Spring 2002 Molecular Biology Exam #2 – Applying the Trade

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 Monday March 25, 2002. **EXAMS ARE DUE AT 11:30 ON MONDAY, MARCH 25**. 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 five pages for this exam, including this cover sheet. The figures were made by me taking digital pictures from journals. To avoid glares, I had to get funny angles, so don't evaluate any figures based on a slanted appearance. All of the figures are at the end of the test and you may not want to print them out and just look at them on the screen. However, you may print them if you prefer. Just be careful about which printer you send it.

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:

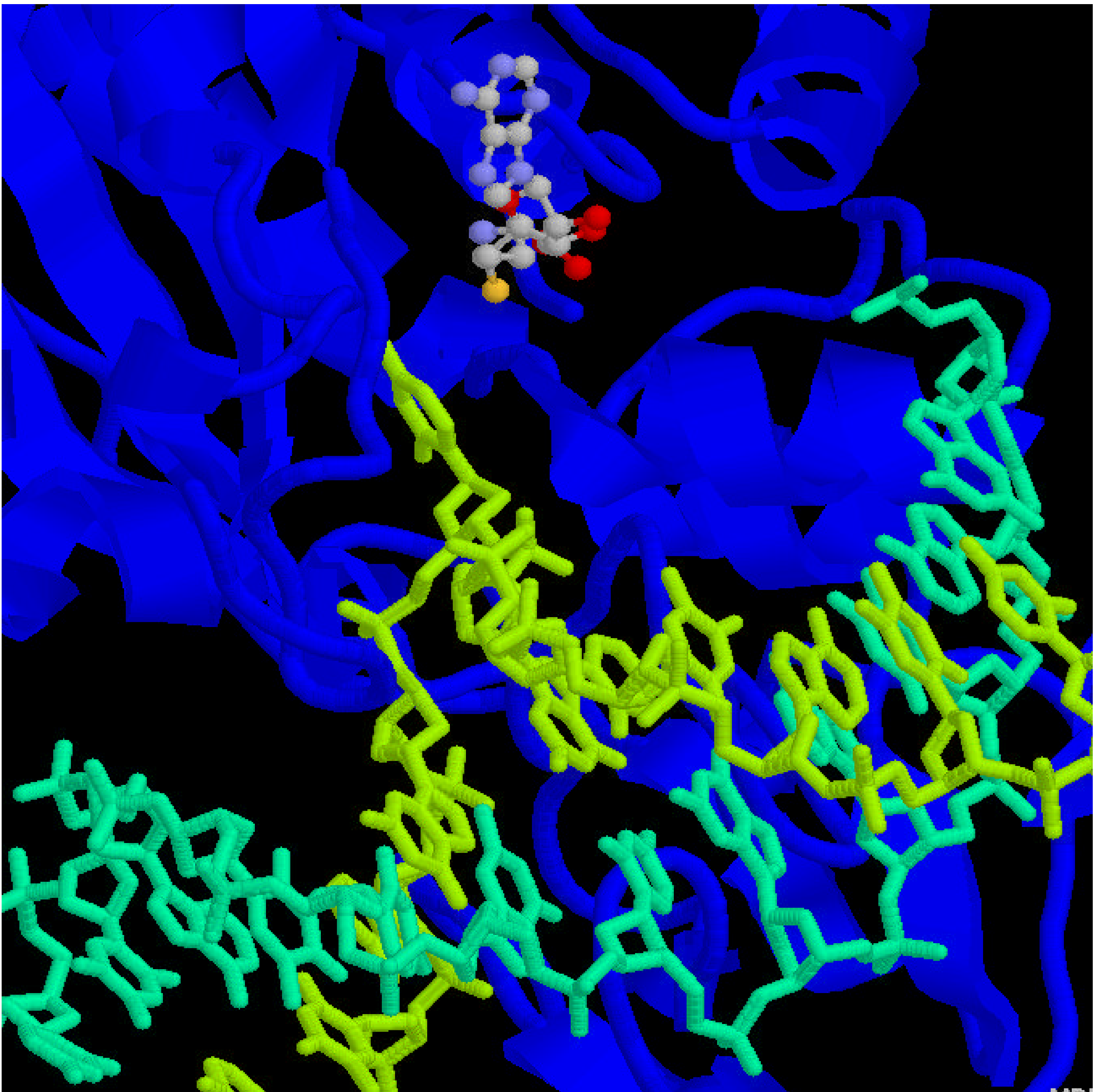
How long did this exam take you to complete (excluding typing)?

