Spring 2005 Molecular Biology Exam #3 – Final Exam

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 2 pm Monday May 9, 2005. **EXAMS ARE DUE AT 2 PM ON MONDAY, MAY 9**. You may use a calculator and/or ruler. The answers to the questions must be typed unless the question specifically says to write/draw 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 inserted in this test, 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.

There are 5 pages, 10 questions, and one bonus question in this exam.

-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):

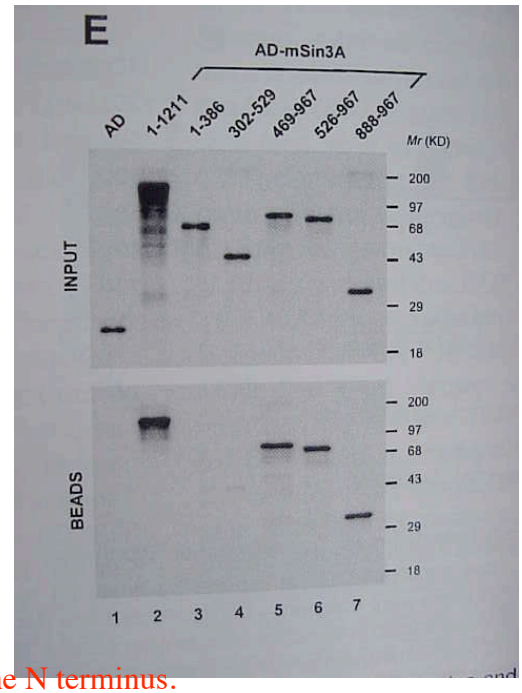
Write out the full pledge and sign:

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)?

12 pts.

1) AD is a part of a transcription factor. MSin3A is the name of another protein that was fused onto AD.



The top panel shows a fluorograph of proteins used in an immunoprecipitation experiment. The bottom panel shows the results of the immunoprecipitation bound to the antibody-covered beads.

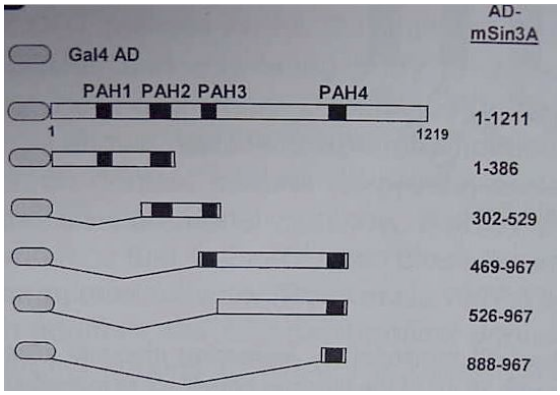
Interpret the data as completely as you can. Use only This figure for your interpretation.

Key points:

- the epitope for the antibody is between aa. 888 and 967
- the epitope is linear
- the antibody does not bind to AD
- the smear in lane 2 above is due to degradation mainly at the N terminus.

8 pts.

2) Now consider the extra information in the figure here→. Improve your answer for question 1 by including the new information shown in the cartoon figure that shows the 4 PAH domains of mSin3A.

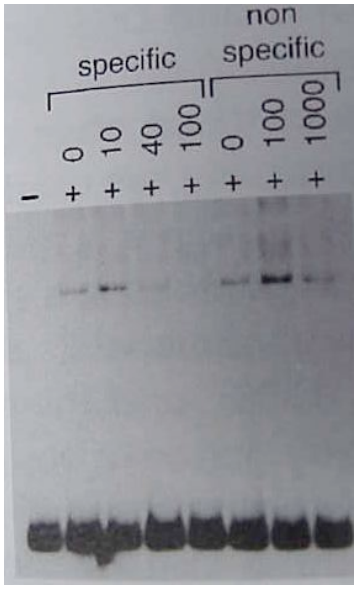


Key points:

The antibody binds to the 4th PA domain.

10 pts.

3) In the third figure, a radioactive DNA probe was incubated with a protein we'll call CWTG, except in the lane marked by "-" in which no protein was added. In the lanes marked by "specific", extra non-radioactive DNA was added of identical sequence to the probe in the amounts indicated (don't' worry about units here). Non-specific means DNA of different from the probe was added to the mixture. Finally, all the mixtures were added to a non-denaturing PAGE and exposed to X-ray film. You are seeing the X-ray film.

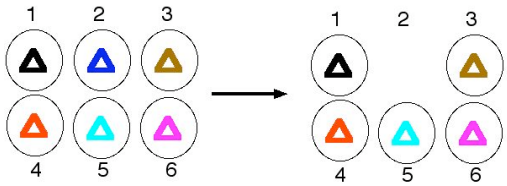


a. Tell me what is demonstrated in this figure.

Key points: CWTG binds to the probe. It looks like only one molecule binds since only one main band is seen. CWTG can be bumped off the radioactive probe by being incubated with non-radioactive probe.

