Biol/Chem 475 Spring 2007 Study Problems for Quiz 2
Quiz 2 (~50 pts) is scheduled for Monday May 14
It will cover

- all handouts and lab exercises to date except the handout/worksheet (yet to be distributed) on computational analysis of DNA sequence data
- basic PCR and dideoxy sequencing (reaction and data display -- not computational stuff)
- questions on ligation handout
- quiz #1 problem set

1. The bacteriophage T4 codes for proteins that can interfere with the metabolic activity of the *E. coli* host and kill the cell. For this reason, the T4 genes coding for these proteins have been historically difficult to clone. Examine the information shown on the **next page** and outline a strategy for cloning one of these T4 genes --identified as Gene Z. **Read the entire problem carefully before you write out your answer. NOTE: you must submit the extra sheet with your exam.**

ON THIS PAGE: OUTLINE your cloning experiment
List the specific steps in your cloning experiment. Number each step.

- Start with **Step 1: Preparation of vector.** Digest with…..
- End with **Step --: Tentative functional confirmation of Gene Z clone(s).**
- Be sure to include steps to optimize finding the clone of interest.
- Next to each step, give a <u>one sentence</u> explanation of its purpose.
- **DO NOT indicate experimental details at this point -- problem continues on the next page**

Step 1: Digest vector with ……
Reason for Step 1

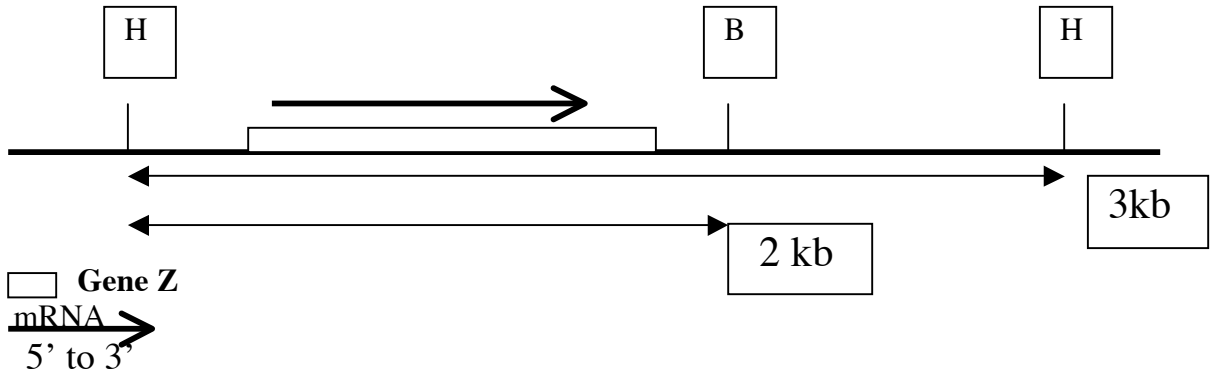
Step 2: Digest T4 genomic DNA with ……
Reason for Step 2:

Step 3:
Reason for Step 3:

Problem 1 continued: For each step, describe experimental details (such as the composition of the agar plates that cells are plated on) that are *critical* to your cloning strategy.

- Organize your answer by Step #
- Emphasize experimental details that are *different* from what we did in class. *For example:* I'm ***not*** looking for recipes for restriction enzyme digests or details on what is in an RE buffer. But you do need to mention by name the enzymes that you use. *Another exaxmple:* I don't need a procedure for transformation, but I do need to know the composition of the agar plates that the transformed cells are seeded on.

Bam HI G↓GATTC = B
Hind III A↓AGCTT = H



Genotype of XL1-Blue strain of *E. coli:*
Bacterial chromosome: recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac
F' *proA*$^{+}$*B*$^{+}$, *lacI*^{q} *lacZΔM15 Tn10 (tet*^{r}*)*
ampicillin kanamycin tetracycline IPTG X-gal