8 **pts.**

1. Go to this web site www.bio.davidson.edu/courses/Molbio/Exams/2002/what.pdb and tell me what is going on here (Netscape will work better than IE). You will need to manipulate the chime model in order to see a very unusual phenomenon. In order to get full credit, you will need to describe what you see happening that was frozen in time. I am not looking for minutia, there is a very striking image here and you will know what I am talking about when you see it properly.

The protein is in blue, DNA in shades of green, and a hetero molecule (SAH) in CPK colors. The most striking feature is one base (a cytosine) has been flipped out of the double helix. The hetero atom has a nitrogenous base but it is not a nucleotide.

This is a methyl transferase and the cytosine is going to be methylated. You did not need to know this to get full credit, but that is what you are seeing.



12 pts.

2. Figure 1 shows some FACS data. One type of cell was either treated with the drug TPA or not, as indicated. Each boxed graph represents a different experiment. For each experiment, cells were incubated with a fluorescently labeled antibody to one of three antibodies (top, middle, bottom) that bind to three different surface proteins (solid line). As a control, identically treated cells were incubated with an antibody that does not bind to the cell at all (dotted line). The Y-axis is the number of cells, don't worry about absolute values. Describe what is happening to the surface proteins in these six boxes.

For the top pair, TPA appears to reduce the number of binding sites on the cells.
In the middle pair, the number of binding sites per cell is increased by TPA treatment.
In the bottom pair, most cells are unaffected by TPA treatment, but a small number of cells increase their binding sites.

8 pts.

3. What can you tell me about the protein in figure 2?

Full credit was given for either: 1 TM domain or a soluble protein produced in the ER due to signal sequence.

14 pts.

4. In figure 3, total genomic DNA was isolated from the family members as shown. A single locus was sequenced for each person. The two women have a disease.

a) What is the molecular cause of this disease?

The females are heterozygous at one base and thus they have a point mutation in one of their two alleles. The male is homozygous wt.

b) Tell me the odds of the mother's grandchildren having this disease?

Because the females are heterozygous and have the disease, this is a dominant mutation. Therefore, the daughter has a 50% chance of passing this on to her children who would have the disease too.

20 pts.

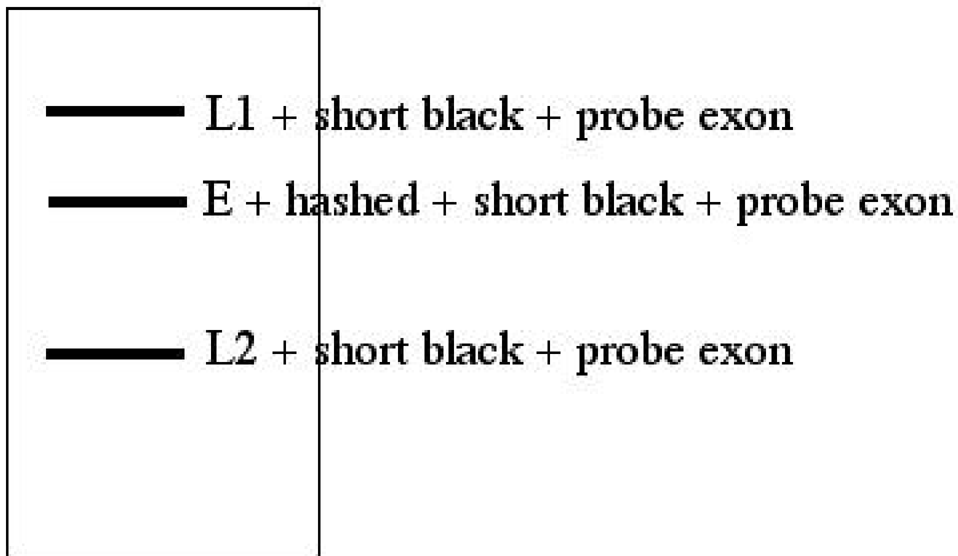
5. In figure 4, we are looking at western blots. The proteins loaded in all four lanes were first immunoprecipitated with anti-SHC antibody (SHC). As shown below the blots, half were probed with either an anti-phosphotyrosine (P-Y) or anti-EGF Receptor antibody (EGFR). Prior to the immunoprecipitation, half of the cells were treated with EGF and half were not. Interpret this figure as fully as you can.

