# Spring 2005 Molecular Biology Exam #1 - Learning the Tools

http://www.bio.davidson.edu/courses/Molbio/Exams/2005/MolExam1_05.doc

There is no time limit on this test, though I have tried to design one that you should be able to complete within 4 hours. You do not have to take this exam in one block of time; you may come and go as you wish. However, if you feel another course presents a conflict of interest, please discuss this with me. 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 Wednesday Feb. 2, 2003. **EXAMS ARE DUE AT 9:30 ON WEDNESDAY, FEBRUARY 2 in DANA 256 lab**. You may use a calculator and/or ruler. The answers to the questions must be typed into this file unless the question specifically says to hand write/draw the answer in the space provided. If you do not place your answers in the appropriate locations, I may not find them.

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):

ANSWER KEY

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)?

**16 pts.**

1. My grandmother has lost her molecular cloning manual and needs some help. She has been doing molecular biology most of her life, but with her age, her mind is not as sharp as it used to be. Please help her by providing the information requested below.

a) How do you make a 400 mL of a 2X stock solution when the 1X solution is 0.15 M Tris; 200 mM NaCl; pH 8.0?

14.52 g Tris (0.3M)

9.36 g NaCl (0.4M)

Dissolve in about 300 mL water, add HCl to pH of 8.0, add water to 400 mL final volume.

b) Grandma needs to electrophorese some DNA from her transgenic tomatoes to see which one she should serve cousin Elmo who refuses to eat GMO's. Remind her how to make a 200 mL gel that is: 0.35% agarose; 1$\mu$g/mLEtBr; 0.5X TBE.

0.7 g agarose

20 mL 5X TBE stock solution

20 $\mu$L EtBr stock solution

180 mL water, add argarose, microwave, cool a bit, add EtBr (ethidium bromide), pour.

c) The molecular grocery store had some stock solutions, but not the ones she is used to. Please help her make 200 $\mu$L of a DNA solution that is 88 $\mu$g/mL from the store brand stock solution that came in 400$\mu$g/mL concentration.

DNA is 4.55X too concentrated.

43.95 $\mu$L DNA stock plus 156.05 $\mu$L water

d) Grandma is ready to digest her DNA stock solution. She wants to digest 150 ng (1 $\mu$g = 1,000 nanograms, ng) of her stock solution you helped her make in c above. The final volume will be 15 $\mu$L, and you need to digest the DNA with EcoRI and BamHI which work well in the same 10X buffer that came with the enzymes. Tell her how to set up this DNA digestion.

1.7 $\mu$L diluted DNA solution (88 $\mu$g/mL concentration)

0.75 $\mu$L BamHI

0.75 $\mu$L EcoRI

1.5 $\mu$L 10X stock solution

10.3 $\mu$L water: add water first and enzymes last

**FWs:** NaCl = 58.5; EcoRI = 125; BamHI = 415; EtBr = 394; EDTA = 416; Tris = 121; HCl = 36.5; agarose = 204. Other raw materials include SDS = stock solution of 10%; TBE = stock solution that is 5X; stock solution of EtBr = 10 mg/mL.

**12 pts.**

2. Someone claims to have cloned human beings and produced a Northern blot to prove his point (Figure 1). RNA has been isolated from whole blood of 3 alleged clones plus the original donor and all 4 samples were subjected to gel electrophoresis. Two probes were used on the same blot.

a. Interpret the data as fully as you can.

First, the actin control demonstrates the same amount of RNA was loaded for donor, clone 2 and clone 3, but a lot more (5 times??) RNA was loaded for clone 1.

Donor and clone 1 have the same molecular weight α hemoglobin; clone 2 has a different version of α hemoglobin mRNA (larger molecular weight); clone 3 is heterozygous for the two versions of α hemoglobin. Therefore, clones 2 and 3 cannot be true clones since their mRNAs are different lengths.

Clone 1 produces about 5 times less α hemoglobin than the donor which is consistent with clone 1 not being a true clone since we would expect a true clone to have the same promoter and thus produce the same amount of mRNA as the donor.

b. Do the data support the claim that one of these three clones are true clones? Explain your answer.

No, the data do not support this claim for the reason given above.

