

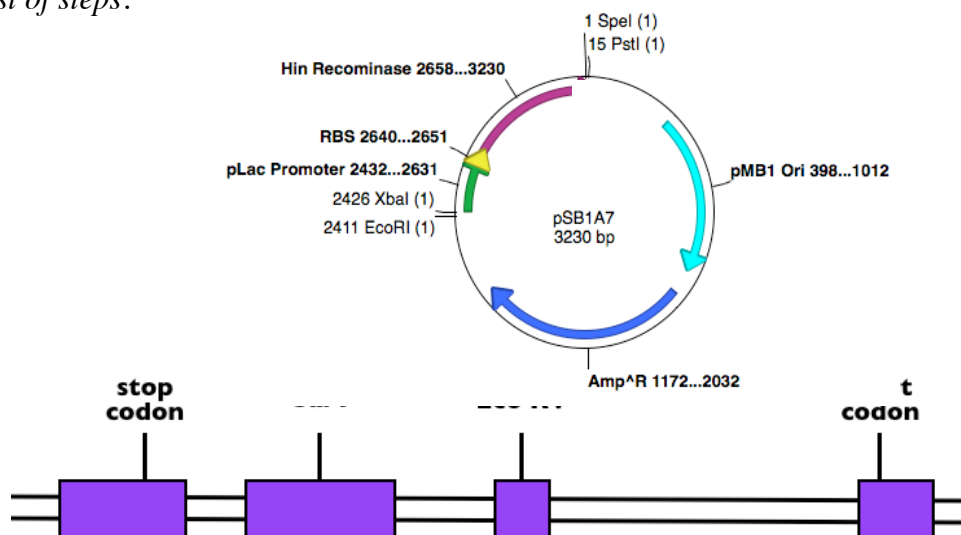
12 pts.

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?
- 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.
- 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?
- 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.

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.

14 pts.

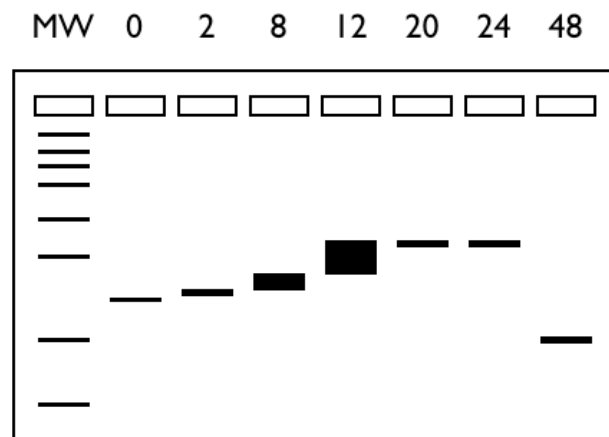
3. You may have heard in the news recently that a new species of frog was discovered in a rainforest. Your goal is to isolate genomic DNA and construct an expression library using λ phage. You are interested in cloning the gene that encodes an enzyme which produces an intense green skin color and you 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. 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.

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.

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.



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. b) Identify all the ligands in this structure. To get credit, you must tell me how you arrived at your answer. 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.

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.

12 pts.

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

Abbreviations	FULL NAME
D	
A	
V	
I	
S	
R	
N	
H	
P	
T	
C	
L	