Homework 4

Problem 1. Figure 1 shows a map of a linear chromosome fragment. The map shows the position of restriction enzyme recognition sites and the locations of three genes, X, Y and Z. Below the map is a drawing representing 8 lanes of an agarose gel. The two outside lanes have molecular weight marker DNAs. The inside 6 lanes are labeled with letter corresponding to restriction enzymes (E= EcoRI, B=BamHI, H=HindIII) used to digest the chromosome shown in the map.

a) Draw the bands that would be seen in each lane if the enzyme (or enzymes) indicated were used to digest the chromosome. DRAW THE BANDS ON A PRINTOUT OF THE MAP AND TURN THAT IN WITH YOUR OTHER WORK.

b) If a solution of this chromosome had an OD260 absorbance value of 0.1, how many chromosome molecules would the solution contain?

c) Based on the expected frequency of EcoRI recognition sites in a random DNA sequence, does this chromosome fragment contain more, less, or the same as the expected number of EcoRI sites? Show the calculation supporting your answer.

d) If you performed a Southern blot of the gel from part (a) and used the entire Gene Y gene as a probe, which bands in each line would hybridize to the probe?

e) You have sequenced the two regions of this chromosome containing the HindIII sites. The sequences are shown on the map in Figure 1. Use these sequences to design 20 nucleotide long PCR primers that could be used to amplify DNA containing Gene Y.

Problem 2. You wish to clone Genes X, Y and Z shown in the map for problem 1 into the plasmid vector, pBIO304. pBIO304 contains three dominant genes that confer resistance to antibiotics to E. coli cells that contain them. AMP-R confers resistance to ampicillin, TET-R confers resistance to tetracycline, and CM-R confers resistance to chloramphenicol. A map of pBIO304 is shown in figure 2. Figure 2 also shows the results of agarose gel electrophoresis of molecular weight markers (outside lanes), four clones discussed in part (a) of this problem (clones 1, 2, 3 and 4), and two clones discussed in part (b) of this problem (clones 5 and 6).

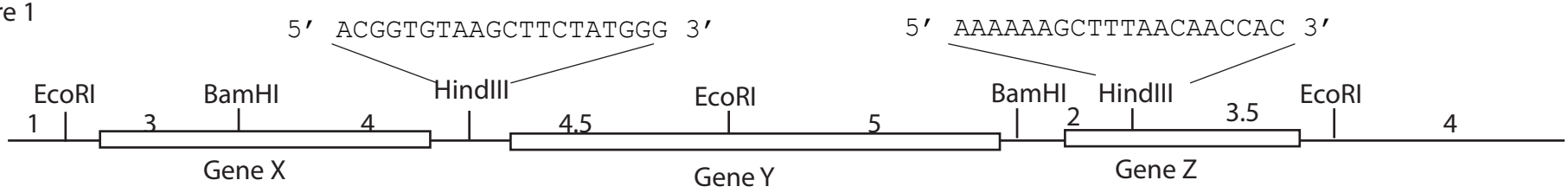
a) You cut the chromosome (see figure 1) and pBIO304 with EcoRI, mix the digested DNAs together, perform a ligation reaction, transform E. coli with a sample of the ligation reaction, and plate the cells on LB+AMP. You isolate plasmid DNA from four clones (clones 1, 2, 3 and 4), digest the plasmid with BamHI, and run each digest in a separate lane as indicated in the figure. Based on these results, which clones contain a complete copy of Gene X, Gene Y, or Gene Z?

b) Draw a map of the recombinant plasmid corresponding to clone 1.

