Exercise 8 – Analysis of DNA using Restriction Enzyme and Electrophoresis:

1. What is nuclease?

DNA held by covalent bond joining adjacent nucleotides in DNA is called a phosphodiester bond. The phosphodiester bond between nucleotide in DNA molecules are very stable unless they are physically stretched or exposed to enzymes name nuclease.

Enzyme capable of breaking (hydrolyzing) phosphodiester bonds in DNA molecules and classified into exonuclease and endonuclease.

2. How does an endonuclease differ from an exonuclease?

Endonuclease digest DNA by breaking phosphodiester bonds in the interior of DNA molecule. Exonuclease enzyme digest nucleotides from the ends of the DNA molecule.

3. What is a restriction endonucleases? Write names of some restriction endonuclease.

Restriction endonucleases are a special class of Endonuclease from bacteria to cut DNA. EcoRI & Hind III. These are enzymes digest DNA by recognizing specific short sequences of bases called palindromic.

4. What are 2 restriction endonuclease (RE) that we used in our lab? Write DNA sequences these RE recognize. Do they produce sticky ends or blunt ends when they cut the DNA molecules?

EcoRI & Hind III. Both produce sticky ends when cut.

5. How does the number of restriction sites relate to the number of fragments produced for linear DNA or circular DNA?

Eukaryotic DNA, always going to have one more or one less fragment than you have restriction sites.

Prokaryotic DNA, the number of fragments will equal the number of restriction sites.

6. What is palindromic DNA sequence?

Reading from the same thing in both direction to read the sequences bases that restriction endonuclease recognizes. For example, M’adam I’m adam.
7. What is electrophoresis? What does agarose gel electrophoresis allow us to do?

It’s a gel that allows move fragment of DNA across by attracting DNA, which is negative (anode) to opposite side (cathode) positive side base on size, and conformation of DNA. It will migrate with current.

8. What is the chemical nature of agarose? Polysacchirdes (lipid) & sea weed.

9. What factors effect the migration rate of DNA through an agarose gel? Size, shape (conformation), and charge.

10. For DNA molecules of equal sizes, how do the different shapes (conformation) of DNA differ in terms of distance traveled through an agarose gel? Supercoil travels the fastest, follow by linear, relax, and nicked supercoil the slowest.

11. In your pAMP electrophoresis experiment, why did you run a DNA ladder (lane 5) and undigested pAMP DNA (lane 4)?

Lane 4 is control of DNA to see what uncut plastmic looks like.

Lane 5 is DNA ladder: DNA digest. Containing known base pair lengths and use to compare with fragments in lanes 1-3.

12. Write some practical applications for use of restriction end nuclease?

LOOK AT EXERCISE 8 page 11, #9 for answer.
13. Examine the following pattern of an electrophoresis gel and draw a restriction map of the DNA. Is this DNA circular or linear? Circular DNA because RE equal fragments.

Also, there are multiply bands on Lane 4.

Lane 1: DNA + EcoRI
Lane 2: DNA + Hind III
Lane 3: DNA + EcoRI + Hind III
Lane 4: DNA only
Lane 5: DNA ladder
14. Examine the restriction map of plasmid pGEX below.
A. How many fragments would be produced if the plasmid is digested with Sal I?
   What size would this/these fragment(s) be? 4,000 bp
B. How many fragments would be produced using EcoRI?
   What size would this/these fragment(s) be? 800, 3,200 bp
C. How many fragments would be produced using EcoRI + Hind III + Sal I?
   What size would this/these fragment(s) be? 600, 800, 1,100 & 1,500 bp

15. Draw the banding pattern for the restriction digestion of the above plasmid as indicated on right hand side below. The migration of a DNA ladder and its fragment-sizes are identified by the arrows.

   A) For Not I only have RE, therefore its 4,000 bp

   B) EcoRI has two fragments of 800 & 3,200 bp

   C) EcoRI and Hind III has 800, 1,100, & 2,100

   D) If incubated with EcoRI, Hind III & Sal I you get 800
      (EcoRI to EcoRI) 1,100 from (EcoRI to Hind III) and
      Sal I is 600 & 1,500 bp.
The following is a real picture of an electrophoresis gel with a particular DNA digested with EcoR I (Lane 1); HindIII (Lane 2); EcoRI & HindIII (Lane 3); No enzyme (Lane 4). Lane five contains DNAs of known sizes.

Estimate the size of the DNA molecule. Is this DNA molecule circular or linear? Draw a restriction map of the DNA at the right hand side of the picture.
16. Examine the following gel. Determine whether the DNA is prokaryotic or eukaryotic. Draw a restriction map of the DNA.

Linear DNA because RE does not equal fragments.
Also, there are no multiply bands on Lane 4.
Restriction Mapping: An example

1. Many copies of a cloned 5.0 kb linear DNA

2. Analyze DNA by restriction enzyme cleavage and agarose gel electrophoresis.

3. Construct calibration curve for markers.

4. Read kb for each fragment from calibration curve, given distance migrated.

5. Results

<table>
<thead>
<tr>
<th></th>
<th>Uncut</th>
<th>EcoRI</th>
<th>BamHI</th>
<th>EcoRI + BamHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>kb</td>
<td>5.0</td>
<td>4.5</td>
<td>3.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>2.0</td>
<td>2.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

6. Interpretation

Uncut

0.5 kb

EcoRI

4.5 kb

BamHI

3.0 kb

Predicted EcoRI and BamHI fragments

3.0, 1.5, and 0.5 kb

7. Construct

Model A

EcoRI

0.5 kb

BamHI

3.0 kb

Model B

EcoRI

0.5 kb

BamHI

5.0 kb

Model B

2.5 kb

2.0 kb

0.5 kb

8. Conclusion

EcoRI and BamHI data indicate that model B is correct.