

2005 Molecular Lab Exams Combined

Midterm One

Please use the following table as needed for questions that follow.

Name	Bacterial Source	Recognizes	BL	BM	BH	BK
EcoRI	Escherichia coli	GAATTC	20	100	100	100
Avall	Anabaena variabilis	GGCC	80	100	20	20
HindIII	Haemophilus influenzae	AAGCTT	60	100	20	100
PstI	Providencia stuartii	CTGCAG	20	60	100	80
PvuII	Proteus vulgaris	CAGCTG	80	100	40	20
HhaI	Haemophilus haemolyticus	GCGC	80	100	100	100
HaeIII	Haemophilus aegyptius	(Pu)GCGC(Py)	60	100	100	100

1. 6 pts. Pipetteman Question:

- You need to pipette 38 microliters. Which pipetteman will you use and how will you set it?
- You need to pipette 5.7 microliters. Which pipetteman will you use and how will you set it?
- You need to pipette 0.42 milliliters. Which pipetteman will you use and how will you set it?

2. 12 pts. Restriction Set-Up Question. You need to set up a triple digest of a mystery DNA that is at 0.25 micrograms/microliter. The enzymes you will be cutting with are Avall, HindIII, and PvuII. Set up the recipe for the reaction below, including all the components that you know need to be added. Assume the final volume is 20 microliters, as was performed in class.

3. 4 pts. each. Some Math Questions:

- Predict how many times HhaI will cut a 55 kb chromosome and how large the predicted fragments will be.
- Predict how many times HaeIII will cut a 80 kb chromosome and how large the predicted fragments will be.

4. 12 pts. Evil Mapping Question: Use the following table of restriction-digest information to draw the original map of the plasmid. Please note that I am not asking you to draw the gel (although that might help you); I want the final plasmid map.

KpnI Alone	SmaI Alone	EcoRI Alone	KpnI/SmaI	KpnI/EcoRI	SmaI/EcoRI	Triple Digest
1.5 kb 3.5 kb	5 kb	5 kb	1.5 kb 1.5 kb 2 kb	1.5 kb 1 kb 2.5 kb	0.5 kb 4.5 kb	0.5 kb 2 kb 1.5 kb 1 kb

5. 8 pts. Plasmid Isolation Reagents. Complete the following table.

Reagent	Purpose? Explain, avoiding repeating terms in reagent name.
Spin Column	
Alkaline Protease Solution	
Nuclease-Free Water	
Cell Resuspension Solution	

6. 8 pts. You have been given the following template and primer to sequence. Draw the resulting gel, indicating which lane is which, and top vs. bottom. Primer – GTACCGTTCC

7. 4 pts. each. Answer the following compare/contrast questions about agarose vs. acrylamide, making clear which is which in your answer.

- a. How is DNA visualized in an each kind of gel?
- b. How is each kind of gel polymerized?
- c. What is the resolution capacity of each kind of gel?
- d. How did we use each kind of gel in this lab so far?

8. General Project-Specific Questions:

- a. Regarding this cloning vector, explain 2 features and how they have been used with portions of labs you have completed so far. 6 pts.
- b. Regarding the insert, what is it and where did it come from? 4 pts

Midterm Two

Answer questions 1-3 based only on the genomic/PCR lab.

1. 6 pts. In terms of genomic DNA preparation, how did you achieve the following? Explain each thoroughly. CELL LYSIS, CRUDE SEPARATION OF DNA FROM PROTEINS, PURE DNA

2. 9 pts. CONTRAST PCR and sequencing in terms of the following categories.

	Monomers Used	Priming	Product(s) – Structure of/Length of?
PCR			
Sequencing			

3. 4 pts. Explain ALL the primers you used during the PCR lab – including what they targeted, the product size(s), and the kind of analysis each product was used for.

Answer questions 4-6 based only on the southern blotting lab.

4. 4 pts. Explain how you will mix the following: 2.5 M Tris-HCl, 3.5 M NaOH, 5.4 M NaCl, pH 7.0

Molecular Weights You May Need

20 g NaOH/1 L

87.66 g NaCl/1L

78.8 g Tris-HCl

Tri-Sodium Citrate/2H₂O – 88g/L

5. 8 pts. Outline the southern blotting procedures – starting from your DNA sample tube. Your answer should include/describe the overall purpose of the procedure.

6. 8 pts. In terms of the Giovannoni et al. paper that you were assigned to read for southern blotting methods, describe 2 things that were different from what you did and 2 things that were the same. Your answer can only address methods up to and including the blotting step (NOT the probe/hybridization procedures).

Answer questions 7-10 based only on the probe/hybridization lab.

7. 12 pts. Draw and describe an entire complex that should have formed for a positive hybridization reaction using the procedures and methods performed in our lab.

8. 2 pts. What SHOULD the probe that you used have bound to?
9. 4 pts. In terms of the Giovannoni et al. paper that you were assigned to read for southern blotting methods, describe 2 ways the probe/hybridization steps used in the paper were different than those we used.
10. 3 pts. What question was Giovannoni et al. attempting to address in their paper?

Answer questions 11-13 based only on the DGGE lab.

11. 9 pts. Compare and contrast DGGE and sequencing gels in terms of the following specific questions: Name/describe 3 ways they are similar; Name/describe 3 ways they are different:
12. 3 pts. You are studying 2 populations of bacteria – the red and the green mat layers. The red layer has 10 different bacteria. The green layer has 4. Draw the predicted DGGE results that would arise from analyzing these 2 samples.
13. 8 pts. In terms of the Sodora et al. paper that you were assigned to read for DGGE methods, answer the following specific questions:

What agent and specific gene was being analyzed via DGGE?
What question were the researchers addressing using DGGE?
What result did the researchers show using DGGE?