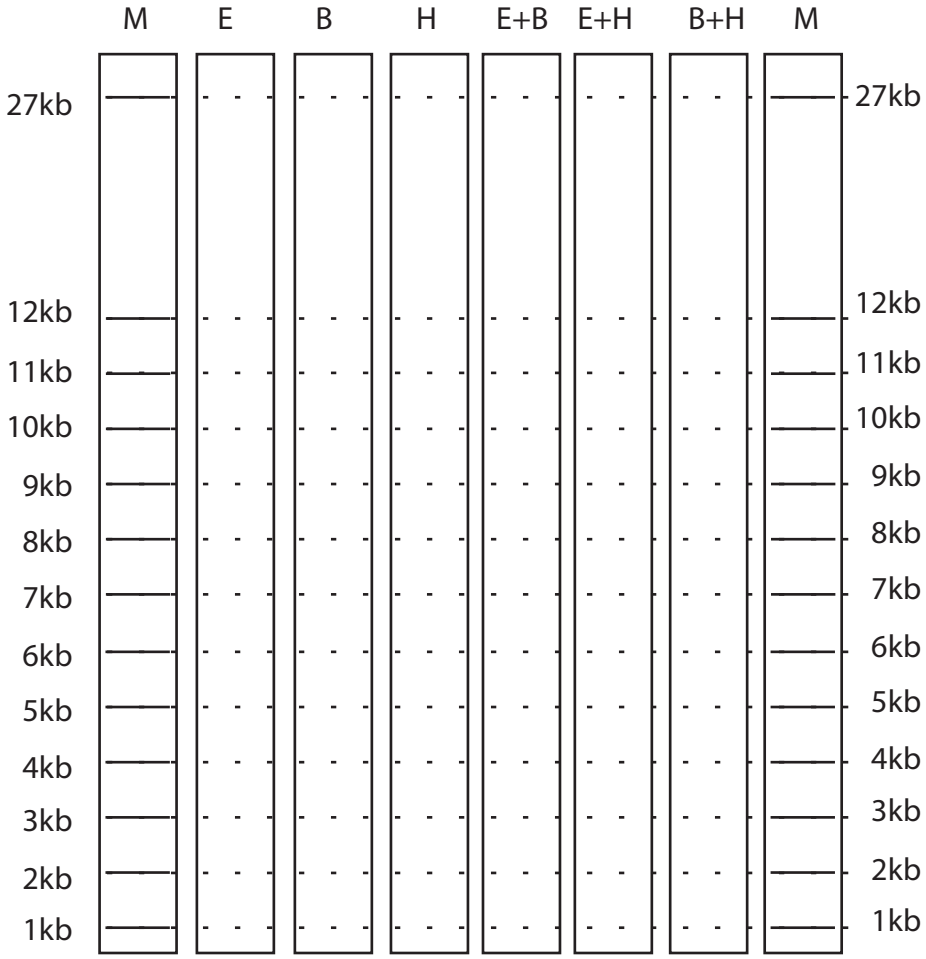
c) You perform a similar cloning experiment as in part (a), except that you use BamHI to cut the chromosome and pBIO304. You isolate plasmid DNA from two clones (clones 5 and 6), digest them with EcoRI, and run each digest in a separate lane as indicated in the figure. Your analysis of these results indicates that both clones contain a complete copy of Gene Y. Why do the results of the restriction digests differ for clones 5 and 6?

d) If you mixed up the E. coli cells that contain clones 1 and 5, how could you tell them apart without isolating DNA from them? Briefly describe what you would do and the expected result for each clone.

