

**7.02/10.702  
RECOMBINANT DNA METHODS  
EXAM**

**KEY**

Regrade requests are due to the instructor in the 7.02 teaching lab by the following Wednesday--NO EXCEPTIONS! Please complete a regrade request form and turn it in with your exam.

### Question 1 (20 points, 2 points each)

1. A
2. B
3. B
4. A
5. C
6. D
7. C
8. F
9. D
10. B

### Question 2 (40 points)

(1 point)

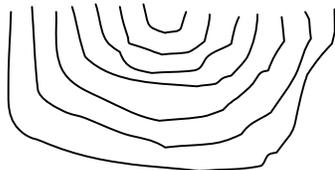
a) primer D

(9 points; 1 point each for name and 2 points each for evidence)

b)

Primer A: forms a hairpin sequence with itself (student should indicate basepairing between bases in the sequence, as shown below)

5' CTTGCAGTAGTACTGCACGC 3'



\*lines indicate H bonds

Primer B: forms a "primer dimer" with the forward primer, as shown below:

forward: 5' CATCAGGCTAAGATTCGGCC 3'

3' GCCGGAACGACATGAGGTAT5' reverse

Primer C: is too short (or has too low G/C) content to be used with the forward primer. To calculate the  $T_m$ :

forward:  $4^\circ\text{C}(11 \text{ G/C basepairs}) + 2^\circ\text{C}(9 \text{ A/T basepairs}) = 62^\circ\text{C}$

reverse:  $4^\circ\text{C}(7 \text{ G/C basepairs}) + 2^\circ\text{C}(10 \text{ A/T basepairs}) = 48^\circ\text{C}$

## Question 2 (continued)

(3 points)

b)

answer: 1.7 to 2 minutes (depending on where you thought the primers were located)

justification: Taq DNA polymerase synthesizes DNA at a rate of 1000 bp/min (1 kb/min) (1 point). Since the primers are ~1.7 kb apart, you need to give the polymerase at least 1.7 minutes to synthesize the new DNA strand, thus generating a new template molecule (1 point).

(9 points)

c) In this key, I assumed that the primers were located just flanking the 1.7 kb piece of DNA.

Template DNA	Product(s) observed on gel? (yes or no)	Size of band(s) in kb?	Explanation of your answer
unaffected	YES  (0.5)	~1.7 kb (darker)  (0.5)	In unaffected persons, both copies of the <i>bld</i> region are wild type (no deletion), and thus have binding sites for both primers. This will lead to exponential amplification and a PCR product that is the size of the distance between the primers + the primer lengths (~1.7 kb). (1 point for both copies have binding site, 1 point for exponential amplification and PCR product size)
carrier	YES  (0.5)	~1.7 kb (lighter)  (0.5)	In carriers, one copies of the <i>bld</i> region is wild type (no deletion), and thus have binding sites for both primers. Amplification from this template will be the same as in "unaffected." The other copy will be mutant (deletion) and will not have binding sites for both primers. (1 point for one copy has binding sites, other doesn't; 1 point for exponential amplification/ size)
affected	NO  (0.5)	N/A  (0.5 for nothing)	In affected persons, both copies of the <i>bld</i> region are mutant (deletion). There will be no binding site for one of the primers, and thus you will never get exponential amplification of the DNA of the <i>bld</i> region. (1 point for both copies have deletion; 1 point for NO binding site and no exponential amplification)

## Question 2 (continued)

(6 points total)

d)

1. The PCR products from "unaffected" and "carrier" individuals will be the same size (~1.7 kb) (2 points). There may be a difference in intensity (unaffected brighter than carrier), but this may be difficult to see on a gel (1 point).
2. The diagnosis of an "affected" person relies on a negative result (lack of a band) (2 points). Negative results are inconclusive, as there are many ways that a PCR reaction could result in "no band." (1 point).

