Analysis of Precut Lambda DNA

Restriction enzymes are a special class of proteins that cut DNA at specific sites and have become an indispensable tool in molecular biology. Restriction enzymes, also known as endonucleases, recognize specific sequences of DNA base pairs and cut or chemically separate the DNA at that site. The specific sequence of DNA recognized by a restriction enzyme is called a restriction site.

These unique enzymes occur naturally in some bacteria and act to protect them from invading viruses. Viruses called bacteriophages, phages for short, attack bacteria by inserting their genetic material into the bacterial cell. The phage commandeers the bacterial cell, replicating rapidly until the bacterial cell lyses and releases more phages to carry out the same infection process in neighboring cells. However, if the bacterial strain has restriction enzymes that recognize restriction sites on the invading phage nucleic acid, then enzymes can destroy the invading genetic material by digesting and inactivating the phage genes. Bacterial cells protect their own DNA from being self-digested by modifying certain nitrogen bases along their genome, this prevents their restriction enzymes from recognizing and digesting their own sequences.

Restriction Enzymes as Molecular Scissors

Scientists use restriction enzymes as tools to assist with DNA splicing. Restriction enzymes are used to cut both the DNA sequence of interest and the host DNA; they act like molecular scissors. Their discovery led to the development of recombinant DNA technology that allowed, for example, the large scale production of human insulin for diabetics using *E. coli* bacteria. Many restriction enzymes have been studied in detail, and more than 600 are available commercially and are used routinely for DNA modification and manipulation in laboratories.

Gel Electrophoresis

Once the DNA has been digested, the “soup” of fragments must be separated in order to learn more about each fragment, such as its size. Gel electrophoresis is a method used to separate DNA fragments based on their sizes by applying an electrical field to an agarose gel containing these DNA fragments. DNA contains many negative electrical charges, and scientists used this property to separate pieces of DNA. Once the fragments are loaded onto the gel, an electrical current is applied causing the negatively-charged DNA molecules to move towards the positive electrode (red). The agarose gel acts as a matrix of tiny pores that allow small particles to move through it relatively quickly. The larger fragments migrate much more slowly through the gel. After a set period of exposure to the electrical current, the DNA fragments are separated by size with the smaller ones located further away from the wells than the larger fragments. Fragments that are either the same or very similar in size will tend to migrate together through the gel. Fragment bands form as a result of the various distances the DNA segments migrate.

Stains to visualize DNA

DNA is colorless and must be visualized with a “stain”. In this laboratory activity, DNA bands may be stained with either Fast Blast™ DNA Stain or SYBR® Safe DNA gel stain then analyzed with Vernier’s bioimaging systems. Fast Blast stained gels can be viewed with the White Light Transilluminator and SYBR Safe stained gels can be viewed with the BlueView.
Transilluminator. Either transilluminator can be used with the ProScope HR and Logger Pro software to capture digital images of the stained gel and perform analysis to determine the sizes of the DNA bands.

**Lambda (λ) DNA**

In this activity, the DNA you will be working with is the genome of a virus that has already been digested into segments by restriction enzymes. Lambda DNA comes from a bacteriophage and is harmless to man and other eukaryotic organisms, and therefore, makes an excellent source of DNA for experimental study. The individual digests of this bacteriophage, a 48,502 base pair linear DNA segment, use three common restriction enzymes: EcoRI, HindIII and PstI.

The restriction sites for each of the restriction enzymes are as follows:

<table>
<thead>
<tr>
<th>Table 1: Restriction sites* recognized by EcoRI, HindIII, and PstI</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’….G[AATTC…….3’</td>
</tr>
<tr>
<td>3’….CTTAA G…….5’</td>
</tr>
<tr>
<td>5’….A[AGCTT…….3’</td>
</tr>
<tr>
<td>3’….TTCGA A…….5’</td>
</tr>
<tr>
<td>5’….CTGCA G…….3’</td>
</tr>
<tr>
<td>3’…G[ACGTC…….5’</td>
</tr>
</tbody>
</table>

*The four DNA bases are Adenine (A); Cytosine (C); Guanine (G); and Thymine (T)

Your task is to separate the DNA fragments based on size using a procedure known as gel electrophoresis and then compare the DNA fragments with those of a standard ladder whose base pair sizes are already known.

