

Spring 2010 Molecular Biology Exam #1 - Learning the Tools

There is no time limit on this test, though I have tried to design one that you should be able to complete in 4 hours or less. You are not allowed to use your notes, any books, any electronic sources except those specified in the exam, nor are you allowed to discuss the test with anyone until all exams have been submitted. **YOUR PRINTED EXAM ANSWERS ARE DUE AT 12:30 ON WEDNESDAY, FEBRUARY 3.** You may use a calculator and/or ruler. The answers to the questions must be typed into a new word file. When you are asked to draw a picture, you may draw in Word, or draw by hand. Make sure you write neatly if you do it by hand – print neatly. If I cannot read it, you don't get points. Submit your typed answers in hard copy please.

There are 4 pages for this exam, including this cover sheet.

-3 Pts if you do not follow this direction:

Please do not write or type your name on any page other than this cover page. Staple all your pages (INCLUDING THE TEST PAGES) together when finished with the exam.

Name (please print here):

Write out the full pledge and sign:

On my honor I have neither given nor received unauthorized information regarding this work, I have followed and will continue to observe all regulations regarding it, and I am unaware of any violation of the Honor Code by others.

How long did this exam take you to complete (excluding typing)?

12 pts.

1. For many years, students who have taken my Molecular Biology course have applied for lab tech jobs right after graduation. I have NEVER had a student fail to get a job before graduation. One reason for this is that all my students learn to make solutions. Here are four problems that could keep you off the unemployed college graduate list.

a) You take an optical density reading of a DNA sample but it is too concentrated to give you an accurate reading. You take $1 \mu\text{L}$ of the DNA sample and dilute it with $14 \mu\text{L}$ of dH_2O . You then take a reading of the DNA on the spectrophotometer and it gives you an OD reading of 0.123. Written above the spectrophotometer is this conversion factor: 1 OD of dsDNA = $50 \mu\text{g}/\text{mL}$ of DNA. **Question:** what was the concentration of your original DNA sample?

92.25 $\mu\text{g}/\text{mL}$

b) You need to prepare some solutions for a Southern blot. You are given 50X SSC, 10% SDS, and 5 M NaOH. **Question:** How would you make 250 mL of a solution whose final concentrations were 1X SSC, 0.25% w/v SDS and 10 mM NaOH? FW of NaOH is 40.0.

5 mL SSC, 6.25 mL SDS, 0.5 mL NaOH the rest with water

c) **Question:** How do you make a 1.25% w/v agarose gel with a final TBE buffer concentration of 0.5X if your gel is 60 mL in volume and your stock TBE solution is 10X?

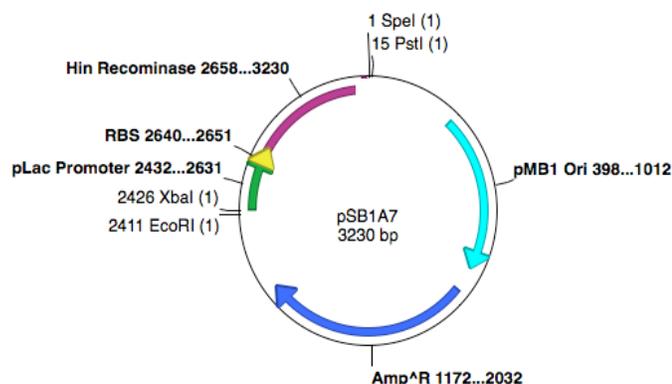
0.75 g agarose, 3 mL TBE, 57 mL water, microwave, pour.

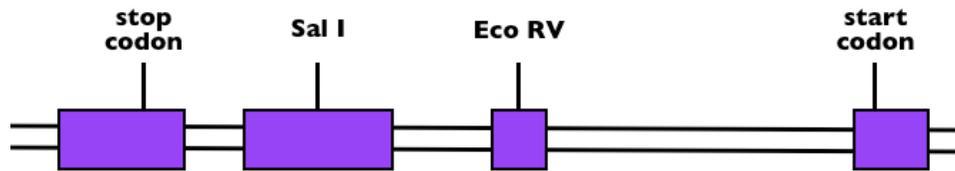
d) How do you make 400 mL of a solution that is 100 mM Tris, 0.05 M NaCl, 1 mM EDTA, and pH of 7.5 using HCl? **FWs:** NaCl = 58.5; EcoRI = 125; BamHI = 415; EDTA = 416; Tris = 121; HCl = 36.5; agarose = 204.

4.84 g Tris, 1.17 g NaCl, 0.1664 EDTA, 300 mL water, dissolve, pH with HCl to 7.5, water to 400 mL

14 pts.

