Spring 2002 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 4 hours, except for typing. 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. 6, 2002. **EXAMS ARE DUE AT 11:30 ON WEDNESDAY, FEBRUARY 6**. You may use a calculator and/or ruler. 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.
There are four pages for this exam, including this cover sheet.

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):

Average = 86.5%
Range = 100 – 40
Points Added = 7

Write out the full pledge and sign:

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

16 pts.

1. Imagine you are a senior looking for a lab job and you have 5 job applications on file at different labs. It is Friday afternoon and you return to your room to get ready for a small party with your best friends which begins at 9 pm. At 8:30, you notice there are some faxes on your desk. The five labs have asked you to perform these calculations and send back your response before midnight on Friday. Please answer these questions correctly so you can get 5 job offers and still go to the party with your friends by 9 pm. A table of formula weights is after the last question.

a. Make 225 mL of a solution that is 2.5 M NaCl, 0.1 M Tris-HCl, pH 7.5, 100 mM EDTA.
32.9 g NaCl + 2.72 g Tris + 9.36 g EDTA; obtain about 170 mL water, add dry stuff, dissolve, pH with HCl to 7.5 and bring volume up to 225 mL

b. Make 50 mL of a 0.5X TBE, 1 X EtBr , and 0.8% w/v agarose when you have 5 L of 10X TBE and 100 mL of a 1000X EtBr stock solutions.
2.5 mL TBE + 50 µL EtBr + 0.4 g agarose: add 47.5 mL water plus 2.5 mL TBE stock, pour in agarose. Microwave, cool add EtBr and pour into gel mold.

c. Make 10 mL of a solution that is 1.5% v/v SDS, 250 mM NaCl when you have stock solutions of 20% SDS and 5M NaCl.
750 µL SDS + 500µL NaCl stock solution: Get 8.75 mL water and add other two stocks.

d. Set up a restriction enzyme digestion of 0.25 µg DNA using EcoRI and BamHI with a final volume of 35 µL. You had a sample of DNA and made a dilution of it by mixing 16 µL of stock and 384 µL water. This dilution was measure at 260 and 280 nm and you obtained these OD's:

OD_{260} = 0.015
OD_{280} = 0.008

Remember that 1 OD_{260} = 50 µg/mL.

0.015 * 50 * 25 = 18.75 µg / mL DNA in original stock
mix in order:
16.2 L water and then add
3.5 µL 10X buffer
13.3 DNA from original stock
1 µL EcoR I
1 µL BamH I
35 µL total volume

e. What can you tell me about the quality of DNA in question d. above?
Because 260:280 ratio was near 2, the purity of the DNA is very high.

FWs: NaCl = 58.5; SDS = 288; TBE = 238; EcoRI = 125; BamHI = 415; EtBr = 394; EDTA = 416; Tris = 121; HCl = 36.5; agarose = 204.

12 pts.

2. One of your job applications went to a cancer lab and they have another task for you. Design PCR primers for the yeast gene CDC26. Be sure to list each primer from 5' to 3' and tell me the melting temperatures of each primer. You may use this web page and its associated links <http://www.bio.davidson.edu/courses/Molbio/Protocols/IDHwebpage.html>.
Tell them how many amino acids are in CDC26 and its molecular weight.
VERY IMPORTANT: Your primers should be designed so you can express CDC26 using pET3a (see attached pdf file) expression vector and you must clone the CDC26 PCR product into the BamHI site. BamHI recognition sequence is GGATCC and the cut site looks like this:

5′ G$_{-OH}$ $_{PO4-}$GATCC 3′
3′ CCTAG$_{-PO4}$ $_{HO-}$G 5′

CDC26 coding DNA:

