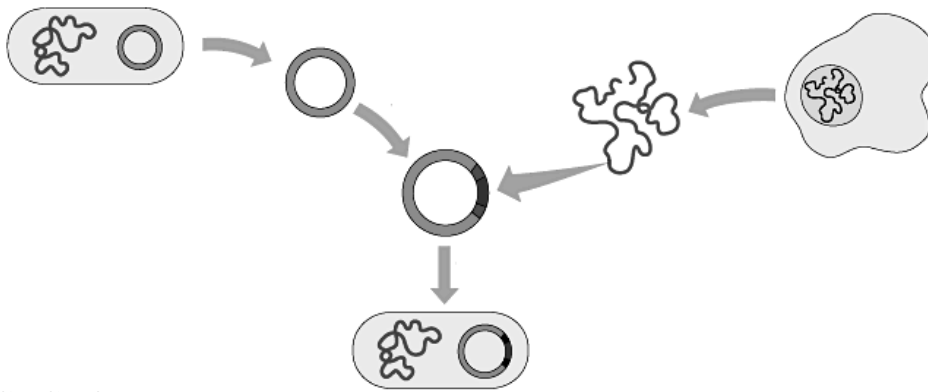


The *E. coli* Insulin Factory



BACKGROUND

Bacteria have not only their normal DNA, they also have pieces of circular DNA called plasmids. Plasmids are a wonderfully ally for biologists who desire to get bacteria to produce very specific proteins. The plasmids conveniently can be cut, fused with other DNA and then reabsorbed by bacteria. The bacteria easily incorporate the new DNA information into their metabolism. This “recombining” of DNA is called RECOMBINANT DNA.

Extracting a gene from one DNA molecule and inserting it into another requires precise “cutting and pasting.” To carry out this procedure, a piece of DNA containing the gene of interest must be cut out of a chromosome and “pasted” into a bacterial plasmid.

The cutting tools for making recombinant DNA in a test tube are bacterial enzymes called *restriction enzymes*, which were first discovered in the late 1960s. In nature, these enzymes protect bacteria against intruding DNA from other organisms and phages. They work by chopping up foreign DNA, a process called restriction because it restricts foreign DNA from surviving in the cell. Other enzymes chemically modify the cell’s own DNA in a way that protects it from the restriction enzymes.

Most restriction enzymes recognize short nucleotide sequences in DNA molecules and cut at specific points within these recognition sequences. Several hundred restriction enzymes and about a hundred different recognition sequences are known.

GOALS

In this activity, a make-believe DNA message for the protein insulin is marked on the cell DNA. Your task will be to find an enzyme that cuts the plasmid once (and only once) and the cell DNA as close as possible on both ends of the insulin gene so that the insulin code can be fused into the circle of the plasmid DNA.

To do this you will need to:

1. Determine which restriction enzyme to use to cut your DNA strand.
2. Determine which antibiotic you would use to determine if your finalized recombined DNA was absorbed by the bacteria or not.

SUMMARY OF PAGES

White = instructions

Pink = plasmid


Goldenrod = cell DNA


Yellow = record keeping/answer sheets

Green = restriction enzymes

 Ampicillin resistance

 Tetracycline resistance

 Kanamycin resistance

 Origin of Replication

 Insulin protein gene

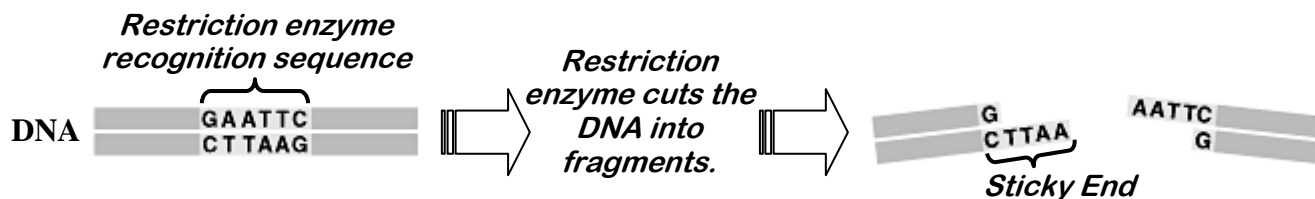
INSTRUCTIONS

Part A.

