Lab 13: DNA Fingerprinting

Introduction:
**Gel electrophoresis** is a laboratory tool that allows scientists to compare DNA from different individuals. Gel electrophoresis uses an electric current to separate fragments of DNA that have been cut by **restriction enzymes**. Every individual’s DNA fragments will be slightly different, and therefore the pattern of cut DNA that is created by gel electrophoresis will be unique to each person. This is why gel electrophoresis is called “DNA fingerprinting” because everyone’s fragment pattern will be different just like everyone’s fingerprints.

The Three Major Steps for DNA Fingerprinting:
1. **Prepare DNA Samples:**
   DNA is extracted from cells and **cut into fragments using a restriction enzyme.** The fragments that will be created will be different depending on which restriction enzyme is used.

2. **Load DNA Samples:**
   Gel electrophoresis uses agarose gel. Agarose gel is a porous, jelly-like substance. For gel electrophoresis, melted agarose gel is put into a form that makes it into a rectangular shape. A comb is inserted to create small slots or wells where the DNA to be “loaded” using a small pipette. Observe the diagram below to see what this looks like.

3. **Separate DNA Samples:**
   The rectangular gel is submersed in an electrophoresis chamber filled with a special salt water that can conduct electricity. An electrical current is applied to the salt water with the gel in it. The electrical current will cause the DNA fragments to move. DNA is a negatively charged molecule that will move toward the positively charged cathode. As the fragments move **smaller** fragments will move faster and more easily through the thick matrix or mesh of the gel, while **larger** fragments will move slower through the gel. This will result in a **banding pattern** that is unique to a specific individual. To “see” the DNA, the gel will be stained with a blue dye to **stain the bands** so they are visible to the naked eye.

![Diagram of Gel Electrophoresis Setup](image)
Lab Part A: Cutting DNA with Restriction Enzymes

**Introduction:** Restriction enzymes are special enzymes harvested from unique bacteria that scientists use to cut DNA. Restriction enzymes are like little “molecular scissors”! They cut DNA but only at specific sequences. Examine the DNA sequence below and find the sequence **G A A T T C** in the top DNA strand. Circle it and the bases below in the sequence.

**Uncut DNA:**

```
TGATCGTGGAAATTCGATGATCGATGCTAGCTGAA
ACTAGCACCCTAAGCTACTAGCTACGATCGACTTT
```

Notice that the sequence below the GAATTC sequence (CTTAAG) is identical, just backwards! A lot of restriction enzymes recognize sequences called “palindromic sequences”. Palindromes are the same backwards as forwards (like the words MOM and DAD and RACECAR).

The restriction enzyme cuts the sequence into two pieces with unpaired bases hanging on the ends. The cuts would look like this:

**Piece 1:**

```
TGATCGTGG
ACTAGCACC
```

**Piece 2:**

```
AATTCGATGCTAGCTGAA
GCTACTACGATCGACTTT
```

**Restriction Enzyme Analysis Questions:**

1. **Why do scientists use restriction enzymes?**

```
___________________________________________________________________________________________
```

2. **What would scientists use to see their newly cut fragments of DNA?**

```
___________________________________________________________________________________________
```

3. **Look at the sequences below. If you add a restriction enzyme (that cuts at GAATTC) to the uncut DNA, how many DNA fragments will result? Circle the fragments.**

**Uncut DNA 1:**

```
TGATCGTGGAAATTCGATGATCGATGCTAGCTGAA
ACTAGCACCCTAAGCTACTAGCTAAGCGATCGACTTT
```

Number of fragments: _____ Length of fragments: ___________ basepairs (ignore ends with unpaired bases)
Honors Biology  
Unit 4: Genetics  
Due Tuesday January 24th

**Uncut DNA 2:**  
TGATCGTGACCTTGCATGATCGAATTGCTAGCTGAATTCAAAAAA  
ACTAGCACCTGAAGCTACTAGCTTAAGCGATGCAGCTTAAGTTTTTT  
Number of fragments: _____ Length of fragments: ___________ basepairs (ignore ends with unpaired bases)

**Uncut DNA 3:**  
TGATCGTGACCTTGCATGATCGAATTGCTAGCTGAATTCAAAAAA  
ACTAGCACCTGAAGCTACTAGCTTAAGCGATGCAGCTTAAGTTTTTT  
Number of fragments: _____ Length of fragments: ___________ basepairs (ignore ends with unpaired bases)

4. You are given a new sample of DNA that matches one of the uncut samples above (1, 2, or 3). How could you use restriction enzyme analysis to match your unknown sample to one of the known samples above? Explain!

___________________________________________________________________________________________  
___________________________________________________________________________________________  
___________________________________________________________________________________________  
___________________________________________________________________________________________

Lab Part B: Applications of Gel Electrophoresis

Gel electrophoresis can be used to compare DNA from different individuals, thus why it is called DNA fingerprinting. DNA is cut using restriction enzymes and then run through gel electrophoresis (see front page for how this works). The “banding” patterns in the gel are unique to each person. Gel electrophoresis can help determine familial relationships. For example, it can be used to determine the parents or relatives of an individual like in a paternity test. Gel electrophoresis can also be applied in forensics. It can be used to compare DNA found a crime scene to the DNA of possible suspects. Lastly, gel electrophoresis can be used to isolate a specific gene that has separated in an individual band. A scientist can cut out a particular band containing a gene to perform further experiments.

