

## EXERCISE 9B

Name \_\_\_\_\_

# How are plasmids used in recombinant DNA technology?

***Day Two: Which unknown plasmid sample had no plasmids, which had non-recombinant pUC18 plasmids, and which had recombinant pUC18 plasmids?***

## Objectives

After completing this exercise, you should be able to:

- ◆ Analyze an agarose gel to determine the number of different-sized DNA fragments present in a sample.
- ◆ Analyze an agarose gel to determine the approximate size of each DNA fragment
- ◆ Determine which agar plates were inoculated with transformed bacteria and which were inoculated with bacteria that were not transformed.
- ◆ Examine colonies of transformed *E. coli*, to identify the colonies that carry a non-recombinant plasmid and the colonies that carry a recombinant plasmid.

## Prelab

**Before you come to lab, read this entire exercise.**

The separation of DNA molecules by electrophoresis is similar to the separation of proteins by electrophoresis. In both cases, the molecules to be separated are loaded onto a support medium and then subjected to an electrical field. The **cathode** (negative electrode) attracts positively charged molecules, while the **anode** (positive electrode) attracts negatively charged molecules. This attraction causes the molecules in the mixture to migrate through the support medium at different rates depending on their size, shape, chemical composition, and electrical charge. As the molecules migrate at different rates, they gradually separate from each other.

The main difference between protein electrophoresis and DNA electrophoresis lies in the composition of the support medium. While polyacrylamide gels are generally used for protein separations, agarose gels are commonly used for DNA separations. Agarose is a polysaccharide that is extracted from seaweed. When agarose is dissolved in a solution at high temperature it has a liquid consistency, but as the solution cools it turns into a gel. Agarose gels are cast by dissolving agarose in a Tris-buffered solvent at a high temperature, and then pouring the solution into a horizontal tray and allowing it to cool. A comb is inserted into the cooling gel so that wells are formed as the gel solidifies. The cooled gel is then transferred into a gel box where it is submerged in a Tris-buffered electrode solution before loading DNA samples into the wells. Since these gels are run horizontally and beneath the electrode buffer, they are sometimes referred to as “horizontal gels” or “submarine gels”.

DNA molecules will migrate towards the anode during electrophoresis because they have a high concentration of negatively-charged phosphate groups in the sugar-phosphate backbone of the double helix. These negative charges provide a fairly uniform charge-to-mass ratio among all DNA molecules. Because of this uniform ratio, the migration rate of DNA molecules during agarose gel electrophoresis is almost entirely a function of size. As with protein electrophoresis, smaller molecules move through the gel more rapidly than larger molecules. Scientists generally measure the size of DNA molecules in terms of **number of base pairs** (bp) rather than **molecular weight** (MW).

Recall that during SDS-PAGE there is a linear relationship between the migration distance of the proteins and log of MW. Similarly, during agarose gel electrophoresis there is a linear relationship between the migration distance of the DNA molecules and log of bp (number of base pairs.) Because of this, agarose gel electrophoresis can be used to estimate the size (number of base pairs) of DNA molecules. In order to do this, a standard curve that shows the relationship between migration distance through the gel and log of bp must be generated. The standard curve is generated by measuring the migration distance of several DNA molecules of known size (number of base pairs), plotting a scatter diagram of migration distance vs. log of bp, and then using linear regression to determine the equation of the “best fit” straight line for the data points. Once this is done, you can substitute the migration distance of any DNA molecule on your gel into the linear regression equation and then calculate the log of bp for that molecule.

DNA, like most proteins, is colorless. Therefore, before DNA samples are loaded onto an agarose gel, they are mixed with a sample buffer that includes a blue tracking dye. During electrophoresis, the tracking dye migrates very rapidly through the gel, along with the smallest of DNA fragments. When the dye approaches the end of the gel, you know it is time to stop the electrophoresis. Glycerol is also included in the sample buffer in order to make the sample denser so that it settles into the well as it is loaded.

After electrophoresis is complete, the gel is stained so that the DNA bands can be seen. The most commonly used dye for staining DNA is ethidium bromide, a dye that is highly specific for binding to DNA or RNA. DNA or RNA bound to ethidium bromide fluoresces strongly under ultraviolet light, so these gels are viewed and photographed using ultraviolet light.

Because both ethidium bromide and ultraviolet light are mutagens, you will be using a less sensitive but safer stain for your DNA gels: methylene blue. Methylene blue is a blue-colored stain that binds to DNA in the same way that Coomassie Blue binds to proteins. The blue color is easily seen using normal visible light

### ***What is the goal of this lab exercise?***

During exercise 9A, you were given 3 unknown plasmid samples labeled A, B, and C. These samples were used to carry out 2 experiments:

- 1) Competent *E. coli* cells were mixed with the 3 unknown samples in order to allow transformation to take place. The *E. coli* cells were then plated on nutrient agar containing ampicillin and Xgal.
- 2) The restriction enzyme EcoRI was mixed with the 3 unknown samples in order to cut any DNA present into fragments. The resulting DNA fragments were then separated using agarose gel electrophoresis.

In this lab exercise, you will view the results of these 2 experiments in order to determine which unknown plasmid sample had no plasmids, which sample had non-recombinant pUC18 plasmids, and which sample had recombinant pUC18 plasmids. In addition, you will use the results of agarose gel electrophoresis to estimate the size of the fragments that were produced when EcoRI digested the DNA in the 3 unknown samples.

## Lab Procedures

### I. Examine your DNA gel in order to determine which unknown plasmid sample had no plasmids, which had non-recombinant pUC18 plasmids, and which had recombinant pUC18 plasmids

1. Place a piece of plastic wrap over the top of a light box and gently slide your gel onto the plastic wrap. Cover your gel with another piece of plastic wrap.
2. Orient your gel so the triangular notch is in the lower right corner. Remember that the wells were assigned numbers counting from left to right.

