Name:_____   Section:_____

7.012 Problem Set 5

Please print out this problem set and record your answers on the printed copy. Answers to this problem set are to be turned in at the box outside by 4:10 Wednesday, October22. Problem sets will not be accepted late. Solutions will be posted on the web October 23, 2003.

*PLEASE NOTE,: Questions 1 and 2 cover material from lectures 14 and 15 and thus would be good practice for Quiz II.

Question 1

Restriction enzymes are extensively used in molecular biology. Below are the recognition sites of two of these enzymes, BamHI and BclI.

a) BamHI, cleaves after the first G:

     5' G|GATCC 3'
     3' CCTAG|G 5'

Does cleavage by BamHI result in a 5' or 3' overhang? What is the sequence of this overhang?

b) BclI cleaves after the first T:

     5' T|GATCA 3'
     3' ACTAG|T 5'

Does cleavage by BclI result in a 5' or 3' overhang? What is the sequence of this overhang?

c) Given the DNA shown below …

       5' ATTGAGGATCCGTAATGTGTCCTGATCACGCTCCACG 3'
       3' TAACTCCTAGGCATTACACAGGACTAGTGCGAGGTGC 5'

    i) If this DNA was cut with BamHI, how many DNA fragment would you expect?
    Write out the sequence of these double-stranded DNA fragments.

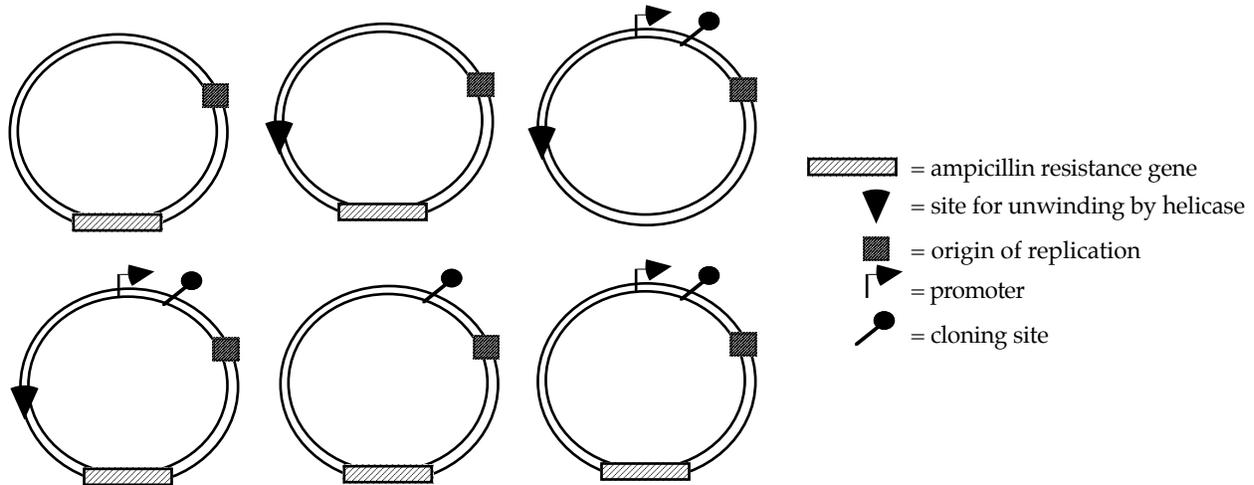## Question 1, continued

c)   ii) If the DNA shown on the previous page in (c) was cut with BclI, how many DNA
        fragment would you expect? Write out the sequence of these double-stranded DNA
        fragments.

d) You can ligate a restriction fragment produced in (c, i) to one produced in (c, ii). Write out
the sequence of the resulting fragment.

e) Could you cut the fragment from (d) with either BamHI or BclI? Explain.

Question 2

You wake up one morning to your roommate exclaiming about her sudden hair growth. She has been supplementing her diet with a strange new fungus purchased at the local farmer's market. You take samples of the fungus to your lab and you find that this fungus does indeed make a protein (the harE protein) that stimulates hair growth. You construct a fungal genomic DNA library in the hope of cloning the *harE* gene. If you succeed you will be a billionaire! You

= ampicillin resistance gene
= site for unwinding by helicase
= origin of replication
= promoter
= cloning site

obtain DNA from the fungus, digest it with a restriction enzyme, and clone it into a vector.

a) Circle the vector that has the MINIMUM features required for your library construction.

b) You clone your digested genomic DNA into this vector. What type of *E. coli* (bacteria) cells do you need to transform to create your library?

c) How do you distinguish bacterial cells that carry a vector from those that do not?

d) Circle on the following lists ALL you would need in order to construct the genomic DNA library. Assume you start with intact genomic DNA.

