

Spring 1998 Molecular Biology Exam #2 - Real Data

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 noon Monday March 23, 1998. **EXAMS ARE DUE AT 8:30 ON MONDAY, March 23.** You must use the web as indicated on question #7 and only for #7. 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 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:

Original
copy

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

8 pts.

1) Figures 1 and 2 show the results when a receptor on the plasma membrane of mammalian cells (FcRII) interacts with its ligand (IgG). There are 2 naturally occurring alleles of FcRII (B1 and B2), and one engineered allele (Tail⁻) that has had its cytoplasmic tail deleted completely. Figure 1 is provided to give you some background information.

Interpret the data from figure 2.

8 pts.

2) A recent discovery was made about individuals who are resistant to the onset of AIDS after they have been infected with HIV. As we all know, there is genetic variation in the population and this included coding as well as non-coding portions of the genome. Some individuals have a chemokine (short protein used in cell to cell communications) that has an altered 3' UT region. In these individuals, the amount of chemokine is higher than in people who do not have the altered 3' UT region.

- A. Formulate an hypothesis to explain what is being observed.
- B. Devise an experiment to test your hypothesis.

8 pts.

3) As you can see in figure 3, you can now buy RNA blots already to probe from companies like Clontech.

- A. What assumption have the experimenters made with this figure? What control would you like to see?
- B. Let's assume that the proper controls have been performed, interpret figure 3.

7 pts.

4) Figure 4 was published in *Cell*. Interpret the results.

7 pts.

5) Interpret figure 5B. Ignore the lines below the graph marked 1, 2, 3.

7 pts.

6) Figure 6 shows a family pedigree and an agarose gel. Interpret these results if an open symbol means wild-type, a spot in the middle means carrier genotype, and a black shape means diseased status for this recessive disease.

10 pts.

7) For this question, I want you to show off your computer skills that might be required of you for a job as a lab tech. You may use the web to figure out how to do this, including this site <http://www.bio.davidson.edu/Biology/Courses/Molbio/NIHsearch.htm>, but you may choose your favorite way.

- A. You work in a lab that studies Okazaki fragments.
Find the RasMol image for Taq DNA polymerase and put it on your web page so that I can click on it from your main page.
- B. You work for Monsanto and have obtained a peptide sequence (IEESQFAIVVFSENY) from a plant protein. Search Genbank and tell me two things.
 - 1) What is the name of the full-length protein and what species did it come from?

- 2) Are there any proteins from other species that have a high degree of sequence similarity. Explain your answer. (You might want to cut and paste some of your Genbank information to support your answer.)

10 pts.

8) Figure 7 deals with a protein called Fgf5 that is involved in the growth of hair. A mutant strain of mice exists that has had this gene mutated so that normal Fgf5 protein is not made (recessive mutation).

A. Interpret the results from figure 7.

B. Given what you have learned in figure 7, hypothesize how the mutant phenotype can be very long hair compared to wild-type.

Questions 9 - 11 are related questions on the same topic. You might want to look at all 3 questions before answering any of them.

8 pts.

9) Figure shows two immunofluorescence micrographs from a familiar lab (see title and authors). Interpret the results from figure 8 if you know that p58 is a protein that is located in the salvage compartment.

8 pts.

10) Figure 9 shows some experiments with lysozyme with modified carboxyl-termini. Three constructs were made that terminate in either KDEL, HDEL, or DDEL. Interpret these results.

8 pts.

11) What do we learn new about *ERD2* from figure 10 if you know that GalT is a protein that is found in the Golgi compartment. (By the way, did you realize that the Golgi body was discovered 100 years ago in 1898?!)

9 pts.

12) Briefly answer these lab questions:

A) Tell me how you would make a 0.5% agarose gel that has a volume of 60 ml, is made of 0.5X TBE (you have 1 L of 5X TBE) and the MW of agarose is 89.

B) What is calf intestine alkaline phosphatase used for (outside a calf, that is)?

C) What is the function of phenol/chloroform in a DNA prep?

