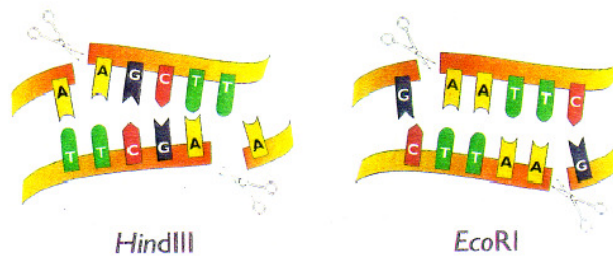


Restriction Enzyme Cutting of Lambda DNA Simulation



Simulating the effects of three restriction enzymes on Lambda DNA

"Lambda" is a virus which preys on bacteria and kills them. It is known as a bacteriophage, a "bacteria eater" or predator. When Lambda is present in bacteria colonies it kills them very effectively.

In this exercise you will use the computer to simulate the Lambda DNA restriction digests that you will be using in the laboratory for electrophoresis.

Save (in your my documents) and then Open the Microsoft Word file on my webpage called Uncut Lambda DNA. 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. (They are all lowercase for this simulation.)

What does each of these letters represent?

Now determine how many letters there are in the genome. To do this: From the EDIT menu, choose SELECT ALL. Under the TOOLS menu, choose WORD COUNT. Note the number of characters.

***Note in Microsoft 2007 and later, there is no edit tab. It has ribbons now. Choose the review ribbon tab. Look in the proofing section for a symbol that looks like ABC with a 123 in a white box below it.

What is the size of the Lambda genome:

- in bases?
- in kilobases? (note: 1 Kb = 1,000 bases)

Click OK to close the window.

Recall that there are a large number of 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, and their respective restriction sites are as follows:

Restriction Enzyme Recognition site (5'→3')

***Bam*HI** G/GATCC

***Eco*RI** G/AATTC

***Hind*III** A/AGCTT

where the six letter sequence represents the nucleotide sequence that the enzyme recognizes, and the slash represents the place where the DNA will be cut by the enzyme. For example, *EcoRI* cuts between G and A whenever it encounters the six base sequence GAATTC.

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. 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^pAATTC
- e. Select REPLACE ALL
- f. Click YES or OK to close the window

This 'cuts' the Lambda DNA at all the *EcoRI* restriction sites by putting 2 paragraph breaks (^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.

1. How many restriction sites were there?
2. How many resulting fragments will there be?

Choose 'OK' and then choose 'close'

Determine the size of each of the fragments:

Highlight the first 'paragraph' of the genome (in reality the first fragment resulting from restriction) by double clicking anywhere in the margin alongside the first few lines.

Choose the WORDCOUNT again. Note the number of characters (i.e. the number of bases) in this fragment and enter this number in table 1 below. Repeat this for all the fragments.

Close the file remembering to answer 'No' to the question 'Do you want to save changes to uncutlambdadna.doc?'

Repeat the above to determine the fragment sizes of the Lambda DNA when cut by the other two restriction enzymes.

Remember where their cuts are and what sequences they recognize.

Table 1: Fragment sizes

Restriction Enzyme	Size of each fragment (number of bases)								Number of fragments
<i>EcoRI</i>									
<i>HindIII</i>									
<i>BamHI</i>									

After you have completed the restriction simulation for all three Restriction Enzymes complete Table 2 below by arranging the fragments produced by each Restriction Enzyme in order of size, starting with the largest.

Table 2: Fragment sizes by length

Restriction Enzyme	Size of each fragment (number of bases)								Number of fragments
<i>EcoRI</i>									
<i>HindIII</i>									
<i>BamHI</i>									

Now simulate electrophoresis.

Draw an electrophoresis gel. Make believe you loaded each of the three lanes of the gel with a different sample of your three restriction enzyme digests: *EcoRI*, *BamHI* and *HindIII*. Label the lanes with the corresponding enzyme that was used. Draw a sketch below of how your electrophoresis gel would look after you ran it for a few hours. 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).