DATA SHEET

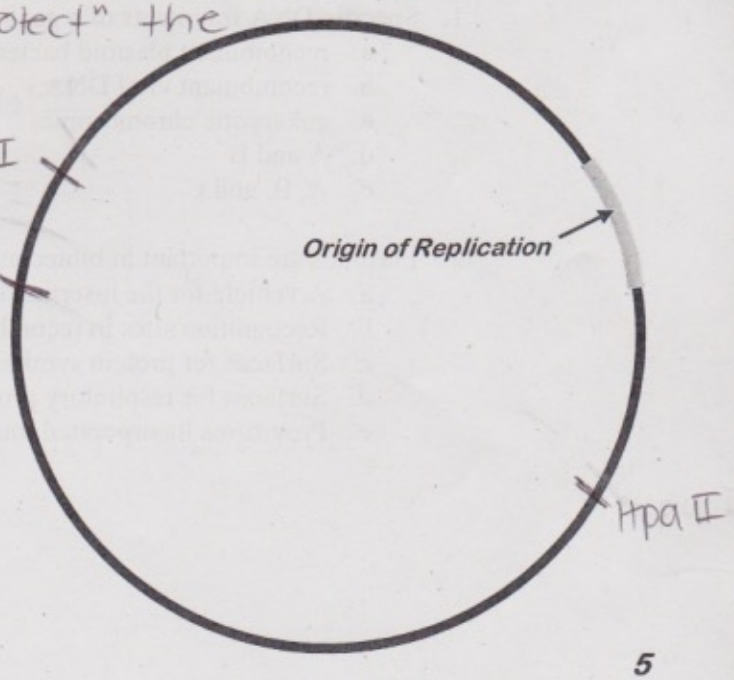
Name of Restriction Enzyme	No. of Cuts on: Plasmid	DNA	USED (ONE) (X)	NOT USED (X)	Exact Reason For Use or Non-use
Ava II	/			X	Plasmid ~~did~~ not cut
Bam HI	/			X	Plasmid uncut by enzyme
Bgl II	1	/		X	DNA not cut by enzyme
Eco RI	1	II	X		Enzyme cut plasmid once, DNA at two places by gene
Hin dIII	/			X	Plasmid uncut by enzyme
Hpa II	1	II		X	Not cut close to gene
Sac I	/			X	Plasmid uncut by enzyme
Xma I	/			X	Plasmid was not cut

1. **Which of the antibiotic resistances does your plasmid contain?**
   Ampicillin and Tetracycline resistance
2. **Which antiobiotic(s) could you use in your growth medium to test for plasmid uptake?**
   Ampicillin and/or tetracycline
3. **Which antiobiotic(s) could NOT be used in your growth medium to test for plasmid uptake? Explain.** Kanamycin could not be used because the plasmid would not "protect" the cell from damage.

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.***

Name: Leonie Hoeher Mod: _____ Date: 5/1/15

Restriction Enzyme Lab

List the Enzymes that Cut the Plasmid once: Bgl II, Eco RI, Hpa II

List the Enzyme you choose to use for this experiment: Eco RI

Discussion Questions

1. Why was it important to find an enzyme that would cut the plasmid at only one site? What could happen if the plasmid were cut at more than one site?
   If it were cut more often than once, it would disperse into fragments of plasmid. It would not be able to re-combine with DNA anymore.
2. Why was it important to discard any enzymes that cut the plasmid at the replication site?
   If the replication site was cut, it would not function. The plasmid DNA would cease to replicate in the cell, thereby not multiplying for further study.
3. Why might it be important to cut the DNA strand as closely to the desired gene as possible?
   Cutting the DNA close to the desired gene is necessary so that undesired sequences are left out and the sticky ends find each other.
4. In this activity, you incorporated an insulin gene into the plasmid. How will the new plasmid DNA be used to produce insulin?
   The plasmid DNA will replicate and the bacterial cell it is put back in to will divide. The insulin gene will be expressed as proteins which can be harvested. (The gene could also be used for study).

Multiple choice practice questions:

1. Specific DNA fragments of a gene library are contained in
   a. recombinant plasmid bacteria
   b. recombinant viral DNA
   c. eukaryotic chromosomes
   d. A and B
   e. A, B, and C

2. Plasmids are important in biotechnology because they are
   a. A vehicle for the insertion of recombinant DNA into bacteria
   b. Recognition sites in recombinant DNA strands
   c. Surfaces for protein synthesis in eukaryotic recombinants
   d. Surfaces for respiratory processes in bacteria
   e. Proviruses incorporated into the host