Solutions to 7.012 Problem Set 5

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?

5' G 3' *Cleavage by BamHI leaves a 5' overhang*
*3' C**CTAG** 5'*

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?

5' T 3' *Cleavage by BclI leaves a 5' overhang*
*3' A**CTAG** 5'*

c) Given the DNA shown below …

5′ ATTGAGGATCCGTAATGTGTCCTGATCACGCTCCACG 3′
3′ TAACTCCTAGGCATTACACAGGACTAGTGCGAGGTGC 5′

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

2 fragments:

A ***B***

5′ ATTGAG 3′ *5′ GATCCGTAATGTGTCCTGATCACGCTCCACG 3′*
3′ TAACTCCTAG 5′ *3′ GCATTACACAGGACTAGTGCGAGGTGC 5′*

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.

2 fragments.

C

5′ ATTGAGGATCCGTAATGTGTCCT 3′
3′ TAACTCCTAGGCATTACACAGGACTAG 5′

D

5′ GATCACGCTCCACG 3′
3′ TGCGAGGTGC 5′

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.

A + B 5′ ATTGAGGATCCGTAATGTGTCCTGATCCGTAATGTGTCCTGATCACGCTCCACG 3′
3′ TAACTCCTAGGCATTACACAGGACTAGGCATTACACAGGACTAGTGCGAGGTGC 5′

or

A + D 5′ ATTGAGGATCACGCTCCACG 3′
3′ TAACTCCTAGTGCGAGGTGC 5′

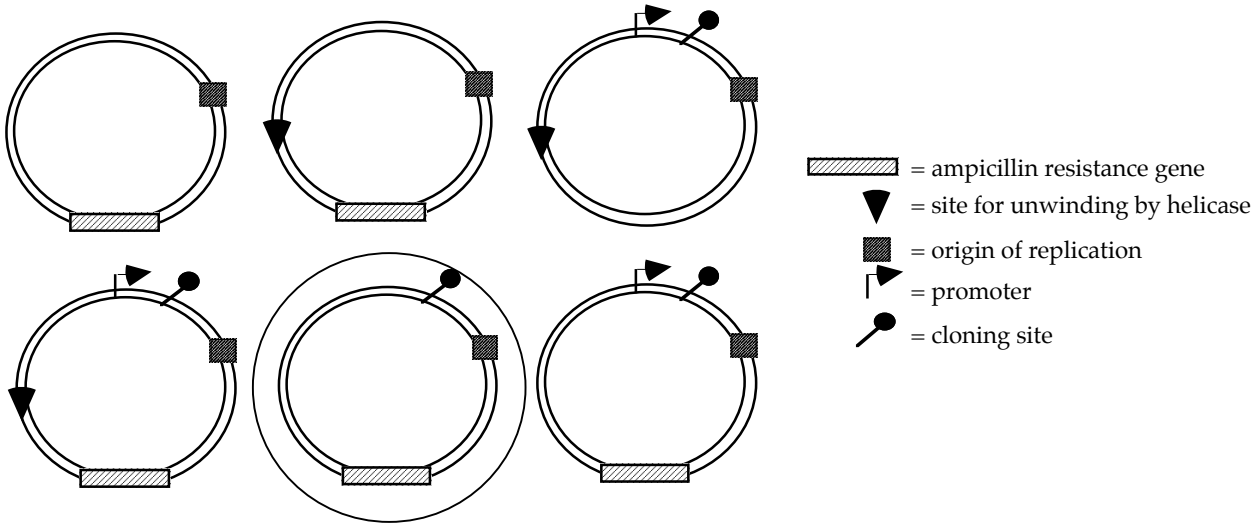
or B + C or B + D

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

No, the recognition sites for both BamHI and for BclI have been destroyed,.

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 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?
You need ampicillin sensitive cells.

c) How do you distinguish bacterial cells that carry a vector from those that do not?
Bacterial cells that carry a vector will be able to grow on ampicillin whereas untransformed cells will 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.