A complex figure but I was looking for key observations:

1) SHC coprecipitates EGFR, p66, p52 and p46 only when EGF is given to cells
2) When EGF is administered, EGFR, p66, p52 and p46 all become phosphorylated on one or more tyrosine residues.
3) p52 and p46 appear to be more heavily phosphorylated than the other 2 proteins.
4) Many of you said it would have been nice to see an anti-SHC blot as well, which as a good point.
5) The faint bands were of little interest, but you could discuss them as well.

16 pts.

6. Figure 5: Draw a picture of what you would predict you'd see if the encoded mRNA from the single gene illustrated here were probed with the exon labeled in red. You should know that exons L1, L2 and E can each be the first exon in their respective mRNAs . Also notice a scale bar is provided so you can estimate lengths. As a follow up question, estimate how long each translated protein would be.



L1 = ~ 655 nt including 200 nt poly-A tail - protein = ~ 92 amino acids
E = ~ 629 nt including 200 nt poly-A tail - protein = ~ 103 amino acids
L1 = ~ 569 nt including 200 nt poly-A tail - protein = ~ 92 amino acids

16 pts.

7. Interpret figure 6 as completely as you can. It is a pair of Northern blots where the top panel was probed with a cDNA of interest and the bottom panel is the same blot probed with a constitutive cDNA.

Key points:

1) 18S ribosomal band as loading control.
2) All lanes are about the same, except 6 may be a bit low.
3) Order of TSL mRNA for each lane is: 4>6>5=1>2>3

6 pts.

8. Figure 7 is interesting. The paper said that neuronal NOS was labeled brown by immunocytochemistry in panels C and D. An enzyme that absolutely requires the product of neuronal NOS (nitric oxide) in order to function was assayed in panels E and F. In this assay, blue is produced inside cells when nitric oxide and the enzyme (NDP) are both present.

There are a couple apparent contradictions I would like you to point out to me in these data. Assume that all experiments were performed properly and all controls worked well.

The major feature I was looking for was that nNOS and NDP staining do not overlap the way the authors indicated. There is NDP blue where there is little or no nNOS. This is an apparent contradiction. The second one was that if NDP can be blue where there is no nNOS, then why is the

brain slice in F lacking blue? I only graded for one of these two contradictions since the question failed to explain the difference between C/E and D/F.

+2 pts.

9. As you were advised in class before spring break…. summarize why Munroe and Pelham rejected two of their proposed models to explain how KDEL proteins are retained in the ER.

Direct binding to KDEL retainer protein – no evidence for this.

Modification of KDEL proteins – no evidence for this.

Figure 7.

