ORIGINAL

1999 Molecular Biology Exam #3 – 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, any books, or electronic sources, nor are you allowed to discuss the test with anyone until Monday May 10, 1999. **EXAMS ARE DUE AT 9 AM ON MONDAY, May 10**. 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.

-3 points 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:

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

10 pts.

1. Figure one shows a DNase footprint assay. R and Y are sequencing lanes for MW standards. All protein sources are derived from mitochondiral protein. Lane 1 used crude homogenate that was performed in a buffer containing a lot of salt and detergent . Lane 2 used the crude homogenate after it was dialyzed with a simple buffer. Lanes 3 – 9 are fractions of protein after the homogenate was run over an affinity column matrix . Used in each reaction are: flow through fraction of protein (lane 3), protein eluted with 0.25 M KCl (lane 4), 0.5 M KCl (lane 5), 0.8 M KCl (lane 6), 1 M KCl (lane 7), and 2.0 M KCl (lane 8). Lane 9 used the buffer alone and no KCl or protein.

Interpret this figure one lane at a time and be sure to include in your answer the following two points where appropriate:

a) the location of binding by this protein or proteins

b) how many proteins are binding here

8 pts.

2. Figure 2 shows a band shift assay. They are trying to determine the function of a *Drosophila* protein called extended (exd). On the left part of the figure, they added 4 increasing amounts of engrailed protein and no exd protein. On the right side, they added a fixed amount of exd and the same four increasing amounts of engrailed. Interpret this figure.

10 pts.

3. On a related, figure 3 shows another band shift assay. This time they used two different probes GTCAATTAAAGCATCAATCAATTTCG (LEFT) or
GTCAATTAAATGATCAATCAATTTCG (RIGHT).

In this experiment, they used protein combinations as indicated by the boxes. en stands for engrailed, ubx stands for ultrabithorax, and exd you know already.

Interpret this figure.

10 pts.

4. A group of clever genetic engineers have designed a cow that produces low lactose milk. They produced the enzyme lactase (normally secreted by intestinal cells) in mammary glands. I would like you to design a cow to produce low lactose milk but don't damage the meat by expressing this enzyme in other cells. Draw a diagram or two on your answer sheet showing me what DNA constructs you would make to generate this cow. Also walk me through any breeding you would deem necessary.

8 pts.

5. Figure 4 shows a large family pedigree and some molecular data related to a genetic disease. PCR and a restriction enzyme were used on genomic DNA, and the results electrophoresed on an agarose gel. What can you tell me about this disease?

10 pts.

6. Figure 5 uses the CAT assay and a wide range of hormone receptors. As you may know, most hormones are hydrophobic and their receptors are located in the cytoplasm. When the hormone binds to its receptor, the receptor changes shape and migrates into the nucleus and becomes a transcription factor to activate a range of genes. Regions of promoters/enhancers that bind to hormone receptors are called "hormone response elements". In this experiment, they wanted to know what affect a protein called SRA would have on the ability of hormone receptors to do their jobs as transcription factors. In the figure, H = hormone. It is interesting to note the use of RU486, the "morning after pill".

a) Tell me what affect SRA has as revealed in this experiment.

b) What is the function of RU486 in this experiment?

34 pts.

7. Three figures for this question can be found on the web at this URL: <http://www.bio.davidson.edu/Biology/macampbell/restricted/data.html>. To view it, type in the URL, then print the figures but make sure you do not leave prints all over campus in case someone in class finds it who has not started taking the test. They are from a paper that was examining a very important organ that most people do not even know exists – the vomeronasal organ (VO) which is what detects pheromones in humans (I'm not making this up). VR $_i$2 is a particular receptor in VO neurons used to detect pheromones and the researchers are trying to make transgenic mice.

a) Read carefully the figure legend that appears on page 4 of this test. The black boxes represent noncoding (i.e. 5' and 3' UT). The IRES section permits the two genes (VR $_i$2 and the reporter gene) to be transcribed as a single mRNA but each protein to be translated separately. Finally, a fusion protein of tau and reporter will result from some of these constructs. The tau portion of the reporter genes causes the fusion proteins to bind to all microtubules, which labels the full length of the axons (www figure 3). Tell me how you would be able to tell which cells contained the DNA shown in lines a, c, d, e, f, and g (www figure 1). You cannot use PCR.

b) What is the difference between the DNA in lines c and d? How was this difference generated and in what cells would it be done?

c) The purpose of this experiment was to create different reporter gene constructs so that mice would be heterozygous for constructs VG and VL (www figure 1). Tell me how you would make such a mouse given these constructs. You do **not** need to list every manipulation, but do tell me which cells and mice and major procedures you would use to generate these mice. Do not duplicate your answer from part b above. Mice containing constructs f and g were used as controls, but we will not consider those here.