2 pts.

13) What unit of measure is used for the molecular weight of proteins? (This is a give-me question to bring the total up to 100 pts.)

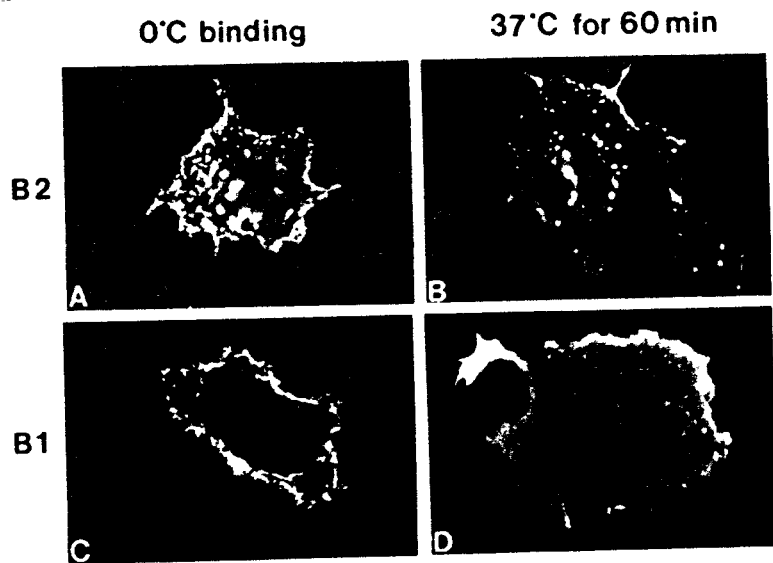


Figure 1. Binding and Internalization of IgG Complexes by COS Cells Transfected with FcRII-B1 and FcRII-B2

Twenty four hours after transfection with FcRII-B1 or -B2 cDNAs, transiently expressing COS-1 cells were incubated with 20 µg/ml IgG complexes for 2 hr at 4°C. After washing with cold PBS-glucose (5 mM), the cultures were fixed using paraformaldehyde-lysine-periodate (A and C) or warmed to 37°C for 1 hr to allow endocytosis prior to fixation (B and D). Fixed cells were permeabilized with saponin and then stained with fluorescein-conjugated goat anti-rabbit IgG to localize surface-bound and/or internalized ligand. A: FcRII-B2, 2 hr 4°C; B: FcRII-B2, 1 hr 37°C; C: FcRII-B1, 2 hr 4°C; D: FcRII-B1, 1 hr 37°C.