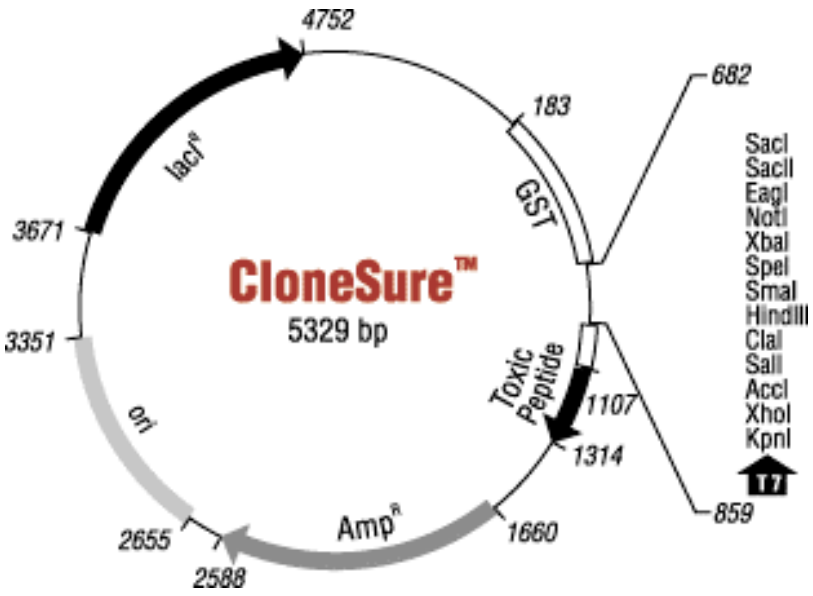
PLUS MAP OF pGEM

2. Examine the CloneSure vector shown below. The multiple cloning site is imbedded in a sequence that codes for a peptide that binds to the *E. coli* origin of replication and prevents initiation of genomic replication. Expression of this peptide is under the control of the *lac* operator. *lac* I^q = lac repressor

a. You use this vector in a subcloning experiment and transform *E. coli* with the ligated plasmid. What do you add to the media that you plate the transformed cells on? Briefly explain.

b. Briefly explain the significance of ***each*** of the claims **by comparing this vector with the pGEM vector and the XL1 Blue strain** [lab handout].

- *Offers true background-free cloning. Eliminates the colony screening step. All colonies are positive.*
- *Works with any strain of E. coli. Is this statement true for pGEM?* ***Explain in detail.***
- *Eliminates the need for* ***complete*** *digestion of the vector.*



CloneSure Hype

100% Positive Clones Everytime

- Offers true background-free cloning
- Eliminates the colony screening step; all colonies are positive
- Works with any strain of *E. coli*
- Eliminates the need for dephosphorylation [see ligation notes] or complete digestion of the vector
- Makes cloning quick and easy

3 . A transformation reaction contains 200µl competent cells, 2.5 µl ligated DNA (20 ng/µl) and 800 µl L-broth. 100 µl of this mix was spread on a plate with X-gal, IPTG, Amp and Tet. After an overnight incubation, 460 blue and 24 white colonies were present on the plate.

a. determine the number of transformants (transformed colonies) per ng of DNA.

b. Determine the % of transformants that carried a recombinant plasmid.

4. Review the genotype of the XL1-blue E. coli strain.

Answer this question for either ***recA 1or endA1:*** .

What is the wild-type function of the gene product?:

one sentence

Why is a mutation in this gene present in the competent cell line?

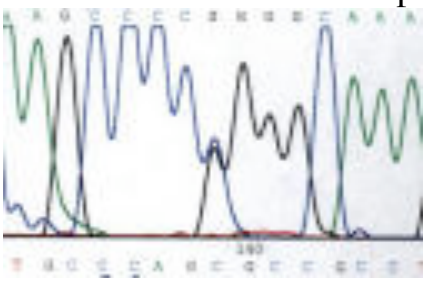
one sentence

5. Examine the map of the pCR TOPO vector on the following page

a. What is the sequence of the M13R primer -- 5' to 3' -- first six bases only?

b. What is the sequence f the T7 primer -- 5' to 3' -- first six bases only?

6. On the dideoxy sequencing chromatogram shown below indicate which pieces of DNA are closer to the bottom versus the top of the acrylamide gel.



7. Here is a portion of the human β globin gene:

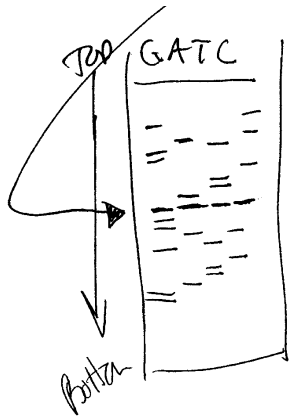
5'AGATTAGTCCAGGCAGAAACAGTTAGATGTCCCCAGTTAA[illegible]CCTCCTATTTGACA
CCACTGATTACCCCATTGATAGTCACACTTTGGGTTGTAAGTGACTTTTTATTTATTT
GTATTTTTGACTGATTAAGAGGTCTCTAGTTTTTTATCTCTTGTTTCCCAAAACCTAA
TAAGTAACTAATGCACAGAGCACA[illegible]TTGATTTGTATTTATTCTATTTTTAGACATAA
TTTATTAGCATGCATGAGCAAATTAAGAAAAACAACAACAAATGAATGCATATATA
TGTATATGTATGTGTGTATATATACACATATATATATATATTTTTTTTCTTTTCTT **3'**

