

## PAPER RECOMBINANT DNA USING BACTERIAL PLASMIDS

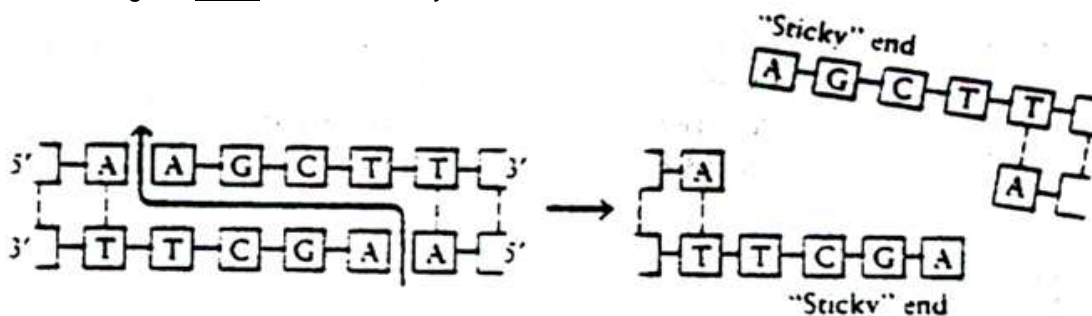
(Modified from several earlier versions)

### BACKGROUND:

Recombinant DNA technology produces large amounts of a selected gene, which controls production of a desired protein. In order to be successful, scientists must first locate (simple now that the Human Genome Project is finished) and then isolate the gene of interest. Once the gene is isolated it is transferred into a bacterial cell. The bacteria multiply and the gene of interest is copied along with the normal bacterial genome. The new population of bacteria then produces large amounts of the desired protein. In this paper activity you will insert the gene for insulin into a bacterial plasmid, and through bacterial transformation will understand how this plasmid is then transferred into a host bacteria cell.

A plasmid is a small circular piece of bacterial DNA. It is not the bacteria's main circular chromosome that it always needs to survive, but a smaller piece that carries only a few genes. Generally, the plasmid genes can give the bacteria proteins that will allow it to survive under particular environmental conditions. These genes are only transcribed/translated when they are needed by the bacteria. The plasmid that you will deal with in this activity has three antibiotic resistance sites (genes) as well as the replication origin site. The replication origin site tells the DNA replication enzyme (DNA polymerase) where to begin replication (remember that the plasmid is a circle). This plasmid confers resistance to ampicillin, tetracycline, and kanamycin (that is, there are genes that produce proteins that will allow the bacteria to survive these antibiotics). A bacterial cell that takes up a plasmid with all of these sites intact will be able to replicate the plasmid, and will become resistant to all three of these drugs.

In order to insert the gene of interest into the plasmid you must learn about restriction enzymes. These enzymes were isolated from bacteria that use them as a defense against foreign DNA that usually enters the bacteria from a virus. Bacteria with these enzymes are able to cut up viral DNA into non-functional pieces. Each restriction enzyme recognizes and cleaves (cuts) only a very specific sequence of DNA. The particular restriction enzymes that you will use cut the DNA in such a way that it produces "sticky ends." These sticky ends are staggered pieces of single stranded DNA that will bind with complementary sticky ends—those that have been cleaved using the same restriction enzyme.



In order to get your gene for insulin into the bacterial plasmid you will have to choose which restriction enzyme will successfully cleave your human DNA, and also cleave the plasmid, in such a way that you do not disrupt the gene of interest. You also have to cut the original plasmid (so the gene of interest's sticky end will match the plasmid's sticky end), without eliminating the replication origin, and without eliminating all of the antibiotic resistance sites.

Once you have successfully created a recombinant plasmid that contains the gene for insulin and still has at least one resistance site, the plasmid is ready to be transferred into the host bacteria (this would be the same procedure that you do in the pGLO lab). Since the original host bacteria have no antibiotic resistance, to check that the plasmid has been successfully taken up by the bacteria, you would grow them on a petrie dish that contains nutrient agar mixed with the antibiotic that the recombinant plasmid can resist. That way you know that any bacteria that survive the antibiotic on the dish, have received the plasmid that you inserted.

In a real lab 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 human insulin (remember that the genetic code is universal, so the proteins produced would be the same regardless that they are coming from a bacterial cell). You could then purify the insulin and sell it so that it could be used by diabetics. This insulin would be exactly the same as human insulin produced in human cells.

- GOALS:
1. determine which restriction enzyme to use to cut your DNA segments.
  2. determine which antibiotic you would use to determine if your finalized recombinant DNA was absorbed by bacteria or not.

## **INSTRUCTIONS** (work in pairs and turn in ONE lab report)

Materials needed: handouts, scissors and tape

### **1. READ AND FOLLOW DIRECTIONS CLOSELY.**

2. Cut out the strips on the sheet of paper labeled Cell DNA. Tape the strips together in the 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 2, etc. Note where the DNA code for insulin (the protein gene) is located.
  3. Cut out the Plasmid strips from the paper labeled plasmid. Tape the strips together in any sequence. Everyone's plasmid DNA can be different. Tape the ends of the strip together so that the plasmid is circular. NOTE: save the key about antibiotic resistance from the bottom of the sheet for future use)
  4. Use the plasmid map on the answer sheet 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.
  5. Cut out the restriction enzymes (ligase is the enzyme needed to help "glue" the sticky ends together completely). 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 sequences the enzyme cuts. Run each enzyme along the plasmid and find its recognition site. As you check each enzyme against the plasmid, mark the Plasmid Map to show where each enzyme would cut (label each on the map). Also note on the table on the answer sheet how many times each enzyme will cut the plasmid. (Remember that each group's results will be different because they have different plasmid sequences)
  6. 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 each end of the gene for insulin. The enzyme must not cut into the gene itself. Mark directly on the 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 each enzyme next to each line you draw.
  7. After you have completed testing each enzyme, select which one enzyme you would use to cut the plasmid and the cell DNA. Remember that you must choose the restriction enzyme that will meet ALL of the following criteria:
    - a. the replication origin on the plasmid is intact
    - b. at least one, but not all antibiotic resistance sites are lost
    - c. the enzyme cuts the plasmid only once
    - d. the enzyme does not cut into the gene of interest (insulin), but does cut the cell DNA at two sites and as close to the gene as possible
    - e. the restriction enzyme selected must leave identical sticky ends around the gene of interest as well as on the plasmid.
- \*\*\*\*IF THE ENZYME DOES NOT MEET ALL OF THE ABOVE, IT CANNOT BE USED**
8. Use tape to splice your insulin gene into the plasmid chain. You have now created RECOMBINANT DNA.
  9. Complete the table on the answer sheet. Be sure to give reasons why you chose your enzyme and why you did not choose the others. Answer all questions.
  10. Staple your recombinant DNA plasmid (only) to the back of the answer sheet and hand in.

Use this map to show the relative positions of the genes for antibiotic resistance AND the approximate location(s) of the cuts that could be made by the 8 enzymes.



plasmid replication site