**Standard Ladder and DNA Fragment Size Determination:**

To determine the sizes of the experimental DNA bands, they must be compared to a standard ladder that has DNA bands with known molecular weights. The standard used during this activity is the HindIII digest of lambda DNA, a commonly used DNA molecular weight marker. The standard ladder is run in a lane next to the experimental lanes under the same conditions. Linear DNA fragment migration distance through a gel is inversely proportional to the log10 of its molecular weight (base pair number). This process is carried out using the Gel Analysis in Logger Pro.
OBJECTIVES

In this experiment, you will

• Perform agarose gel electrophoresis using three different predigested samples of lambda DNA and uncut lambda DNA.
• Stain the gel.
• Document and examine gel results with an imaging system.
• Construct a standard curve and determine the size of the DNA fragments from the gel using Logger Pro.

MATERIALS

| Computer | lambda DNA samples:*
| Vernier interface | lambda DNA – uncut
| Logger Pro | lambda DNA – EcoRI digest
| Vernier Blue Digital Bioimaging System | lambda DNA – HindIII digest
| or White Digital Bioimaging System | lambda DNA – PstI digest
| electrophoresis chamber & power supply | electrophoresis buffer, 50x, TAE*
| adjustable micropipette, 2–20 µL and tips | sample loading dye*
| adjustable micropipette, 20–200 µL and tips | fast Blast DNA stain*
| microwave oven or hot plate | multicolor micro test tubes*
| microcentrifuge | foam micro test tube holders*
| gel support film | agarose, 5 g*
| rocking platform | staining trays*
| water bath or heat block | permanent markers
| ruler, millimeter | laboratory tape (not regular sticky tape)

* Included in Bio-Rad kit

PRE-LAB ACTIVITY

1. Make a gel. Note: You do not need to do this step if you are using a pre-cast gel.
   a. Clean the lab table surface, wash your hands, glove, set the lab mat, and review lab safety procedures.
   b. Measure out 0.5 g of agarose and pour the powder into the flask.
   c. Measure out 50 mL of 1X TAE (Tris-Acetate- EDTA) buffer and transfer volume to the flask containing the agarose. Swirl gently and place a funnel, stem first, into the top of the flask.
   d. Place the flask with its cover in a microwave and heat on high for 40 seconds or until the solution starts to boil. The agarose solution must be crystal clear and void of suspended granules, use additional ten-second blasts of the microwave until this condition is attained.
   e. With hot-gloves, remove the hot flask and transfer the container to your lab space.
   f. While the flask is cooling, prepare the gel tray by taping the two open ends of the gel tray with lab tape (masking tape and Scotch tape will not work) then place the eight-toothed comb in position at one end of the tray. The tray needs to be placed level on the surface of the lab mat.
   g. Allow the solution to cool until the flask can comfortably be placed on the back of your palm (60° to 55°C).
**PROCEDURE**

Quick Guide for Analysis of Precut Lambda DNA Kit

<table>
<thead>
<tr>
<th>Part A Sample Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Obtain a set of colored micro test tubes and label as follows:</td>
</tr>
<tr>
<td>yellow ( L ) = lambda DNA</td>
</tr>
<tr>
<td>violet ( P ) = ( PstI ) lambda digest</td>
</tr>
<tr>
<td>green ( E ) = ( EcoRI ) lambda digest</td>
</tr>
<tr>
<td>orange ( H ) = ( HindIII ) lambda digest</td>
</tr>
<tr>
<td>2. Using a fresh tip for each sample, pipet 10 ( \mu L ) of DNA sample from each stock tube and transfer to the corresponding colored micro test tube.</td>
</tr>
<tr>
<td>3. Add 2 ( \mu L ) of sample loading dye to each tube. Mix the contents by flicking the tube with your finger.</td>
</tr>
<tr>
<td>4. Optional: Heat the DNA samples at 65(^\circ)C for 5 minutes.</td>
</tr>
<tr>
<td>5. Pulse-spin the tubes in the centrifuge to bring all of the liquid to the bottom or tap them gently on the benchtop.</td>
</tr>
<tr>
<td>6. At this point you can put the DNA samples into the refrigerator and run the agarose gel during the next class or you can continue with Step 7 and run the agarose gel today. Ask your instructor if you are unsure what to do.</td>
</tr>
</tbody>
</table>
### Part B Agarose Gel Electrophoresis

7. Remove the agarose gel from the refrigerator (if necessary), remove the plastic wrap, and place the gel in the electrophoresis chamber. Fill the electrophoresis chamber and cover the gel with approximately 275 mL of 1x buffer.

