Isolation and Characterization of an Unknown Phagemid
(20 Points)

Name: _____
Partner's Name: _____
Lab Section: _____
Unknown #: _____

1. Attach a copy of your gel photo, indicating the four lanes corresponding to your samples and what sample is specifically represented in each lane (i.e., label "1. uncut", "2. HindIII", "3. BamHI", and "4. both").

2. Complete the attached DATA SHEETS. All measurements should be made in "cm" using significant figures to the *hundreth position*.

3. Answer the following questions relating to agarose gel electrophoresis:
   a. In general, do shorter or longer pieces of double-stranded DNA travel farther on an agarose gel? _____
   b. Do DNA samples, like protein samples, have to be specially treated to ensure migration through a gel based solely upon molecular weight? Explain why or why not. Assume in your answer that the DNA is NOT exhibiting supercoiling.
   _____
   _____
   _____
   _____
   c. Toward which terminal do DNA samples migrate? Explain why, in terms of the primary structure of DNA molecules.
   _____
   _____
   _____
   _____

4. a. Based upon a visual inspection of the agarose gel photo, is supercoiling evident in <u>your</u> native phagemid? _____
   b. If present, how many supercoiled forms were exhibited? _____ On your gel photo, <u>circle</u> all DNA bands which you believe to represent supercoiled forms.
   c. Which of your sample lane(s) (1-4) in this experiment would indicate the presence or absence of supercoiling? *Explain*.
   _____
   _____
   _____
   _____

5. Generate a plot of log(Mwt) vs. distance traveled for each of the lambda molecular weight markers. Attach it to your lab report, and use it to address the following questions:
   a. Using this graph, determine the molecular weight of the original pALTER-1 phagemid, NOT INCLUDING THE GENE INSERT. Show *on your graph* how you made this determination,

   Molecular Weight = _____

b. Which of the sample lane(s) (1-4) in your experiment do you use to determine the molecular weight of the phagemid? Explain.

_____
_____
_____
_____

c. Using this graph, determine the molecular weight of the gene insert. Show *on your graph* how you made this determination.

Molecular Weight = _____

d. Which of the sample lane(s) (1-4) in your experiment do you use to determine the molecular weight of the gene? Explain.

_____
_____
_____
_____

e. On average, the molecular weight of double-stranded DNA is 650 g/mole per base pair. Use this number as a conversion factor to predict the base length of your gene insert. Show your work below:

f. Based *solely on the length of your gene insert*, what are the possible identities of your unknown gene? Circle all possible answers:

B. stearothermophilus PFK *E. coli* PFK RNase T1

[WARNING: Each of the gene products generated by the restriction digests will actually be larger than predicted based only upon individual gene length. This is because the promoter region has also been cut out along with the gene by the restriction enzymes used in this experiment.]

6. Based on the pattern of DNA fragments on the gel photo resulting from the three separate restriction digests, how was the gene in your phagemid originally inserted into pALTER-1? In other words, what restriction enzymes surround your gene insert?

_____

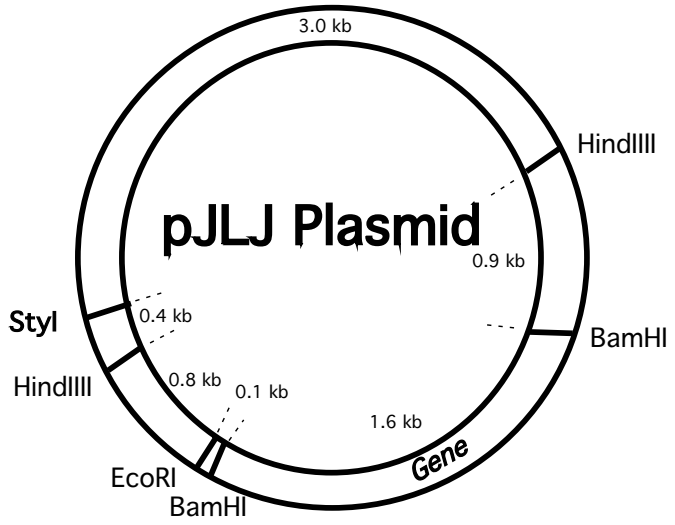
Based *solely on the restriction enzyme digests*, what are the possible identities of your unknown gene? Circle all possible answers:

B. stearothermophilus PFK *E. coli* PFK RNase T1

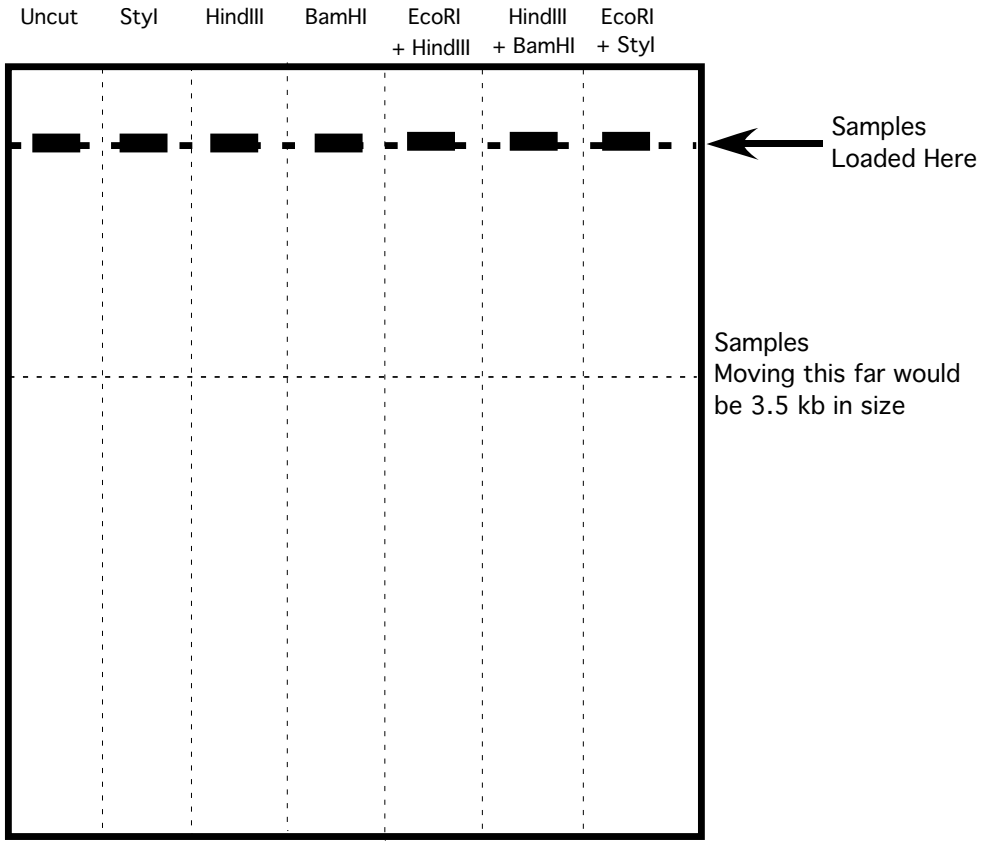
7. Why is <u>agarose</u> used as the polymer for separating DNA fragments in *digestion experiments*, whereas <u>polyacrylamide</u> gel electrophoresis is commonly used to *sequence* DNA?

_____
_____
_____
_____

8. Consider the following restriction enzyme map of the pJLJ plasmid:



a. Sketch the band pattern that you would anticipate for the following agarose gel. In the uncut lane, represent one population of DNA corresponding to a relaxed form, one population corresponding to differently supercoiled forms, and one population of "nicked" DNA.



b. Circle the DNA band that corresponds to <u>only</u> the gene (1.6kB) cut out of the pJLJ plasmid.

c. Place a square around the DNA band corresponding to uncut, relaxed, chimeric plasmid.

DATA SHEET
(page 1)

MOLECULAR WEIGHT STANDARDS*

Fragment No.	Molecular Weight (grams/mole)	Log (Mwt)	Distance Migrated from Well (cm)
1	_____	_____	_____
2	_____	_____	_____
3	_____	_____	_____
4	_____	_____	_____
5	_____	_____	_____
6	_____	_____	_____
7	_____	_____	_____
8	_____	_____	_____
9	_____	_____	_____
10	_____	_____	_____
11	_____	_____	_____
12	_____	_____	_____
13	_____	_____	_____
14	_____	_____	_____

*Note: Bands with similar molecular weight may be difficult to distinguish, though it *should* be possible to estimate the center of each individual DNA band. Bands 13 & 14 may or may not be visible.

DATA SHEET
(page 2)

SAMPLE LANES**

Sample Identity	**Distance Migrated from Well (cm)**
Lane 1: Uncut Phagemid	_____ _____ _____
Lane 2: HindIII Digested Phagemid	_____ _____
Lane 3: BamHI Digested Phagemid	_____ _____
Lane 4: HindIII & BamHI Digested Phagemid	_____ _____

**Note: Each sample may not contain as many bands as there are blanks provided.