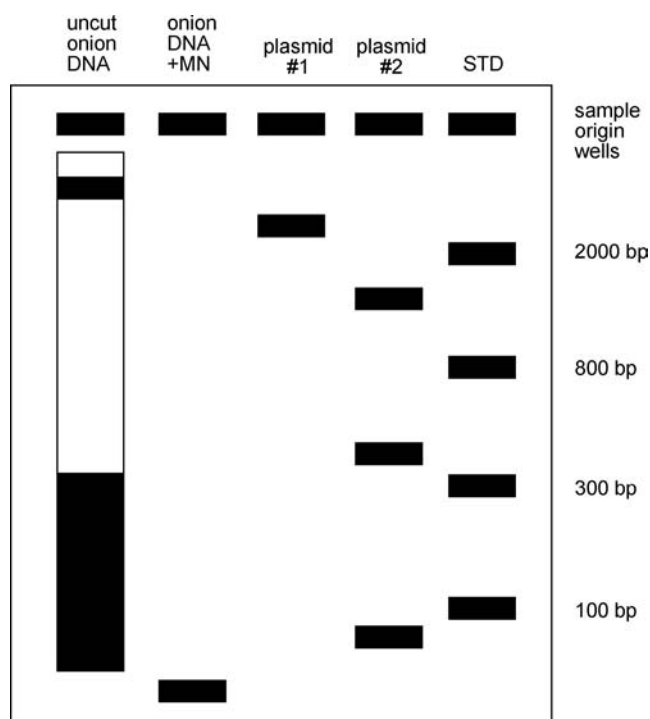


Figure 1.4 Computer-Generated Gel.

Table 1.1 Values of the Standard.

Band	Distance	Size (b.p.)
1		
2		
3		
4		

2. Using 3-cycle semilog graph paper, graph distance traveled vs. fragment size (in base pairs) of the standard. Fragment sizes are labeled on the gel.
 - a. Label linear-axis in mm increments representing distance traveled.
 - b. Mark log axis as DNA fragment sizes (represented as number of base pairs). The first cycle will represent 10-100 base pairs; the second cycle will represent 100-1,000 base pairs; the third cycle will represent 1000-10,000 base pairs.
 - c. Draw the best fit straight line with your plotted points.
3. Measure the migration distance in mm for each band on the computer-generated gel and determine the size of the fragments in each band using the standard curve. Record your results below.

Table 1.2 Values for Unknown Bands.

Band	Onion DNA + MN		Plasmid #1		Plasmid #2	
	Distance	Size (b.p.)	Distance	Size	Distance	Size (b.p.)

4. From the photograph of your gel determine the fragment sizes for the onion DNA and plasmid DNA samples as homework.

END OF PART A AND PART B DISCUSSION

Where are restriction endonucleases naturally found and how are they used?

What kind of ionic charge does DNA have and what gives it this charge?

Explain how a gel works to separate DNA fragments of various sizes during electrophoresis.

What do the bands on the gel represent?

Why is a standard used during electrophoresis?

Why does the onion DNA appear as a smear on the gel?

Why is *semi-log* paper used when graphing migration distance vs. size of DNA?

Explain some of the applications of the electrophoresis technique.

Why do we wear gloves during the lab?

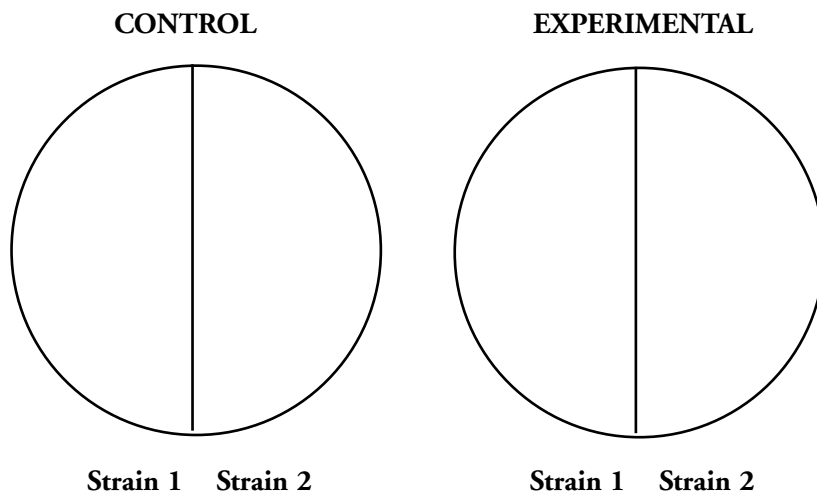
PART C: GENE EXPRESSION (WEEK 2)—INTERPRETING RESULTS

Day 2 (One week later)

1. Allow the plates to reach room temperature.
2. Observe the plates and record the results below and on the data table on the board. (Which strains grew on which plates?)
3. Discard plates according to instructions. ** Autoclave bags will be available—DO NOT DISCARD IN TRASH**

Results

Draw and label the growth pattern for both plates.



END OF PART C DISCUSSION

What happened with bacterium Strain 1 in the presence of ampicillin? Strain 2?

Based on your results, which bacterial strain do you think contained the ampicillin resistance plasmid?

What do the results of this experiment reveal to you about the importance of the expression of the gene for Beta Lactamase?

Antibiotic resistance is a real human health concern. Can you explain why some bacteria survive on nutrient agar plates containing antibiotics while others cannot? How might this affect someone who becomes infected with resistant bacteria? What would you have to do to treat this person?

How could the recombinant DNA technique be used medically to help people with hemophilia? diabetes? AIDS?