

Spring 2010 Molecular Biology Exam #2 – Applying Lessons

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 all exams are submitted on Monday March 22. **EXAMS ARE DUE AT 12:30 ON MONDAY, MARCH 22.** You may use a calculator and/or ruler. The answers to the questions must be typed under each question unless the question specifically says to do otherwise. If you do not type your answers on the appropriate pages, I may not find them unless you have indicated where the answers are. I would like you to print your exam with your interwoven answers. Color ink is not required.

-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):

Anne Swerkey

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)?

Average was 84.5%

Added 8 pts to everyone's

8 pts.

1. Tell me how to make the following two solutions. You must show your work to receive partial credit for wrong answers.

a. Make 25 mL of a 12% w/v EDTA, 2X TBE solution.

Dissolve 3 g of EDTA in about 15 mL water. Then add 5 mL 10X TBE solution. Bring up to 25 mL with water.

b. Your DNA solution is 1.2 $\mu\text{g}/\mu\text{L}$ in concentration. You need 100 μL of this DNA at a new concentration of 36 $\text{ng}/\mu\text{L}$. Tell me a good way to make this new DNA solution.

Take 3 μL of the stock DNA solution and put it into 97 μL water. Done.

FWs: NaCl = 58.5; EtBr = 394; EDTA = 416; Tris = 121; HCl = 36.5; agarose = 204. Other raw materials include SDS = stock solution of 20%; TBE = stock solution that is 10X;

8 pts.

2. Identify the origin of this DNA sequence. Name the species and gene.

GCATTGAAATACAGTTGTAATCTTCCCATTCCTTTTTGACCTGGAGATCAAGAGTGTAT
GAGGGATCCATCAACGAAGAAAGACTACACCACGTTCAAAGATGGTTTATGCTAATTAAT

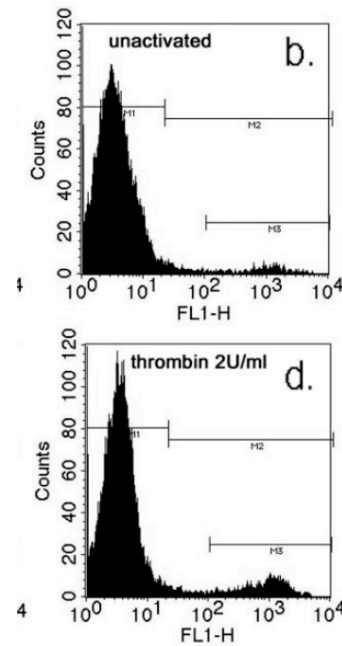
Erd1 from yeast, *Saccharomyces cerevisiae*.

3 pts.

3. In 1983, Hugh Pelham and his graduate student walked into the lab to discuss some research. During their talk, they failed to notice a gel running in the back of the room. Before they could reach the door, it exploded and burned down the lab. It was during this traumatic time, that Pelham shouted one of the most famous lines in molecular biology which we discussed in class prior to spring break. What was that now famous line? Love the shirt! A version of this story was told in class the Friday before spring break.

12 pts.

4. To the right, you see data from an experiment where cells were either untreated or treated with 2 units of thrombin (you don't have to know what thrombin is). Afterwards, cells were incubated with fluorescent ligand (FL1-H) and subjected to flow cytometry. Describe the two populations of cells in zones M1, M2 and M3 for panels b and d. No more than two sentences for each of the 6 cell groups (2 panels x 3 sections each).



Key points:

M1 – b has about the same number of cells as d. Panel b is wider but panel d is taller. Hard to know for sure unless you measured the area under the curve, not consider the height of the peaks.

M2 – about the same if you exclude cells in region M3.

M3 – More cells in panel d than panel b. This tells us that thrombin activates a few cells to have very high concentrations of the receptor for FL1-H.

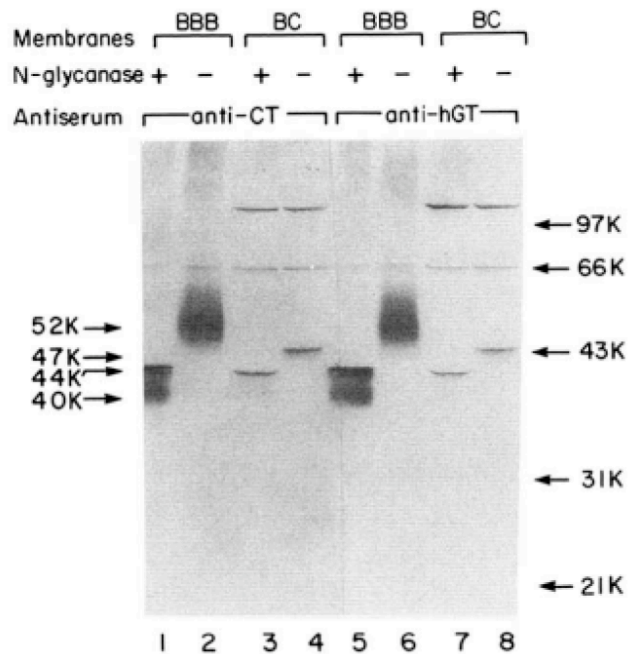
Order of fluorescence M1 < M2 < M3.