(12 points total)

e) Experimental design parameters were as follows:

1. Primer location (3 points total)

- The primers should bind to sites flanking the *bld* gene (0.5 pts per primer)
- The forward primer should bind to the 3'→5' strand near the beginning of the gene, and the reverse primer to the 5'→3' strand near the end of the gene. (1 point)
- DNA synthesis should be in the 5'→3' direction (arrows facing each other) (1 point)

2. The components of the PCR reaction are (5 points total):

- Taq polymerase (1 point)
- dNTPs (1 point)
- template DNA (cDNA generated from patients' genomic DNA) (1 points)
- buffer containing Mg<sup>2+</sup> (1 point)
- forward and reverse primers (1 point)

3. Extension time is equal to / greater than the distance between the two primers (1 point)

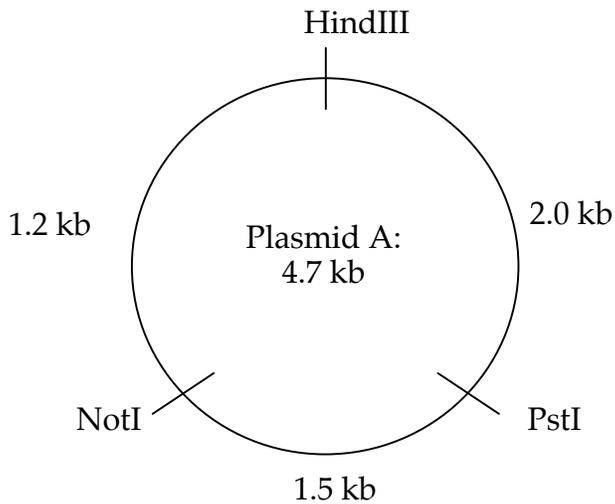
4. Experimental data (3 points total)

- Unaffected: 2.3 kb (assuming primers outside of gene) (1 point)  
Carrier: 2.3 kb, 2.1 kb (1 point)  
Affected: 2.1 kb (1 point)

### Question 3 (20 points)

(7 points)

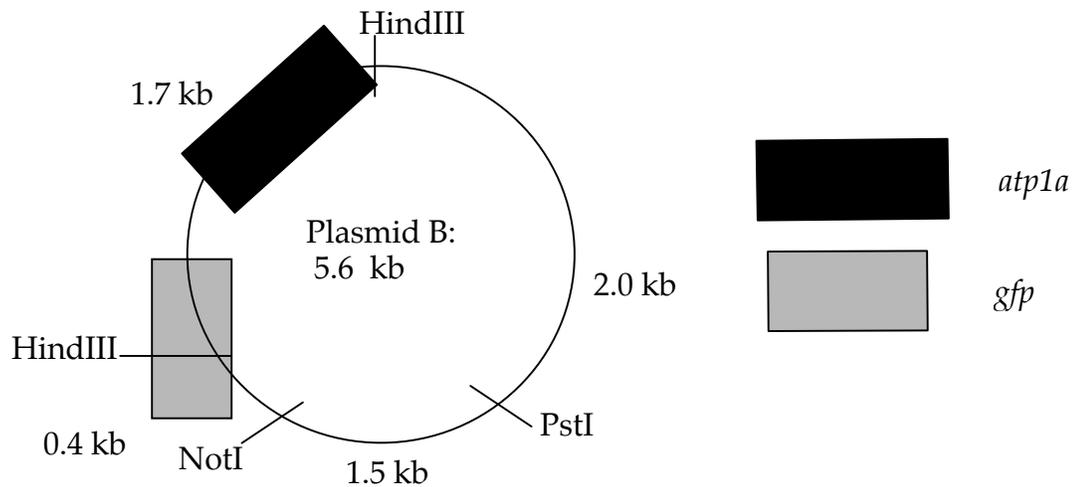
a)



- 1 point for sites in order (3)
- 1 point each for distances in order (3)
- 1 point for total size

(7 points)

b)



- 2 points for placing a new HindIII between existing HindIII and NotI sites (and not affecting the rest of the plasmid)
- 2 points each for placing the "new" distances (1.7 and 0.4) in the correct places (4 total)
- 1 point for total size (1)

### Question 3 (continued)

(1 point)

c) Plasmid B contains the *gfp* insert.