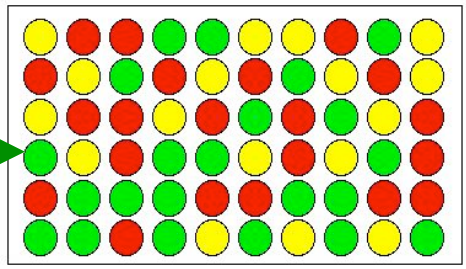
b. What was the purpose of the non-specific DNA?
Key points: non-specific DNA indicates that the loss of band shift when adding cold probe was not simply a consequence of adding so much DNA but a consequence of competition due to the same DNA sequence binding to CWTG.

8 pts.



4) Joe DeRisi sent me an email problem and said if any Davidson students wanted to go to UCSF for graduate school, I'd have to answer his question. Please help me! Hundreds of different knockout strains of *Plasmodium* were created, each with a different gene deleted (only 6 are shown here). In place of the deleted gene, they inserted a unique segment of DNA that could be amplified by PCR. The parasites were grown in a blood culture plus a new drug his lab created. Only 5 of the strains survived (see figure).

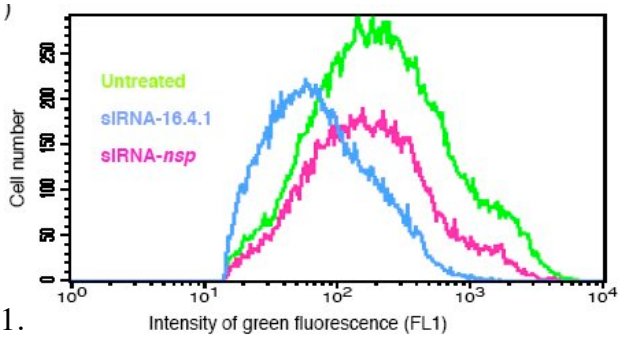
Joe's lab group performed DNA microarrays with all unique segments amplified and colored green from the starting pool of strains (only 6 are shown here). At the end of the experiment, they amplified the remaining unique segments and colored the DNA red (only 5 are shown here). The two colors of DNA were incubated with a new Plasmo-Chip and the results shown below. Each spot represents a different unique segment of DNA.



a. Draw an arrow pointing to a spot that could represent strain #2 from the figure above.
Key points: green = 6 starting strains
red = final 5 strains
b. What color would you expect to see if a strain was resistant to the new drug? Explain your answer.
Key points: you would expect resistant strain to grow faster than others. Therefore the spot should be red (faster than average growth and thus disproportionately over represented in second sampling).

9 pts.

5). A cell line was created that had GFP coding DNA fused onto the 3' end of another gene called 16.4.1.
Three populations of the cells were treated as shown in this figure (nsp indicates the siRNA targeted an unrelated transcript; the two colors of green do not match, but they should have).

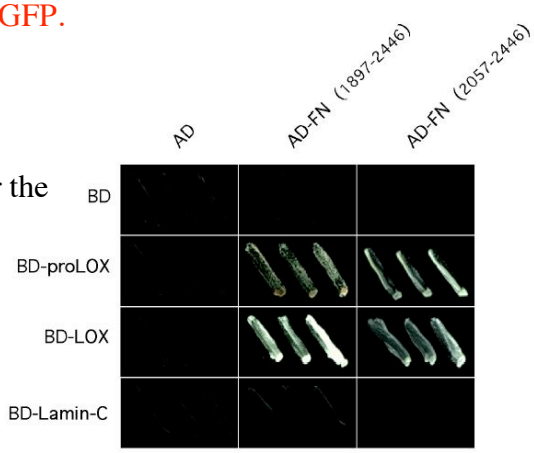


Summarize the experiment and results to someone in Bio111.
Key points: - Untreated shows you the amount of GFP produced normally.
- siRNA-16.4.1 shows the effects of siRNA silencing the GFP mRNA through RNAi (about 4 fold reduction in GFP production as shown by shift to the left from 200 to 50 FL1).

- siRNA-nsp shows the non-sequence specific consequences of adding extra siRNA to a cell. There are fewer cells, but these cells contain about the same amount of GFP.

10 pts.

6) A yeast two-hybrid experiment was performed using the parts of two proteins shown in the figure (FN and LOX or the unprocessed version called pro-LOX).



The white streaks are yeast cells growing without added histidine and 12 different petri dishes without histidine were used for the 12 different combinations.

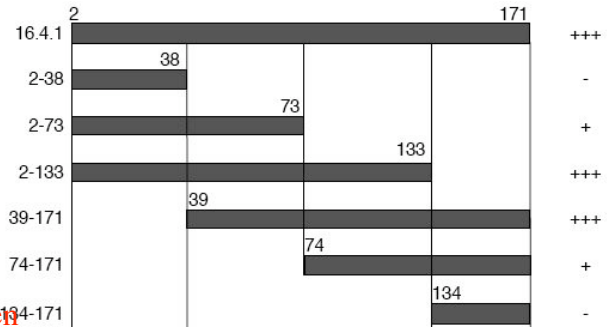
Summarize the conclusion(s) based on these data.

