Pre/Post-quiz Assessment Study Biol. 462 Lab

[Correct answer is italicized. Blue questions are in post-quiz only.]

Students: to assess whether or not the laboratory of this course helps you learn concepts and skills in molecular biology, I have made up this "instrument" (sorry for the use of assessment jargon). Please try to answer the questions as correctly as possible, but do NOT stress yourself. As you see, it is anonymous! Please do not use any books, papers, or enlist help from anyone else in answering the questions. Thank you!

Circle the best answer, but if you have NO IDEA what the answer is, then just leave it blank.

1. What is a common use for ethidium bromide?
   a. crystallizing DNA
   b. staining nucleic acids
   c. additive to gels to make them harden
   d. stabilizes DNA while in solution
   e. sequencing DNA

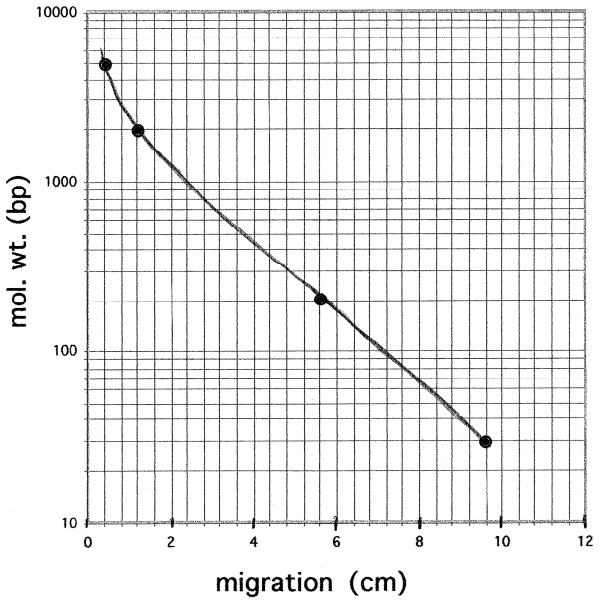
2. What volume of 20X SSC (a common buffer) would you add with water to make a total volume of 800 ml of 4X SSC?
   a. 50 ml b. 500 ml c. 16 ml *d. 160 ml* e. 20 ml

3. How many base pairs (bp) is a fragment that is 2.3 kb (kilo base pairs)?
   a. 0.0023 b. 23 c. 230 *d. 2300* e. 23,000

4. A P20 pipetteman reads vertically: 1-8-1. What is the volume the pipetteman is set to measure?
   a. 1.81 µl *b. 18.1 µl* c. 18.1 ml d. 1,810 µl e. 181 ml

5. Which is the most reasonable sequence of events for isolating and purifying DNA from bacterial cells?
   a. Lyse nuclei, destroy cell membrane, ethanol precipitate, Southern hybridize, phenol/chloroform extract
   b. Break open cell membrane, lyse nuclei, phenol/chloroform extract, Southern hybridize
   c. Lyse cells, phenol/chloroform extract, ethanol precipitate, spin, dry pellet, dissolve in buffer
   d. Ethanol precipitate, phenol/chloroform extract, dry pellet, dissolve in buffer
   e. Ethanol precipitate, phenol/chloroform extract, Southern hybridize

6. Below is a standard curve (semi-log graph paper) from a gel showing the migration distances of known sized DNA fragments. If on the exact same gel a fragment of unknown size migrated 4.8 cm, how many bp is it (extrapolate from the graph)?



   a. 110 bp b. 120 bp
   c. 300 bp d. 400 bp
   e. 600 bp

7. A Southern hybridization of *Eco*RI-digested genomic DNAs from wild-type flies and mutant flies is performed with a 5.0 kb probe isolated from the wild-type genomic DNA with *Eco*RI. On the blot are detected a 5.0 kb band in the wild-type lane and a 2.0 kb band in the mutant lane. No other bands are detected on the blot. Which is the most reasonable explanation for these results?

a. The mutant genome has a deletion of 3.0 kb in the region complementary to the wild-type probe.

b. The wild-type genome has an insertion of 5.0 kb, and the mutant genome has an insertion of 2.0 kb.

c. A 3.0 kb portion of the mutant genome (in the region of the probe) has been mutated (by base substitution) so that it no longer is complementary to the probe.

d. A 2.0 kb transposon has traded places with the 5.0 kb wild-type region in the mutant.

e. There is an extra *Eco*RI cut site in the mutant in the region complementary to the probe, producing a smaller fragment.

