Name _____   Date_____

DNA RESTRICTION ENZYME SIMULATION

Introduction:

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, available at the following link:
   http://goo.gl/0nQ4c
2. Save the file to your computer, to a disk, or to your Google Docs Account. You will need three versions of this file during the course of the lab.
3. Open the saved file in Microsoft Word, Google Docs or your own word processor of choice. 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 REVIEW menu, choose WORD COUNT
   c. Note the number of characters (without spaces).

> **Question 2: What is the size of the Lambda genome:**
>
> a. in bases? _____
>
> b. in kilobases? (note: 1 Kb = 1,000 bases) _____

5. 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 use, and their respective restriction sites are as follows:

Endonuclease	Recognition site (5′→3′)
*Eco*RI	G ↑ AATTC
*Bam*HI	G ↑ GATCC
*Hin*dIII	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, EcoRI 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:

    For MS Word:

    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

    For Google Docs:

    a. Place the cursor at the beginning of the Lambda sequence
    b. Open the EDIT menu and select FIND & REPLACE
    c. In the box 'Find:" type in gaattc
    d. In the box "Replace with:" type in GAATTC
    e. Click NEXT
    f. When the first sequence is found, it will be highlighted. Keep the window open, place the cursor in the restriction sequence between the G and the A, and hit the ENTER key twice.
    g. Click NEXT.
    h. Repeat the cleavage process again for each of the remaining restriction sequences.
    i. Once you have cleaved all of the restriction sequences, click the X box in the FIND & REPLACE window to close the window

This 'cuts' the Lambda DNA at all the *Eco*RI restriction sites by putting 2 paragraph breaks (^p^p in word, done manually in Google Docs) 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 (this is not done in the Google Docs procedure). Scroll through the document:

Question 3:

How many restriction sites were there? _____

How many fragments will be produced? _____

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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 (REVIEW) menu, select WORD COUNT and note the number of words. This represents the number of fragments that were produced.

> **Question 4:**
>
> Does your number of fragments agree with what you predicted in Q3? (YES or NO)

10. Enter the number of fragments in the right-hand column of Table 1 below.
11. Click OK to close the window

Determining the size of each fragment:

12. 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
13. Open the TOOLS (REVIEW) menu and select WORD COUNT. Note the number of characters (characters = bases) in this fragment and enter it in Table 1 below.
14. Now select CANCEL or OK
15. 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.
16. Repeat this process until you reach the end of the genome
17. Download a new copy of the uncut lambda DNA.doc file for the next activity.

Restriction digest with *Bam*HI and *Hin*dIII enzymes:

18. Open the document, and repeat the restriction simulation process and analysis (steps 6- 16) for *Bam*HI and *Hin*dIII. 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 below.
19. 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 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
*Eco*RI		
*Bam*HI		
*Hin*dIII		

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

Restriction Enzyme	Size of each fragment produced (in bases), ordered according to size from largest to smallest.	Total Number of Fragments
*Eco*RI		
*Bam*HI		
*Hind*III		

20. 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: *Eco*R1, *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 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).
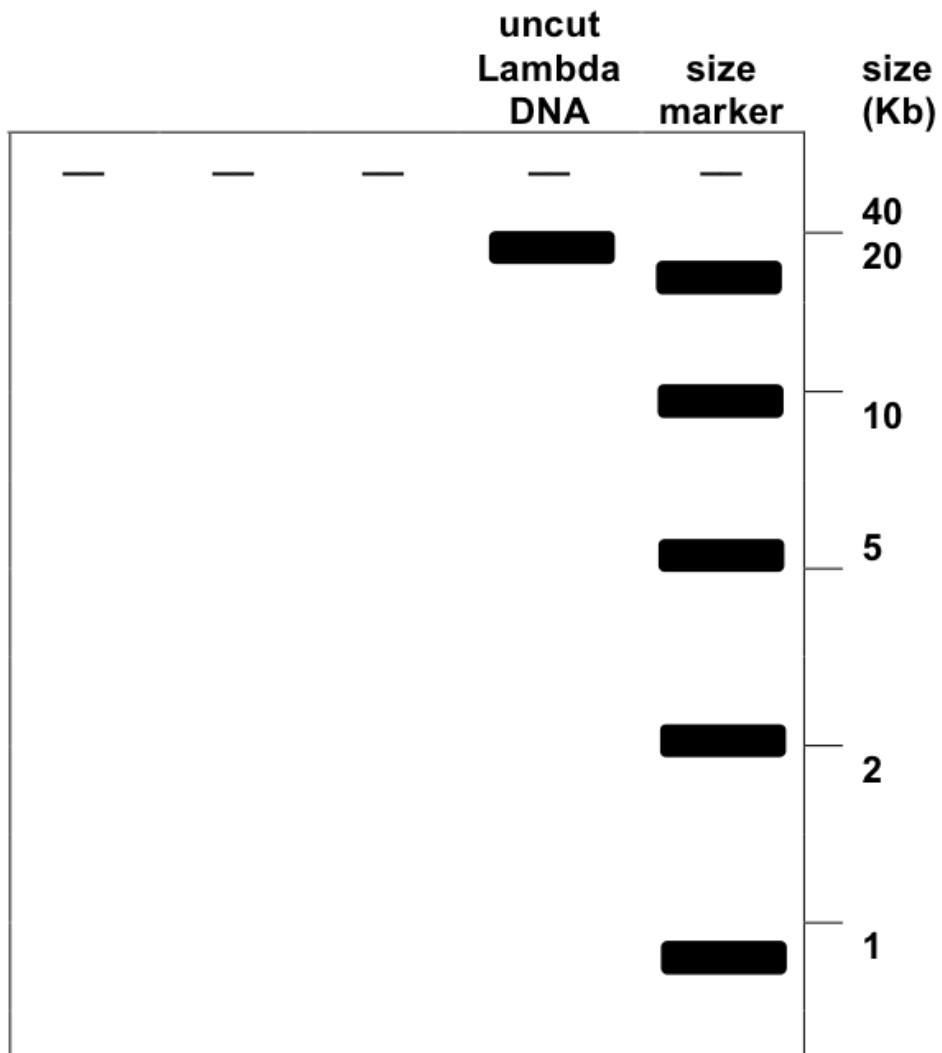
Question 5:

After we run an actual electrophoresis gel in the lab, please indicate below how many distinct bands were visible in each of your digests. (Enter the totals in Table 3 below).

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

Table 3. Comparison of the number of fragments observed on the gel versus the actual number of fragments produced (simulation total) by each restriction enzyme.

	Number of fragments observed (visible on your gel)	Number of fragments produced (simulation total)
*Eco*RI		
*Bam*HI		
*Hind*III		
Uncut		

Question 6:

Explain *why* you might not see all of the fragments that were produced. (In answering this, indicate the probable sizes of the fragments that you do see)

_____

_____

_____

_____

_____

_____

_____

_____

Score: _____ / 30