Investigation #9: Restriction Enzyme
### Pacing/Teacher's Notes

**Teacher's Notes**

Lab procedure adapted from College Board AP Biology Investigative Labs: An Inquiry Approach Teacher's Manual

[Click here for CB AP Biology Teacher Manual](#)

<table>
<thead>
<tr>
<th>Day (time)</th>
<th>Activity</th>
<th>General Description</th>
<th>Reference to Unit Plan</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 (HW)</td>
<td>Pre-lab Activity I</td>
<td>Reading &amp; Activity 1</td>
<td>Cells Day 6 HW</td>
<td>Depends on your schedule; may wish to skip virtual lab and have students pour gels instead in preparation for running gels tomorrow.</td>
</tr>
<tr>
<td>Day 2 (HW)</td>
<td>Pre-lab Activity II &amp; III Activity 1 &amp; 2</td>
<td>Cells Day 7 HW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3 (80)</td>
<td>Guided Practice</td>
<td>Prepare and run gel</td>
<td>Cells Day 8</td>
<td>Stain gels overnight</td>
</tr>
<tr>
<td>Day 4 (40)</td>
<td>Analysis</td>
<td>Analyze mock gel; analyze run gels for HW</td>
<td>Cells Day 9</td>
<td>HW may be skipped if bands on gel are not visible</td>
</tr>
<tr>
<td>Day 5 (20)</td>
<td>Assessment</td>
<td>Lab Quiz</td>
<td>Cells Day 10</td>
<td>Consider assigning an essay promoted by &quot;Thinking About Your Results&quot; questions</td>
</tr>
</tbody>
</table>
Pre-Lab

Question/Objectives

How can we use genetic information to identify and profile individuals?

In this lab we will:

· Use restriction enzymes and gel electrophoresis to create genetic profiles.

Pre-Lab Questions

Read the scenario and background information and answer the following questions in your lab notebook.

After reading Activity I: Restriction Enzymes

5'-AAAGTCGCTGGAATTCACTGCATCGAATTCCCGGGGCTATATATGGAATTCGA-3'

1. What is the sequence of the complementary DNA strand?
2. Assume you cut this fragment with the restriction enzyme EcoRI. The restriction site for EcoRI is 5'-GAATTC-3', and the enzyme makes a staggered ("sticky end") cut between G and A on both strands of the DNA molecule. Based on this information, draw an illustration showing how the DNA fragment is cut by EcoRI and the resulting products.
Pre-Lab Questions

After reading Activity II: DNA Mapping Using Restriction Enzymes

1. Can you make a prediction about the products of DNA from different sources cut with the same restriction enzyme? Will the RFLP patterns produced by gel electrophoresis produced by DNA mapping be the same or different if you use just one restriction enzyme? Do you have to use many restriction enzymes to find differences between individuals? Justify your prediction.

2. Can you make a prediction about the RFLP patterns of identical twins cut with the same restriction enzymes? How about the RFLP patterns of fraternal twins or triplets?

Pre-Lab Questions

After reading Activity III: Basic Principles of Gel Electrophoresis

1. Why do DNA fragments migrate through the gel from the negatively charged pole to the positively charged pole?

Safety

Never handle gels with your bare hands.

An electrophoresis apparatus can be dangerous because it is filled with highly conductive salt solution and uses DC current at a voltage strong enough to cause a small shock. Always turn the power supply switch "OFF" and wait 10 seconds before making any connection.

Connect BOTH supply leads to the power supply (black to black and red to red) BEFORE turning on the power supply. After use, turn off the power supply, and then disconnect BOTH leads from the power supply.
Guided Investigation

Materials

Your Workstation
- 20 microliter vials of DNA fragments prepared using restriction enzymes
- Rack for holding samples
- 3 plastic bulb transfer pipettes
- Permanent marker
- Gel electrophoresis chamber
- Power supply
- Staining tray
- Semi-log graph paper
- Ruler
- Lab notebook

Common Workstation
- 0.8% agarose solution (or gel, if prepared ahead of time)
- 1 x TAE (tris-acetate-EDTA) buffer
- Methylene blue stain

Casting Agarose Gel

Step 1  Seal the ends of the gel-casting tray with tape, dams, or any other method appropriate for the gel box that you are using. Insert the well-forming comb. Place the gel-casting tray out of the way on the lab bench so that the agarose poured in the next step can set undisturbed.

Step 2  Carefully pour the liquid gel into the casting tray to a depth of 5-6 mm. The gel should cover only about one-half the height of the comb teeth. While the gel is still liquid, use the tip of a pipette to remove any bubbles.