**Analyzing DNA Band Patterns:**

**Example 1:**

<table>
<thead>
<tr>
<th>Sample #</th>
<th># of Bases in Sample Fragments</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Lane 2)</td>
<td>1000 bases &amp; 300 bases</td>
<td>1 has two fragments that are 1000 and 300 bases long</td>
</tr>
<tr>
<td>2 (Lane 3)</td>
<td>500 bases</td>
<td>2 has only one fragment that is 500 bases long</td>
</tr>
<tr>
<td>3 (Lane 4)</td>
<td>2000 bases, 800 bases, &amp; 300 bases</td>
<td>3 has three fragments that are 2000, 800, and 300 bases long</td>
</tr>
<tr>
<td>4 (Lane 5)</td>
<td>1000 bases &amp; 100 bases</td>
<td>4 has two fragments that are 1000 and 100 bases long</td>
</tr>
</tbody>
</table>

In this example, a control lane has been set up to compare band sizes of the four samples. In the table below, the band sizes based on the number of bases or nucleotides are given.

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Modified from Bethany Lau © 2014
Example 2:

In this example, two unknown samples from a crime scene are in lanes 1 and 2. Lane 3 contains the banding pattern of the victim’s DNA. Lanes 4 and 5 contain the banding pattern of two different suspects. The table below gives the analysis of this gel electrophoresis result. Note that in this example, the number of bases in each fragment isn’t the focus, but rather the unique banding pattern found in each individual.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crime Scene Sample #1</td>
<td>Sample matches victim’s DNA</td>
</tr>
<tr>
<td>Crime Scene Sample #2</td>
<td>Sample matches suspect B DNA, therefore suspect B was present at the scene</td>
</tr>
<tr>
<td>Victim’s DNA</td>
<td>Victim’s DNA matches crime scene sample #1</td>
</tr>
<tr>
<td>Suspect A DNA</td>
<td>Suspect A DNA does not match either sample, suspect A was not at crime scene or did not leave DNA</td>
</tr>
<tr>
<td>Suspect B DNA</td>
<td>Suspect A DNA matches crime scene sample #2, therefore suspect B was present at the scene</td>
</tr>
</tbody>
</table>

Observation & Analysis of Class Demo Gel Electrophoresis:

Mrs. H just “looooves” when kids chew gum in her class. Especially when she finds it on the floor. In this crime, Mrs. H found gum under a desk. She took it for a DNA sample. She chose three suspects that are most typically chewing gum in class to see if any of them are the culprit in this gross gum crime.

In the gel diagram provided, draw the bands that you observe in the gel provided to you. Be sure to label the lanes with the appropriate sample name! Then complete the table to analyze these banding patterns. Then answer the questions that follow.
1. Which suspect is most likely the person who left the gum on the floor? ____________________________________

2. What is your evidence to support your hypothesis from question 1? ____________________________________

3. If two of the suspects were genetically identical twins, what do you think the banding patterns might look like for these two suspects? Explain! ____________________________________________________________

4. What causes the DNA fragments to move within the gel? ____________________________________________________________


6. What are two possible uses for gel electrophoresis? ____________________________________________________________

**Gel Analysis Problems:**

1. Use the gel below to determine the fragment sizes for each sample. The lengths are written on the left of the gel. If a particular band is not exactly lined up with one of the given lengths, estimate the length. For example, if a band is approximately halfway between length 100 bases and length 50 bases, then the unknown band is approximately 75 bases long.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fragment Sizes in Bases/Nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
2. You work at a sandwich shop. Some customers have complained that workers have been sneezing in their sandwiches! Gross. You decide to figure out who are these gross people. You take a sample of the sneeze juices (mucus and saliva) from two sandwiches and samples from four coworkers. Given the table with the fragment sizes, draw the bands that you would observe in this gel electrophoresis. Put a box or boxes around the bands of the culprit(s) who have been sandwich sneezing!

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fragment Sizes in Bases/Nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandwich 1</td>
<td>1000, 700, 250</td>
</tr>
<tr>
<td>Sandwich 2</td>
<td>950, 400</td>
</tr>
<tr>
<td>Gregory</td>
<td>800, 600, 250, 100</td>
</tr>
<tr>
<td>Tuione</td>
<td>950, 400</td>
</tr>
<tr>
<td>Rossi</td>
<td>1000, 700, 250</td>
</tr>
<tr>
<td>Henderson</td>
<td>1000, 700, 100</td>
</tr>
</tbody>
</table>

Part A

<table>
<thead>
<tr>
<th>Score:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description:</td>
<td>Some or no questions are answered are attempted.</td>
<td>All questions are answered, may not be thorough, and at least 50% accurate.</td>
<td>All questions are answered thoroughly and at least 90% accurate. Demonstrates understanding of information.</td>
<td>All questions are answered thoroughly and 100% accurate. Demonstrates understanding of information.</td>
</tr>
</tbody>
</table>

Part B

<table>
<thead>
<tr>
<th>Score:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description:</td>
<td>Most problems have been completed and analyzed thoroughly and thoughtfully. All questions answered but lack accuracy. Questions are not answered in complete sentences.</td>
<td>All problems have been completed and analyzed thoroughly and thoughtfully. All questions answered with 50% accuracy and thoroughly. Most questions have been answered in complete sentences.</td>
<td>All problems have been completed and analyzed thoroughly and thoughtfully. All questions answered with 90% accuracy and thoroughly. Questions have been answered in complete sentences.</td>
<td>All problems have been completed and analyzed thoroughly and thoughtfully. All questions answered with 100% accuracy and thoroughly. Questions have been answered in complete sentences.</td>
</tr>
</tbody>
</table>
Set Up:
Prep agarose for 12 gels (will provide 1 gel per lab group [max of 9 per class] plus three extra)
Prep TAE buffer
Prep stain & trays
Prep destain (di water) & trays

Used:
Cat130. used 130 A-E.