# Spring 2005 Molecular Biology Exam #2 – Applying Lessons

**Answer Key**

There is no time limit on this test, though I have tried to design one that you should be able to complete within 5 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 21. **EXAMS ARE DUE AT 9:30 ON MONDAY, MARCH 21**. You may use a calculator and/or ruler. The answers to the questions must be typed unless the question specifically says to hand 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.

For the figures, I took photographs from journals, so you may detect warps or angles that seem odd. This is due to my need to reduce glare and shoot form about a 45 degree angle. Do not take this odd perspective into consideration for your answers. Ignore the tilted angles of the figures.

**-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):

Average = 83.1%
Range = 55 – 99
Added 8 points to all scores

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)?

**6 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 275 mL of a 1.5% SDS, 1X TBE solution.

27.5 mL 10X TBE

20.63 mL 20% SDS

226.87 mL water

b. Make 450 mL of 2M NaCl, 500 mM Tris, 0.01 M EDTA.

52.65 g NaCl

27.23 g Tris

1.87 g EDTA

Dissolve in 350 mL water, bring up to 450 mL with water after dissolved.

**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 this protein: `CMAVMAIHLILLTAGTALLLIQVLNL`. Tell me the name of the protein, the species from which it came and what method you used to reach your answer.

macrophage receptor with collagenous structure [*Mus musculus*]

Used modified BLAST "• Search for short, nearly exact matches" There are other valid methods.

**10 pts.**

3. Calculate the molecular weights of the two bands in figure 1. Do receive credit for this answer, you must show me the graph you used to generate your answer. You may use Excel for this answer. Use the 2 dash marks at the top as the edge of the wells in the gel.

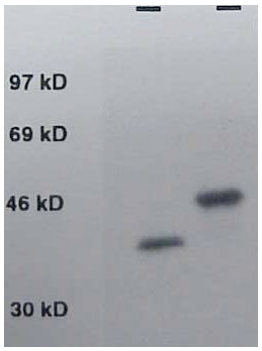
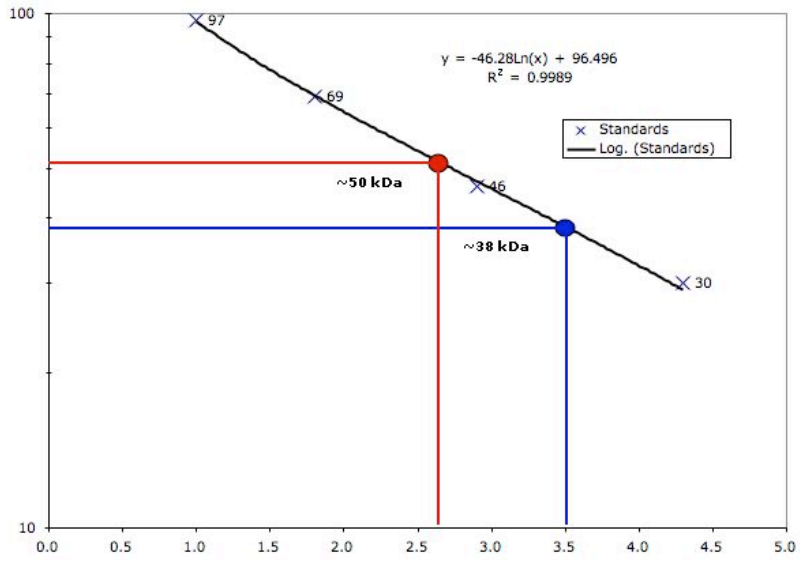



Figure 1.



**16 pts.**

4. A series of experiments were performed to understand what binds to the Tyro3 receptor on the surface of some cells. Left side: Cell expressing Tyro3 were bathed in different media contain various unknown components. When the receptor binds its ligand, it autophosphorylates. Right side: They engineered cells to secrete a soluble form of Tyro3 (i.e. no transmembrane domain; called sTyro3) or a soluble form of an unrelated receptor called TrkB (i.e. sTrkB).

a. Describe the main point for the left side of this figure.
b. What effect did sTyro3 have on phosphorylation of the normal Tyro3?
c. What was the purpose of creating sTyro3?
d. What effect did sTrkB have on phosphorylation of the normal Tyro3?
e. What was the purpose of creating sTyro3?