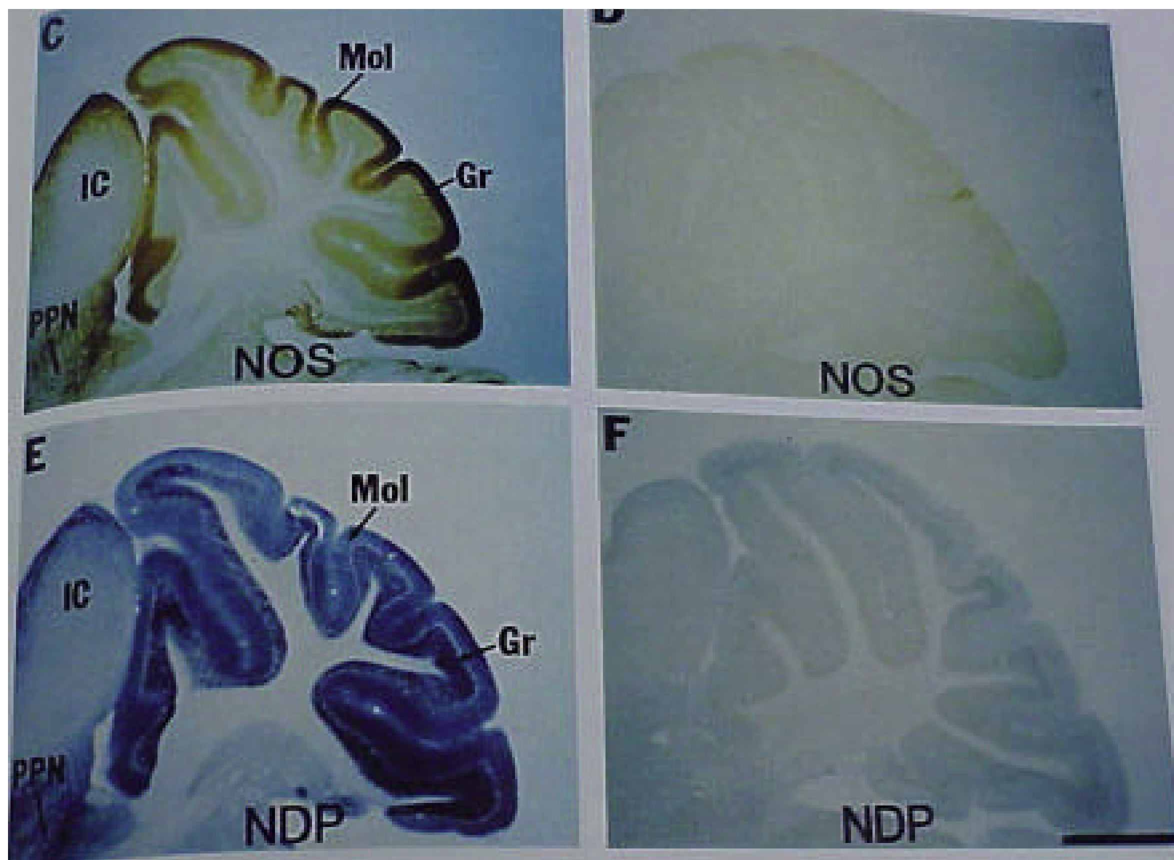


Figure 1

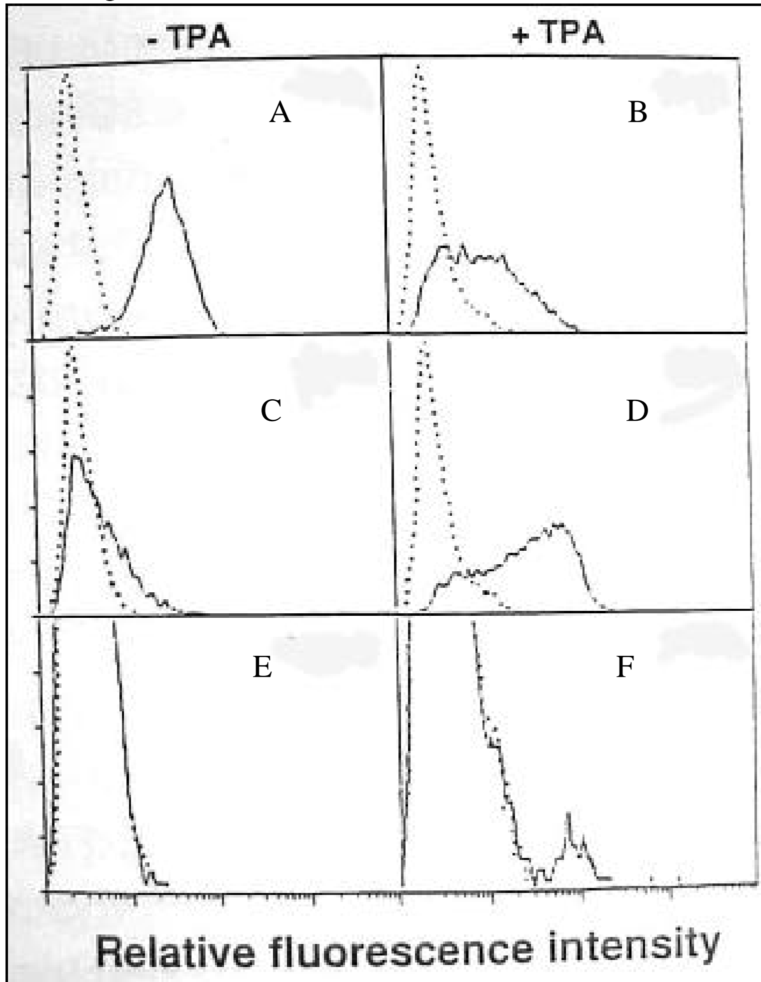


Figure 2