a. You are planning to set up a PCR reaction to amplify the 154 bp region between the ⧓'s. The PCR product should include all of (but not extend beyond) the designated region. List the first five bases of each primer. Your primer sequences must read in the 5' to 3' direction.
Primer A:
Primer B:
b. The template DNA for your PCR is total human genomic (nuclear) DNA. What is the size of the human genome in base pairs?
c. What biochemical "limitation" of DNA polymerase ensures that, among the vast sea of sequences in genomic DNA, only the β globin sequences will be amplified ? **one sentence**

8. A PCR reaction is set up with the following primers. (Note: primers are typically longer than those shown here.)
5' GAAGTTCTC 3' 5' TGTCTCTAA 3'
Draw a cartoon of the PCR product, which is about 200 base pairs long. Indicate the sequence of both strands at the ends of the product. Be sure to label the 5' and 3' ends. Assume that you are looking at a typical product late in the c ycling process.

9. Examine the map of the pCR2.1 vector. For reasons apparent only to yourself, you decide that you want to generate a PCR product that corresponds to the **vector** DNA including the M13Reverse and the T7 promoter sites and the ampicillin resistance gene (etc) but excluding the region between bases 221 and 364. Examine the DNA sequence of vector and give the first six bases for each of the primers (5' to 3') that you would order.

10 a. In an "old-style" ^{32}P sequencing reaction, each sequencing run requires four lanes on a gel. A single initial reaction is set up with ^{32}P labelled dNTPs. After the reaction has incubated for a short period of time, it is divided into four separate tubes, each with a different ddNTP. Each lane on the sequencing gel corresponds to a separate reaction with one of the four ddNTPs. *The cycle sequencing reaction with dye terminator requires only one lane on a gel. Why?*
b. In the "old-style" sequencing, hairpins in the template (intra strand base pairing) would cause the polymerase to fall off, terminating DNA synthesis independent of a dideoxy terminator. This resulted in bands in all four lanes at a given nucleotide position. In the new style sequencing with dye terminaters, hairpins in the template don't result in the fluorescent equivalent of all four dyes superimposed on the readout. Briefly explain why.