Enzymes	Reagents
Restriction enzyme	Size separation gel
Ligase	Okasaki fragments
DNA Polymerase	ATP, TTP,CTP,GTP
RNA Polymerase	ddATP, ddTTP, ddCTP,
Transcriptase	ddGTP
Reverse Transcriptase	Primers
3' to 5' exonuclease	Replication fork
Cloning vector	Human cells
	Virus

Question 3

You find a plasmid that you think carries the *harE* gene, but you need to confirm that indeed the target gene has been inserted. When you made your library, you cut your genomic DNA with EcoRI and cloned it into a unique EcoRI restriction site in the vector.

a) How can you use the EcoRI restriction enzyme to tell you if the gene has been inserted?

Suppose you find that the *harE* gene is in the plasmid, but now you want a restriction map of the recombinant plasmid. You take three individual samples of the plasmid and digest one sample with EcoRI, the second sample with HindIII, and the thrird sample with both EcoRI and HindIII. Then you run the digested DNA on a gel to see the fragments.

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                                              EcoRI
            EcoRI         HindIII               +
                                              HindIII

   5.3       ▬▬▬

   4.1                     ▬▬▬

                                              ▬▬▬    3.0
   2.7                     ▬▬▬

                                              ▬▬▬    2.3

   1.5       ▬▬▬

                                              ▬▬▬    1.1

                                              ▬▬▬    0.4
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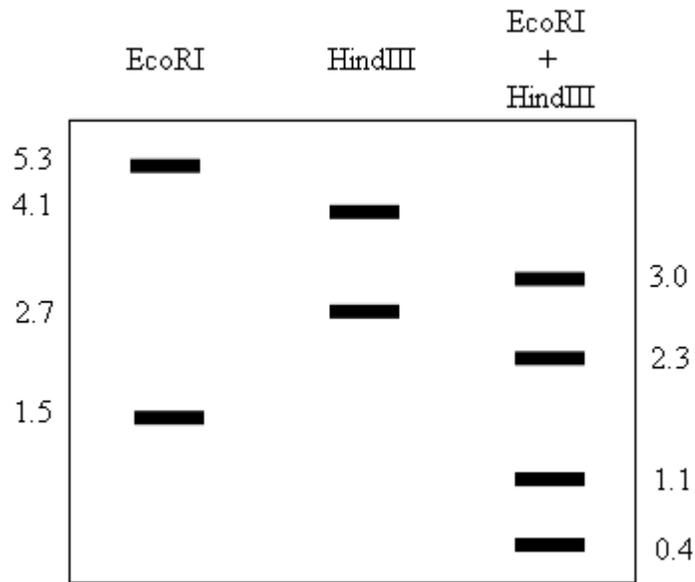
b) Considering that the *harE* gene is smaller than the vector,

   i) Circle the fragments on the gel that contain all or part of the *harE* gene.

   ii) Draw the restriction map of this recombinant plasmid.
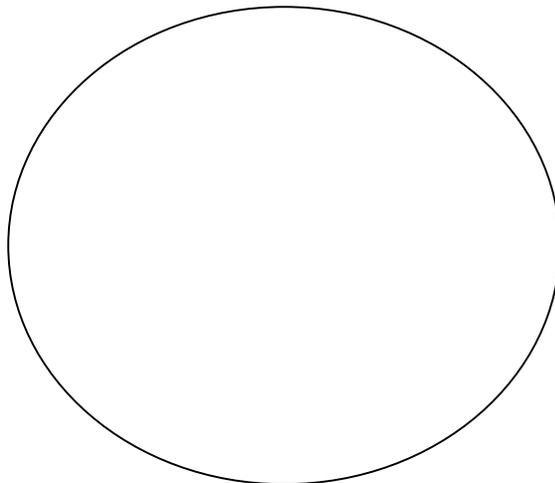
Question 4

Working in mice, you discover a gene, LC1, that when mutant decreases the incidence of cancer in mice. This gene is normally expressed in the lymphatic tissues of mice. You know the sequence of this gene, and you really want to see if there is a similar gene is in humans. So, you set out to find the human homolog of LC1 by screening a human library.

a) What kind of human library would you use? Explain.

b) How would you screen the human library for the homolog of LC1?