Several of the lanes should contain blue bands. The blue bands are areas where DNA has been stained with methylene blue. Because the migration rate of the DNA fragments through the gel is based on size, all of the fragments in a single band should have the same size. Also, because you did not load any sample into well 6, this lane should have no bands.

3. How many bands are visible in the lane that was loaded with unknown sample A + *EcoRI*? \_\_\_\_\_  
How many bands are visible in the lane that was loaded with unknown sample B + *EcoRI*? \_\_\_\_\_  
How many bands are visible in the lane that was loaded with unknown sample C + *EcoRI*? \_\_\_\_\_
4. Based on your observations, which unknown sample contained no plasmids? Explain your answer.

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Which unknown sample contained plasmid pUC18 with no “foreign DNA” insert (non-recombinant plasmids)? Explain your answer.

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Which unknown sample contained plasmid pUC18 with the phage  $\lambda$  DNA insert (recombinant plasmids)? Explain your answer.

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## II. Measure the migration distance of the DNA fragments in your gel

1. "Marker DNA" is a commercially available preparation that contains many DNA fragments of known length. The marker DNA that you used contained DNA fragments with the following lengths:

<b>784 bp(base pairs)</b> <b>1120 bp</b> <b>2040 bp</b> <b>3621 bp</b>
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Which fragments do you expect to travel farthest from the well? \_\_\_\_\_

Which fragments do you expect to travel the least distance from the well? \_\_\_\_\_

Explain:

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2. Using a transparent millimeter ruler, measure the distance from the middle of each well to the middle of each DNA band on your gel. There should be four bands in the two "DNA marker" lanes and fewer bands in the A, B, and C lanes.

### Distance each DNA fragment traveled (mm)

Lane 1 Sample A	Lane 2 Marker DNA	Lane 3 Sample B	Lane 4 Marker DNA	Lane 5 Sample C
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____

## III. Use size and migration distance of the 4 DNA markers to prepare a standard curve

1. Using either a sheet of graph paper or a computer with spreadsheet program, make a scatter diagram that shows the relationship between log of bp and migration distance for the 4 DNA markers. Plot migration distance on the x-axis and log of bp on the y-axis.

**Make sure your scatter diagram is big enough so that it fills an entire 8.5" x 11" sheet of paper. See Appendix E for more detailed descriptions of graphing techniques.**

2. Using either a hand-held calculator or a computer with spreadsheet program, carry out linear regression to determine the equation for the "best fit" straight line for your data points. Write the equation on your graph. Next to the equation, write down the linear correlation coefficient. If the absolute value of the linear correlation coefficient is less than 0.95, ask your instructor for help.

**If you used a calculator, do not clear its memory at this point. You will use the stored data in Part IV.**

#### IV. Estimate the size of the fragments that were produced when EcoRI digested the DNA in your 3 unknown samples

1. Use your linear regression equation to estimate the size of the DNA fragments listed below. Make sure you include appropriate units of measure:

plasmid pUC18 \_\_\_\_\_

phage  $\lambda$  DNA \_\_\_\_\_

#### V. Observe your *E. coli* cultures

1. Retrieve the Petri dishes that you inoculated with *E. coli*.
2. Record the color of the colonies (if any) in each plate:

Plate A \_\_\_\_\_

Plate B \_\_\_\_\_

Plate C \_\_\_\_\_

#### Clean up

Dispose of any materials that had contact with *E. coli* cells in a **biohazard bag**.

Dispose of your excess solutions as directed by instructor.

Remove label tape and any marks made with a marking pen from all glassware. Wash and rinse all glassware, give it a final rinse with dH<sub>2</sub>O, and leave it inverted at your work area in order to drain.

All disposable glassware goes into the special glass disposal receptacle.

All instruments should be turned off and unplugged.

Wipe off your workspace with disinfectant.

Make sure everything that you have used is clean, put away, or discarded. Leave your work area in the same order that you found it in.

Wash your hands and ask your instructor to check your work area before you leave.

## Postlab:

1. From an examination of your agarose gel, how did you know which of your unknown plasmid samples contained the pUC18 plasmid with the phage  $\lambda$  DNA insert? Explain in detail.
2. From an examination of your agarose gel, how did you know which of your unknown plasmid samples contained the pUC18 plasmid without the phage  $\lambda$  DNA insert? Explain in detail.
3. According to your calculations, what is the approximate size of the pUC18 plasmid? How does your calculation compare to the known length of pUC18?
4. According to your calculations, what is the approximate size of the phage  $\lambda$  DNA fragment that was inserted into the pUC18 plasmid?
5. Each of your A, B, and C agar plates was inoculated with *E. coli*, but the results were very different.
  - a) Describe the results you observed on plate A. Also, provide a **detailed explanation** of your results in terms of the composition of the nutrient agar that was in the plate, the genotype of the *E. coli* cells that were inoculated on this plate, and the genotype of the plasmids that were absorbed by the *E. coli* cells (if any).
  - b) Describe the results you observed on plate B. Also, provide a **detailed explanation** of your results in terms of the composition of the nutrient agar that was in the plate, the genotype of the *E. coli* cells that were inoculated on this plate, and the genotype of the plasmids that were absorbed by the *E. coli* cells (if any).
  - c) Describe the results you observed on plate C. Also, provide a **detailed explanation** of your results in terms of the composition of the nutrient agar that was in the plate, the genotype of the *E. coli* cells that were inoculated on this plate, and the genotype of the plasmids that were absorbed by the *E. coli* cells (if any).