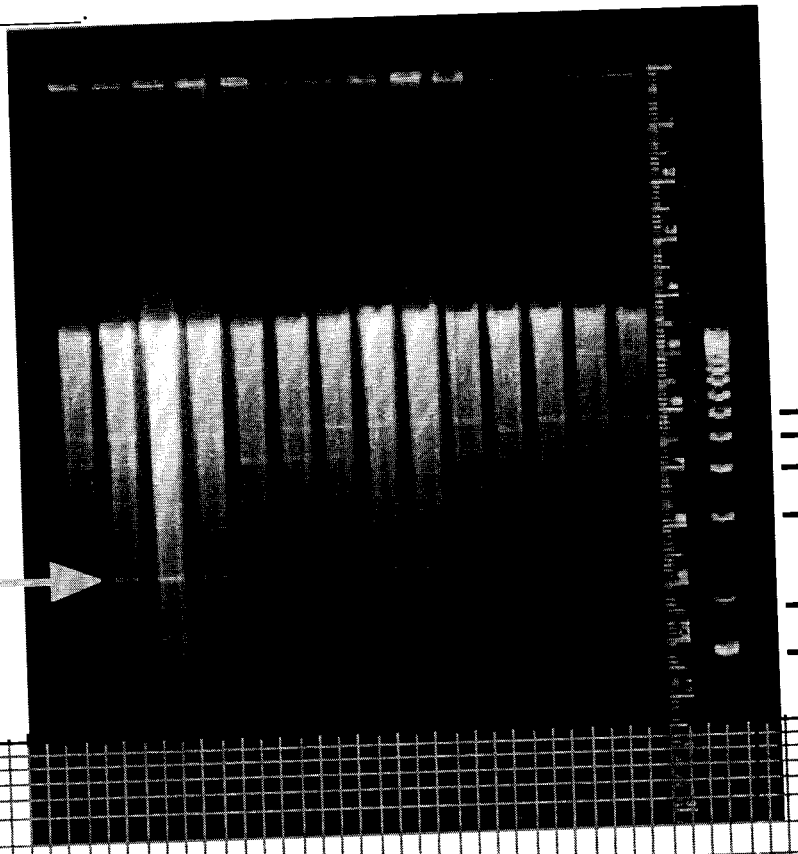
d) In this experiment, they wanted only neurons from the vomeronasal organ to express the tau-reproter fusion proteins. How was this accomplished?