Figure 1

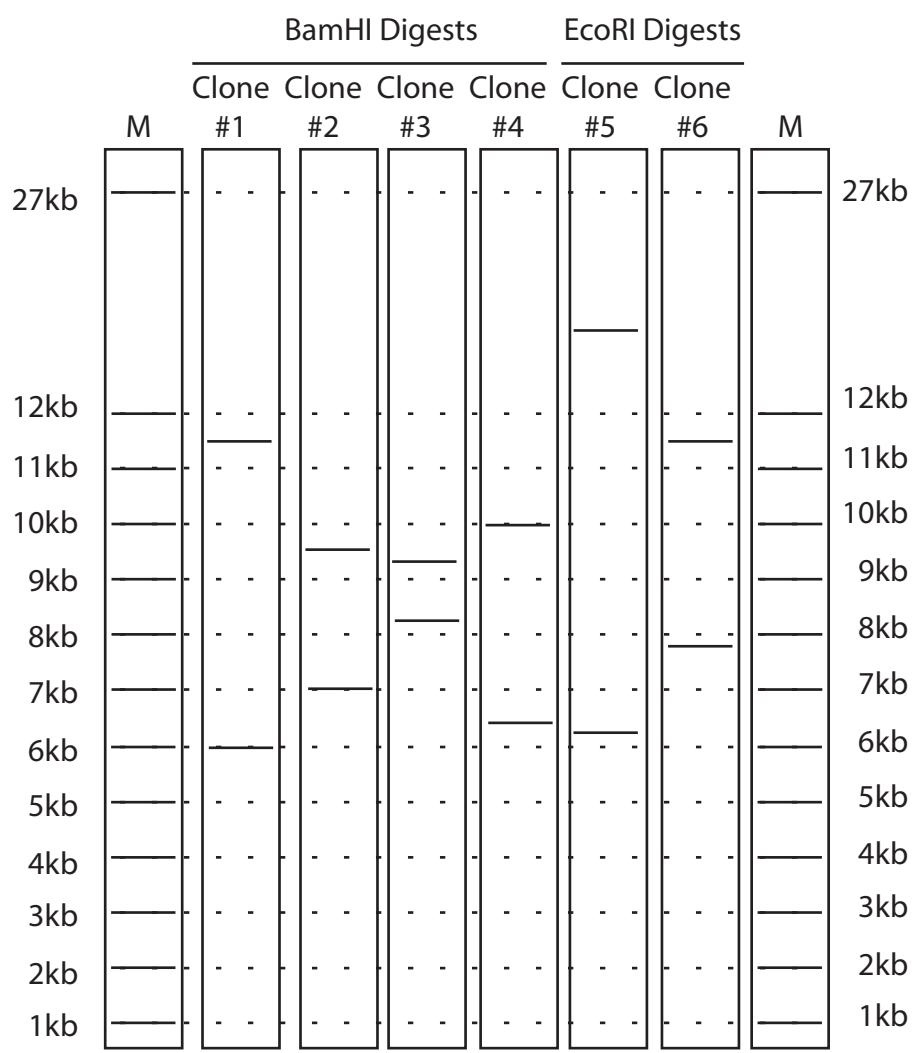


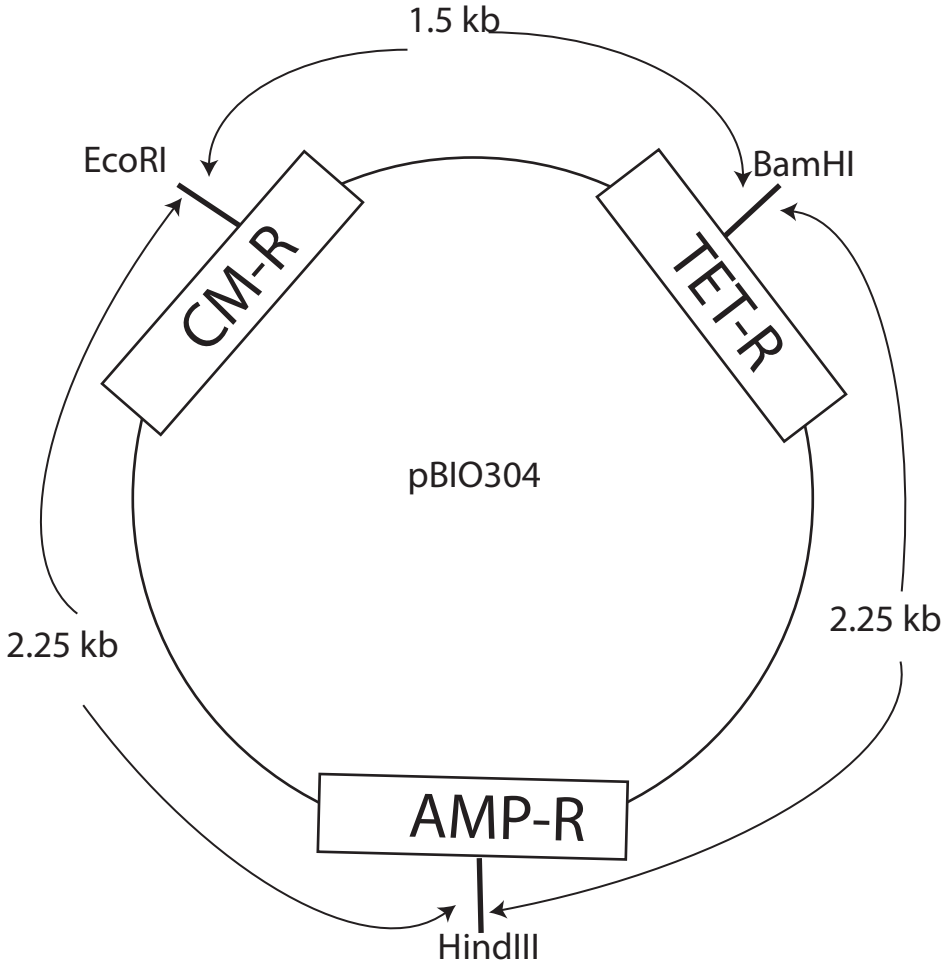
Numbers indicate distance in kb between successive restriction sites
Rectangles correspond to genes. The sequence above the HindIII sites is the sequence of the top strand of DNA at those sites.



Each rectangle is a lane of an agarose gel. The lanes labeled "M" contain molecular weight marker DNA, with the size of each band indicated in kb.
The lane labeled E is for you to draw the results of an EcoRI digest of the chromosome.
The lane labeled B is for you to draw the results of a BamHI digest of the chromosome.
The lane labeled H is for you to draw the results of a HindIII digest o fthe chromosome.
The lane labeled E+B is for you to draw the results of a digest where both EcoRI and BamHI are used.
The lane labeled E+H is for you to draw the results of a digest where both EcoRI and HindIII are used.
The lane labeled B+H is for you to draw the results of a diges where both BamHI and HindIII were used.

Figure 2


BamHI Digests
EcoRI Digests
Clone #1
Clone #2
Clone #3
Clone #4
Clone #5
Clone #6
M
M
27kb
12kb
11kb
10kb
9kb
8kb
7kb
6kb
5kb
4kb
3kb
2kb
1kb


1.5 kb
EcoRI
BamHI
CM-R
TET-R
pBIO304
2.25 kb
2.25 kb
AMP-R
HindIII