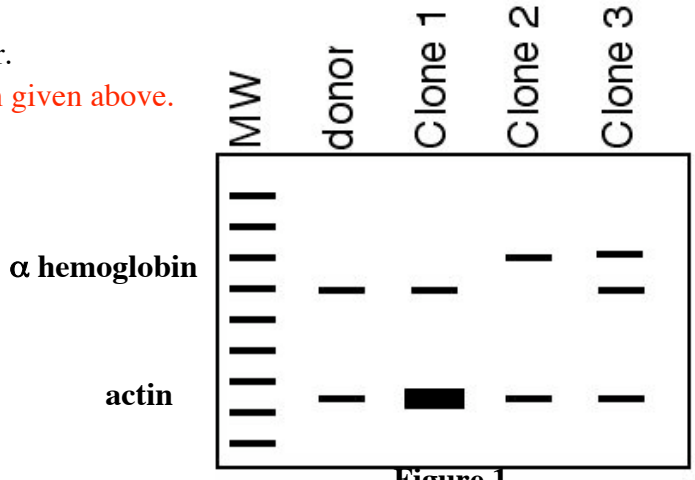



**Figure 1.**

**12 pts.**

3. It turns out there are two kinds of people in the world: those who have a Mickey Mouse (MM) protein and those who have a Donald Duck (DD) protein in their toenails and these two proteins are very different from each other. As it turns out, heterzygotes are lethal *in utero*. A new constitutional amendment is being proposed that MM's cannot marry DD's and there is a need for a quick screen. Explain how you would:

a) produce an antibody for each protein (use outline format only)

1) clip toenails from DD people, grind up the clippings.
2) Inject clippings into mouse, boost 10 days later.
3) Isolate spleen from mouse, fuse with myeloma, produce hybridomas by growing in HAT media.
4) Screen hybridomas for secretion of antibody that binds to DD.
5) Mass produce anti-DD hybridoma.

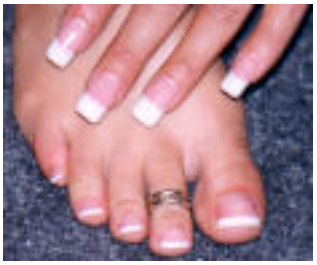
I would do the same thing for MM protein, in parallel and with a different mouse.

b) verify your antibodies using a method that incorporates DNA (use outline format only)

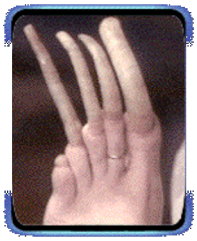
1) Isolate mRNA from are of toe that produces toenails

2) Produce cDNA from said mRNA.
3) Clone cDNAs into vector that can produce an expression library of all the cDNAs.
4) Screen the library with the DD (or MM) antibody to isolate the cDNA that encodes DD (or MM) antigen.
5) Sequence the cDNA and deduce the amino acids.
6) Use anti-DD antibody to affinity purify the DD protein (or anti-MM and MM protein, respectively).
7) Sequence the DD or MM protein and compare with the cDNA-derived amino acid sequence. They should match.

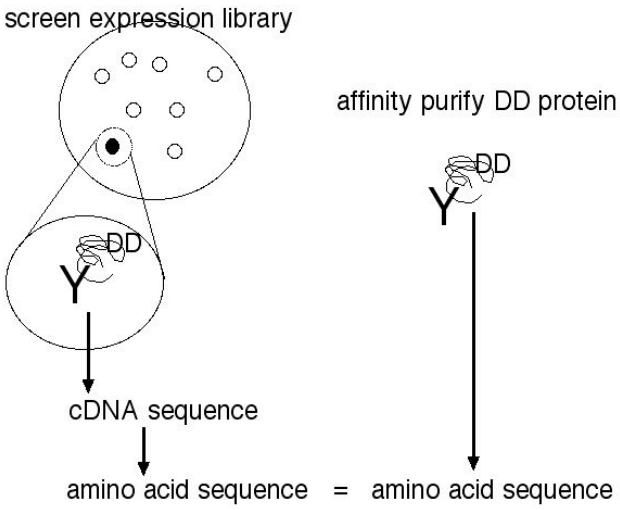
c) Draw a picture of the experimental data from b above.



or……



now you see the problem.