Fig 1

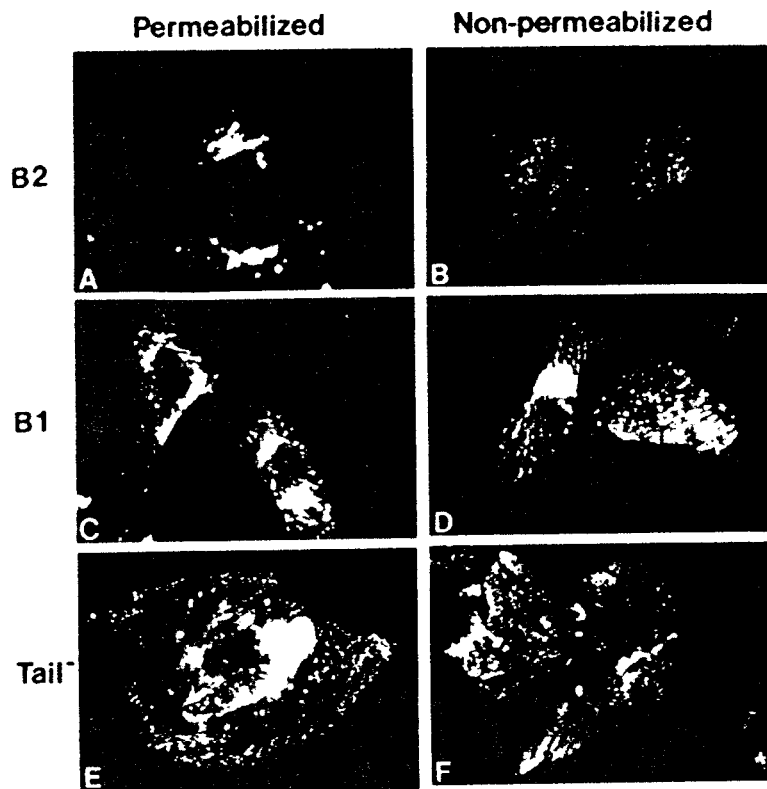


Figure 2. Immunofluorescence Determination of Ligand Internalization by CHO Cell Lines Stably Expressing FcRII-B1, -B2 or -tail-minus

Permanent CHO cell lines transfected with FcRII-B2, -B1, or the FcRII-tail-minus mutant were incubated with 20 µg/ml IgG complexes for 2 hr at 4°C. The cells were washed with cold PBS-glucose (5 mM), incubated at 37°C for 1 hr, and fixed at room temperature with paraformaldehyde-lysine-periodate. To visualize both surface-bound and intracellular ligand, fixed cells were permeabilized with saponin prior to adding a fluorescein-conjugated goat anti-rabbit IgG (permeabilized). To visualize ligand remaining on the plasma membrane selectively, parallel cultures of fixed cells were incubated directly with fluorescent second antibody without detergent addition (non-permeabilized). A: FcRII-B2, permeabilized; B: FcRII-B2, non-permeabilized; C: FcRII-B1, permeabilized; D: FcRII-B1, non-permeabilized; E: FcRII-tail-minus, permeabilized; F: FcRII-tail-minus, non-permeabilized.

Fig 2

Missense Mutations in the Adhalin Gene Linked to Autosomal Recessive Muscular Dystrophy

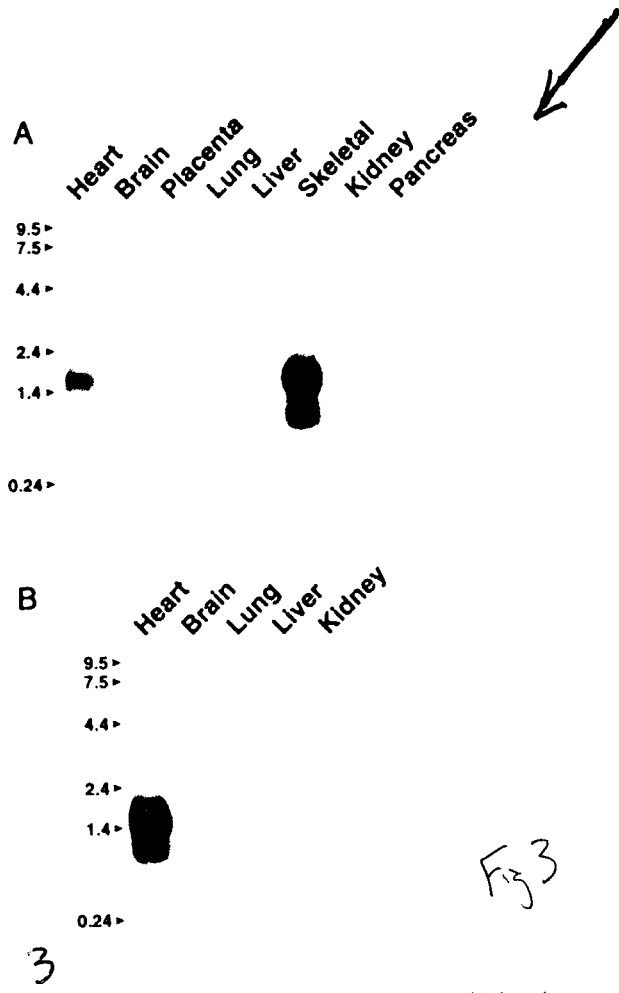


Figure 3 Tissue-Specific Expression of Adhalin mRNA in Adult and Fetal Human Tissues

(A) An RNA blot (Clontech) containing 2 µg of poly(A)⁺ RNA from each of eight human tissues, as indicated, was hybridized with a human adhalin cDNA probe. The autoradiograph was exposed for 48 hr.