8. Check that the wells of the agarose gels are near the black (–) electrode and that the bottom edge of the gel is near the red (+) electrode.

9. Load 10 µL of each sample into separate wells in the gel chamber in the following order:
   - Lane 1: L (yellow tube)
   - Lane 2: P (violet tube)
   - Lane 3: E (green tube)
   - Lane 4: H (orange tube)

10. Place the lid on the electrophoresis chamber carefully. Connect the electrical leads into the power supply, red to red and black to black.

11. Turn on the power and run the gel at 100 V for 30 minutes.

12. When the electrophoresis run is complete, turn off the power and remove the top of the chamber.
Part C Staining the Gel

13. Your teacher will let you know which of the four staining options you will use.

**Option 1**: Overnight staining using 1x Fast Blast stain  
**Option 2**: Quick staining using Bio-Rad’s 100x Fast Blast stain (requires 12-15 minutes)  
**Option 3**: Vernier SYBR Safe Stain post-electrophoresis  
**Option 4**: Vernier SYBR Safe Stain pre-electrophoresis

<table>
<thead>
<tr>
<th>Option 1: Overnight staining using 1x Fast Blast stain</th>
<th>Option 2: Quick staining using Bio-Rad’s 100x Fast Blast stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>14. Stain the gel using 1x Fast Blast Stain.</td>
<td>14. Stain the gel using Bio-Rad’s 100x Fast Blast stain.</td>
</tr>
<tr>
<td>a. Remove the gel and tray from the gel box. Be careful because the gel is very slippery. Slide the gel into the staining tray.</td>
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</tr>
<tr>
<td>b. Add 120 mL of 1x Fast Blast stain into a staining tray (2 gels per tray).</td>
<td>b. Add 120 mL of 100x Fast Blast stain into a staining tray (2 gels per tray).</td>
</tr>
<tr>
<td>c. Let the gels stain overnight, with gentle shaking for best results. No destaining is required.</td>
<td>c. Stain the gels for 2 minutes with gentle agitation. Save the used stain for future use.</td>
</tr>
<tr>
<td>d. Pour off the water into a waste beaker.</td>
<td>d. Transfer the gels into a large washing container and rinse with warm (40–55°C) tap water for approximately 10 seconds.</td>
</tr>
<tr>
<td>e. Proceed to the Analysis section.</td>
<td>e. Destain by washing twice in warm tap water for 5 minutes each with gentle shaking for best results.</td>
</tr>
<tr>
<td></td>
<td>f. Proceed to the Analysis section.</td>
</tr>
</tbody>
</table>
## Option 3: SYBR Safe Stain post-electrophoresis stain

   a. Remove the gel and tray from the gel box. Be careful because the gel is very slippery.
   b. Place the gel in a gel staining tray and measure out 50 mL or enough of the SYBR Safe DNA Gel Staining solution (0.5x) to cover the gel in the tray.
   c. Obtain a piece of Aluminum foil to cover and keep light from directly shining on the gel in the staining tray. The gel will be left in this staining solution for 30 minutes.
   d. Place gel on rocker or periodically swirl the solution and gel in the staining tray to obtain a thorough and uniform stain of the DNA fragments.
   e. No destaining is necessary once the thirty-minute period has elapsed; rinse the gel with water and proceed to the Analysis section.

## Option 4: SYBR Safe Stain pre-electrophoresis stain

14. Your gel has already been stained. Remove the gel and tray from the gel box. Be careful because the gel is very slippery. Proceed directly to the Analysis section.

## ANALYSIS

### Photodocumentation of Gel

1. Connect the transilluminator to AC power and turn it on.

2. Prepare the ProScope for use.
   a. Connect the 1–10x lens to the ProScope.
   b. Connect the ProScope to the USB port of your computer.
   c. Mount the ProScope to the stand and position the stand next to the transilluminator, opposite the side with the hinge.
   d. Level the ProScope so that its lens is parallel to the surface of the transilluminator.

