ORIGINAL

1999 Molecular Biology Exam #2 – Using Molecular Tools

There is no time limit on this test, though I have tried to design one that you should be able to complete within 2.5 hours, except for typing. You are not allowed to use your notes, or any books, nor are you allowed to discuss the test with anyone until Monday March 22, 1999. **EXAMS ARE DUE AT 10:30 ON MONDAY, March 22**. 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.

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:

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

6 pts.
1. Figure one shows a Southern blot using genomic DNA from four mice. Tell me the genotype for these four mice if you know that the goal was to convert wild-type mice to mice that lack the gene for craving cheese. The mutation was to be created by deleting a large portion of the appropriate chromosome.

9 pts.
2. In figure 2, the stringency of the conditions for a Southern blot were altered.
a) What is the difference between high and low stringency as far as the results are concerned?
b) What experimental conditions were manipulated to alter the stringency?
c) Tell me "in plain English" what formamide does given that it was left out in the low stringency condition.

9 pts.
3. Figure three shows a series of 2 control dots and 7 experimental dots on a dot blot. In this experiment, The dots on the membrane are copies of DNA that will bind to one of two alleles (either the M. musc. allele or the M. spr. allele). mRNA was isolated from the 7 experimental individuals and converted to radioactive cDNA by RT-PCR. The PCR products were made single stranded and half of each cDNA was incubated with the two types of dots. The seven individuals were all heterzygous for the two alleles.
a) Interpret these results.
b) What is the function of the M and S lanes in this figure ?
c) Do you think that function (from question b) was successfully carried out? Explain your answer.

6 pts.
4. Figure 4 shows the results of a FACS experiment comparing two cell populations: wild-type and a mutant called 13Δ. Tell me what is going on in these two cell types if ethidium bromide was used to label the cells. Do not break your answer down to an account for each hour but just summarize what is happening over the 13 hours shown here. You might want to use a ruler.

8 pts.
5. Figure five is a well controlled experiment, but the MW standards did not photocopy well. Please interpret figure 5 D for me.

12 pts.
6. Figure 6, believe it or not, shows one line of sequence that was left out of a paper. You must use the WWW to answer this question and you may use any pages from the Davidson Biology web site that might help. Please tell me:
a) the name of the protein
b) what journal published the sequence first
c) what species this sequence is from
d) the first three and last three amino acid names from this line in figure 6
e) how big the mRNA is that encodes this protein
f) what chromosome this gene is on

10 pts.

7. Figure 7 did not photocopy perfectly. In the original, TRAF1 does not touch hTNF-R2.
a) Design an experiment to determine whether TRAF1 does or does not bind to hTNF-R2.
b) Design a second experiment to clone the cDNA for the protein that TRAF1 interacts with in the cytoplasm (indicated by a dark question mark below the white arrow).

10 pts.

8. On a related topic, Figure 8 shows an immunprecipitation experiment. GST is a generic protein that was used to fuse onto hTNF-R2 (ignore the icd part). Various fusion proteins were made that deleted a number of different amino acids of the cytoplasmic tail of hTNF-R2, as indicated in the figure. For example, "-16" means that the first 16 amino acids were deleted. D304-345 means that only those amino acids were deleted, while 384-424 means that only those amino acids were used in the experiment. All variations of hTNF-R2 were fused to GST and the antibody recognized GST only. The arrows indicated bands that are not the GST fusion proteins.
Interpret these results.

6 pts.

9. Figure 9B shows some analysis of the WASP protein. What can you tell me about this protein based off of figure 9B?

8 pts.

10. Here's an unusual question. Figure 10 shows the sequencing gels for a wild-type mom and a mutant dad. On your copy of figure 10, below the lanes, label each lane properly for each nucleotide.

8 pts.

11. Figure 11 is a Northern blot.
a) Tell me everything you can about this blot if you were told that probe X was used.
b) What is missing from this figure?

8 pts.

12. The last question is related to an abstract I saw. A gene that causes a disease has been localized to a small region of the X chromosome that you have cloned in cosmid (let's say 40 kb in length). This disease is caused by a lack of an unknown protein and results in immunodeficiency (loss of B and T cell function even though the cells are present). Tell me how you might clone this disease causing cDNA. Be sure to devise a way to demonstrate that you have the correct cDNA.

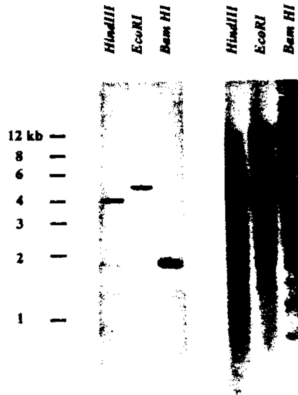
Figure 1

6.4 kb
5.2 kb

Fig 1

Southern blot analysis of genomic EcoRI-digested tail DNA hybridized with a ^{32}P-labeled 705 bp probe.