(B) An RNA blot (Clontech) containing 2 µg of poly(A)⁺ RNA from each of five fetal human tissues, as indicated, was hybridized with a human adhalin cDNA probe. The autoradiograph was exposed for 6 days.

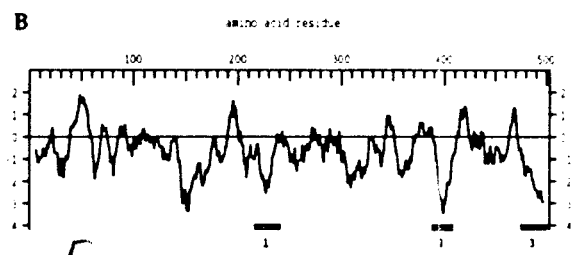


Figure 5 WASP cDNA Sequence, Predicted Polypeptide Sequence, and Hydropathy Plot

(A) DNA sequence of WASP and translated amino acid sequence. The polyadenylation signal sequence ATG TAA and the putative nuclear localization signal are underlined. The nucleotides involved in mutations are circled (211 and 291).

(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 positions of the putative nuclear localization signal (1), the highly basic region (2), and the acidic C-terminus (3) are indicated.

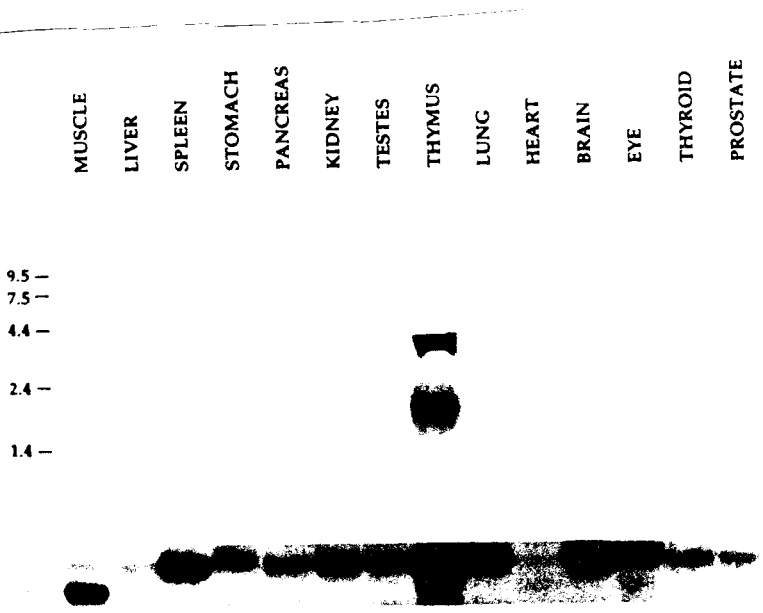


Figure 4 Expression of WASP in Human Tissues

RNA was derived from tissues from a 20-week-old human male fetus. M5.5 was used as a probe. [REDACTED]

[REDACTED]

[REDACTED]

(data not shown). The size of RNA markers is given in kilobases, and a control hybridization with β-actin is illustrated below.