Step 3  The gel will become cloudy as it solidifies (15-20 minutes). Do not disturb or touch the gel while it is solidifying.
### Casting Agarose Gel

**Step 4** When the agarose has set, carefully remove the ends of the casting tray and place the tray in the electrophoresis gel box so that the comb is at the negative (black) end.

**Step 5** Fill the box with 1 x TAE buffer, to a level that just covers the entire surface of the gel.

**Step 6** Gently remove the comb, taking care not to rip the wells. Make sure that the sample wells left by the comb are completely submerged in the buffer.

**Step 7** The gel is now ready to be loaded with your DNA samples. (If you preparing the gel ahead of time, close the electrophoresis chamber to prevent drying of the gel.)

### Loading the Gel

**Step 1** Load 15 - 20 microliters of each sample of DNA into a separate well in the gel, as shown below.

**Step 2** Slowly draw up the contents of the first sample tube into the pipette.

**Step 3** Using two hands, steady the pipette over the well you are going to load.

**Step 4** Expel any air in the end of the pipette before loading the DNA sample.

**Step 5** Dip the pipette tip through the surface of the buffer, position it just inside the well, and slowly expel the mixture.

**Step 6** Draw the pipette tip out of the buffer.

**Step 7** Using a clean loading device for each sample, load the remaining samples into their wells.
**Electrophoresis**

**Step 1** Close the top of the electrophoresis chamber and then connect the electrical leads to an appropriate power supply, positive (+) electrode to positive (+) electrode (black to black) and negative (-) electrode to negative (-) electrode (red to red).

**Step 2** Turn on the power supply and set the voltage as directed by your teacher.

**Step 3** Shortly after the current is applied, you should see loading dye moving through the gel toward the positive pole of the electrophoresis apparatus.

**Step 4** Allow the DNA to electrophorese until the loading dye band is about 1 cm from the end of the gel.

**Step 5** Turn off the power supply, disconnect the leads form the power supply, and remove the lid of the electrophoresis chamber.

**Step 6** Carefully remove the casting tray and slide the gel into a staining tray labeled with the name of your group.

**Step 7** Take your gel to your teacher for staining instructions.

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**Analyzing "Ideal" Gel**

Using the "ideal" gel pictured below, measure the distance (in cm) that each fragment migrated from the origin (well). Enter the measured distances into the data table.

![Image of ideal gel]

<table>
<thead>
<tr>
<th>Distance (bp)</th>
<th>Fragment A</th>
<th>Fragment B</th>
<th>Fragment C</th>
<th>Fragment D</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td></td>
<td><em>200</em></td>
<td></td>
<td><em>200</em></td>
</tr>
<tr>
<td>150</td>
<td></td>
<td><em>150</em></td>
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<tr>
<td>100</td>
<td></td>
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<td>50</td>
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<td><em>50</em></td>
<td><em>50</em></td>
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</table>

*Data points marked with an asterisk (*) represent fragments that are present in the gel.*

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For the "ideal" gel, ensure that these two bands appear as a single band instead of resolving into separate bands.

*These bands do not appear on the ideal gel and likely will not be seen.*
Analyzing "Ideal" Gel

Plot the standard curve using the data from the DNA sample cut with HindIII. Connect the data points with a best-fit line. However, you should ignore the point plotted for the 27,491/23,130 doublet. When using 0.8% agarose gel, these fragments appear as one.

Now use the standard curve to calculate the approximate sizes of the EcoRI and BamHI fragments.

### Table 1: DNA Fragment Migration Distance

<table>
<thead>
<tr>
<th>Distance Plotted</th>
<th>HindIII BP Length</th>
<th>BamHI BP Length</th>
<th>EcoRI BP Length</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>27,491</em></td>
<td>0.484</td>
<td>0.484</td>
<td>0.484</td>
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<tr>
<td><em>23,130</em></td>
<td>0.406</td>
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<tr>
<td>4,991</td>
<td>0.015</td>
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</tr>
<tr>
<td>3,005</td>
<td>0.007</td>
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<tr>
<td>2,019</td>
<td><em>0.004</em></td>
<td><em>0.004</em></td>
<td><em>0.004</em></td>
</tr>
</tbody>
</table>

*For this "slide" gel, ensure that these two bands appear as a single band instead of reaching into separate bands.
**These bands do not appear on the ideal gel and likely will not be seen.*

Independent Inquiry

Measure the distance traveled by your DNA fragments. Use the standard curve to estimate the length (in bp) of your DNA fragments.
Evaluating Results

1. What are some possible challenges you had in performing your investigation?
2. What are some possible sources of error in the electrophoresis procedure? How can you minimize any potential sources of error?