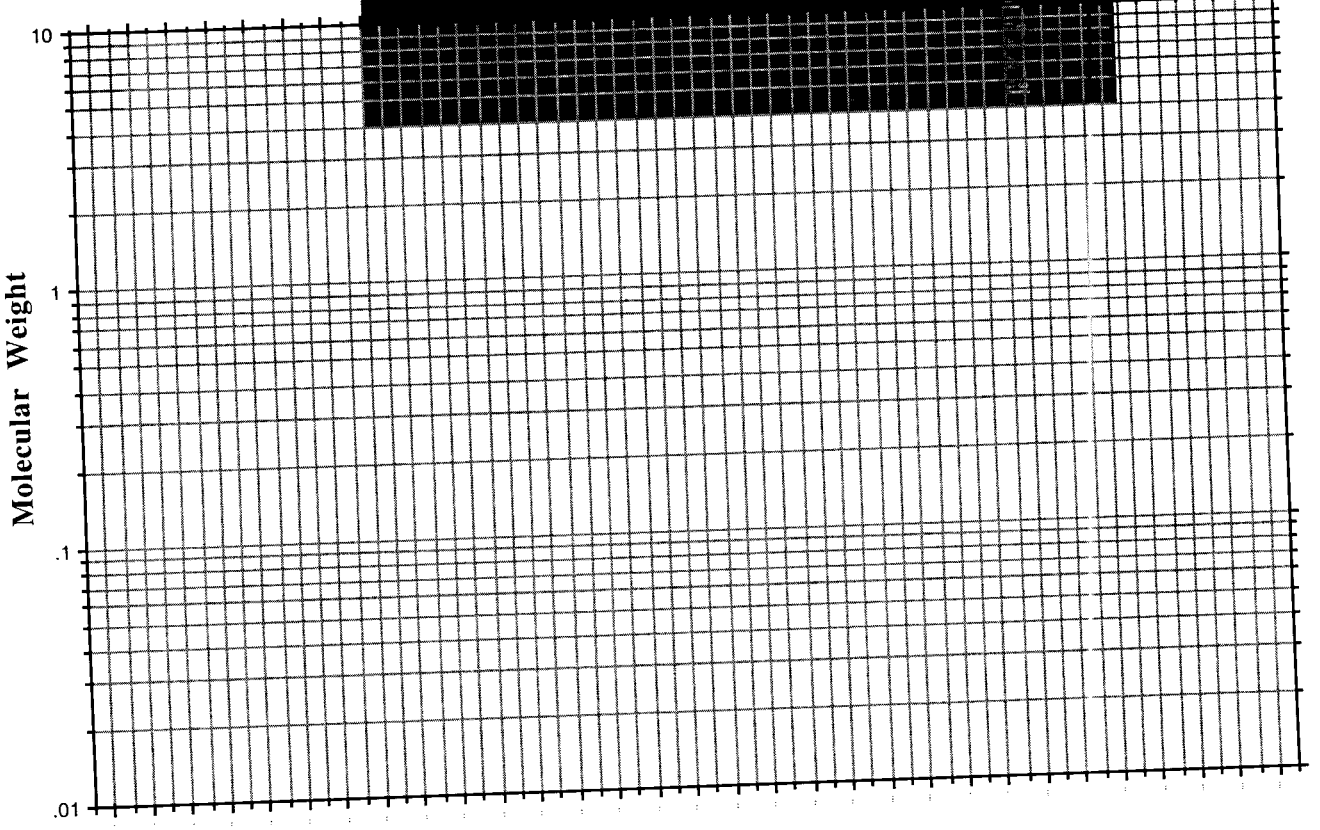
e) They generated these VG/VL mice, and found that vomeronasal neurons expressed either GFP or β-galactosidase, but never both (www figure 2). Interpret these results.

10 pts.

8. The last question... Determine the molecular weight of the band indicated by the arrow. The standards on the right side of the gel are in kilobases. You MUST use the graph provided here and put your answer in the blank below.

The answer is _____.





(a) Wild-type *VR2* locus. The black boxes represent the noncoding regions. The coding region is in yellow. Restriction sites are indicated for BamHI (B) and HindIII (H). "ATG" is the start codon; "TGA" is the stop codon.
(b) VR2-IRES-taulacZ-LNL targeting vector. The white box labeled "i" represents the *IRES* sequence, the blue box represents the coding sequence of *taulacZ*, and the gray box labeled "neo" represents the *neo*-selectable marker *LNL* flanked by loxP sites (indicated by red triangles).
(c) *VR2* locus after homologous recombination with the VR2-IRES-taulacZ-LNL targeting vector. The probe used to detect homologous recombination in Southern blots is represented as a horizontal bar on the right.
(d) *VR2* locus after Cre-mediated excision of the *neo* cassette, which leaves a single loxP site behind.
(e) VR2-IRES-tauGFP mutation, after Cre recombination.
(f) (ΔVR2) GFP-IRES-taulacZ mutation, after Cre recombination.
(g) M71—VR2-IRES-taulacZ mutation, after Cre recombination.

Fig 1

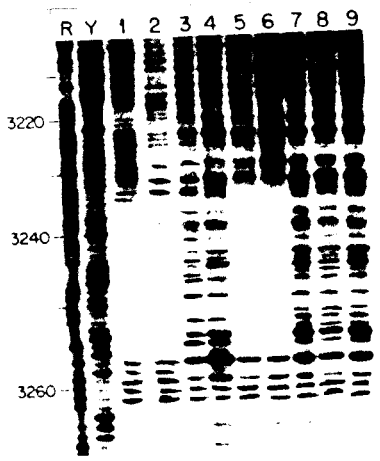
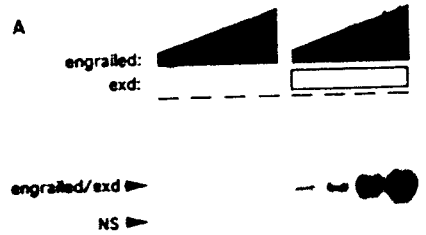