1. This activity requires cooperation between yourself and a lab partner. Each pair of students will turn in one lab report. This activity will take almost two class periods.
2. **READ THE FOLLOWING DIRECTIONS CLOSELY. YOUR CLASS NOTES ON PLASMIDS MAY HELP YOU REFRESH YOUR MEMORY ON HOW THEY FUNCTION.**
3. Obtain scissors and a roll of tape (or a strip of tape about 8 inches long.)
4. Cut out the PLASMID (pink strips). Discard ANY TWO of the strips (except for the strip which contains the “origin of replication” site (see code at bottom of pink sheet). Shuffle the strips and tape the end of one to the end of another in any random fashion (as long as the letters are going in the same direction). After you have taped the four strips into one long strip, tape the two remaining ends together, to form one long circular paper plasmid. Save the key about antibiotic resistance, from the bottom of the pink sheet for later use. Save extra plasmid pieces until you have successfully completed step #8.
5. As one partner works on step 4, the other should cut out the CELL DNA (goldenrod) strips. They must be taped together in order indicated at the bottom of each strip. That is, strip 2 is taped to the bottom of strip 1, strip 3 is taped to the bottom of strip two, etc. Note where the DNA code for insulin (the protein gene) is located.
6. After completing steps 4 and 5, use the PLASMID MAP on the yellow answer sheets to map the relative locations of the DNA code for each of the antibiotic resistances. Use a pencil to mark in the positions of the genes for antibiotic resistance that your plasmid contains. Label each.

YOU ARE NOW READY TO BEGIN ONE OF THE TWO MAJOR PARTS OF THIS ACTIVITY!

7. It is time to begin testing the various restriction enzymes that you have in your laboratory. Cut out ENZYMES (green). There are 8 restriction enzymes given for cutting the DNAs and one ligase fusing the DNAs together when done. Note that on each of the restriction enzyme rectangles, there is the name of the enzyme (such as *Ava II*) and a short DNA sequence that shows exactly what sequence that enzyme cuts.
8. Your job as a biochemist is to find a restriction enzyme that will cut open your plasmid at ONE site only (this may or may not be possible depending upon how you constructed your plasmid). The same enzyme should be able to cut your cell DNA at TWO SITES, one above the and one below the gene for insulin. It is very important that you find an enzyme cuts as close to the insulin gene as possible. The cells have genes for other unnecessary proteins that need to be cut off leaving “sticky” ends that look like this:



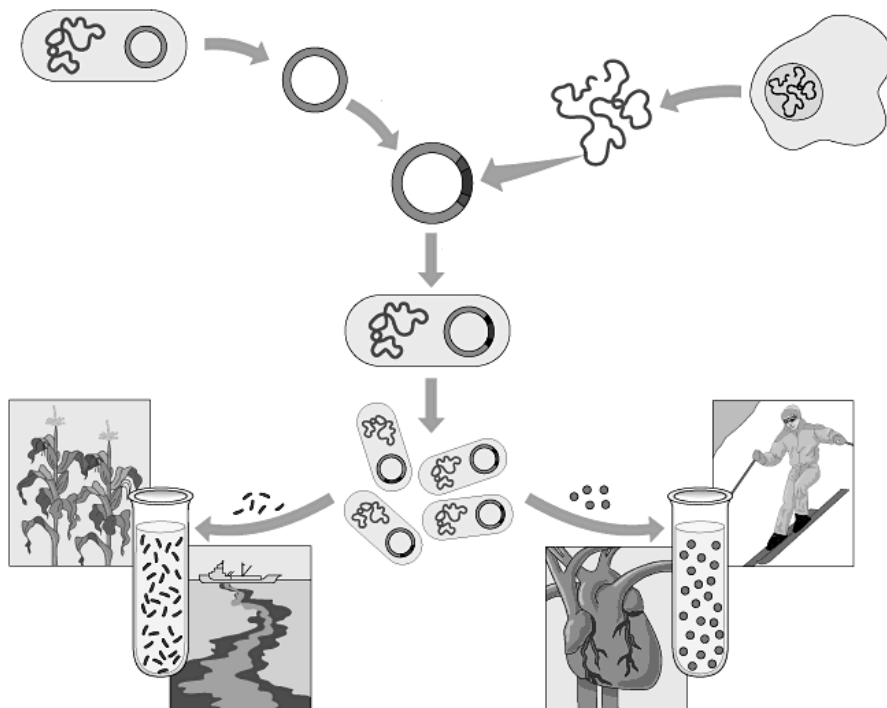
9. You and your partner have 8 restriction enzymes to choose from. Some of the enzymes cannot cut open your plasmid, some can. Some of the enzymes cannot cut your cell DNA at two sites, some can. If they don't meet your needs they are not usable.
10. One partner should take an enzyme, for example, *Ava II*, and check the pink plasmid for a location or locations which can be cut by this enzyme. The other partner should use a pen to mark on the PLASMID MAP (yellow answer sheets) where the enzyme will cut. Label each. Also, note on the table on your yellow answer sheets how many times it will cut the plasmid. **REMEMBER THAT YOUR GOAL IS TO FIND THOSE ENZYMES THAT WILL CUT THE PLASMID ONCE AND ONLY ONCE.** Continue this procedure until all 8 enzymes have been tried. Each group's results will be different because they different plasmid sequences. If you have no enzymes that will cut your plasmid only once, then reconstruct your plasmid.
11. Once you have selected those enzymes that cut the plasmid once, start checking these enzymes against the cell DNA strand. **REMEMBER, THE GOAL IS TO FIND AN ENZYME THAT WILL MAKE A CUT CLOSE TO THE GENE FOR INSULIN, ONE ABOVE AND ONE BELOW.** It is essential that the enzyme not cut into the insulin gene on the DNA. Mark directly on the goldenrod cell DNA strip where the enzymes will cut. Draw the line accurately showing exactly where the bases will be cut apart (and leave the "sticky" ends). Write the name of the enzymes next to each line you draw.
12. After you have completed testing the enzymes, select which ONE enzyme you would use to cut the plasmid and the cell DNA. Use scissors to make the cut in your plasmid and the cell DNA. **CAUTION: Scissors are sharp and easily cut through human flesh!** Be careful to make the cuts in the staggered fashion made by the actual enzyme. This will expose the "sticky" ends where joining will be possible. (since one enzyme was used, all "sticky" ends will be compatible). Use tape to splice your insulin gene into the plasmid chain. You have created **RECOMBINANT DNA!!!**
13. Fill in the rest of the appropriate spaces in the table on the yellow answer sheet. Give exact reasons why you did not use certain enzymes and the reason why you chose the one enzyme you did.

Part B.

In a real situation, you would mix your recombinant DNA plasmids with the bacteria of your choice. These bacteria would absorb the plasmids out of their environment and act as the hosts. These host bacteria should then begin producing insulin. You could purify the insulin and sell it so that it can be used by diabetics.

Early in this activity, you were asked to note and record which of the antibiotic resistances you had on your plasmid. This knowledge is extremely useful in determining if and which bacteria took up the plasmid as they were suppose to. The host bacteria are normally killed by the antibiotics (kanamycin, ampicillin and tetracycline). However, IF the recombinant plasmids were actually taken up by the bacteria, the plasmids may have contained a DNA gene for resisting the effects of one or more antibiotics. Therefore, if the host bacteria are placed in a growth medium containing an antibiotic to which they have a resistant gene in their recombinant plasmid DNA, they will survive. Any bacteria that failed to take in recombinant plasmids would die in the growth medium. **THEREFORE...**the host bacteria that survive have taken in the recombinant plasmids, those that do not survive have not taken it in.

1. Check your records to see which antibiotic resistances your plasmid contains and record on the yellow answer sheets.
2. Answer the rest of the question on the yellow answer sheets.
3. Staple your recombinant DNA plasmid (only) firmly to the back of the yellow answer sheet.
4. Hand in the yellow answer sheet.



