

LAB 12: 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. From the EDIT menu, choose SELECT ALL
 - b. Under the TOOLS menu, choose WORD COUNT
 - c. Note the number of characters.

Question 2

What is the size of the Lambda genome:

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

5. Click OK 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 you used, and their respective restriction sites were as follows:

Endonuclease	Recognition site (5'↑3')
<i>Bam</i> HI	G↑GATCC
<i>Eco</i> RI	G↑AATTC
<i>Hind</i> III	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. For example, *Eco*RI cuts between G and A whenever it encounters the six base sequence GAATTC.

6. For *Eco*RI 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. Open the EDIT menu and select REPLACE
 - c. In the box 'Find what:' type in gaattc
 - d. Move the cursor to the "Replace with:" and type in G^p^pAATTC
 - e. Select REPLACE ALL
 - f. Click YES or OK to close the window

This 'cuts' the Lambda DNA at all the *Eco*RI restriction sites by putting 2 paragraph breaks (^p^p) 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. 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 *Eco*RI as the restriction enzyme, and how large each fragment was (in bases and kilobases).

Determining the number of fragments produced

8. From the EDIT menu, choose SELECT ALL
9. From the TOOLS menu, select WORD COUNT and note the number of words. This represents the number of fragments that were produced. Does it agree with your prediction in Q4 above? Enter the number of fragments in the right-hand column of Table 1 below.
10. Click OK to close the window

Determining the size of each fragment

11. Go to the top of the document and highlight the first 'paragraph' (= the first fragment produced from restriction) by double clicking anywhere within the margin alongside the first few lines
12. Open the TOOLS menu and select WORD COUNT. Note the number of characters (characters = bases) in this fragment and enter it in Table 1 below.
13. Now select CANCEL or OK
14. 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.
15. 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)**

16. 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.

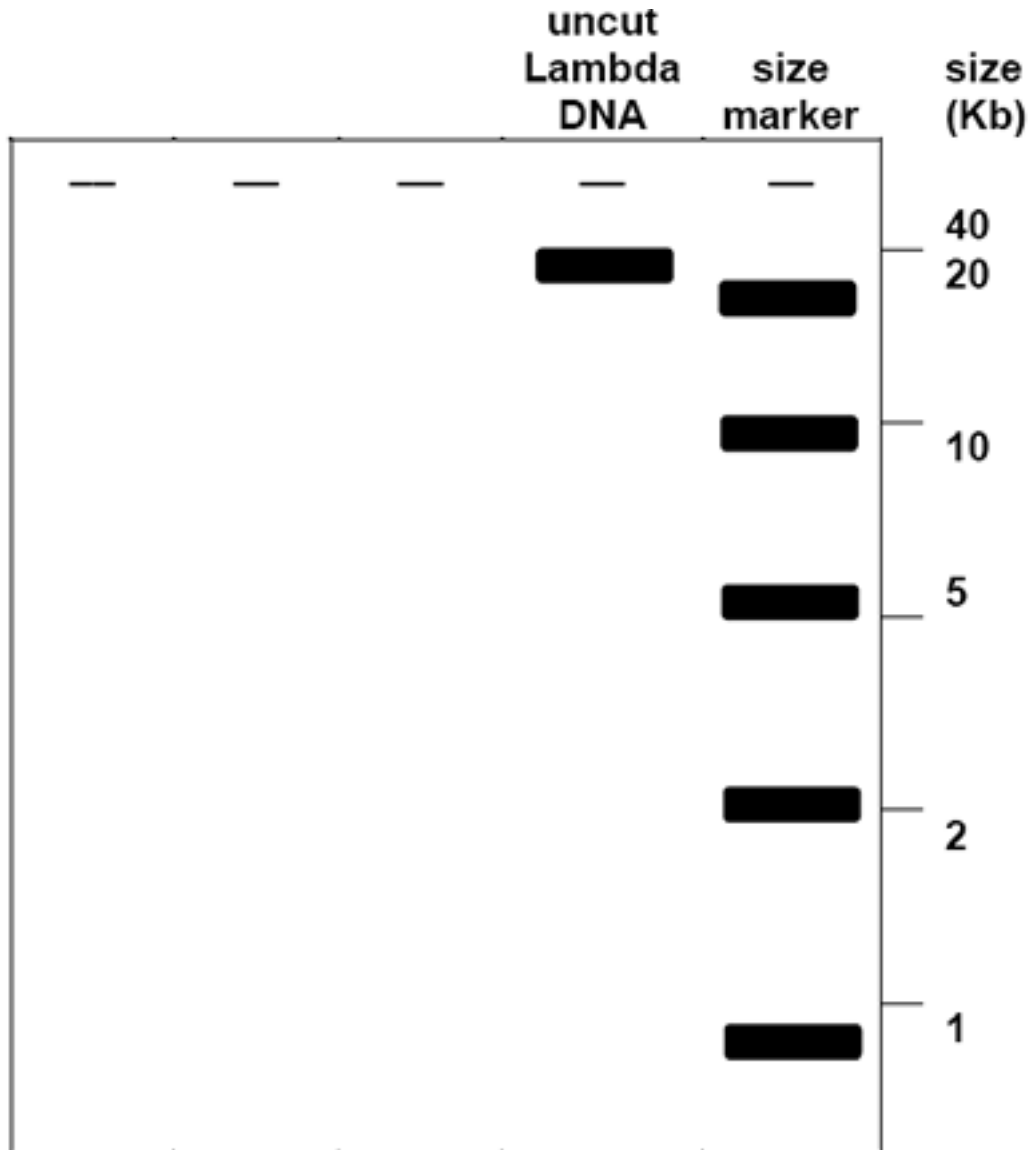
Restriction digest with *Bam*HI and *Hind*III enzymes

17. Reopen the document, and repeat the restriction simulation process and analysis (steps 6-16) for *Bam*HI and *Hind*III. 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 above.
18. After you have completed the restriction simulation for all three endonucleases complete Table 2 below by 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								

19. 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. (In answering this, indicate the probable sizes of the fragments that you would not see as individual bands—HINT!)