Fig 2



(A) Exd binds cooperatively to DNA with *engrailed*. In vitro translated exd and engrailed were mixed with radiolabeled probe [redacted] and analyzed [redacted]. The added proteins for each lane were as follows: lane 1, .5 μl of engrailed and 3.5 μl of reticulocyte lysate; lane 2, 1 μl of engrailed and 3 μl of reticulocyte lysate; lane 3, 2 μl of engrailed and 2 μl of reticulocyte lysate; lane 4, 3 μl of engrailed and 1 μl of reticulocyte lysate; lane 5, 1 μl of exd, .5 μl of engrailed, and 2.5 μl of reticulocyte lysate; lane 6, 1 μl of exd, 1 μl of engrailed, and 2 μl of reticulocyte lysate; lane 7, 1 μl of exd, 2 μl of engrailed, and 1 μl of reticulocyte lysate; lane 8, 1 μl of exd and 3 μl of engrailed. NS indicates nonspecific complexes present in the lysate. Proteins present in each lane are indicated. Open boxes represent the presence of exd, closed triangles represent increasing amounts of engrailed.

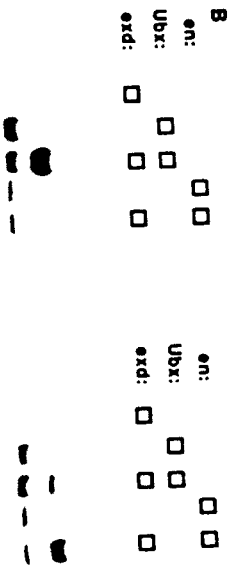
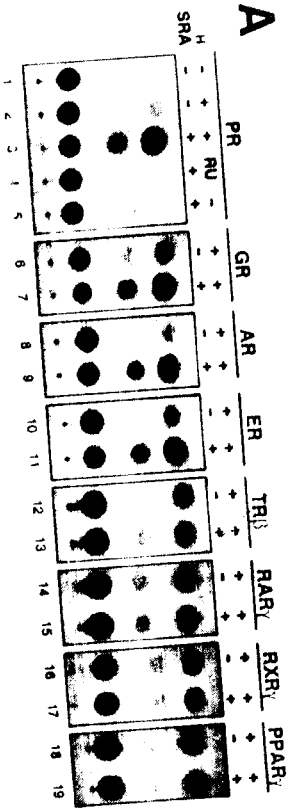


Fig 3

(−). HeLa cells were transiently transfected with plasmids encoding the human receptors for glucocorticoid (GR), androgen (AR), estrogen (ER), thyroid hormone (TRβ), all-*trans* retinoic acid (RARγ), 9-*cis* retinoic acid (RXRγ), or with peroxisome proliferator–activated receptor (PPARγ) along with their cognate hormone response element coupled to a TATA-CAT reporter gene and induced with their appropriate ligands or the PR antagonist RU486.