Key points: - FN and LOX appear to interact.

- LOX interacts with FN better than proLOX based on the greater number of cells.
- LOX can interact with aa. 2057 – 2446, but appears to interact a bit better with the longer construct.
- all negative controls appear to have worked fine, thus we can rely on these positive results.
- Lamin-C does not interact with the portions of FN used in this experiment.

9 pts.

7) A CAT assay was performed using various parts of the promoter for gene 16.4.1 as shown to the right.



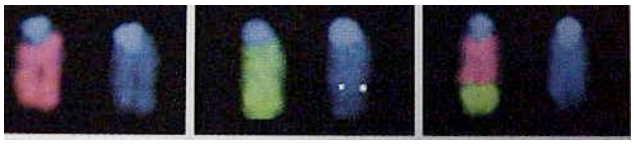
Summarize the main conclusion(s) based on the outcome for the constructs as shown to the right of this table.

Key points: The promoter's most potent section lie between bases 39 and 133. The two sections 39-73 and 74-133 work weakly compared to the same two parts in conjunction with each other.

8 pts.

8) FISH was performed on *wt* human chromosomes 12 and 15 as indicated. The blue versions were stained with a universal DNA dye. Patient JD was tested and these results were found. Explain what has happened to JD.

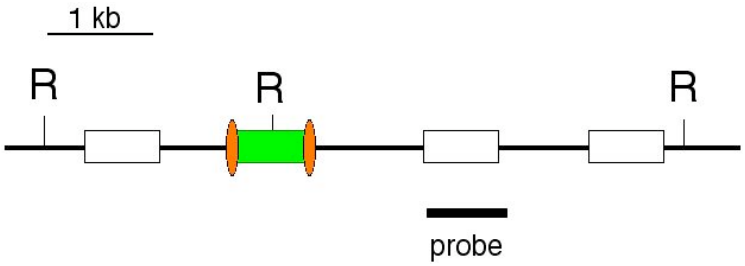
wt Chrm. 12 *wt* Chrm 15 Patient JD



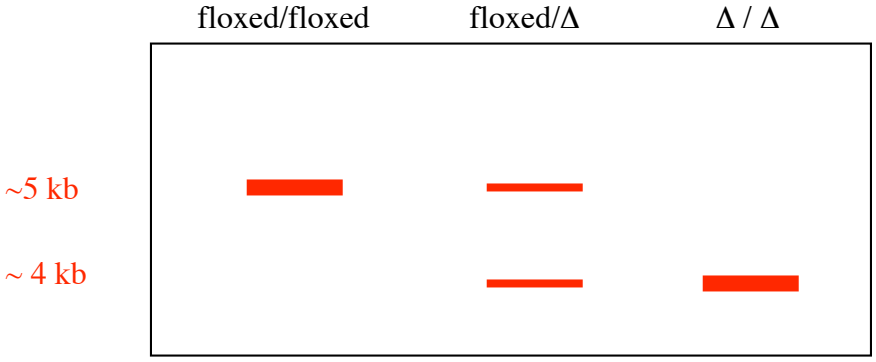
Key points: JD appears to have suffered recombination between Chromosome 12 (pink) and 15 (green). One of JD's chromosome has about 2/3 12 and terminal 1/3 from chromosome 15.

14 pts.

9) A gene was modified by homologous recombination with the results shown here. The 3 exons were left intact but a forth exon was inserted so GFP would be fused into the normal protein but GFP would **not** alter the reading frame for any of the other exons. The GFP exon has been floxed.



Draw the results for a Southern blot using the probe shown above. Include MW sizes for each band that you draw.



12 pts.

10) To get your first job, you'll need to be able to make these solutions:

a) How do you make a 1.3% (w/v) 125 mL gel with 0.75X TBE and 1 μg/mL of ethidium bromide if your starting stocks are 5X TBE, agargose (FW = 149), and a 10 mg/mL stock of ethidum bromide?

b) Make 300 mL of a solution that is 0.5% SDS (v/v), 1.4 M NaCl and 10 mM Tris.

7.5 mL of 20% SDS

24.6 g NaCl

0.36 g Tris – dissolve in about 200 mL of water, add SDS and then water to 300 mL final volume.

c) Produce 0.5 L of a wash solution that is 1 M NaOH, 1.5 M NaCl, and 7% (w/v) powdered milk.

20g NaOH + 43.9g NaCl + 35g powdered milk; dissolve in 400 mL water and add water to 500 mL final volume.

FWs: NaCl = 58.5; EtBr = 394; EDTA = 416; Tris = 121; HCl = 36.5; agarose = 204. Other raw materials include SDS = stock solution of 20%; NaOH = 40.

+2 Bonus Points

10) When the first microarray paper was published in 1997, Joe DeRisi was the first author. What did his PI (principle investigator and thus head of the lab) Pat Brown say when the paper appeared in *Science*? GO JOE!