DATA SHEET

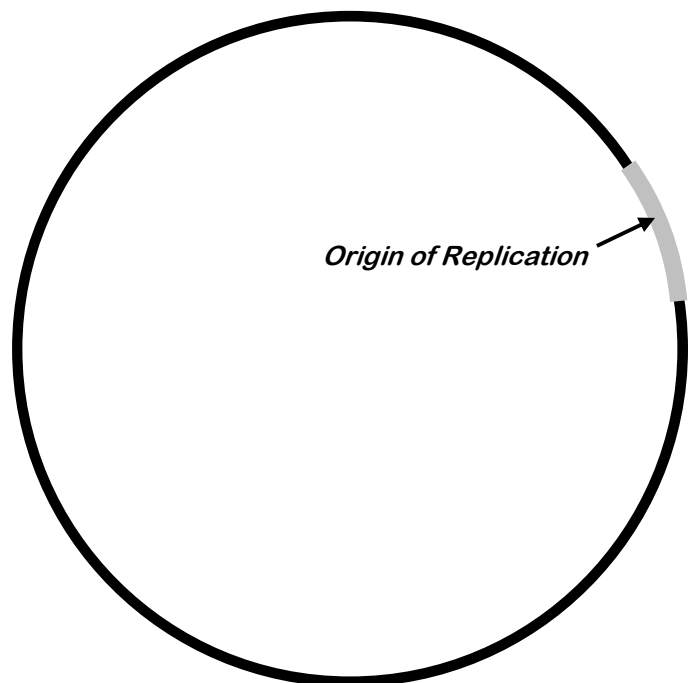
Name of Restriction Enzyme	No. of Cuts on:		USED (ONE) (X)	NOT USED (X)	Exact Reason For Use or Non-use
	Plasmid	DNA			
<i>Ava II</i>					
<i>Bam HI</i>					
<i>Bgl II</i>					
<i>Eco RI</i>					
<i>Hin dIII</i>					
<i>Hpa II</i>					
<i>Sac I</i>					
<i>Xma I</i>					

1. Which of the antibiotic resistances does your plasmid contain?
2. Which antibiotic(s) could you use in your growth medium to test for plasmid uptake?
3. Which antibiotic(s) could NOT be used in your growth medium to test for plasmid uptake? Explain.

PLASMID MAP

Use this map to show:

1. The relative positions of the genes for antibiotic resistance.
2. The approximate location(s) of the cuts that could be made on the plasmid by the 8 restriction enzymes.



NOTE: Be sure to staple your recombinant plasmid to this sheet before turning it in for credit.

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Ampicillin resistance



Tetracycline resistance



Kanamycin resistance

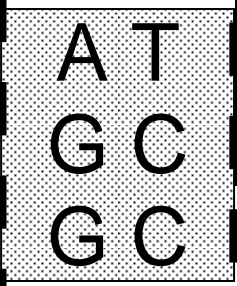
Origin of Replication

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Origin of Replication

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A T
C G
C G

(Print on pink paper).

**C
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D
N
A**



= protein gene

T	A	G ¹	C	T ²	A	T ³	A	G ⁴	C	T ⁵	A
G	C	A	T	T	A	T	A	T	A	T	A
G	C	G	C	C	G	C	G	A	T	C	G
G	C	A	T	G	C	G	C	A	T	G	C
C	G	T	A	A	T	T	A	T	A	A	T
C	G	T	A	A	T	C	G	A	T	A	T
T	A	C	G	G	C	A	T	T	A	C	G
A	T	T	A	G	C	T	A	T	A	G	C
G	C	T	A	T	A	G	C	C	G	G	C
G	C	A	T	A	T	T	A	C	G	G	C
C	G	A	T	C	G	C	G	T	A	G	C
A	T	G	C	A	T	A	T	C	G	C	G
C	G	T	A	T	A	C	G	C	G	C	G
A	T	C	G	A	T	A	T	T	A	C	G
G	C	A	T	A	T	T	A	T	A	T	A
G	C	A	T	C	G	T	A	A	T	A	T
G	C	G	C	G	C	T	A	A	T	G	C
C	G	C	G	T	A	A	T	G	C	G	C
C	G	A	T	C	G	A	T	A	T	A	T
C	G	G	C	T	A	A	T	A	T	C	G
G	C	G	C	C	G	T	A	T	A	C	G

1 2 3 4 5 6 7

(Print on golden rod paper).

Restriction Enzymes

<p>C G C G T A <u><i>Ava II</i></u> G C G C</p>	<p>T A T A C G <u><i>Hin dIII</i></u> G C A T A T</p>	<p>C G C G T A <u><i>Bam HI</i></u> A T G C G C</p>
<p>T A C G T A <u><i>Bgl II</i></u> A T G C A T</p>	<p>G C G C <u><i>Hpa II</i></u> C G C G</p>	<p>C G T A T A <u><i>Eco RI</i></u> A T A T G C</p>
<p>C G T A C G <u><i>Sac I</i></u> G C A T G C</p>	<p>G C G C G C <u><i>Xma I</i></u> C G C G C G</p>	<p>LIGASE</p>

(Print on green paper)