Name: _____

Modeling Recombinant DNA Technology

Materials
- Paper plasmid strips
- Cell DNA with gene of interest strips
- Restriction enzyme sheet
- Tape
- Scissors

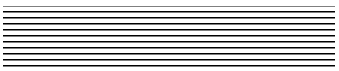
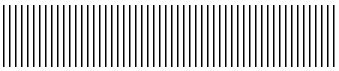
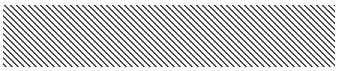
Background

Bacterial have circular plasmids that are usually several thousand base pairs in length. A plasmid will have an origin of replication site and may also contain genes for antibiotic resistance. The antibiotic resistance is useful for helping to screen out bacteria that did not take up the plasmid in an experiment. Plasmids are used in recombinant DNA technology to transfer genes from one organism to another.

Recombinant DNA contains DNA from more than one organism. For example, the gene for human insulin can be inserted into bacteria with the help of a plasmid. The bacteria will then secrete human insulin that can be harvested economically and with great purity. The millions of diabetics throughout the world can then use the insulin.

You have been provided with a paper "plasmid" and a piece of cellular DNA. Your task today is to find the best restriction enzyme to cut out the gene of interest (shaded on the cellular DNA) and to cut the plasmid so the gene of interest can be inserted into it.

Directions

Sequence	Shading on Plasmid
Ampicillin	
Kanomycin	
Tetracycline	
Origin of Replication	

1. Study your plasmid DNA. Notice there are sequences that give antibiotic resistance to bacteria from antibiotics. There is also an origin of replication site. If any of these sequences are cut, the ability of the bacteria to reproduce or to have antibiotic resistance will be destroyed.

2. Construct your plasmid by cutting the strips of the plasmid DNA along the lines. Tape the ends together to form a circular plasmid. Tape them together in the same order they are on the paper. For example, the bottom of strip number 1 would be taped to the top of strip number 2.

3. Construct your strip of cellular DNA by cutting out the strips and taping them together in a linear piece. Again, be sure to tape them in the order they are on the sheet.

4. Cut out your restriction enzyme site cards.

5. Run each restriction enzyme card along the plasmid. Mark any matching restriction sites you find. Colors can help keep these sites organized. The restriction enzymes may cut once, more than once, or not at all. Complete the table in analysis question #1 as you work.

6. Run each restriction enzyme card along the cellular DNA. Mark any sites you find. Colors can help keep these sites organized. The restriction enzymes may cut once, more than once, or not at all. Complete the table in analysis question #1 as you work.

7. Now you must decide which restriction enzyme to use in your pretend transformation. Remember, you want to use a restriction enzyme that can cut out the gene of interest (shaded on the cellular DNA). You want to cut as close to the gene of interest on the cellular DNA as you can so that little extraneous DNA will be included. You must also choose a restriction enzyme that can cut the plasmid *only once* so the gene can be inserted into it. Chose a restriction enzyme that does not cut in a gene for antibiotic resistance or in the origin of replication. You must use the same restriction enzyme to cut the plasmid as you use to cut out the gene of interest. This is necessary so the sticky ends will pair up correctly.

8. Now take a scissors and cut the plasmid at the restriction enzyme site selected. Then, cut out the gene of interest from the cellular DNA at the sites selected.

9. Insert the gene of interest into the plasmid and use "ligase" tape to tape it into place. You should now have a recombinant plasmid that has new DNA inserted into it.

Name: _____

Analysis Questions

1. Complete the following table as you work:

Restriction Enzyme	Number of Cuts Made on Plasmid	Number of Cuts Made on Cellular DNA
Ava II		
HindIII		
Bam HI		
Bgl II		
Hpa II		
EcoR I		
Sac I		
XmaI		

2. Which restriction enzyme did you chose to use? Why?

3. Specifically explain why <u>each</u> of the other restriction enzymes was not selected for use.

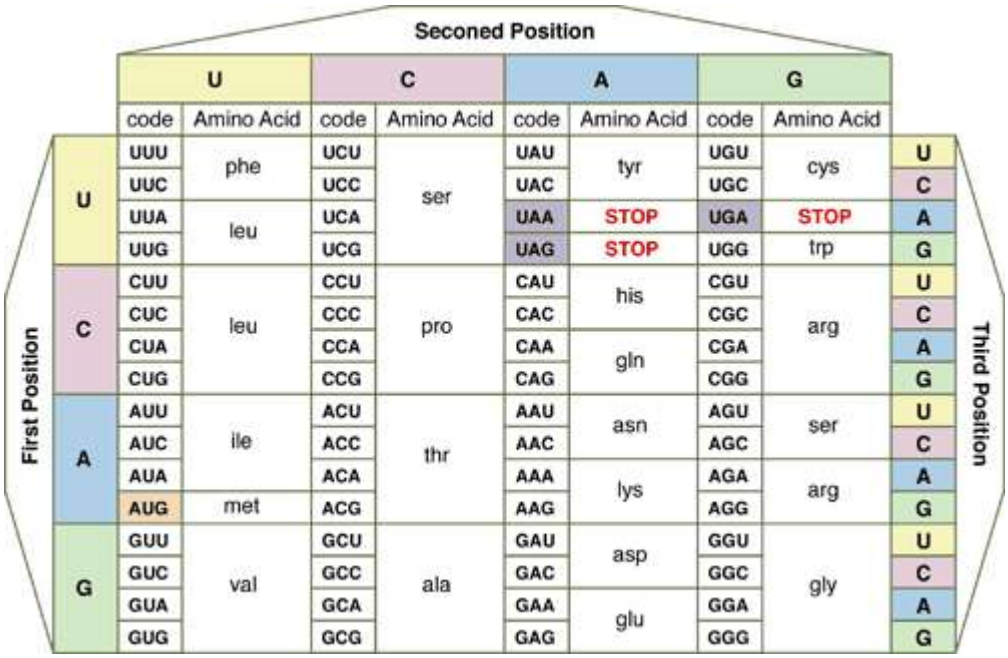
4. The gene of interest is made up of a series of triplet codons. Each three base pairs correspond to an amino acid or a start or stop codon. Look at the ends of your gene of interest. One has the DNA sequence TAC and the other ATT. Look at the codon table and determine what these triplet codons code for. Remember, the table is in mRNA.