Fig. 2



Stringency: High Low

Figure 2. Presence of Arabidopsis

Wild-type DNA (1 μg/lane) was digested with the indicated restriction enzymes, fractionated by agarose gel electrophoresis, and blotted onto GeneScreen Plus filter (New England Nuclear). The filters for the left and right panels are duplicates. The blotted filter was probed with a DNA fragment corresponding to the N-terminal half of the gene under high (left) and low (right) stringency conditions. For high stringency conditions (left), the blotted filter was hybridized to the probe in 1% SDS, 2× SSC (0.3 M NaCl, 30 mM trisodium citrate), 10% dextran sulfate, 50% formamide, 0.1 mg/ml sheared salmon sperm DNA at 42°C. The filter was finally washed with 1% SDS, 2× SSC at 65°C and then with 0.1× SSC at 65°C. For low stringency conditions (right), the hybridization conditions were the same as for the high stringency conditions except that the buffer contained no formamide. The filter was finally washed with 1% SDS, 6× SSC at 50°C. Under high stringency conditions, only the predicted bands corresponding to gene were detected.

Fig 3

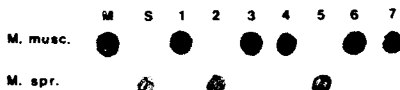


Fig 4

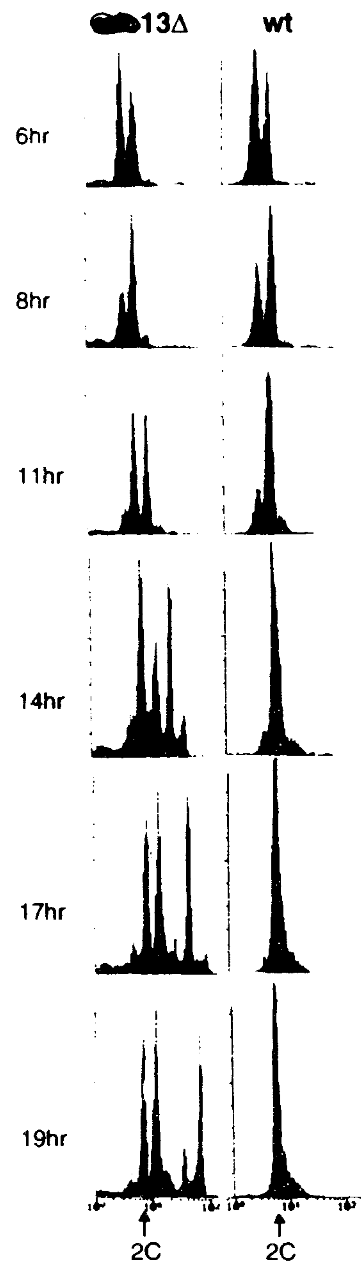
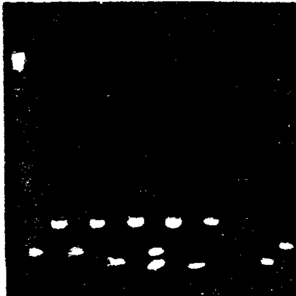


Fig 6

1261 AAAGGGCTCAGCCCTGCCTGGTTCCTGCAGCACGTCATCGTCAGGGACCTGCAGACGGCA 1320
421 K G L S P A W F L Q H V I V R D L Q T A 440

The sequence submitted to GenBank (accession number L33243) is correct.

Figure 5



(D) The expression of *Ich-1*$_L$ and *Ich-1*$_S$ in mouse tissues. The cDNA templates were reverse transcribed from mRNA isolated from thymus (lanes 1 and 2), adult heart (lanes 3 and 4), adult kidney (lanes 5 and 6), embryonic day 15 brain (lanes 7 and 8), and adult brain (lanes 9 and 10). Lanes 11 and 12 are negative controls (no DNA template). Lane 13, *Ich-1*$_L$-positive control Lane 14, *Ich-1*$_S$-positive control. *Ich-1*-specific primers were used in lanes 1, 3, 5, 7, 9, 11, 13, and 14. Actin primers were used in lanes 2, 4, 6, 8, 10, and 12. M, λ phage DNA HindIII digest as molecular weight standard. Actin, *Ich-1*$_S$ (S), and *Ich-1*$_L$ (L) are marked by arrows.