**12 pts.**

4. More news from the field…. salmon can produce mad salmon disease in humans if the meat is eaten raw (the first symptom is an urge to run up the down-escalators to eat more sushi). The cause of the disease is a fish version of a prion protein found in cattle. Explain how you could:

a) clone the salmon gene (outline format)

1) Isolate the bovine cDNA sequence, use it as a probe for screening a salmon genomic library (not cDNA).
2) Isolate any vectors with inserts that bound to the bovine prion probe.
3) Sequence the full gene to verify the coding DNA looks similar to the bovine version.

b) demonstrate which tissues in the fish transcribe the prion gene (outline format with diagram of data)
1) Isolate mRNA from each major salmon tissue
2) Run a gel and produce a Northern blot of the mRNA
3) Probe the blot with the salmon gene that I just cloned.
4) Also probe for actin or similar standard to control for mRNA loading.
5) Identify lanes (tissues) where bands appear for both prion and actin. Ignore lanes that have no actin bands.

**10 pts.**

5. Figure 2 shows the results of a poorly planned cloning experiment. The sad student had not taken Molecular Biology and therefore did not have a full tool belt at her disposal. As you can see, a PCR product was cloned backwards into an expression vector (pDOG). The start codon is near the P site and the stop codon is between the B and K sites at about 3 o'clock.

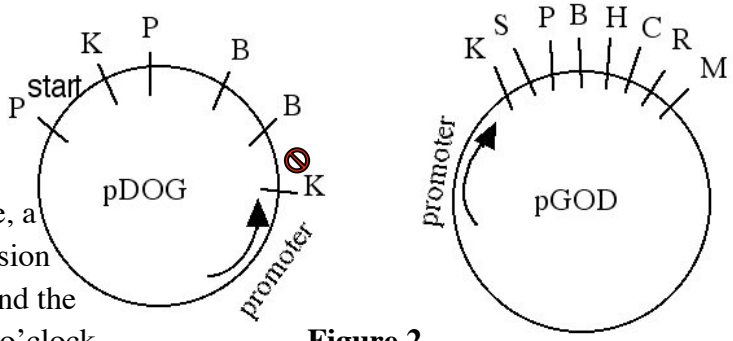



**Figure 2**

Please help this unprepared student clone the ORF (from P → K, including the P and K cut sites) into pGOD so the encoded protein will be expressed properly. You may use any method you want, but you must explain all critical steps. To get full credit, you must experimentally verify your cloning has been successful. You may use diagrams and/or outlines. If you hand write anything, make sure I can read your writing.

Cloning:
1) Use PCR primers that bind upstream of the P site near start and downstream of the K site near the stop. Amplify the DNA. Design the primers so they have restriction sites in them different from the internal cut sites. I suggest S and C, for example.
2) Cut the PCR product with S and C. Do the same double digestion with pGOD.
3) Mix the cut PCR product with the cut plasmid, add ATP, ligase, and buffer.
Verify:
1) Express the protein to see if you get what you expected.
or
1) Digest pGOD with new insert using K, or P, or B, etc to match the restriction pattern with the expected pattern.

**12 pts.**

6. People are making a stink about driving over skunks in the road and they want something done about this smelly problem. The Division of Roadkill (DoK) has developed a plan, all they need is a transgenic skunk that produces GFP in the fur of the white stripe. Design a recombinant gene that will perform this function. Lucky for you, the cDNA that encodes the white pigment has recently been