← Fig 5 ↓



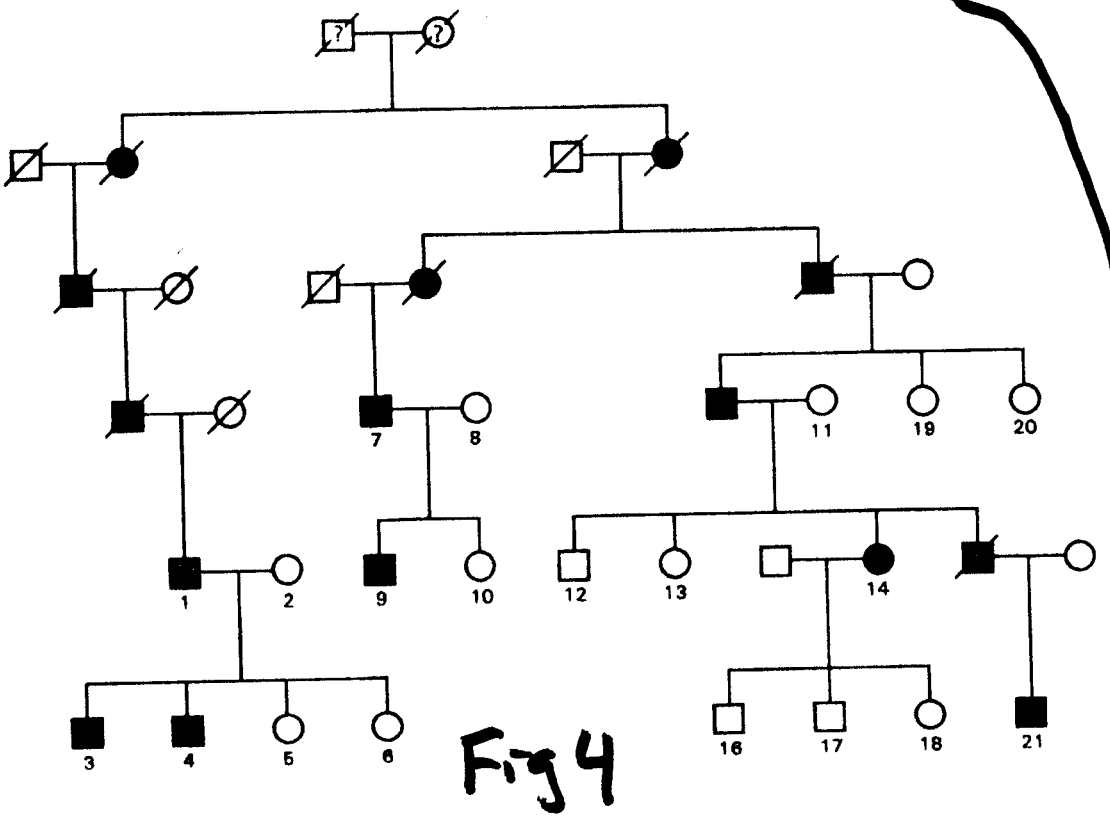
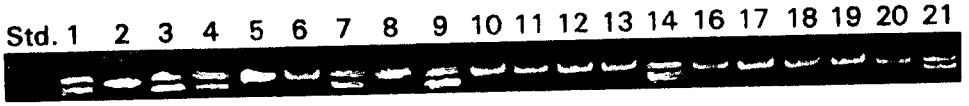


