**Activity: Creating Insulin**  Name :\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Goal: To produce insulin using a bacterial cell**

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 accomplish 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 recombinedDNA was absorbed by the bacteria or not.

**Instructions**

**Part A: Prepping the DNA**

1. Refer to your notes on restriction enzymes and recombinants to help you complete this exercise.
2. Obtain scissors and a roll of tape.
3. Obtain your Plasmid DNA sequence. Notice there are 6 separate sequences numbered 1-6.
4. Color code your plasmid DNA to identify the origin of replication and antibiotic resistance genes. Mark in pencil the name of the gene on your plasmid. Your teacher should display a color coded plasmid.
5. Cut out the PLASMID as strips. You should end up with 6 strips. Discard ANY TWO of the strips (except for the strip which contains the “origin of replication” site. 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 extra plasmid pieces until you have successfully completed the exercise.
6. As one partner works on step 5, the other person should obtain the CELL DNA.
7. Color code the insulin gene yellow on the CELL DNA. Mark in pencil the name of the gene on your plasmid. Your teacher should display a color coded CELL DNA.
8. Cut out the CELL DNA strips. They must be taped together in order indicated to the right of each strip. That is, strip 2 is taped to the right of strip 1, strip 3 is taped to the right of strip two, etc. Note where the gene for insulin (the protein gene) is located.
9. After completing steps 4 and 5, use the PLASMID MAP on the 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.

**Part B: Cutting the DNA**

1. Determine the restriction sequence for each enzyme listed in table 1 on your answer document by going to <http://rebase.neb.com/rebase/rebase.html>. Enter the enzyme name in the “Go directly to enzyme field”. For example, for enzyme AvaII, you will retrieve the sequence below. The arrows indicate where the enzyme will cut to create sticky ends. A W indicates that any nucleotide (ATGorC) can be in that position. Complete Table 1 NOW!

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 5' ..3' .. |  | cutblank3Gcutblank3C | cutdown3 | cutblank3Gcutblank3C |  | cutblank3Wcutblank3W |  | cutblank3Ccutblank3G | cutup3 | cutblank3Ccutblank3G |  | 3' ..5' .. |

1. As one member is recording the sequences, the other group member should be looking for these sequences within that cut your PLASMID DNA ONE TIME! Read below before you start looking through all of those letters.
2. 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 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.
1. 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.
2. 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.
3. 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 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.
4. 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. 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!!!
5. Fill in the rest of the appropriate spaces in the table on answer sheet. Give exact reasons why you did not use certain enzymes and the reason why you chose the one enzyme you did.

**Answer Sheet: Creating Insulin Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Table 1: Restriction Sites**

|  |  |
| --- | --- |
| **Enzyme Name**  | **Restriction Sequence with arrows** |
| **AvaII** |  |
| **HindIII** |  |
| **BamHI** |  |
| **BgIII** |  |
| **HpaII** |  |
| **EcoRI** |  |
| **SacI** |  |
| **XmaI** |  |

**Table 2: Plasmid Data**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name of Restriction Enzyme** | **No. of Cuts on:** | **USED (ONE) (X)** | **NOT USED****(X)** | **Exact Reason For Use or Non-use** |
| **Plasmid** | **DNA** |
| ***Ava II*** |  |  |  |  |  |
| ***BamHI*** |  |  |  |  |  |
| ***Bgl II*** |  |  |  |  |  |
| ***EcoRI*** |  |  |  |  |  |
| ***Hindi*** |  |  |  |  |  |
| ***Hap II*** |  |  |  |  |  |
| ***Sac I*** |  |  |  |  |  |
| ***Exam I*** |  |  |  |  |  |

 **Questions**

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 antibiotics(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.

Origin of replication

Cell DNA sequence

ACCCGGATCCGTGTCCCGGGCCTCTAAGAATTCAGTTCGTCC 1 TGGGCCTAGGCACAGGGCCCGGAGATTCTTAAGTCAAGCAGG

Cell DNA sequence

AAGCTTCCATGTATTGCAGAGAAGCAGTACACGGAAAATTTA 2 TTCGAAGGTACATAACGTCTCTTCGTCATGTGCCTTTTAAAT

Cell DNA sequence

CATTATAAGGAGGAATTCTTAAAGCTTGCCCCGGGATCCTGG 3 GTAATATTCCTCCTTAAGAATTTCGAACGGGGCCCTAGGACC

Cell DNA sequence-KEY

The insulin gene is colored YELLOW

ACCCGGATCCGTGTCCCGGGCCTCTAAGAATTCAGTTCGTCC 1 TGGGCCTAGGCACAGGGCCCGGAGATTCTTAAGTCAAGCAGG

Cell DNA sequence

AAGCTTCCATGTATTGCAGAGAAGCAGTACACGGAAAATTTA 2 TTCGAAGGTACATAACGTCTCTTCGTCATGTGCCTTTTAAAT

Cell DNA sequence

CATTATAAGGAGGAATTCTTAAAGCTTGCCCCGGGATCCTGG 3 GTAATATTCCTCCTTAAGAATTTCGAACGGGGCCCTAGGACC

Plasmid DNA sequence-1

CGGGTCTCAAAGAATTCCAGA GCCCAGAGTTTCTTAAGGTCT

Plasmid DNA sequence-2

TCTTTTACACACAGGTCATCC AGAAAATGTGTGTCCAGTAGG

Plasmid DNA sequence-3

ATCCGGGGGAAAAATCCCTGA TAGGCCCCCTTTTTAGGGACT



**Plasmid DNA Sequence KEY:** Display this document to the students to use as a key for color coding their plasmid DNA

Plasmid DNA sequence-1

CGGGTCTCAAAGAATTCCAGA GCCCAGAGTTTCTTAAGGTCT

AMPICILLIN RESISTANCE

TETRACYCLINE RESISTANCE

KANAMYCIN RESISTANCE

ORIGIN OF REPLICATION

Plasmid DNA sequence-2

TCTTTTACACACAGGTCATCC AGAAAATGTGTGTCCAGTAGG

Plasmid DNA sequence-3

ATCCGGGGGAAAAATCCCTGA TAGGCCCCCTTTTTAGGGACT