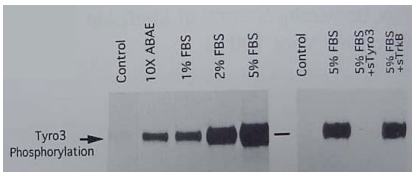



Figure 2.

a. This is to demonstrate a dose-dependent effect and that more than one source of ligand is available. It also serves as a positive control so we know what to look for.
b. It blocked phosphorylation by binding the ligand before it could bind to the receptor.
c. This demonstrates the specificity of the ligand for this one receptor. Protein S is extracelluar ligand.
d. sTrkB had not effect on Tyro3 phosphorylation.
e. This was intended as a negative control in that you expect nothing to happen differently. It demonstrates that excess soluble protein does not block phosphorylation because this receptor does not bind the right ligand.

**12 pts.**

5. Eventually, the investigators fond the protein that is the Tyro3 ligand and they very cleverly called it protein S. They cloned the protein S gene and transfected the protein S gene into COS cells. Describe the data in figure 3.

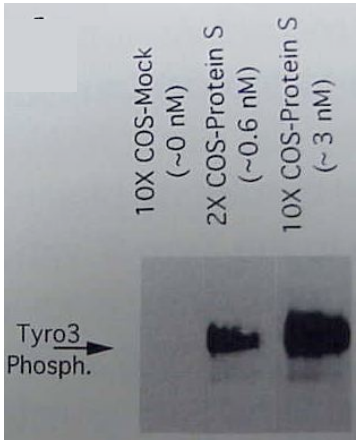



Figure 3.

The more S protein you supply, the more Tyro3 can be phosphorylated. There is a dose-dependant level of phosphorylation. Also, we know that Protein S must be a secreted protein since it stimulates the phosphorylation of Tyro3.
COS cells (derived from monkey kidneys) express Tyro3 proteins.

**10 pts.**

6. Figure 4 shows two northern blots with the indicated probes. Assume that good RNA was loaded in every lane. Interpret these data taking into consideration the data from question 5. Do not describe every lane, just the main lesson or lessons from this figure. The abbreviations are: THYmus, SPLeen, LIVer, KIDney, LuNG, Skeletal Muscle, HeaRT, SKiN, INTestines, UTeRus, OVaRY, PLAcenta, TeSTes, Adult BRain. Support your conclusions with data.

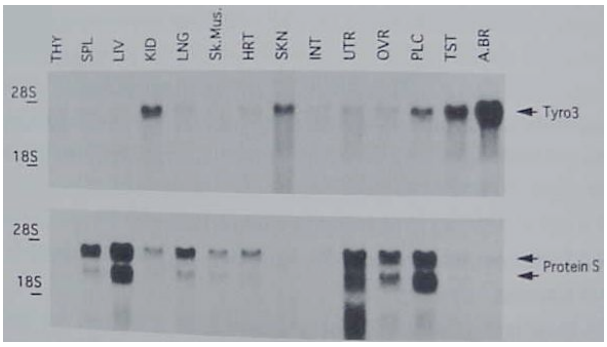



Figure 4.

The interesting fact is that Protein S is not transcribed in the same cells as Tyro3. Therefore, protein S is made at a distance from Tyro3 and moves through the circulatory system (blood and/or lymph) from its site of production to its site of action in brain, testes, placenta, skin, kidney (as in COS cells) and a bit in the uterus and ovaries. Also, there are two forms of Protein S, but we don't know if both of them are equally effective on Tyro3.

**12 pts.**

7. Write down the two sequences that appear in figure 5, from 5' to 3'. After you have written down the two sequences on separate lines, Write the deleted base or bases missing in the mutant.

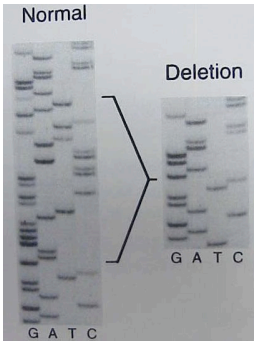



Figure 5.