Fig 4



Figures for Molecular Biology Final Exam, 1999

Figure 1

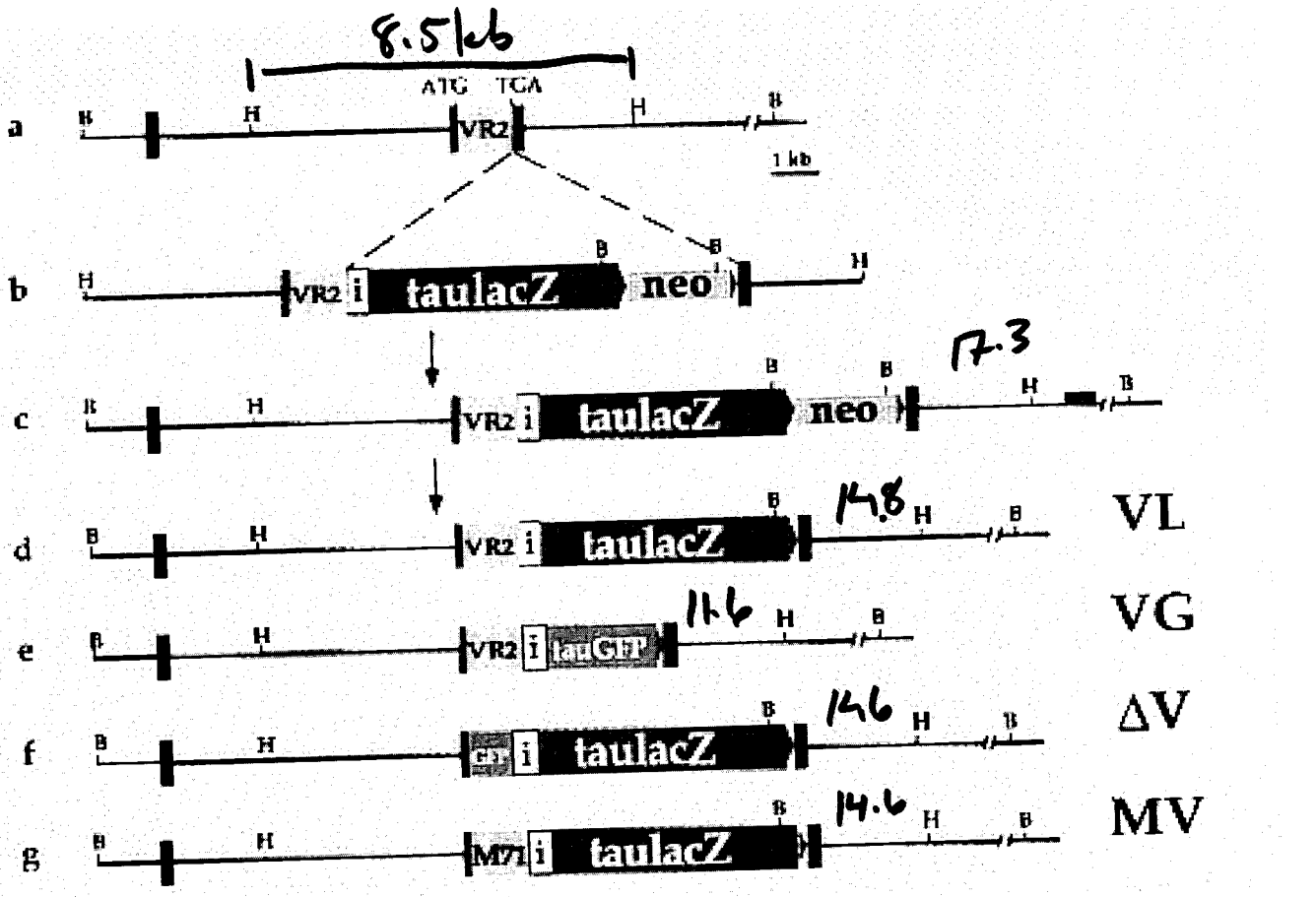
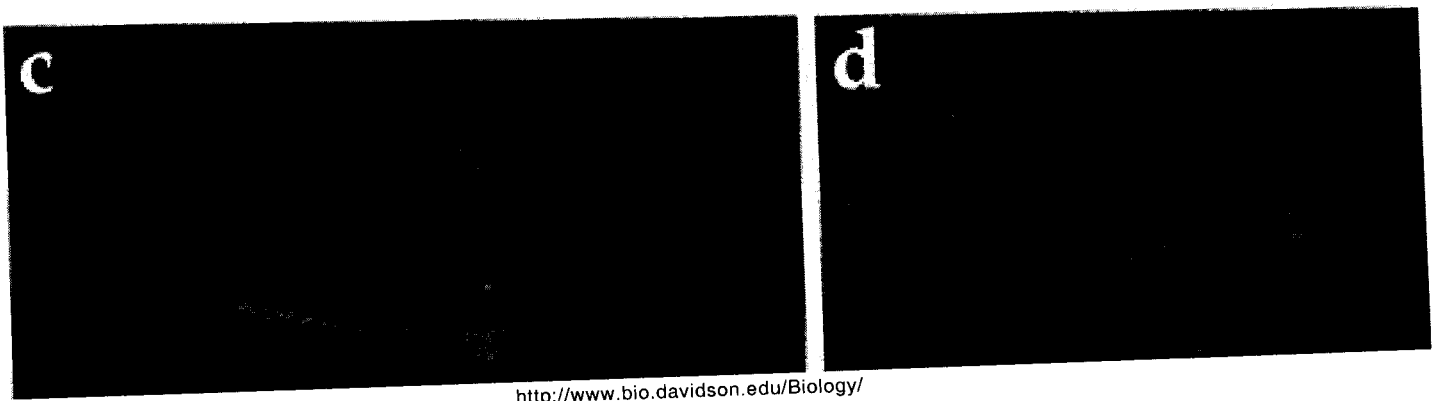