15 pts.

5. On the next page, you will see the results of two western blots using antibodies against either human glucose transporter (hGT) or the carboxyl terminus of the human glucose transporter (CT). BBB stands for blood brain barrier meaning that the source of proteins was from purified blood vessels of a human brain. BC indicates proteins isolated from brain cells (neurons only).

a) What do you think N-glycanase does? Look at the word to speculate on its function.

b) Summarize the data from this western blot. To get full credit for this question, number the proteins from 1 – 6 starting with the largest protein and summarize what you learned about each protein. Your answer should be a numbered list and keep the answers to no more than 3 sentences per number.



a) Removes sugars from asparagiNe amino acids.

b)

1. largest about 110 kDa. In neurons (BC) only. Not glycosylated. Large version of this protein.

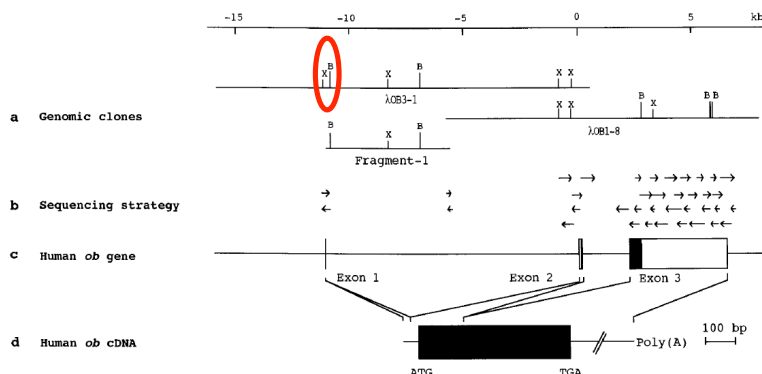
2. about 66 kDa. All lanes, may be artifact to be ignored.

3. 52 kDa in BBB. Smear that goes away with glycanase, therefore a glycoprotein.

4. 47 kDa protein in BC that is glycoprotein.

5. 44 in all lanes probably the same protein 52 → 44 and 47 → 44.

6. 40 kDa protein may be degradation product or shows that 52 kDa was actually two different proteins.



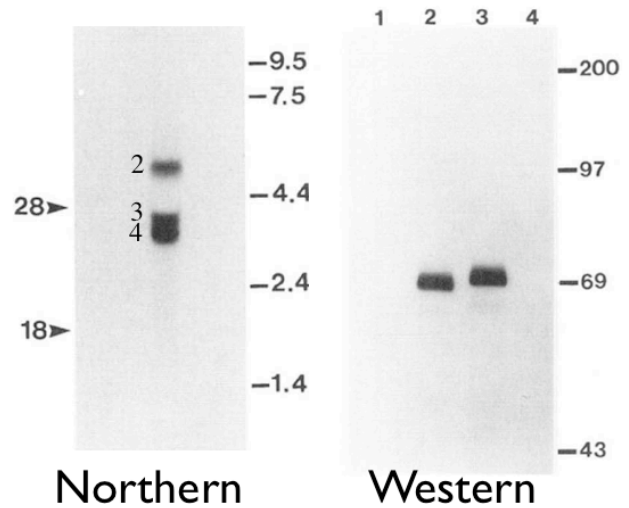
11 pts.

6. The map above shows the final outcomes from a series of experiments designed to define the boundaries of a eukaryotic gene. Draw a circle around the restriction fragment you would use as a probe for a northern blot to detect the existence of alternative splicing that included or excluded exon 1. Explain why you chose this fragment in 3 sentences or less.

Smallest restriction fragment that binds only to exon 1. Don't want intro and don't want other exons. Not ideal to get a negative result with this probe when exon 1 spliced out, but exon 1 is too small to see size shift in mRNAs.

12 pts.