3. Prepare Logger Pro for use.
   a. Start Logger Pro.
   b. Choose Gel Analysis►Take Photo from the Insert menu.
   c. Check Close Window and Auto Arrange as the Photo Actions.
4. Take a photo of the gel.
   a. Transfer the gel to the central portion of the transilluminator platform.
   b. Orient the gel and platform so that the wells are at the top of the picture in Logger Pro.
   c. Orient and focus the ProScope so both the bands and lane numbers are clear and sharp. Note: Lowering the brightness by clicking Camera Settings may improve visibility of the gel.
   d. Place the Imaging Hood over the ProScope and the transilluminator. Reach through the flap of the hood to make final adjustments for best position, focus, and resolution.
   e. Once satisfied with the image, click [Take Photo]. The screen should now resemble Figure 1. Note: You may want to re-size the photo and graph to increase the size of the photo for ease of analysis.

5. Indicate the position of the wells on the photograph.
   a. Click Set Origin, [Set Origin].
   b. Click the photograph just to the left of the first well. A yellow coordinate system will appear.
   c. Position the x-axis directly along the bottom edge of the wells. You can move the origin by clicking either axis and dragging it to the desired location. The axis can be rotated by clicking the round handle on the x-axis.

6. Convert the units of distance from pixels to millimeters.
   a. Click Set Scale, [Set Scale].
   b. Click and drag to draw a line that is one centimeter long using the ruler on the gel tray as your guide. For example, click and drag between the 1 cm mark and the 2 cm mark to create a line that is one centimeter long.
   c. Enter the distance value and units as millimeters and click [OK].

7. Identify the bands and base pair values of the standard ladder using the HindIII digest lane base pair values in Table 2.
   a. Click Set Standard Ladder, [Set Standard Ladder].
   b. Click the center of the second band in the HindIII digest lane (the fourth lane).
   c. Enter the number of base pairs for this band using the values in Table 2. Click [OK].
   d. Click the center of the next band in this lane and enter the base pair value. Click [OK].
   e. Repeat this process for each visible band of the standard ladder. Logger Pro will automatically create a standard curve on the graph.

8. Identity the bands in the remaining lanes.
   a. Click Add Lane, [Add Lane] and select Add Lane.
   b. Click the center of the second band in the EcoRI digest lane (the third lane). Notice that when you click, three things happen: a marker with a distinct shape and color appears on the photograph, a matching marker appears on the standard curve of the graph, and the distance and number of base pairs are added to the data table.
c. Click the center of the next band in this lane.
d. Continue this process for each visible band in the EcoRI digest lane.

9. Repeat Step 8 for the PstI digest lane (the second lane). You will start with the first band in this lane. Note: Do not add a lane for the uncut lambda DNA. It contains only one band, which contains approximately 48,000 base pairs.

10. Record the base pair values for the experimental lanes in Table 2. Not all cells will be filled.

11. (optional) Save and print the results of the gel analysis.

**DATA**

Record your results in the table below to reflect the migrations patterns on your gel and determine the size of the fragments. Some lanes will have fewer than six bands.

<table>
<thead>
<tr>
<th>Table 2 Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>L = uncut lambda DNA</td>
</tr>
<tr>
<td>Distance (mm)</td>
</tr>
<tr>
<td>Band 1</td>
</tr>
<tr>
<td>Band 2</td>
</tr>
<tr>
<td>Band 3</td>
</tr>
<tr>
<td>Band 4</td>
</tr>
<tr>
<td>Band 5</td>
</tr>
<tr>
<td>Band 6</td>
</tr>
</tbody>
</table>

**QUESTIONS**

1. What is a restriction enzyme?

2. How are restriction enzymes useful to bacteria in nature?

3. Describe the palindromic property of restriction sites.

4. How are the DNA fragments separated from one another after a restriction digest has been performed?

5. What is a molecular weight standard (also known as marker or ladder) and what is its role?

6. How is the DNA visualized?
Vernier Lab Safety Instructions Disclaimer

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- Directions for preparing solutions
- Important tips for successfully doing these labs