You are about to perform a procedure known as DNA fingerprinting. The data obtained may allow you to determine if the samples of DNA that you will be provided with are from the same individual or from different individuals. For this experiment it is necessary to review the structure of DNA molecules.

DNA consists of a series of nitrogenous base molecules held together by weak hydrogen bonds. These base pairs are in turn bonded to a sugar-phosphate backbone. The four nitrogenous bases are adenine, thymine, guanine, and cytosine (A, T, G, and C). Remember the base-pairing rule is A - T and G - C. Refer to the figure below of a DNA molecule.

The Structure of DNA

The schematics above represent a very small section of DNA from three different individuals. In this representation of DNA the symbol system is as follows:

**Backbone:**

S = Five carbon sugar molecule known as deoxyribose

P = Phosphate group

**DNA Nucleotide Bases:**

A = adenine    C = cytosine    G = guanine    T = thymine

Analysis of the three DNA samples above (see next page) might help us detect similarities and differences in samples of DNA from different people.
Pre-Lab Focus Questions: Introduction to DNA Fingerprinting

Consideration  What is the structure of DNA?

1. Compare the “backbone” of the sugar-phosphate arrangement in the side chains of all three figures. Are there any differences?

2. In the above figure, do all three samples contain the same bases? Describe your observations.

3. Are the bases paired in an identical manner in all three samples? Describe the pattern of the base pair bonding.

4. In your attempt to analyze DNA samples from three different individuals, what conclusions can you make about the similarities and differences of the DNA samples?

5. What will you need to compare between these DNA samples to determine if they are identical or non-identical?
Lesson 1  Restriction Digestion of DNA Samples

Consideration  How can we detect differences in base sequences?

At first sight, your task might seem rather difficult. You need to determine if the linear base pair sequence in the DNA samples is identical or not! An understanding of some historically important discoveries in recombinant DNA technology might help you to develop a plan.

In 1968, Dr. Werner Arber at the University of Basel, Switzerland and Dr. Hamilton Smith at the Johns Hopkins University, Baltimore, discovered a group of enzymes in bacteria, which when added to any DNA will result in the breakage [hydrolysis] of the sugar-phosphate bond between certain specific nucleotide bases [recognition sites]. This causes the double strand of DNA to break along the recognition site and the DNA molecule becomes fractured into two pieces. These molecular scissors or "cutting" enzymes are restriction endonucleases.

Two common restriction enzymes (endonucleases) are EcoRI and PstI which will be provided to you in this lab procedure. To better understand how EcoRI and PstI may help you in performing your DNA fingerprinting experiment, first you must understand and visualize the nature of the "cutting" effect of a restriction endonuclease on DNA:

The line through the base pairs represents the sites where bonds will break if the restriction endonuclease EcoRI recognizes the site GAATTC. The following analysis questions refer to how a piece of DNA would be affected if a restriction endonuclease were to "cut" the DNA molecule in the manner shown above.

1. How many pieces of DNA would result from this cut? ___________

2. Write the base sequence of the DNA fragments on both the left and right side of the "cut".

   Left: _____________________________

   Right: ____________________________

3. What differences are there in the two pieces?
4. DNA fragment size can be expressed as the number of base pairs in the fragment. Indicate the size of the fragments [mention any discrepancy you may detect].
   a) The smaller fragment is ___________ base pairs (bp).
   b) What is the length of the longer fragment? ______________

5. Consider the two samples of DNA shown below - single strands are shown for simplicity:

   
   **Sample #1**
   
   C A G T G A T C T C G A A T T C G T A A C G T T

   **Sample #2**
   
   T C A T G A A T T C C T G G A A T C A G C A A A T G C A

   If both samples are treated with the restriction enzyme EcoRI [recognition sequence **GAATTC**] then indicate the number of fragments and the size of each fragment from each sample of DNA.

   **Sample # 1**
   
   # of fragments:________

   **Sample # 2**
   
   # of fragments:________

   List fragment size in order: largest ——> smallest

   **Sample # 1**
   
   **Sample # 2**
Lesson 1  Restriction Digestion of DNA Samples

Review Questions

1. Before you incubated your samples, describe any visible signs of change in the contents of the tubes containing the DNA after it was combined with the restriction enzymes.

2. Can you see any evidence to indicate that your samples of DNA were fragmented or altered in any way by the addition of EcoRI/PstI? Explain.

3. In the absence of any visible evidence of change, is it still possible that the DNA samples were fragmented? Explain your reasoning.

4. (Answer the next day—after the restriction digest)

   After a 24 hour incubation period, are there any visible clues that the restriction enzymes may have in some way changed the DNA in any of the tubes? Explain your reasoning.
Lesson 2  Agarose Gel Electrophoresis

Review Questions

1. The electrophoresis apparatus creates an electrical field with positive and negative poles at the ends of the gel. DNA molecules are negatively charged. To which electrode pole of the electrophoresis field would you expect DNA to migrate? (+ or -)? Explain.

