LABORATORY WORKSHEET: HOW IS DNA ANALYZED?

Background Information

Biotechnology is the manipulation of the biological capacity of cells and their components. For thousands of years people have used biotechnology when they used yeast to make flour into bread and grape juice into wine. Today we are using biotechnology to study the basic processes of life, to diagnose illnesses, and to develop new treatments for diseases.

Some of the tools of biotechnology are natural components of cells. Restriction enzymes are made by bacteria to protect themselves from viruses. They inactivate the viral DNA by cutting it in specific places. DNA ligase is an enzyme that exists in all cells and is responsible for joining together strands of DNA. Scientists use restriction enzymes to cut DNA at specific sequences called recognition sites. They then rejoin the cut strands with DNA ligase to make new combinations of genes. Recombinant DNA sequences contain genes from two or more organisms.

In this worksheet you will simulate the cutting of DNA with restriction enzymes. You will also model gel electrophoresis to analyze the DNA fragments produced.

Objectives:
1. To use models to simulate the technique of using restriction enzymes to cut long DNA chains into smaller sequences.
2. To understand the principle underlying gel electrophoresis to analyze DNA fragments.

Materials:
Looseleaf paper
Pencil or pen

Part I: Restriction Enzymes
A. There are now about 200 known restriction enzymes that cut DNA at specific recognition sites. For example, the restriction enzyme Hind II recognizes the base sequence G T C G A C.

1. Copy the sequence below on your looseleaf paper and underline the Hind II recognition sequence.
   T A A G C C G T C G A C T C C

2. Write out the DNA sequence complementary to the one in question 1. Read the complementary strand in reverse and underline the Hind II recognition sequence on it.

B. When the restriction enzyme Hind II recognizes the sequence GTTCGAC, it will cut the DNA strand between the cytosine (C) and guanine (G) on both strands. Therefore, it will leave blunt ends on the fragments:
   -G T C * G A C-  -G T C  G A C-
   -C A G * C T G-  -C A G  C T G-
   Blunt ends
The restriction enzyme Eco RI cuts its recognition site at nonadjacent points on the DNA molecule, leaving "sticky" ends. Eco RI recognizes the base sequence G A A T T C and cuts this sequence between the guanine (G) and adenine (A) bases:

\[
\begin{align*}
-G\text{ A A T T C-} & \quad -G\underbrace{\text{A A T T C}} \quad G- \\
-C\text{ T T A A* G-} & \quad -C\text{ T T A A} \\
\end{align*}
\]

Sticky ends can bind to similar sticky ends from other Eco RI-digested fragments. After recombining, the ends are joined by DNA ligase to form a new pattern of bases. By cutting DNA from two different organisms with the same enzyme and recombining with DNA ligase, scientists make recombinant DNA.

3. Copy the sequence given below and complete the strand complementary to it. On both strands indicate the Eco RI restriction sites with arrows. Remember to read the complementary strand in reverse.

\[
\text{G C C T C T A A G A A T T C A G T T C G}
\]

4. Once the Eco RI has cut the above DNA chain, how many fragments of DNA would there be? Would the ends be blunt or sticky? How many bases would there be in each fragment? Note: When counting the length of a DNA fragment, count only the number of bases in the upper strand.

C. Below you will see two sequences of DNA—DNA IA and DNA IB. (DNA IB is a mutant variation of DNA IA.)

**DNA IA**

```
TTG CAA GTC AGA AGA ATT CAA CCT AGG AAT TCT AAG CGC
AAC GTT CAG TCT TCT TAA GTT GGA TCC TTA AGA TTC GCG
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**DNA IB**

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TTG CAA GTC AGA AGA AGT CAA CCT AGG AAT TCT AAG CGC
AAC GTT CAG TCT TCT TCA GTT GGA TCC TTA AGA TTC GCG
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5. What is the difference between the two sequences?

6. Copy the sequences onto your paper. Identify the Eco RI recognition sites on both sequences and mark the sites where Eco RI would cut with arrows. (You will use these sequences again in Part II.)

7. How many fragments of DNA were made from each sequence after digestion with Eco RI?

8. What are the lengths (in basepairs) of the fragments from the DNA IA and DNA IB digestions?

9. Can you recombine any of the "sticky" ends of DNA IA and DNA IB to make a new sequence of DNA? If so, write out the sequence for one such recombination.
Part II: Gel Electrophoresis

D. Scientists identify differences in DNA sequences by measuring the length and number of fragments created by digestion with restriction enzymes. A technique called gel electrophoresis is used to separate fragments according to length. DNA fragments (cut with an appropriate restriction enzyme) are placed on one end of a specially-prepared block of agarose called a gel. An electric current is applied across the agarose which causes the strands to migrate through the gel. (Since DNA molecules are negatively charged, they migrate towards the positive electrode.) The agarose is like a sponge with small holes in it. Therefore, the smaller DNA fragments can move through the gel at a faster rate than larger fragments. This means that the larger fragments are found nearer the point of origin. Scientists then use a special stain to make the DNA fragments visible as bands. By counting the number of bands researchers can tell how many fragments exist. By observing the distance each fragment has migrated, they can determine how big each fragment is.

Model of Gel Electrophoresis Migration

A. Restriction Enzyme Digestion
   Two different strands of DNA 1,000 base pairs (bp) long, cut with the same restriction enzyme (RE).

   DNA X
   | |  DNA Y
   RE RE

10. How many fragments are produced by cutting DNA X? By cutting DNA Y?

B. Migration Distances of DNA In Gels
   The size of the DNA fragments is proportional to the distance traveled.

<table>
<thead>
<tr>
<th>Fragment Size (bp)</th>
<th>Distance from Origin (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>600</td>
<td>1</td>
</tr>
<tr>
<td>400</td>
<td>3</td>
</tr>
<tr>
<td>300</td>
<td>4</td>
</tr>
<tr>
<td>200</td>
<td>5</td>
</tr>
<tr>
<td>100</td>
<td>6</td>
</tr>
</tbody>
</table>
C. Gel Electrophoresis
Appearance of DNAs X and Y after digestion and separation by gel electrophoresis.

<table>
<thead>
<tr>
<th>Distance Migrated</th>
<th>X</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 cm</td>
<td></td>
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<tr>
<td>4 cm</td>
<td></td>
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</tr>
<tr>
<td>5 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 cm</td>
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</tbody>
</table>

11. The fragments generated from DNA X and DNA Y were then analyzed by gel electrophoresis. From the data in figures B and C, calculate the sizes of the fragments.

12. On your paper draw a simulated gel. Then draw bands corresponding to the positions to which the Eco RI digests of DNA IA and DNA IB would migrate. The fragments of each type of DNA should be in separate lanes and at distances from the origin proportional to the numbers of bases in each fragment. From the following distances, determine how far your fragments will migrate: 40 base pairs will migrate 2.5 cm; 30 bp will migrate 5 cm; 20 bp will migrate 7.5 cm; and 10 bp will migrate 10 cm. Make sure all the DNA is accounted for.

13. If a mutation causes one of the base pairs in a recognition site to change, what effect will it have on the ability of the restriction enzyme to cut at that site?

14. Explain how scientists use restriction analysis to determine that one sequence of DNA is different from another.