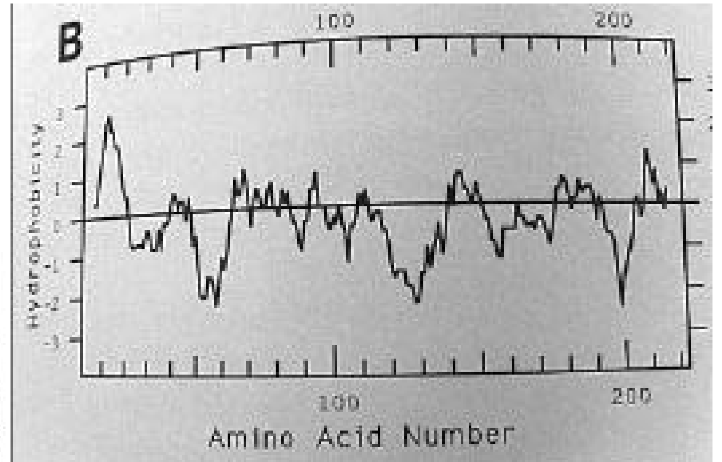


Figure 3

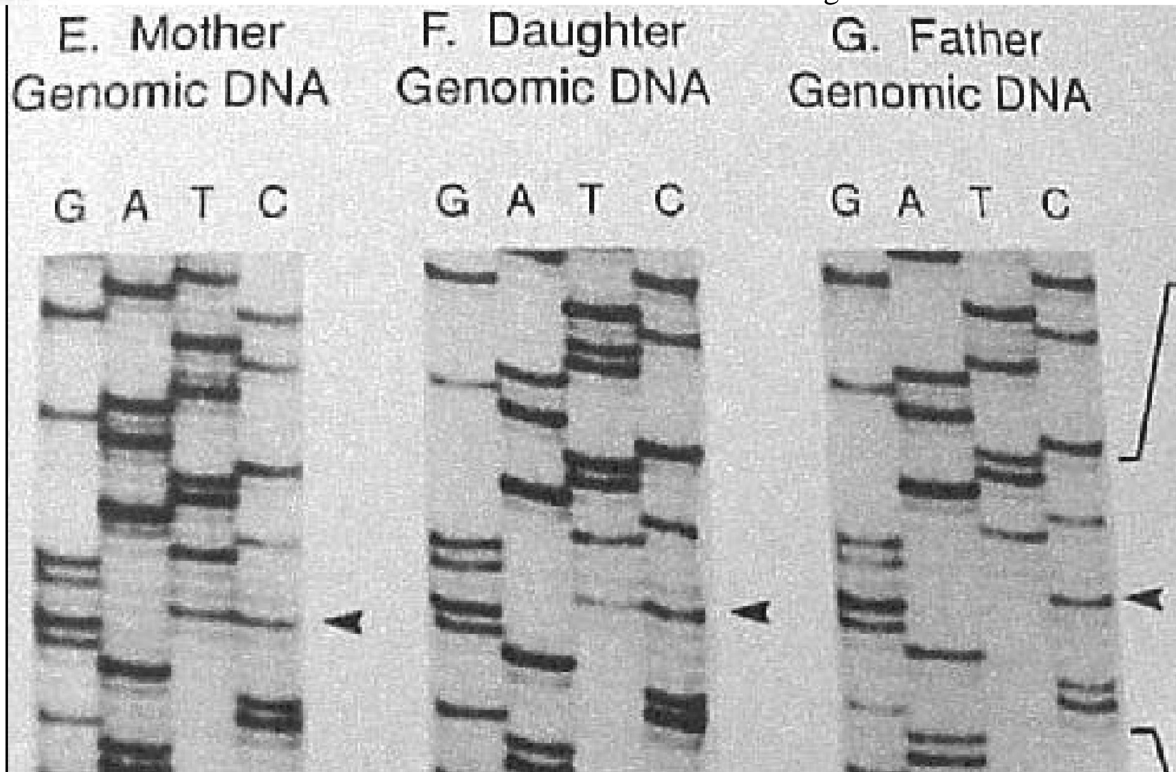


Figure 4