2. What color represents the negative pole?

3. After DNA samples are loaded into the sample wells, they are “forced” to move through the gel matrix. What size fragments (large vs. small) would you expect to move toward the opposite end of the gel most quickly? Explain.

4. Which fragments (large vs. small) are expected to travel the shortest distance from the well? Explain.
Post-Lab: Thought Questions

1. What can you assume is contained within each band?

2. If this were a fingerprinting gel, how many samples of DNA can you assume were placed in each separate well?

3. What would be a logical explanation as to why there is more than one band of DNA for each of the samples?

4. What caused the DNA to become fragmented?

5. Which of the DNA samples have the same number of restriction sites for the restriction endonucleases used? Write the lane numbers.

6. Which sample has the smallest DNA fragment?

7. Assuming a circular piece of DNA (plasmid) was used as starting material, how many restriction sites were there in lane three?

8. From the gel drawing on page 35, which DNA samples appear to have been “cut” into the same number and size of fragments?

9. Based on your analysis of the example gel drawing on page 35, what is your conclusion about the DNA samples in the drawing? Do any of the samples seem to be from the same source? If so, which ones? Describe the evidence that supports your conclusion.
Post-Lab: Analysis of Results

If the overnight staining protocol was used to stain gels, record your results and dry gels as described in the gel staining procedures in Lesson 2 page 38.

Attach the plastic sheet tracing of the banding patterns from the DNA electrophoresis below.

Tracing of electrophoresis gel

Attach the dried gel showing the banding patterns from the DNA electrophoresis below.

Dried electrophoresis gel
Quantitative Analysis of DNA Fragment Sizes

If you were on trial or were trying to identify an endangered species, would you want to rely on a technician's eyeball estimate of a match, or would you want some more accurate measurement?

In order to make the most accurate comparison between the crime scene DNA and the suspect DNA, other than just a visual match, a quantitative measurement of the fragment sizes needs to be completed. This is described below:

1. Using a ruler, measure the distance (in mm) that each of your DNA fragments or bands traveled from the well. Measure the distance from the bottom of the well to the center of each DNA band and record your numbers in the table on the next page. The data in the table will be used to construct a standard curve and to estimate the sizes of the crime scene and suspect restriction fragments.

2. To make an accurate estimate of the fragment sizes for either the crime scene or suspect DNA samples, a standard curve is created using the distance (x-axis) and fragment size (y-axis) data from the known HindIII lambda digest (DNA standard). Using both linear and semilog graph paper, plot distance versus size for bands 2–6. On each graph, draw a line of best fit through the points. Extend the line all the way to the right-hand edge of the graph.

Which graph provides the straightest line that you could use to estimate the crime scene or the suspects' fragment sizes? Why do you think one graph is straighter than the other?

3. Decide which graph, linear or semilog, should be used to estimate the DNA fragment sizes of the crime scene and suspects. Justify your selection.

4. To estimate the size of an unknown crime scene or suspect fragment, find the distance that fragment traveled. Locate that distance on the x-axis of your standard graph. From that position on the x-axis, read up to the standard line, and then follow the graph line to over to the y-axis. You might want to draw a light pencil mark from the x-axis up to the standard curve and over to the y-axis showing what you've done. Where the graph line meets the y-axis, this is the approximate size of your unknown DNA fragment. Do this for all crime scene and suspect fragments.

5. Compare the fragment sizes of the suspects and the crime scene.

Is there a suspect that matches the crime scene?

How sure are you that this is a match?
### Electrophoresis Data

Measure the distance (in millimeters) that each fragment traveled from the well and record it in the table. Estimate its size, in base pairs, by comparing its position to the HindIII lambda DNA standards. Remember: some lanes will have fewer than 6 fragments.

<table>
<thead>
<tr>
<th>Band</th>
<th>Size Standard</th>
<th>Suspect 1</th>
<th>Suspect 2</th>
<th>Suspect 3</th>
<th>Suspect 4</th>
<th>Suspect 5</th>
<th>Crime Scene</th>
</tr>
</thead>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.416</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>6.517</td>
<td></td>
<td></td>
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<td></td>
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</tr>
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<td>2.027</td>
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</tr>
</tbody>
</table>
Post Lab: Interpretation of Results

1. What are we trying to determine? Restate the central question.

2. Which of your DNA samples were fragmented? What would your gel look like if the DNA were not fragmented?

3. What caused the DNA to become fragmented?

4. What determines where a restriction endonuclease will “cut” a DNA molecule?

5. A restriction endonuclease “cuts” two DNA molecules at the same location. What can you assume is identical about the molecules at that location?

6. Do any of your suspect samples appear to have EcoRI or PstI recognition sites at the same location as the DNA from the crime scene?

7. Based on the above analysis, do any of the suspect samples of DNA seem to be from the same individual as the DNA from the crime scene? Describe the scientific evidence that supports your conclusion.