8. The two different sized bands detected in the Southern described above can be called:

a. SNRPs *b. RFLPs* c. SNPs d. AFLPs e. microarrays

9. In which gel type and percentage would a 100 bp fragment be best resolved (diffuse the least)?

a. 2% agarose b. 0.8% agarose *c. 8% polyacrylamide (29:1)*
d. 5% polyacrylamide (29:1) e. 0.5% polyacrylamide (29:1)

10. How many ml are in 504 µl?

a. 0.000504 ml *b. 0.504 ml* c. 5.04 ml
d. 504 ml e. 504,000 ml

Please give your honest opinion:

11. I feel self-assured in the molecular biology lab--I am competent and confident in my pipetting skills, running equipment such as the microfuge, handling small tubes, and performing protocols safely and efficiently.

a. Strongly agree b. Agree
c. Neutral/Ambiguous/Not Sure d. Disagree e. Strongly Disagree

12. Molecular biology research (in the lab) is what I want to do in the future.

a. Strongly agree b. Agree
c. Neutral/Ambiguous/Not Sure d. Disagree e. Strongly Disagree

13. I have no concerns about my skills and abilities in this lab.

a. Strongly agree b. Agree
c. Neutral/Ambiguous/Not Sure d. Disagree e. Strongly Disagree

14. For the following, please rate the laboratories based on a). difficulty; and b). interestingness.

	Very Easy				Too Difficult	Not Interesting				Very Interesting
Isolate Genomic DNA	1	2	3	4	5	1	2	3	4	5
E.coli transformation	1	2	3	4	5	1	2	3	4	5
Mini-plasmid prep.	1	2	3	4	5	1	2	3	4	5
Labeling DNA	1	2	3	4	5	1	2	3	4	5
RE digests and mapping	1	2	3	4	5	1	2	3	4	5
Southern Hybridization	1	2	3	4	5	1	2	3	4	5
RFLP project	1	2	3	4	5	1	2	3	4	5
RNA Isolation	1	2	3	4	5	1	2	3	4	5
Lab Manual	1	2	3	4	5	1	2	3	4	5

15. My personal safety was never compromised--I had the needed protective equipment and clothing, and everyone in the lab was safety conscious.

a. Strongly agree b. Agree c. Neutral/Ambiguous/Not Sure
d. Disagree e. Strongly Disagree

16. What did you find was most interesting about the Molecular Biology Lab? (Can be specific or general.)

17. What did you find was the least interesting about the Molecular Biology Lab? (Can be specific or general.)

18. Other comments or suggestions for the laboratory or laboratory manual?

Examples of Mid-Semester Exam Questions to Assess Laboratory Skills and Analytical Skills

Early Semester

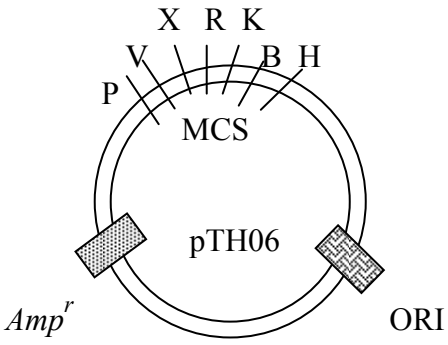
a. How many grams of Ammonium Persulfate (F.W. = 228.20) would you need to make 5 ml of a 100 mM solution? What is the **percentage** of this solution?

b. Given the following stock solutions: 1 M Tris, pH 8 25% glycerol 0.5 M EDTA, pH 8

How would you make 75 ml of a buffer that is composed of 15 mM Tris, pH 8.0, 1% glycerol, and 1 mM EDTA? Be sure to include volumes of all stock solutions (in both **microliters** (µl) and in **milliliters** (ml)), and a **brief description** of how you would mix the ingredients to the proper volume and concentrations. (Hint: See pg. 7 of your lab manual for help.)