(1 point)

d) The *gfp* cDNA is 0.9 kb in size.

(4 points)

e) Here is what was required to get full credit:

- *atp1* cDNA is located only in the 1.7 kb part of the map B (upstream of *gfp*) and right next to the HindIII site (see problem description)
- the *gfp* sequence flanks the HindIII RE site closest to Not site

### Question 4 (20 points)

(11 points total)

a) For each vector, circle YES (good choice) or NO (bad choice). For each "NO" answer, explain your reasoning in one or two sentences.

pEX1:

YES NO (1 point)

IF NO, WHY NOT:

1. No drugR gene (AmpR) (1 point)-->can't select for cells containing plasmid (1 point)
2. No promoter, so can't express gene (1 point)

pEX2:

YES NO (1 point)

IF NO, WHY NOT

1. No T7 promoter (1 point)-->can't express gene (make RNA) (1 point)

pEX3:

YES (1 point) NO

pEX4:

YES NO (1 point)

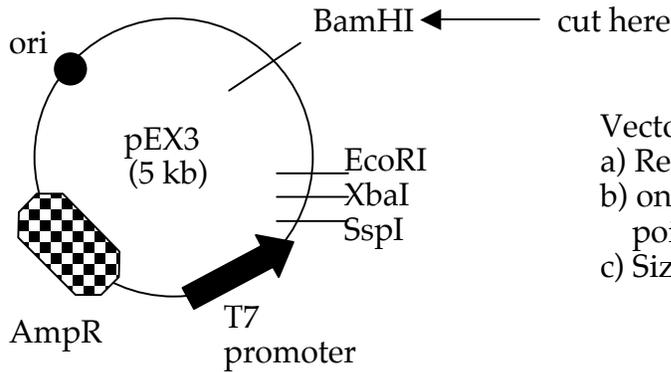
IF NO, WHY NOT:

Gene will be ligated in the wrong orientation (1 point), so won't get *lal* mRNA made (no *lal* transcription) (1 point)

## Question 4 (continued)

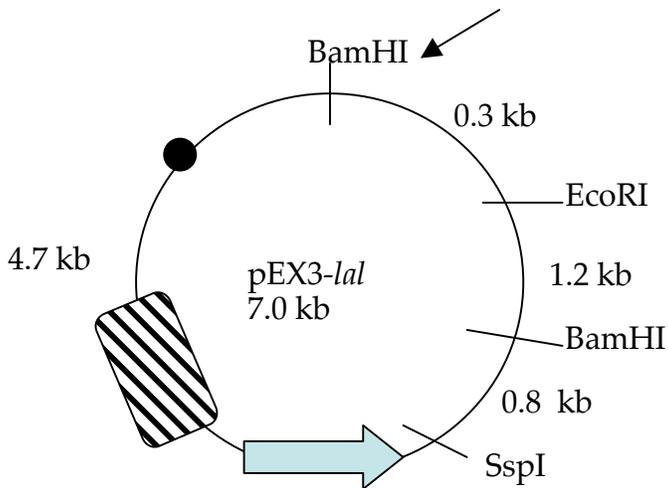
(9 points total)

b) What size band(s) will tell you that you have isolated religated vector DNA? Vector DNA with the *lal* insert? Justify your answers by drawing restriction maps of the plasmids and indicating how each band arises.



Vector alone (3 points total):

- Restriction map: 1 point
- one band when cut at single BamHI site: 1 point
- Size of the band is 5 kb band: 1 point



Vector + insert (6 points total):

- Restriction map: 3 points
  - 1 point for indicating the size of the BamHI/EcoRI fragment of vector as 0.3 kb and/or the 4.7 kb between BamHI and SspI
  - 2 points for having the insert in the correct orientation (with 1.2 kb fragment closest to 0.3 kb fragment)

- Two BamHI sites-->Two bands (1 point)
- Band sizes of 5.5 kb and 1.5 kb (2 points)