7. The paired figures to the right show you a northern blot and a western blot for the same gene/protein. There is only one lane for the northern blot, but 4 lanes for the western blot. Lane 1 is a negative control for the western blot. The other 3 lanes in the western blot were produced by expressing in COS cells the cDNAs corresponding to the 3 numbered bands from the Northern blot. Interpret the data from the western blot but limit your answer to no more than 6 sentences total.



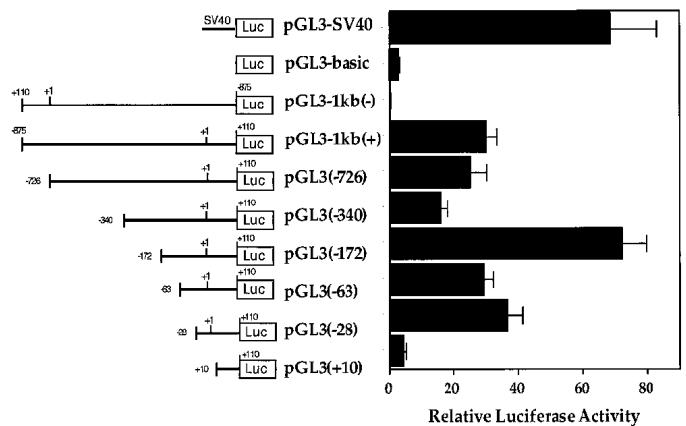
#2 mRNA biggest, protein smallest. Alt. splicing with a long 3' UT region.

#3 mRNA middle, protein bigger than #2. Different exon combination that makes a longer protein.

#4 mRNA smallest, protein is AWOL. Perhaps epitope spliced out or protein not made/stable. Hard to be sure since negative result.

16 pts.

8. The figure to the right shows you a series of deletion mutations made to a promoter. The coding gene downstream of the promoter encodes luciferase (Luc). Explain these data using a numbered list from 1 – 10 with 1 being the SV40 construct and going down the constructs in order. You are not allowed to write more than 2 sentences for each number.



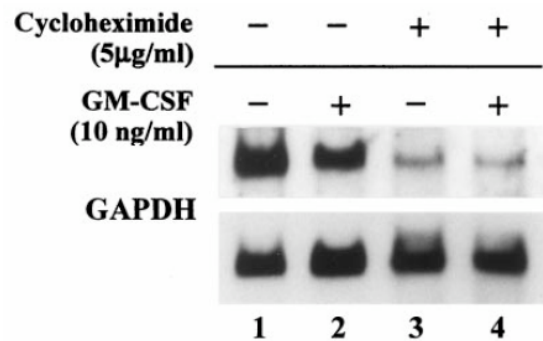
1. SV40 viral promoter, strong, + control.

2. no promoter, - control.

3. inverted promoter, - control.
4. wt promoter = + control and baseline for promoter strength.
5. short deletion about the same as wt promoter
6. bigger deletion gives slightly reduced output.
7. bigger deletion gives strongest output. Probably deleted a repressor binding site but retained an activator of some sort.
8. bigger deletion returns to wt level (#4) so probably deleted the activator uncovered in #7.
9. bigger deletion similar to 4, 5, and 8.
10. too much deleted, similar to #2.

15 pts.

9. A mystery gene was being studied, we'll call it gene E for short. Bone marrow cells were treated as indicated in the figure with + indicating the presence of the compound and - indicating the absence of the compound. GAPDH is a house keeping gene meaning its transcription is unaffected by any of the treatments. GM-CSF is a growth factor normally secreted in the bone marrow.



a) Estimate a relative amount of gene E mRNA in this Northern blot for lanes 1 – 4. Use relative numbers like 1, 10, 100, not words like little, some, lots.

Something similar to:

1, 0.5, 0.1, 0.05.

b) What is the purpose of including GAPDH in this experiment?

Loading control for comparison against Gene E.

c) What conclusions can you draw about gene E?

Requires protein production to be transcribed, probably an transcription factor. One person speculated a positive feedback loop of E producing more E. I like that idea. +1 point.

Gene E is repressed about 50% when exposed to GM-CSF.

+2 Bonus points: If you remember what cycloheximide is, then you do not need to email me. If you want to know what cycloheximide is but you cannot remember, then email me and I will tell you what it does but you will lose your 2 bonus points. You can email me during working hours at my college account or my personal account amalcolm.campbell@gmail.com in off hours. But be aware that I keep normal hours, I have a family I enjoy being with on the weekend, so plan your email accordingly.

Inhibits ribosome and blocks translation.