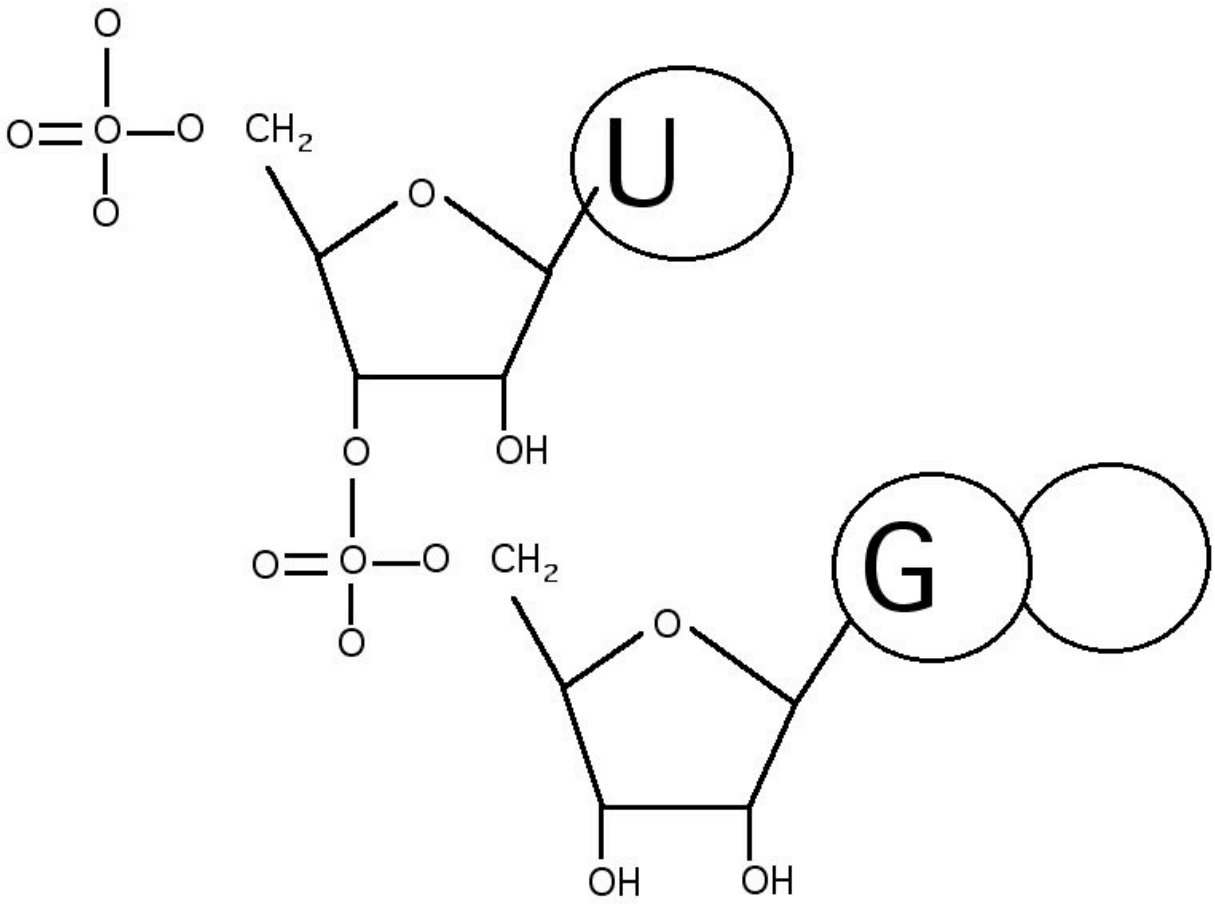
cloned. You do not have to tell me how to get it into the skunks, only DNA design. Getting the DNA into the skunk is the responsibility of DoJYDW.

1) Use the White Stripe (WS) cDNA to probe a skunk genomic library and isolate the WS promoter.

2) Splice the WS promoter upstream of the GFP cDNA. And there you have the DNA construct.


**14 pts.**

7. On the back of this page, draw an RNA dinucleotide with U as the fist base and G as the second base added to the growing polymer. Do not take any shortcuts in the atoms except in the two bases. For the bases only, you can draw circles as appropriate, but you do not need to fill in all the carbons or nitrogens, etc. for the bases. You do need to include all other atoms.



**12 pts.**

8. Using the table provided, hand PRINT in the name for each amino acid. DO NOT TYPE.

| Abbreviations | FULL NAME |
|---|---|
| W | tryptophan |
| I | isoleucine |
| L | leucine |
| D | aspartic acid (aspartate) |
| T | threonine |
| H | histidine |
| N | asparagine |
| G | glycine |
| K | lysine |
| V | valine |
| E | glutamic acid (glutamate) |
| Y | tyrosine |