Name: _____ TOC#

Recombinant Technology

Introduction:

Today you will put together information about recombinant DNA and restriction enzymes to create a plasmid with new functions. The objective is to simulate gene cutting and splicing technology by inserting a "gene" from a piece of "Human DNA" into a "Bacterial Plasmid."

Background:

DNA fragments cannot function all by themselves. They must become part of the genetic material of living cells before the genes they contain can be activated. DNA fragments are incorporated into part of the recipient cell's genetic material.

For example, DNA fragments may be combined with bacterial DNA so that they can later be inserted into a bacterial cell. Bacteria often contain small circular DNA molecules known as **plasmids** in addition to their chromosomes. These plasmids can be removed from bacterial cells and cut with the same restriction enzyme used to produce the other "foreign" DNA fragments. The cuts made by the restriction enzyme produce matching "sticky ends" on the DNA fragments and the cut plasmids. These sticky ends are the sites at which a DNA fragment and a plasmid can be joined end to end, thereby forming a new plasmid that contains a piece of foreign DNA

DNA formed by fusing a DNA fragment and a plasmid consists of parts from different kinds of organisms. IN genetic engineering, molecules of combined DNA are known as chimeras because they are produced by combining DNA from different species. Combined DNA is also known as recombinant DNA, since DNA from two sources have be recombined to produce it. When this is inserted back into a living organism, we call that organism transgenic.

Procedure:

Follow the steps below to combine a piece of human DNA that codes for insulin production into a bacterial plasmid. Then when this is complete, the plasmid will be able to express the gene for human insulin and create insulin diabetic people can use to regulate their blood sugar.

1. Fill in the missing bases on the sheets labeled Human DNA, Plasmid DNA, and enzyme sheets.
2. Label genes on Human DNA and Plasmid DNA using the legends at the bottom of the sheet.
3. Make a long strand of human DNA by:
    a. Cut strips of DNA and tape them IN ORDER#1-6 so that you have one long strip of human DNA
4. Make a circular plasmid by:
    a. Cut strips of plasmid DNA and tape them into a circle in ANY order
    b. All strips are note needed (you can use anywhere between 2 and 6 pieces)
    c. You MUST include the strip with the replication site.
    d. Tape your selected strips into a circle
5. Choose one restriction enzyme from the sheet provided to cut the plasmid and the human DNA with.
    a. Using that enzyme as a template mark, with a pencil, any recognition site on your plasmid.
    b. *You make have chosen a restriction enzyme that does not have a restriction site on your plasmid. If this happens, choose another restriction enzyme.*
    c. Using the same restriction enzyme, make the recognition sites on your Human DNA.
        i. Your goal is to isolate or "cut out" the gene for insulin production.
6. "Digest" the pieces of DNA by cutting along the lines of your recognitions sites on both your plasmid DNA and Human DNA.
7. Insert your DNA fragment into the open plasmid
    a. Use the ligase to covalently bond sugars and phosphates
    b. Tape together the matching sticky ends of each fragment
    c. This will form a solid piece of recombinant DNA.
8. If this were a real lab, you would then insert this newly engineered Plasmid into a bacterial cell. You would provide it with the appropriate conditions and nutrients and the bacterium would divide as often as every 20 minutes. The growing colony of bacterial cells would use ribosomes, tRNA, mRNA, and the enzyme RNA polymerase to carry out protein synthesis. The bacteria would then produce Human insulin.

Analysis Questions:

1. What is critical about these pieces fitting together?

2. Why must the Human and Plasmid DNA be cut with the same restriction enzyme?

3. What product would you collect from your newly engineered bacteria? Explain how this is possible.

4. Why was it important that the plasmid contain the replication site?

5. Could the bacterial cell produce the product if the plasmid had not been inserted? Why or why not?

6. Could the plasmid produce the product without being put into a bacterium?

7. Looking at your plasmid, you may or may not have included genes other than the replication site such as various antibiotic resistance genes. Why do you think these may be important for the plasmid to contain?

8. Biotech companies like Genentech and BioRad actually carry out this process in South San Francisco. Why do you think this form of the protein produced here is important for diabetics?