Middle Semester

A gel-purified DNA fragment with a *Sal*I-digested overhang on one end and a *Kpn*I-digested overhang on the other end was ligated to and cloned into the multiple cloning site (MCS) of a 3.2 kb *Xho*I/*Kpn*I-double digested vector named pTH06. The map of the vector (undigested and without the insert) is shown below. (Remember: Restriction enzyme sites found within the MCS are a negligible distance apart unless separated by an insert, and they are unique in the vector). All of these enzyme sites, including that of *Sal*I, are listed on pages 77-78 of your laboratory manual.



H = *Hin*dIII; K = *Kpn*I; S = *Sal*I;
R = *Eco*RI; V= *Eco*RV; B = *Bam*HI;
X = *Xho*I; P = *Pst*I

Restriction enzyme sites must be mapped onto the insert. To construct the map, the plasmid DNA (i.e., the pTH06 vector with the insert cloned as described above) is digested with different combinations of the enzymes *Hin*dIII (H), *Eco*RV (V), *Eco*RI (R), and *Bam*HI (B). After subjecting the plasmid digests to gel electrophoresis alongside standard-sized fragments, the molecular weights of the fragments are determined. Below is a list of the resulting fragments in kb and their corresponding enzyme digests. d = doublet

H: 3.5, 2.0, 1.3, 0.7	H/B: 3.5, 1.1, 0.9, 0.7 (d), 0.6
R: 6.6, 0.9	H/V: 3.2, 2.0, 1.0, 0.7, 0.3 (d)
B: 4.4, 2.5, 0.6	R/B: 4.3, 1.7, 0.8, 0.6, 0.1
V: 4.2, 3.3	R/V: 4.2, 1.3, 1.1, 0.9
H/R: 3.5, 1.3, 0.9, 0.7, 0.8, 0.3	B/V: 3.2, 2.1, 1.2, 0.6, 0.4

a. What is the molecular weight in kb of the **insert** of the recombinant plasmid? _____

b. Using a series of annotated drawings, illustrate the strategy of cloning the *Sal*I/*Kpn*I-digested fragment into the *Xho*I/*Kpn*I-digested pTH06 plasmid vector. Show how the pTH06 vector had to be linearized to accept the fragment for insertion. Show how the fragment was attached to the vector (show the actual bases in restriction enzyme-digested ends). Show the location of the restriction enzyme sites in the MCS relative to the insert.
Answer these questions. The answers are critical to your being able to construct a map of your insert.:
Which enzyme sites (if any) were eliminated in the digestion of the vector before its ligation to the insert?

Which enzyme sites in the vector (if any) were restored after ligating the insert to the vector? Which sites (if any) were destroyed?

c. Illustrate the restriction enzyme map of the plasmid's insert showing the insert's orientation relative to the restriction enzyme sites in the vector's MCS. Be sure to include distances between the sites in kb. Illustrate the insert as a straight line on graph paper--there is no need to show the entire vector DNA, only its MCS. Hint: Use the known location of enzyme sites present in the plasmid's MCS as part of your mapping strategy, and remember that if a site for an enzyme is found in the MCS, there is no other site for that enzyme anywhere else in the vector portion of the plasmid. Rule of thumb: When a plasmid is digested with an enzyme that has a site in the MCS, then any fragment from this digestion that is smaller than the vector, must be from the insert portion of the plasmid. Be sure that you make obvious what is vector DNA and what is insert DNA on your illustration.

Beginning of Last Third of the Semester
(Hint: Please read Appendix III of your lab manual for help in your analysis of the following problem). Dodder is a parasitic plant that is attracted to volatiles emitted by other plants like tomato. It grows towards the plants, and wraps itself around and attaches to the plants' stems and leaves, draining them of nutrients. As the U.S. Department of Agriculture's top 10 noxious weeds, Dodder may reduce agricultural productivity by as much as 90%. Apparently, wheat plants emit a chemical that repels Dodder. The following problem is a hypothetical example of how RFLP analysis can be used to clone the gene that encodes this potentially useful protein in wheat that is either an enzyme that produces this repellent or is the repellent itself. (See Pennisi (2006) Science *313*: 1867 for more information about Dodder.) Scientists have discovered a mutant wheat strain that is unable to repel Dodder. Because it has a high reversion rate, scientists performing research on this mutant plant believe that the mutation was caused by the insertion of a transposable element in a gene they named *dodder chow* (*docho*, for short). Classical genetics and molecular analysis indicate that all or part of the *docho* gene can be found in a 4.3 kb *Hin*dIII/*Eco*RV-digested fragment within the wild-type (non-mutant) wheat genome. This 4.3 kb fragment was cloned from the genome, and its complete restriction enzyme map is shown below.

