

LAB 13 – Restriction Enzyme Simulation

Objective:

In this exercise you will use the computer to simulate the Lambda DNA restriction digests that you will also perform in the laboratory. Using the results from the computer simulation and your actual restriction digests, you will answer a series of questions designed to help you interpret the results of your DNA digests.

1. Download and SAVE the Lambda DNA sequence from the class website:
2. Save the file to your computer or to a disk.
3. Open the saved file in Microsoft Word. It contains the entire phage Lambda DNA sequence, or genome. Browse through the file. Notice how the file is simply a long string of the letters A, C, G and T.

Question 1

What does each of these letters represent?

4. Now determine how many letters there are in the genome. To do this:
 - a. SELECT ALL (Ctrl + A) the characters or double click anywhere in the letters.
 - b. Look at the lower left of the screen. It will say Word 1/1. Click on it...
 - c. Note the number of characters (letters).

Question 2

What is the size of the Lambda genome:

- a. in bases? _____
- b. in kilobases? (note: 1 Kb = 1,000 bases) _____

5. Click CLOSE to close the window.

Simulating the Effects of Restriction Enzymes

Recall that there are a large number of restriction endonucleases (restriction enzymes), and that each recognizes a specific sequence of DNA nucleotides and cuts at a specific point within that sequence. The three restriction enzymes we will use, and their respective restriction sites, are as follows:

Endonuclease	Recognition site (5'↑3')
<i>EcoRI</i>	G↑AATTC
<i>BamHI</i>	G↑GATCC
<i>HindIII</i>	A↑AGCTT

...where the six letter sequence represents the nucleotide sequence that the enzyme recognizes, and ↑ represents the place where the DNA will be cut by the enzyme on that one strand. For example, *EcoRI* cuts between G and A whenever it encounters the six base sequence GAATTC. (Don't forget, these restriction enzymes do sticky end cuts on the double helix – this is just showing where *one* of the strands is being cut)

6. For *EcoRI* perform a single digest simulation and count the number of fragments produced using the following procedure:
 - a. Place the cursor at the beginning of the Lambda sequence.
 - b. Go to the HOME tab and look towards the right. Click on REPLACE (or hit Ctrl + H).
 - c. In the box "Find what:" type in **gaattc**
 - d. Move the cursor to the "Replace with:" and type in **G^p^pAATTC** (^p = a break)
 - e. Select REPLACE ALL
 - f. Close the window.

This 'cuts' the Lambda DNA at all the *EcoRI* restriction sites by putting 2 paragraph breaks (which is what the ^p^p is) at each point where the sequence has been cut. It also converts the recognition sequence of the six bases to all UPPERCASE LETTERS so that you can see them more easily in the entire text. Scroll through the document:

Question 3

How many restriction sites were there? _____

How many fragments will be produced? _____

7. Now use the computer to determine how many fragments were produced using *EcoRI* as the restriction enzyme, and how large each fragment was (in bases and kilobases).

Determining the Number of Fragments Produced

8. SELECT ALL (Ctrl + A). Now, look at the lower left of the screen for the Word Count. It will say Word X/X. Click on it... and note the number of words. This represents the number of fragments that were produced. Does it agree with your prediction in Question 3 above? Enter the number of fragments in the right-hand column of **Table 1** below.
9. Click OK to close the window

Determining the Size of Each Fragment

10. Go to the top of the document and highlight the first 'paragraph' (the first fragment produced from the restriction enzyme) by double clicking anywhere within the margin alongside the first few lines.
11. Now, look at the lower left of the screen for the Word Count. It will say Word X/X. Click on it... Note the number of characters (characters = bases) in this fragment and enter it in **Table 1** below for the respective "word".
12. Close the window.
13. Move the cursor to the start of the next paragraph, highlight as before, and repeat – noting the number of characters (i.e. bases) in the table below.
14. Repeat this process until you reach the end of the genome

Table 1: Fragment sizes (in bases) resulting from a restriction digest of Lambda phage DNA using different restriction endonucleases.

Restriction Enzyme	Size of each fragment produced (in number of bases)								Total Number of Fragments
<i>EcoRI</i>									
<i>BamHI</i>									
<i>HindIII</i>									

Question 4

Does your number of fragments agree with what you predicted in **Q3**? **(YES or NO)**

15. Save the file on the computer as EcoR1.doc. Close the file. Download a new copy of the uncut lambdaDNA.doc file for the next activity.