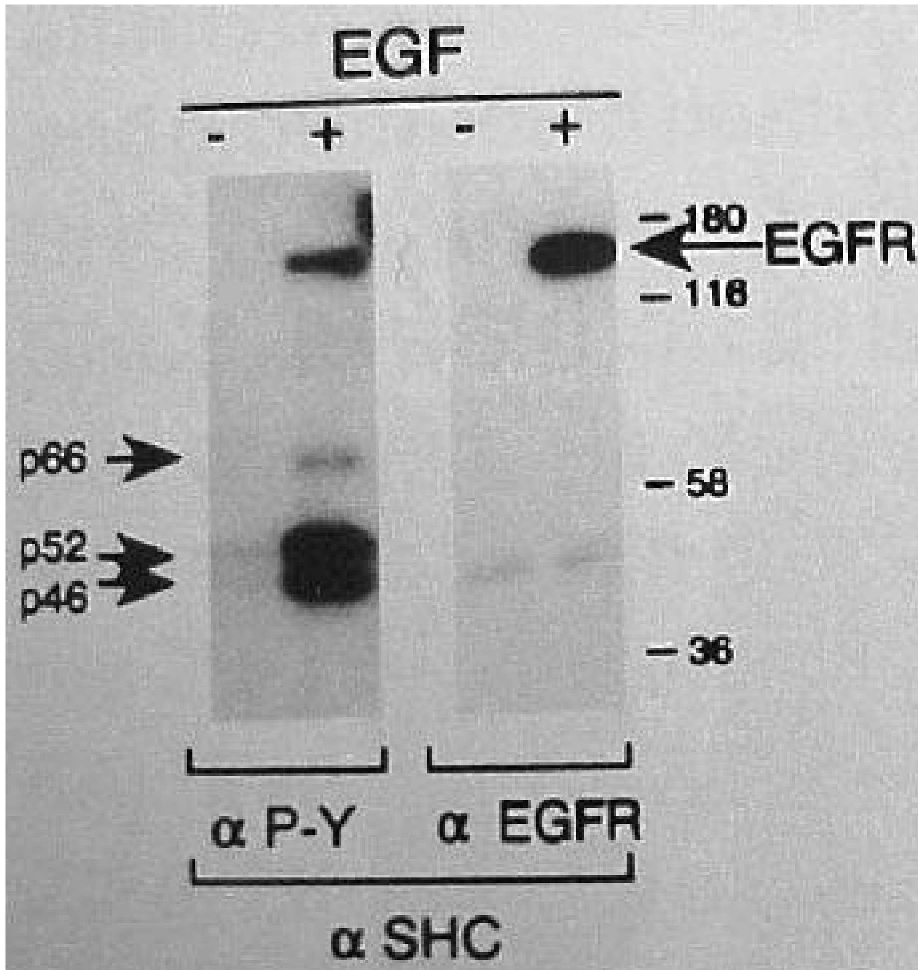


Figure 5

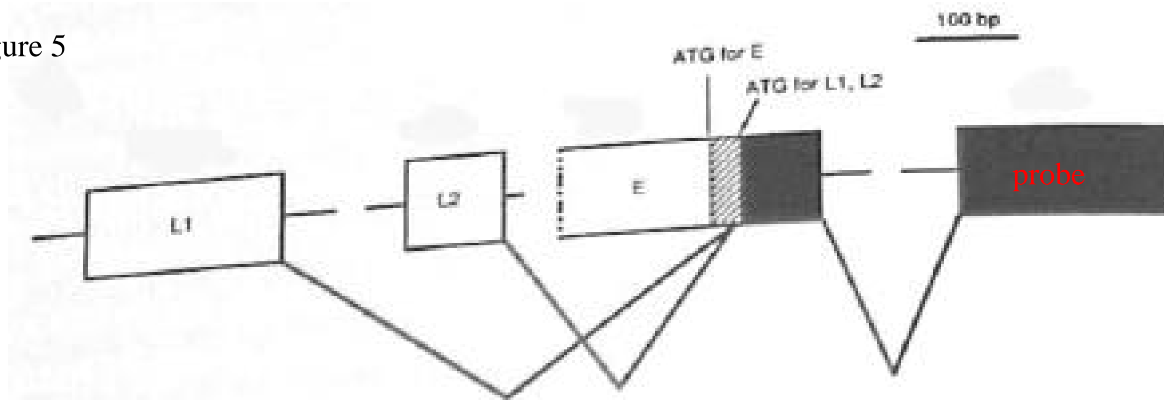


Figure 6