Figure 2



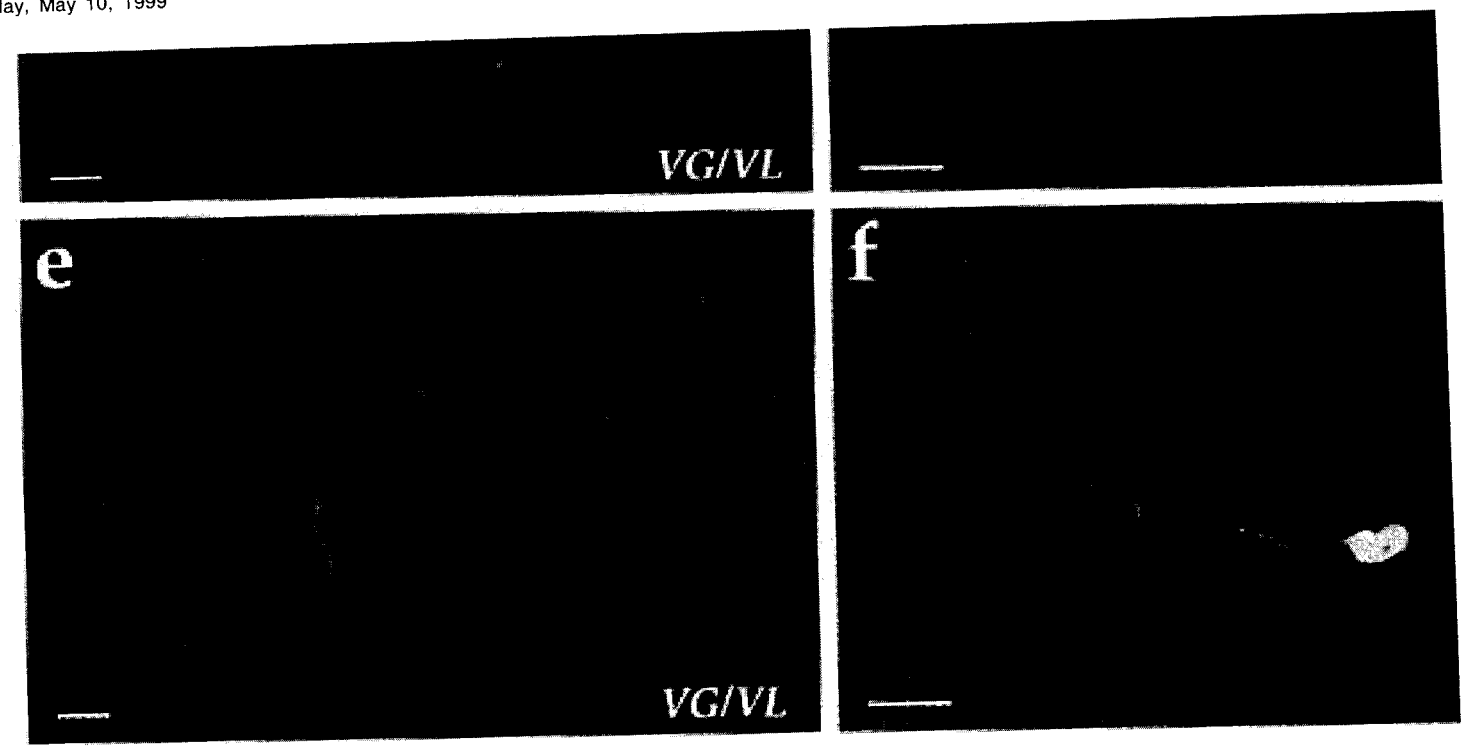
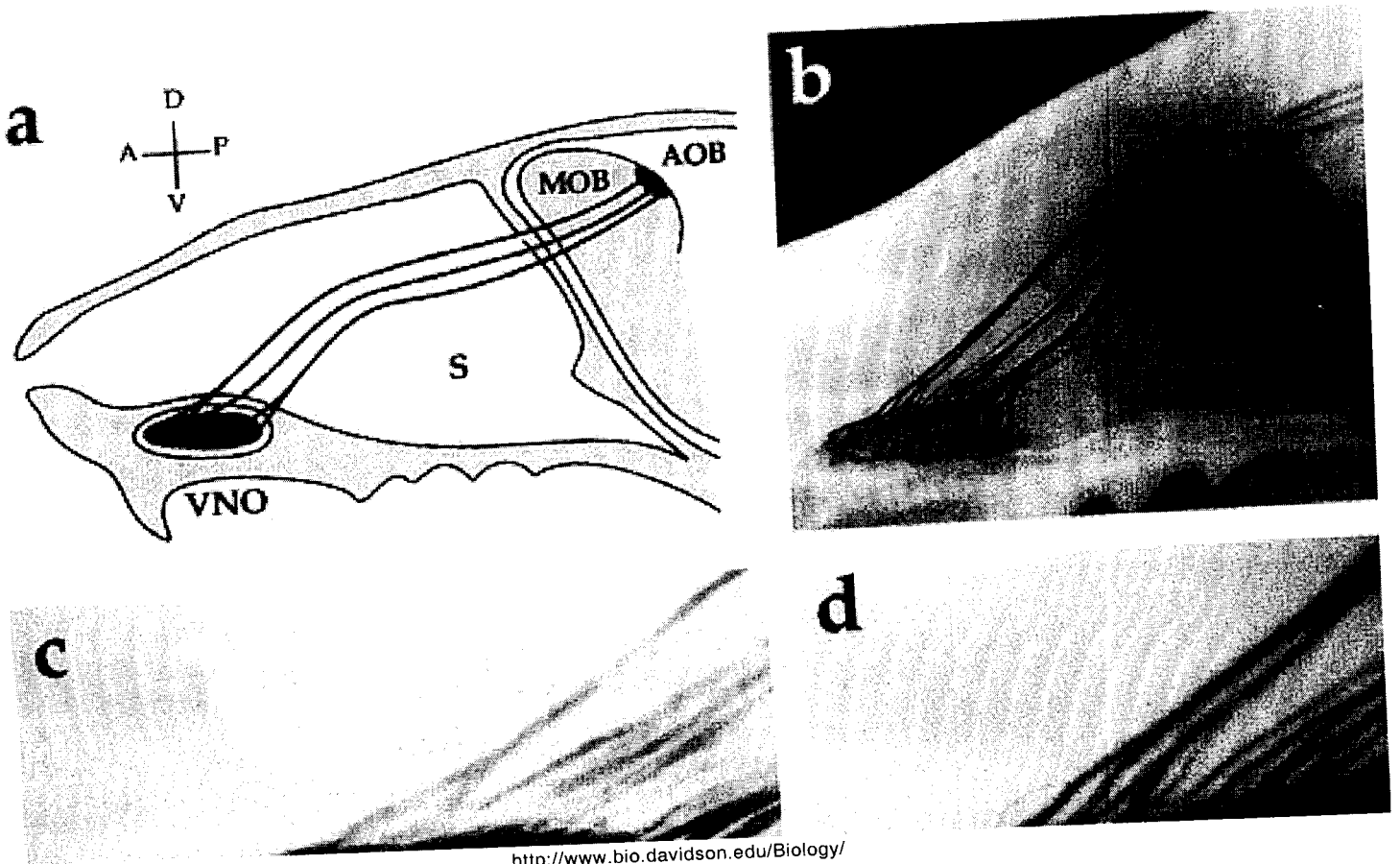