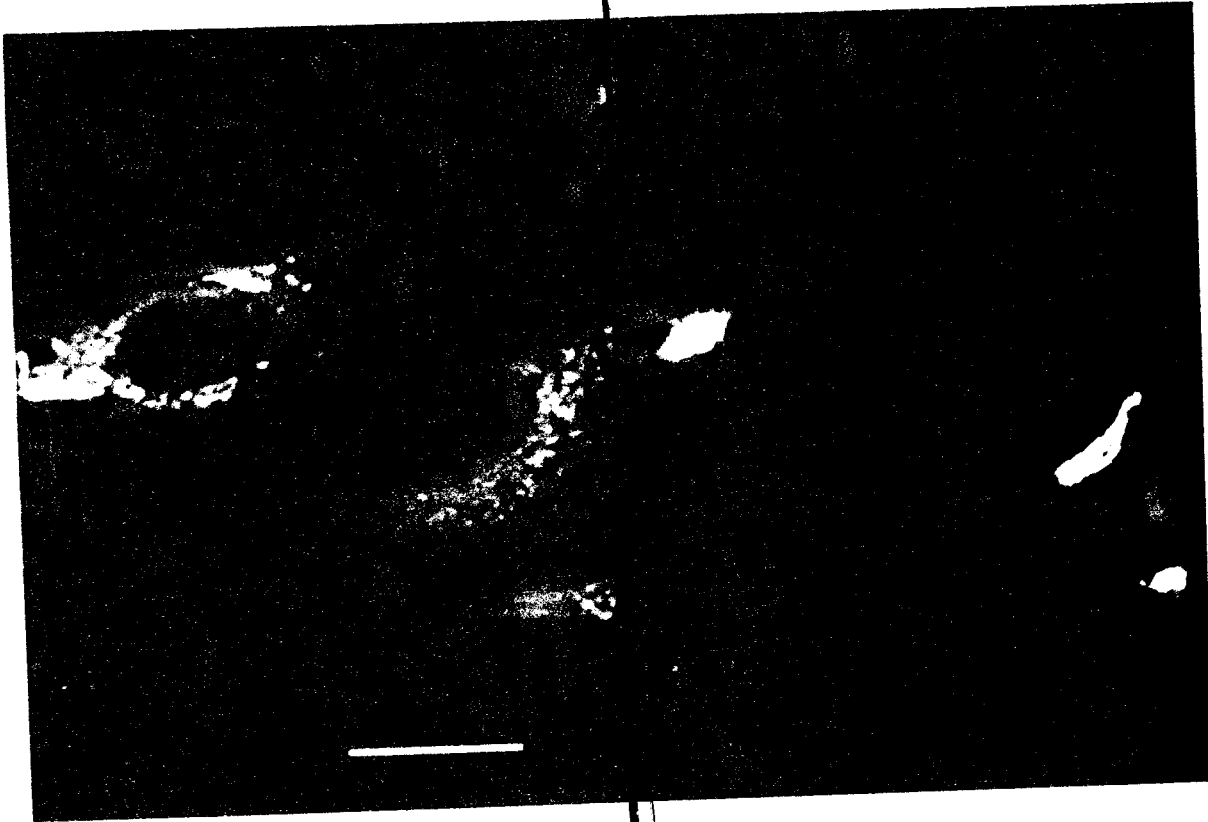
Fig 4

Human KDEL

Receptor from the Golgi Complex to the Endoplasmic Reticulum

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England

← Same cells ↓



8

p58

hERD2

Figure 8 Expression of hERD2 in Mouse Cells
Stably transformed cells expressing c-myc-tagged hERD2 were prepared for double-label immunofluorescence using rabbit anti-p58 (an intermediate compartment marker) and the anti-myc monoclonal antibody 9E10. ~~Micrographs were prepared using a Zeiss 10A fluorescence microscope with a 100x objective lens and a 1024x1024 pixel camera. The bar corresponds to 25 μ m.~~