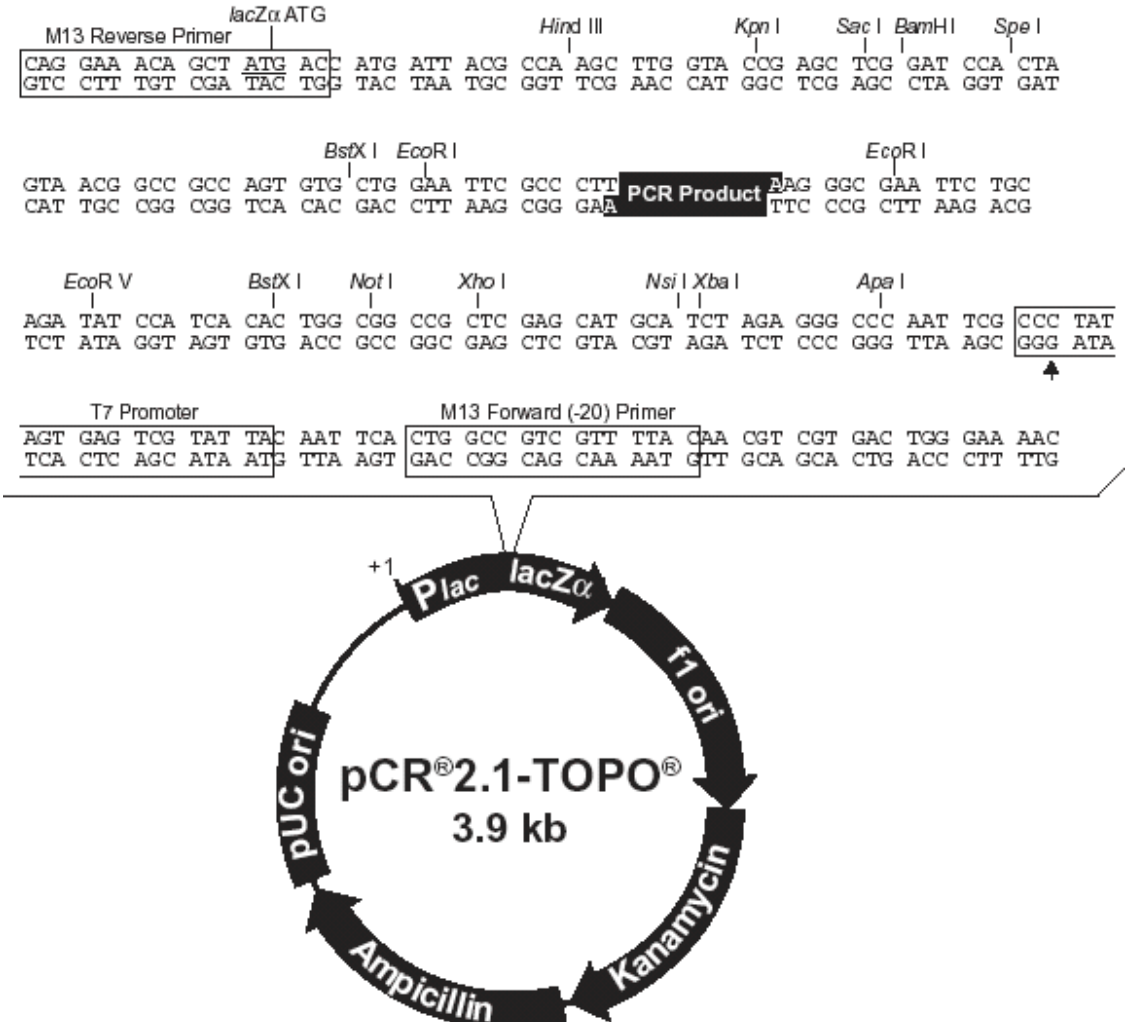
11. You perform a cloning experiment with a PCR product using the PCR2.1 TOPO vector and the TOP10F' E. coli as host (next page page). After transformation and plating you have hundreds of discrete colonies, but they are all white. You think that you may have made a mistake in executing the experiment. **** FOR THIS QUESTION ASSUME THAT THE BLUE-WHITE SCREEN IS BEHAVING ITSELF.****

Fill in the Table. By each possibility indicate YES (it is consistent with all white colonies) or NO (it would not explain the all white colonies). Then give a brief (one sentence only) explanation.

Problem -- everything else okay except:	**Yes/No**	**Very CONCISE explanation**
No IPTG in medium		
No X-gal in medium		
forgot to add PCR product to ligation mix		
*retrieved wrong cell line from freezer: cell line is *endA*$^+$		
*retrieved wrong cell line from freezer: cell line is *lac+ WT for lac operon*		
*retrieved wrong cell line from freezer: cell line is *missing the F' plasmid*		
*retrieved wrong cell line from freezer: cell line is *missing the lambdoid prophage*		

* genotype is otherwise as indicated on the next page

Map of pCR 2.1 TOPO vector



Comments for pCR®2.1-TOPO® 3931 nucleotides

*Lac*Za fragment: bases 1-547
M13 reverse priming site: bases 205-221
Multiple cloning site: bases 234-357
T7 promoter/priming site: bases 364-383
P_{lac} = lacZ promoter (lac operator is nearby)

Genotype of TOP 10 F' cells:

Bacterial chromosome: *ϕ80 lacZΔM15* ΔlacX74 recA1 endA1*

**lambdoid prophage carrying lacZ mutation with small N-terminal deletion*

F' factor: *lacI^q Tn10 (tet^r)*

12.

Reagent (Stock conc.)	vol per _____ Rxns	vol per 20 μl Rxn	final conc
H_20			
10X PCR Buffer			1X
dNTP's (2.5mM)			0.25mM
Mg^{++} (25mM)			2 mM
BSA (10mg/ml)			0.2 μg/μl
primer 1 (50 μM)			0.5μM
primer 2 (50 μM)			0.5μM
Taq polymerase		0.5 μl	
Template		1 μl	

You are setting up 10 PCR reactions with 10 different templates. The volume of template added to each reaction will be 1 μl. The final volume of each reaction will be 20 μl.

Complete the table and briefly explain (two sentences or a diagram) exactly how you would set up this PCR run.