Figure 3



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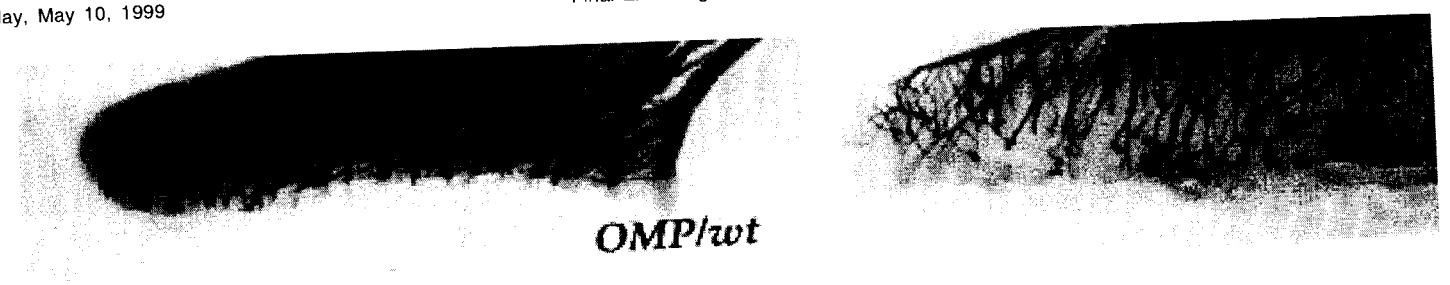

Send comments, questions, and suggestions to: **macampbell@davidson.edu**
or (704) 892 - 2905