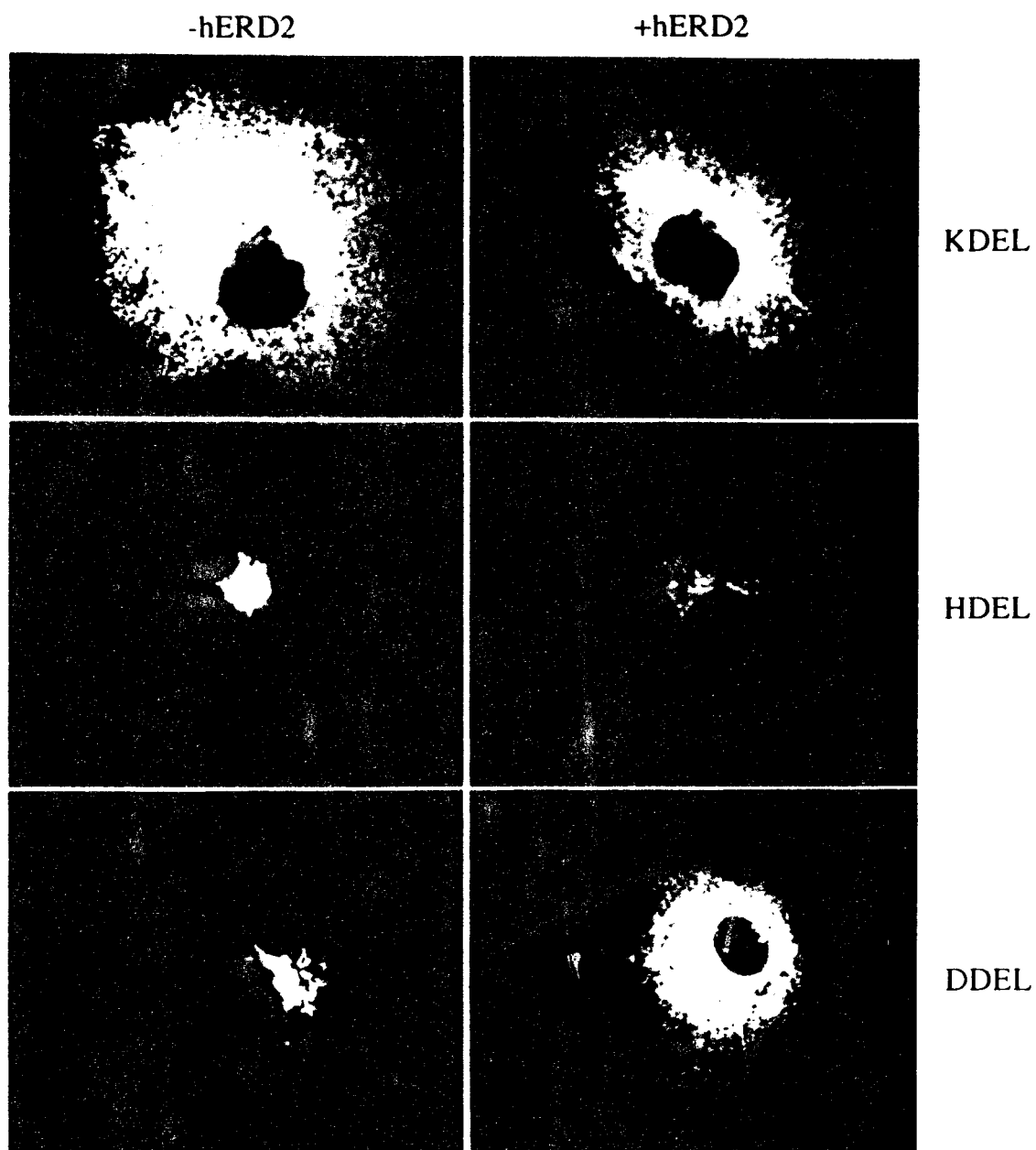
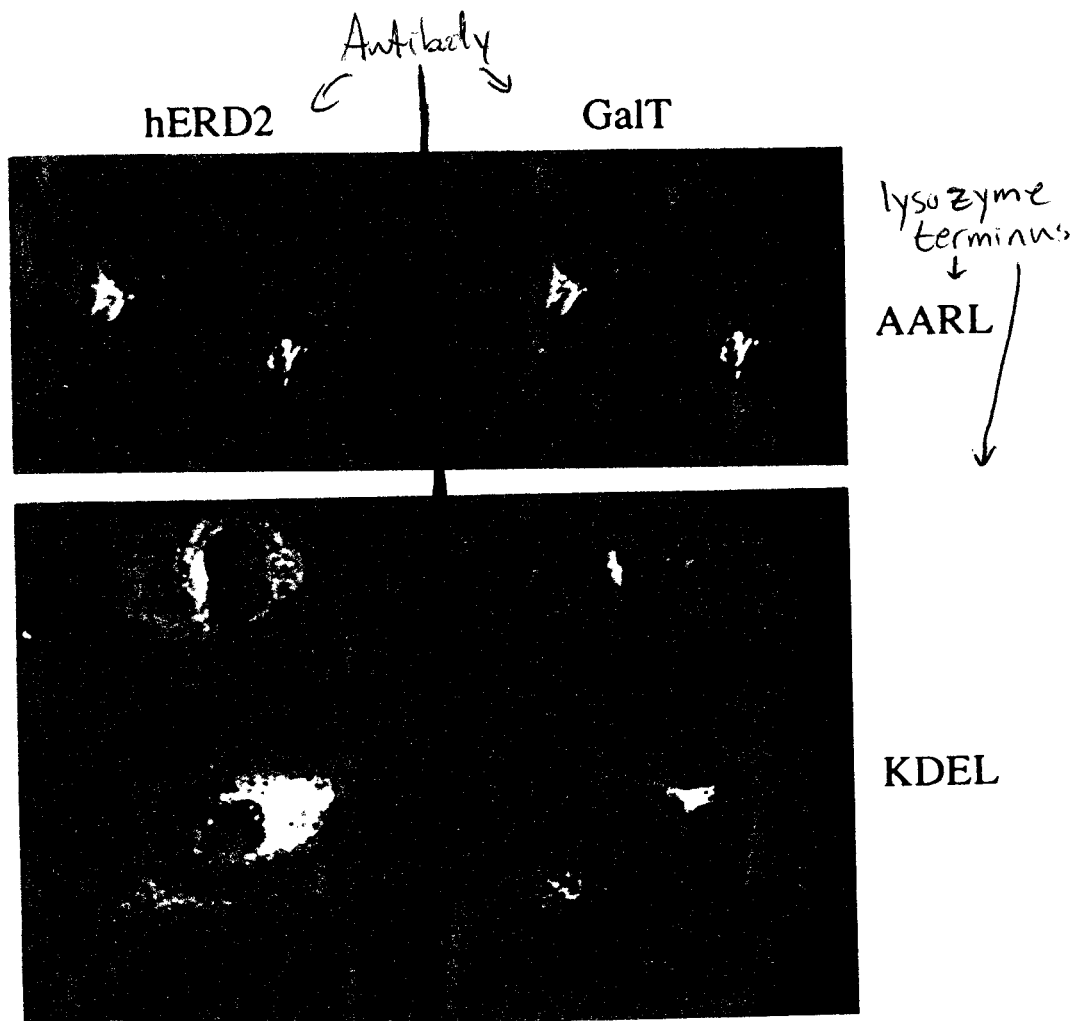


Figure 1. Immunofluorescent Staining of Lysozyme in Typical Cells for ~~hERD2~~. The C-terminal sequences of the lysozyme constructs are indicated; cells in the right-hand column also expressed hERD2, while those on the left did not. The lysozyme constructs that were not retained in the ER were concentrated in the Golgi and also in lysosomes. Lysosomal staining was seen with all unretained constructs, whether or not hERD2 was present, but in this figure it is prominent only in the cell expressing hERD2 and lysozyme-HDEL. The width of each panel corresponds to approximately 140 μm .



10
 Figure 10
 COS cells expressing myc-tagged hHERD2 and lysosome-AARL or -KDEL (as indicated) were stained with anti-myc (left) and anti-galactosyl transferase antibodies (right). To preserve the immunoreactivity of the galactosyl transferase, the cells were fixed with methanol and acetone, which does not preserve ER structure well. The top panel shows cells expressing myc-tagged hHERD2 and lysosome-AARL (left and right, respectively). The bottom panel shows cells expressing myc-tagged hHERD2 and lysosome-KDEL (left and right, respectively). The galactosyl transferase is localized in the lysosome in both panels.