5. Each three base pairs codes for an amino acid in a protein. How many amino acids does your gene code for (excluding start and stop)?

Name: _____

6. Look at the left hand side of your gene of interest. Complete the table below to record the sequence of bases that would be found on the messenger RNA and the sequence of amino acids.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
DNA	TAC													
mRNA	AUG													
Amino Acid	Start / Met													

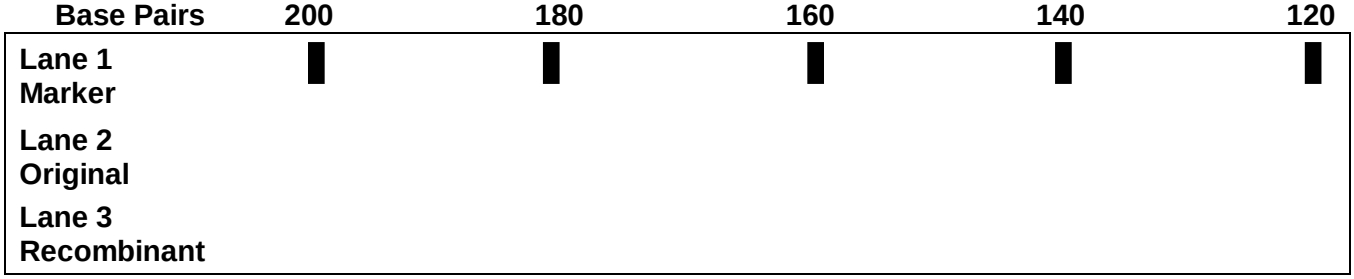


7. Was the strand you translated (the left hand side of the gene of interest) the sense or anti-sense strand of the DNA?

8. How many base pairs long is the gene of interest (including the start and stop triplets)?

9. What is the base pair size of the recombinant plasmid you constructed?

10. In order to make sure the inserted length of DNA is actually in the plasmid, you would need to run an electrophoresis gel of the recombinant plasmid and the original plasmid. Mark where the DNA fragment sizes would show up for the original and the recombinant plasmid on the gel below:



Name: _____

CELL DNA

T	A	G	C	T	A	T	A	G	C	T	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	G	C	T	A	G	C
A	T	G	C	A	T	C	G	C	G	C	G
C	G	T	A	T	A	C	G	C	G	C	G
A	T	C	G	A	T	T	A	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	

Name: _____

PLASMID

1		2		3		4		5		6	
G	C	A	T	T	A	C	G	T	A	T	A
C	G	G	C	A	T	G	C	G	C	A	T
C	G	A	T	G	C	A	T	G	C	A	T
C	G	A	T	G	C	G	C	T	A	G	C
A	T	A	T	C	G	T	A	G	C	C	G
G	C	A	T	C	G	T	A	G	C	C	G
A	T	T	A	C	G	A	T	G	C	G	C
G	C	G	C	C	G	A	T	G	C	T	A
T	A	T	A	C	G	C	G	G	C	A	T
T	A	G	C	T	A	C	G	C	G	G	C
T	A	T	A	T	A	T	A	A	T	G	C
C	G	G	C	T	A	A	T	A	T	T	A
T	A	T	A	T	A	G	C	G	C	T	A
T	A	C	G	T	A	G	C	G	C	C	G
A	T	C	G	A	T	A	T	T	A	G	C
A	T	A	T	G	C	G	C	T	A	A	T
G	C	G	C	G	C	G	C	A	T	A	T
G	C	T	A	G	C	G	C	T	A	C	G
T	A	A	T	A	T	C	G	A	T	T	A
C	G	G	C	C	G	C	G	C	G	C	G
T	A	G	C	T	A	C	G	T	A	C	G

Name: _____

ENZYMES (add cut sites)

C G C G T A G C G C	Ava II	T A T A C G G C A T	Hind III	C G C G T A A T G C G C	Bam HI
T A C G T A A T G C A T	Bgl II	G C G C C G C G	Hpa II	C G T A T A A T A T G C	Eco RI
C G T A C G G C A T G C	Sac I	G C G C G C C G C G C G	Xma I		