**Normal**

GAG CAG GTC GAA GGG GAT GGC GGC CAC CAT GTC GAT GAG GAA CCA GCC

**Deletion**

T GAG CAG GTC GAG GAA CCA GCC

**Deleted bases**

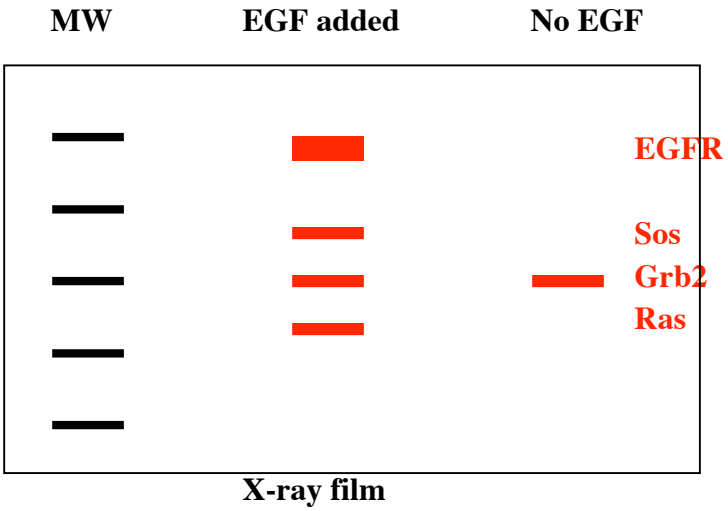
GAA GGG GAT GGC GGC CAC CAT GTC GAT

**16 pts.**

8. Figure 6 shows what an investigator thinks the EGF receptor looks like when it has bound EGF (epidermal growth factor). When the ligand is bound, the heterotrimer of Grb2, SoS and Ras bind to EGFR.

Draw the results from an immunoprecipitation experiment if all the proteins were labeled with $^{35}S$, separated by SDS-PAGE, and imaged via fluorography

on X-ray film. Use the blank film below to draw the data if the antibody used binds to Grb2.



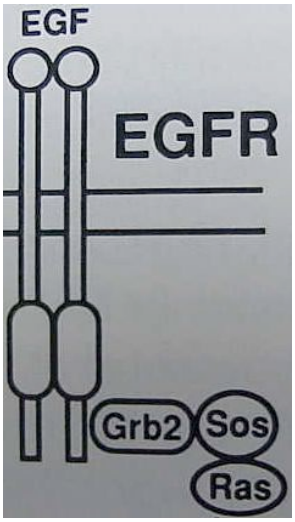




Figure 6.

**10 pts.**

9. A population of identical cells was measured by flow cytometry. The data are presented in Figure 7, with fluorescence intensity on the X-axis and number of cells on the Y-axis. Explain the data for the 5 surface proteins as labeled by fluorescent antibodies in 5 different experiments.

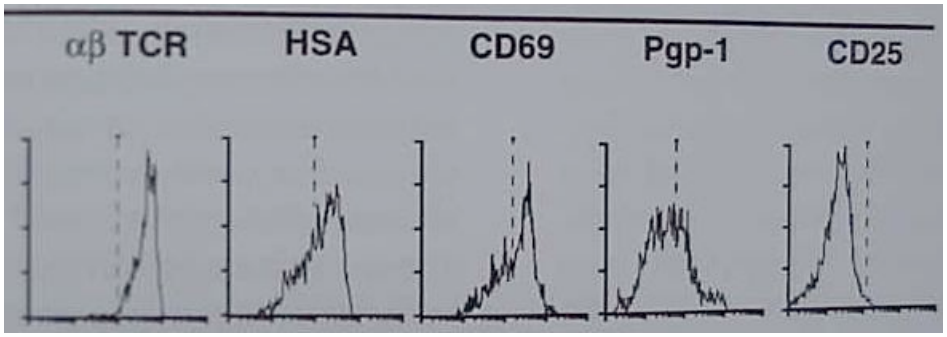



Figure 7.

The cells are identical, so there is no genetic variation. Therefore the only thing left to do is rank the 5 proteins in order of amount of protein and range of protein.

- TCR has the narrowest range and the highest expression level.
- HSA has the next highest level, but its range is very broad.
- CD69 is the next most abundant and its range is also fairly broad.
- Pgp-1 is the next most abundant with the broadest peak of the 5 surface proteins.
- CD25 has the lowest abundance with a fairly narrow range of expression levels.