

Spring 2000 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 within 3 hours, except for typing. You are not allowed to use your notes, or any books, any electronic notes or old tests, nor are you allowed to discuss the test with anyone until Wednesday Feb. 9, 2000. **EXAMS ARE DUE AT 11:30 ON WEDNESDAY, FEBRUARY 9.** You may use a calculator and/or ruler and you must use the internet for one question but you are NOT allowed to use any of the computers in the molecular lab for this question. The answers to the questions must be typed on a separate sheet of paper unless the question specifically says to write the answer in the space provided. If you do not write your answers on the appropriate pages, I may not find them unless you have indicated where the answers are.

-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:

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

14 pts.

1. Starting with this peptide (MENAHTKTVEEV LGHFGVNESTGLSLEQ), answer the following questions. Use the web page from lab and the internet to answer these questions:
 - a. From what protein and species is this peptide derived?
 - b. Find the cDNA online and design two PCR primers to clone the ORF. Write down the sequences of your primers.
 - c. What was the vector that contained this clone?
 - d. From what tissue was this cDNA produced?

10 pts.

2. In the space provided, draw a TTP. You may write just the letter “T” for the base.

10 pts.

3. List the steps you would need to perform if you wanted to do a Southern blot of your lab partner to see if she or he were diploid or haploid. Do not worry about details (e.g. specifically which enzyme you might use) but make sure you tell me the common steps in all Southern blots. Also, describe the probe you would use. You will not know the exact probe, but tell me the general properties you would want in said probe.

8 pts.

4. Explain how the pET expression system works. You MUST draw a picture of the pET plasmid and label all its parts to receive full credit.

8 pts.

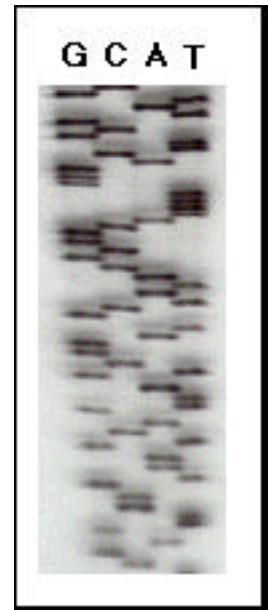
5. Design an experiment to determine the molecular weight of the only human Mg channel that is found in the basement membrane of toenails. You must determine the molecular weight by an experiment with real protein; you cannot use only electronic information. All you know at this point is that mice have the same protein and that mouse cDNA has been cloned and sequenced.

4 pts.

6. Describe how blue/white selection works when cloning DNA.

12 pts.

7. Read this sequencing gel. Write down the **template** sequence from which this radioactive DNA was derived. Make sure you write your answer from 5' to 3'.



12 pts.

8. Go to this URL:

www.bio.davidson.edu/Biology/Courses/Molbio/Exams/2000/exam1fig1.gif

You will see a picture of two DNA-binding proteins binding to DNA (DNA is the two shades of blue). Find the two amino acids binding in the minor groove. Now go to this URL

www.bio.davidson.edu/Biology/aatable.html.

- Explain to me why it makes sense that these amino acids are both R.
- Pick a conservative substitution that you think would not disrupt the quaternary structure depicted. Explain your answer.
- Choose the most disruptive amino acid substitution you can imagine and explain your choice.

12 pts.

9. The following requests have been picked up from Mars. It appears that a life form on Mars has taken the Mars landers hostage until the following answers can be obtained. (You see, Martians cannot take molecular biology and they are mean, green, and angry!)

- Make 350 mL of a solution that is 70 mM KCl, 10% w/v sarkosyl, and 0.15 M Tris-HCl (pH 7.8). (FW KCl = 74.5; FW Tris = 121; FW sarkosyl = 293)
- Make 125 mL of a 0.45% agarose gel in 0.5X TBE when you are given 10X TBE stock solution. (FW agarose 66; FW TBE = 123)
- What is the concentration of a stock solution of DNA when the OD_{260} of some DNA reads 0.025 and this DNA was made by diluting 3 μ L of the stock solution into a final volume of 400 μ L?
- Tell me how to make 600 mL of a solution that is 0.015M NaCl, 5% v/v SSC, and 250 mM NaOH. (FW NaCl = 58.44; FW NaOH = 40; you have a stock solution of SSC).

10 pts.

10. Figure out how to subclone the shaded piece of DNA into a new copy of the same plasmid. Remember to include an experiment to verify that you have cloned the correct piece. In the diagram, K, S, R, X, P, N all represent different restriction sites. The sizes of the fragments are given in kb. Notice that this big fragment has been cloned into a polylinker where the different restriction sites are very close to one another.