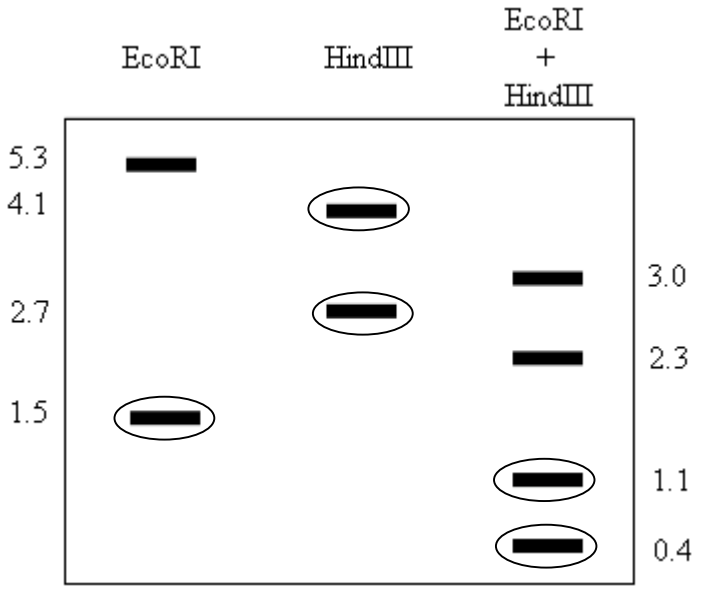
Enzymes	Reagents
Restriction enzyme	Size separation gel
Ligase	Okasaki fragments
DNA Polymerase	ATP, TTP,CTP,GTP
RNA Polymerase	ddATP, ddTTP, ddCTP,
Transcriptase	ddGTP
Reverse Transcriptase	Primers
3' to 5' exonuclease	Replication fork
Cloning vector	Human cells
	Virus

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?
You can cut the plasmid with EcoRI and look for two fragments, one that represents the vector and one that represents the insert. You would not know for sure that the insert is the harE gene without further tests. To confirm that the insert is the harE gene, you would need to know some amino acid sequence of the harE protein. You could then make a degenerate nucleic acid probe that would be complementary the harE gene sequence.

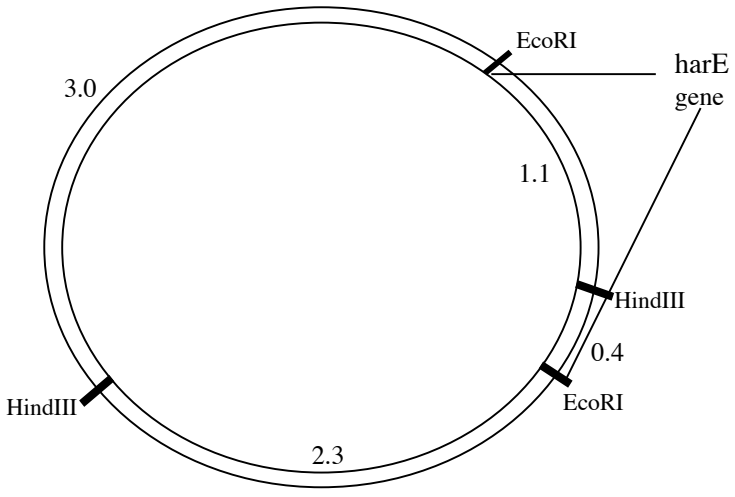
Suppose you find that the *harE* gene is in the plasmid, but now you want a restriction map of the recombinant plasmid. You take three individual samples of the plasmid and digest one sample with EcoRI, the second sample with HindIII, and the third sample with both EcoRI and HindIII. Then you run the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



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.
Because the LC1 gene may only be expressed in the lymphatic tissues of mice, you may choose to screen a genomic library. If you were to screen a cDNA library, you would need to make sure that lymphatic specific mRNA's were represented.

b) How would you screen the human library for the homolog of LC1?
Because you know the sequence of the LC1 gene from mice, you could make a probe complementary to the mouse sequence. A human homolog, by definition, would have significant sequence similarity. The hybridization could be performed at a temperature that would allow probe binding to similar but not necessarily identical sequence.