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       1  ATGATCAGAA GGGCCCCTAC CACCTTGCAG CTCAGTCACG ACGACGTAAC
      51  CTCTCTGATC GATGACCTGA ACGAGCAGAA ACTCAAGCAG CAGCTGAATA
     101  TCGAGAAGAC AAAATACTTC CAAGGAAAAA ATGGCGGATC GCTGCACTCC
     151  AATACAGACT TTCAGGACAC ATCGCAGAAT ATCGAAGACA ACAATAACGA
     201  TAACGATAAC GATATCGATG AAGATGACGA CATGTCATCT TACAACGACA
     251  AAGCAGCCTC GGTAGCGCAC ACCAGAGTCC TCAATTCCTT GCATCTGTCC
     301  ACCGACAGCA ATACCGCCCA CGAGACGTCC AATGCAAACG ACAACCACAA
     351  CCCCTTCTAC ATCCGCGAGG AATAA
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Forward Primer = gcatggatccATGATCAGAAGGGCCCCTAC Tm = 66° C
Reverse Primer = gcatggatccTTATTCCTCGCGGATGTAGA Tm = 63° C
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124 amino acids and MW = 14 kDa (13.9 kDa mature protein)

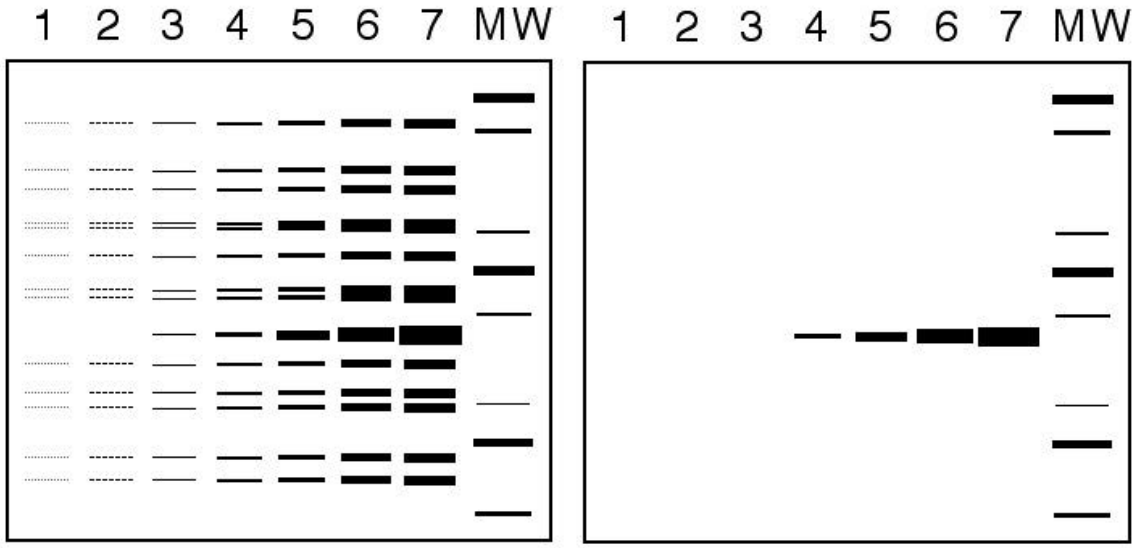
10 pts.

3. Read the 5' most 25 bases you can read from this gel and then tell me the template's sequence from 5' to 3'. <http://www.bio.davidson.edu/course s/genomics/seq/ezytwo.gif>. These two gels contain the same sequencing reaction but have been run for different lengths of time. The one on the left was run for only 2 hours while the right gel was run for 4 hours.

template: 5′ C CTT CAC ATG GAT GTT CTC CCT CGT 3′

10 pts.

4. Interpret these results. An experiment was performed where cells grown in tissue culture were pulse-labeled with ^{35}S for different amounts of time. At the end of the experiment, all the protein were electrophoresed on a gel which was later exposed to X-ray film. At the same time, an identical set of cells were grown but not pulse-labeled. They were used for a western blot with an antibody that binds to the calcium pump.