(B) Coprecipitation of GST–hTNF-R2icd fusion proteins in CT6 cell extracts. GST and GST fusion proteins containing the complete and mutant cytoplasmic domains of hTNF-R2 were incubated with lysates from CT6 cells, as described in Experimental Procedures. Arrows indicate bands

Figure 7

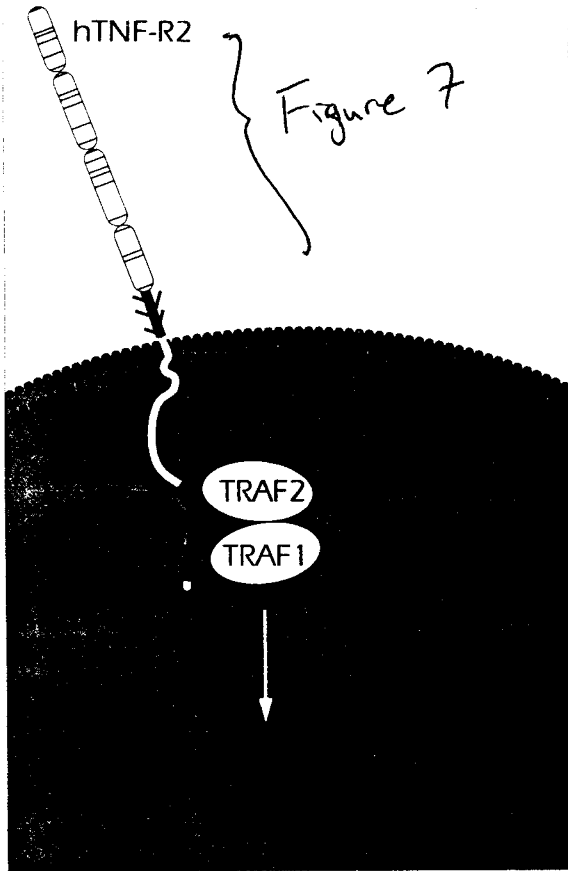


Figure 7. A Model for the Interaction among TRAF1, TRAF2, and the Cytoplasmic Domain of TNF-R2

See text for details.

Figure 8 both

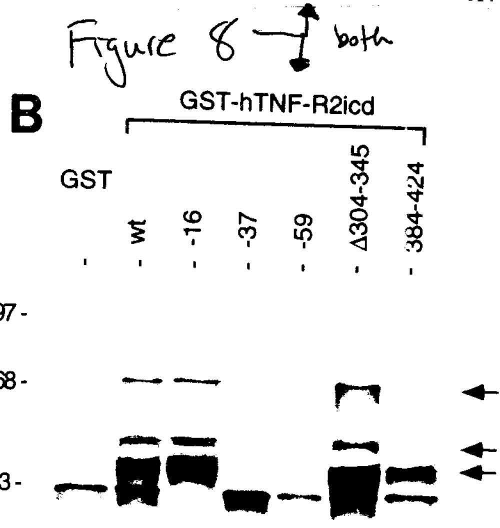


Fig. 9

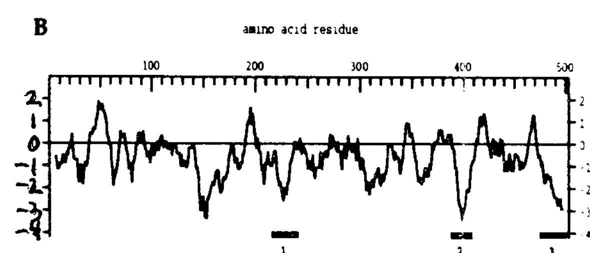


Figure 9. *WASP* cDNA Sequence, Predicted Polypeptide Sequence, and Hydropathy Plot

(A) DNA sequence of *WASP* and translated amino acid sequence. The polyadenylation signal sequence AATAAA and the putative nuclear localization signal are underlined. The nucleotides involved in mutations are circled (T211 and G291).

(B) Kyte–Doolittle hydropathy profile of the predicted amino acid sequence of WASP. The hydropathy plots were obtained by standard computer-assisted analysis, using the algorithm and hydropathy values of Kyte and Doolittle (1982). The

Figure 11

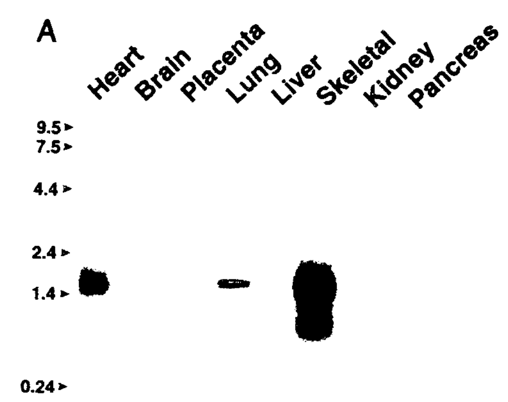


Fig 10