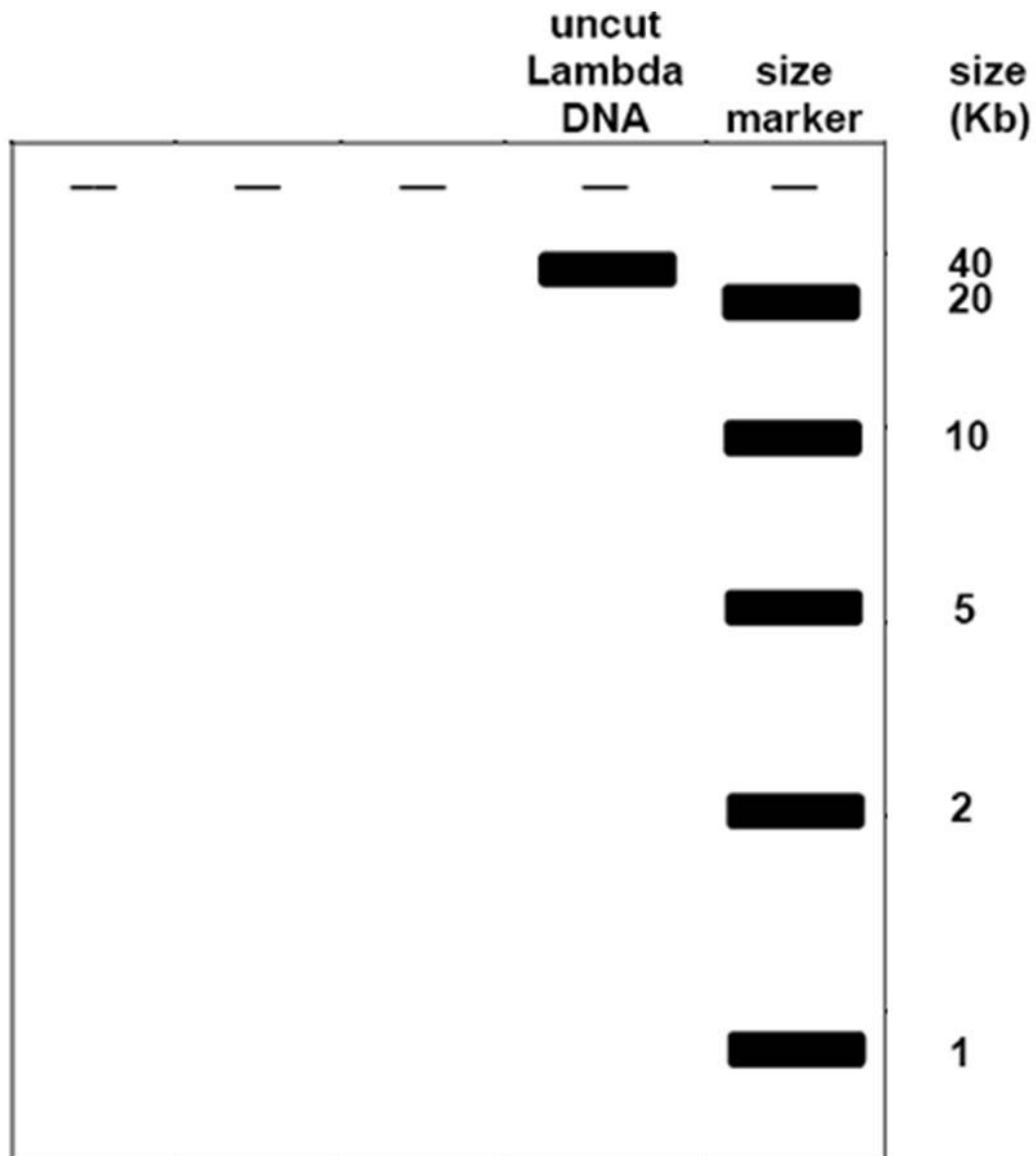
Restriction Digest with *Bam*HI and *Hind*III Restriction Enzymes

16. Reopen the document, and repeat the restriction simulation process and analysis (steps 6-16) for *Bam*HI and *Hind*III – **using the correct restriction sequence!** Make sure to use the appropriate recognition sequence for each restriction enzyme and the appropriate “replace” sequence. Use the *Eco*R1 system you just completed as your model. Enter the resulting number of fragments and fragment sizes in **Table 1** on the previous page.
17. After you have completed the restriction simulation for all three restriction endonucleases, complete **Table 2** below by simply arranging the fragments produced by each endonuclease in order of size, starting with the largest.

Table 2: Lambda phage DNA fragments produced by different endonucleases, ordered according to size (in bases)

Restriction Enzyme	Size of fragments produced (in bases), ordered from largest to smallest							
<i>Eco</i> RI								
<i>Bam</i> HI								
<i>Hind</i> III								

18. Now we are going to simulate a gel electrophoresis on the diagram below. Make believe you loaded each of the three lanes of the gel with a different sample of your three restriction enzyme digests: *EcoR*I, *Bam*HI and *Hind*III. Label the lanes with the corresponding enzyme that was used. Now "turn on" the power supply and watch your DNA move down the gel. Draw a rough sketch below of how your electrophoresis gel would look after you ran it for a while. Draw the bands of DNA fragments where they would settle on the gel, according to size order, using the "size marker" lane as your DNA fragment size guide (your ruler).



Question 5

Explain *why* you might not see all of the fragments that were produced when actually running a gel. (HINT: In answering this, indicate the probable sizes of the fragments that you would not see as individual bands...)