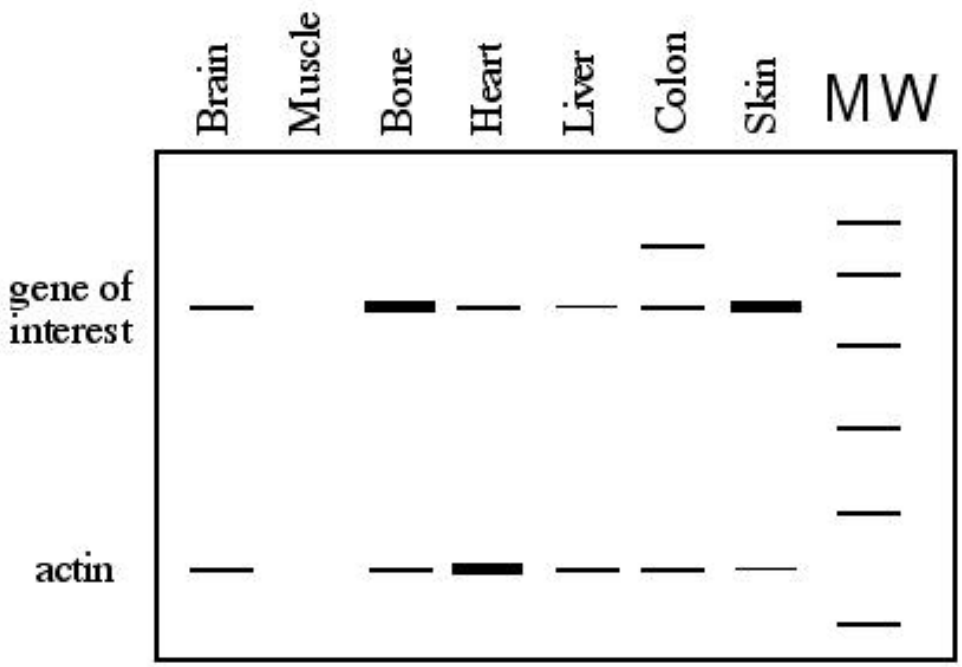


data from pulse-labelling

data from western blot

Key points:
proteins were labeled with ^{35}S and more proteins accumulated label over time 1➡ 7.
Calcium pump was induced at time point 3 but was not detected by the antibody until time point 4 in the western blot. Therefore blot is less sensitive than labeling in this experiment.
Once the pump was experessed, it accumulated faster than other proteins.
We can determine the molecular weight of the pump.

14 pts.
5.



data from Northern blot

Interpret the data from this Northern blot above as completely as you can.
Key Points:
Brain: gene of interest (goi) was expressed about the same as actin
Muscle: technical problem since no actin band – ignore this lane
Bone: more goi expressed than actin

Heart: less goi expressed than actin
Liver: less goi expressed than actin
Colon: goi about the same as actin plus a new band – either closely related gene or alternative splicing product
Skin: much more goi expressed than actin (highest of all tissues)

10 pts.
6. LIST the amino acids abbreviated here: MLK DAY WISH
methionine, leucine, lysine, aspartate, alanine, tyrosine, tryptophan, isoleucine, serine, histidine.

15 pts.
7. You have finally landed the job of your dreams. You work in Paris as a lab tech with a window overlooking the Eiffel Tower. To keep this job and your office with a window, you have to solve this problem. There is a need to produce a lot of human factor VIII for hemophiliacs but a new method is needed. Design an expression system that allows you to overproduce the protein in the green leaves of kudzu. You do not want to express factor VIII in any cells except those of the leaves so be careful how you design your DNA. You do not need any sequence information for this design but you should combine diagrams with text to explain your idea. Do not worry about how you will get the DNA into the cells, just know that you can get the DNA into a kudzu seed and it will propagate into every cell of the plant.

Key features:
Need leave specific promoter upstream of human factor VIII gene (without its normal promoter) or cDNA. Gene related to chlorophyll production or some hypothetical gene was fine.

8 pts.
8. Well, your kudzu idea was such a hit, you have been promoted to technician level III. You have been given a technician of your own to do the grunt work which frees you up for more creative solutions to the world's problems. Now the lab is in a jam again and needs your input. Design a protocol to clone the calcium pump cDNA from a black rhino. There is no information on this protein or gene but you know all muscles make a lot of this protein. You also know that many calcium pumps have been cloned and sequenced from many other organisms including yeast, plants, and animals. You have been given this assignment on a Friday and the answer is expected on Monday morning. *Bon chance*.
One possible answer among many acceptable ones:
Get calcium pump sequence from closely related species (elephant, etc.)
Extract mRNA from rhino muscle
Use elephant cDNA as probe on either rhino Southern or Northern blot to verify it will bind
If elephant binds rhino, use it to probe a rhino muscle cDNA library
Pull out calcium pump cDNA and sequence. Verify its the right by comparing to elephant sequence.