V=*Eco*RV; H=*Hin*dIII; B=*Bam*HI; P=*Pst*I; S=*Pst*I; Sizes in kb.

To characterize the *docho* gene, the smallest genomic fragment that encompasses at least part of this gene needs to be identified from the fragment of DNA above. This can be done by restriction fragment length polymorphism (RFLP) analysis. Genomic DNAs from wild-type (+) wheat plants and DNA from the *docho* mutant are isolated, and equal amounts are digested with different restriction enzymes. These digests are subjected to electrophoresis through a 0.8% agarose gel. The gel is blotted onto a nylon membrane that is then hybridized with the radioactively labeled (nick-

translated) 4.3 kb *Hin*dIII/*Eco*RV-digested fragment (map shown above). The membrane is exposed to x-ray film and the size of the bands determined by extrapolation with a standard curve. The sizes in kilobases (kb) of the bands for each digest and wheat plant strain (wild-type (+) or *docho*) are given below. (f = band is significantly more faint compared to other bands in the same set of digests; dk = band is significantly more intense (darker) compared to other bands in the same set of digests). [Note that intensity of hybridized bands relates to what proportion of the probe is complementary to the DNA in the band (i.e., "probe overlap") as well as the number of molecules of complementary DNA there are in a band (which is ultimately determined by the (µg) amount of DNA loaded in each well of the gel)--see Appendix III].

*Bam*HI		*Hin*dIII		*Pst*I		*Sal*I		*Eco*RV		*Pst*I/*Sal*I	
+	*docho*	+	*docho*	+	*docho*	+	*docho*	+	*docho*	+	*docho*
2.9	5.1	5.4(dk)	9.2	4.4(dk)	4.4(dk)	2.7	3.5	6.1(dk)	8.2	2.7	2.7
2.2(dk)	2.2(dk)			3.6	4.2	2.3(dk)	2.7		1.7	1.3	1.6
1.0	1.6(f)				1.4	1.1(f)	2.6(f)			1.1	1.4
0.3(f)	1.0						1.1(f)			1.0	1.3

Use these data above and the map of the 4.3 kb probe to answer the following questions:

a. Show the restriction enzyme map of the appropriate region of the wild-type genomic DNA including the restriction enzyme sites that you are able to locate in the genome on either side of the DNA corresponding to the 4.3 kb probe. (Be sure to indicate distances between the sites in kb, and make it obvious which sites are not in the probe—i.e., the sites that you mapped).

b. What is the best estimate for size (kb) of the transposon? Briefly, but specifically, explain how you arrived at this answer.

c. Determine the smallest wild-type restriction enzyme fragment from the 4.3 kb probe that contains all or part of the *docho* gene. You can accomplish this task by determining in which restriction enzyme fragment the transposon inserted, reasoning that the *docho* gene must have been disrupted by the insert to cause the *docho* mutant phenotype. Justify your answer briefly. Note: The smallest restriction fragment may not be listed in the hybridization results above.

d. According to the RFLP analysis, what restriction site(s) does the transposon contain? How many of these sites are there? Are there any sites that you know do not exist in the transposon? Briefly explain your answers.

e. Below, show a map of the mutant genome in the area of the *docho* gene. Pay particular attention to the restriction enzyme site map of the transposon relative to the sites within the 4.3 kb area corresponding to the probe. Be sure to make the distances between the sites and the borders of the transposon obvious. Feel free to briefly explain the derivation of your map. For clarity, you may assume that the transposon inserted in the exact center (middle) of the restriction enzyme fragment you determined in part c above.

f. To best confirm your conclusion of part c (above) and your map of the genome (part e), with what restriction enzyme(s) (one single or double digest is sufficient) would you digest the genomic DNAs (wild-type and mutant), and what size restriction enzyme fragments would you expect to find in the wild-type and *docho* lanes after RFLP analysis using the 4.3 kb probe?