2. Below are a restriction map of plasmid and a human gene of known sequence. Your task is to clone the human liver cDNA for this gene so that the start codon is closer to Eco RI site than Pst I site of the polylinker, but get rid of the promoter+RBS+Hin insert already in the plasmid. Explain how you would accomplish the cloning using a series of numbered steps. *Do NOT write in paragraph form. I want a numbered list of steps.*





Note that in the plasmid, the (1) refers to the frequency of these sites in the plasmid plus existing insert. In the gene's restriction map, all the relevant restriction sites are indicated if they are present.

Cut plasmid with Pst and EcoRI

Gel purify plasmid away from old insert

Make cDNA from liver cells

Amplify known cDNA using PCR primers that have EcoRI site on forward primer and PstI site on reverse primer.

Digest PCR product with EcoRI and PstI.

Ligate amplified insert to plasmid. Transform, select for amp resistance.

Verify insert size with EcoRI and PstI. Verify orientation with EcoRI and SalI. Run on gels.

14 pts.

3. You may have heard in the news recently that a new species of frog was discovered in a rainforest. Your goal is to isolate genomic DNA and construct an expression library using λ phage. You are interested in cloning the gene that encodes an enzyme which produces an intense green skin color and you have an antibody that you think will work. **Questions:** a) Design a well controlled experiment to demonstrate that your antibody will work for the library screening. Draw a picture of your expected experimental results as part of your answer.

The key was to show the antibody works and not to use the antibody to clone the cDNA to prove it worked. Best answers had a western blot or ELISA with +control from skin, - control from some other tissue/species.

b) Propose 2 reasonable explanations why you might not be able to isolate the DNA even though you plated 3 genomes worth of DNA in your screening.

Many reasons including size, reading frame, splicing, post-translational modifications, etc.

12 pts.

4. As I had predicted in class, we had a big snow storm this weekend. What I want you to do is to engineer some grass for my yard so that my grass never lets snow accumulate on it. To do this, you need to produce arctic antifreeze protein on the surface of the leaves and no other tissue. Draw a picture of the DNA you would engineer to put in my grass.

Needed 3 parts: promoter for leaves, or cells on the surface of leaves,

Signal sequence before coding of antifreeze protein

antifreeze protein

10 pts.

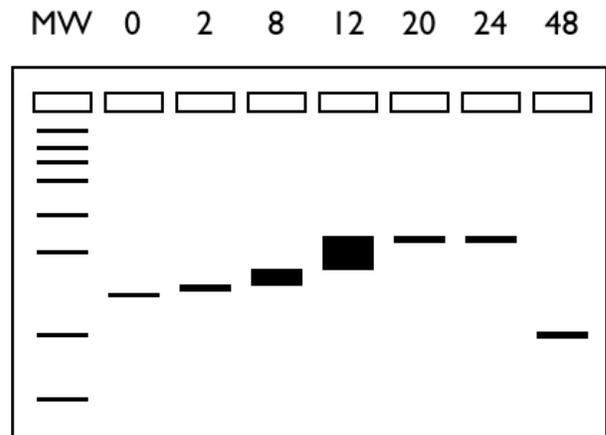
5. Interpret these data from a western blot using a very specific monoclonal antibody. The different lanes indicate the number of minutes after inducing an expression vector with IPTG. Assume that the same amount of total protein was loaded in each lane of the gel.

Key points included: increasing amount of protein (thicker bands)

Larger molecular weights (shifted upwards)

Reduced size at the end (protease??)

Smearing bands/broader bands



12 pts.

6. [View this structure in Jmol](#). (← click on that sentence.) You are NOT allowed to use any other web pages to answer these questions. Questions: a) How many subunits are in this protein? To get credit, you must tell me how you arrived at your answer. 2, several ways to visualize.

b) Identify all the ligands in this structure. To get credit, you must tell me how you arrived at your answer. 5 NO₃, 6 coppers, 1 non-standard amino acid that required clicking on ligands above and below.

c) View this structure so that you can count how many alpha helices and beta strands. How many of each are there? To get credit, you must tell me how you arrived at your answer.

Show structure gave you 15ish alpha helices and 8 beta strands.

14 pts.

7. Draw the molecular structure for AMP. You must show every atom except for those in the base – you can just spell out what the letter A stands for. Draw a box around the 3' carbon. Draw a circle around the atom where the next nucleotide will bind to this one in a growing chain polymerized by a polymerase.

Needed to ID correct parts and show all hydrogens.

12 pts.

8. Copy and past this table into your answer file. Type out the amino acids for these abbreviations.

Abbreviations	FULL NAME
D	No one missed any of these.
A	
V	
I	
S	

R	
N	
H	
P	
T	
C	
L	