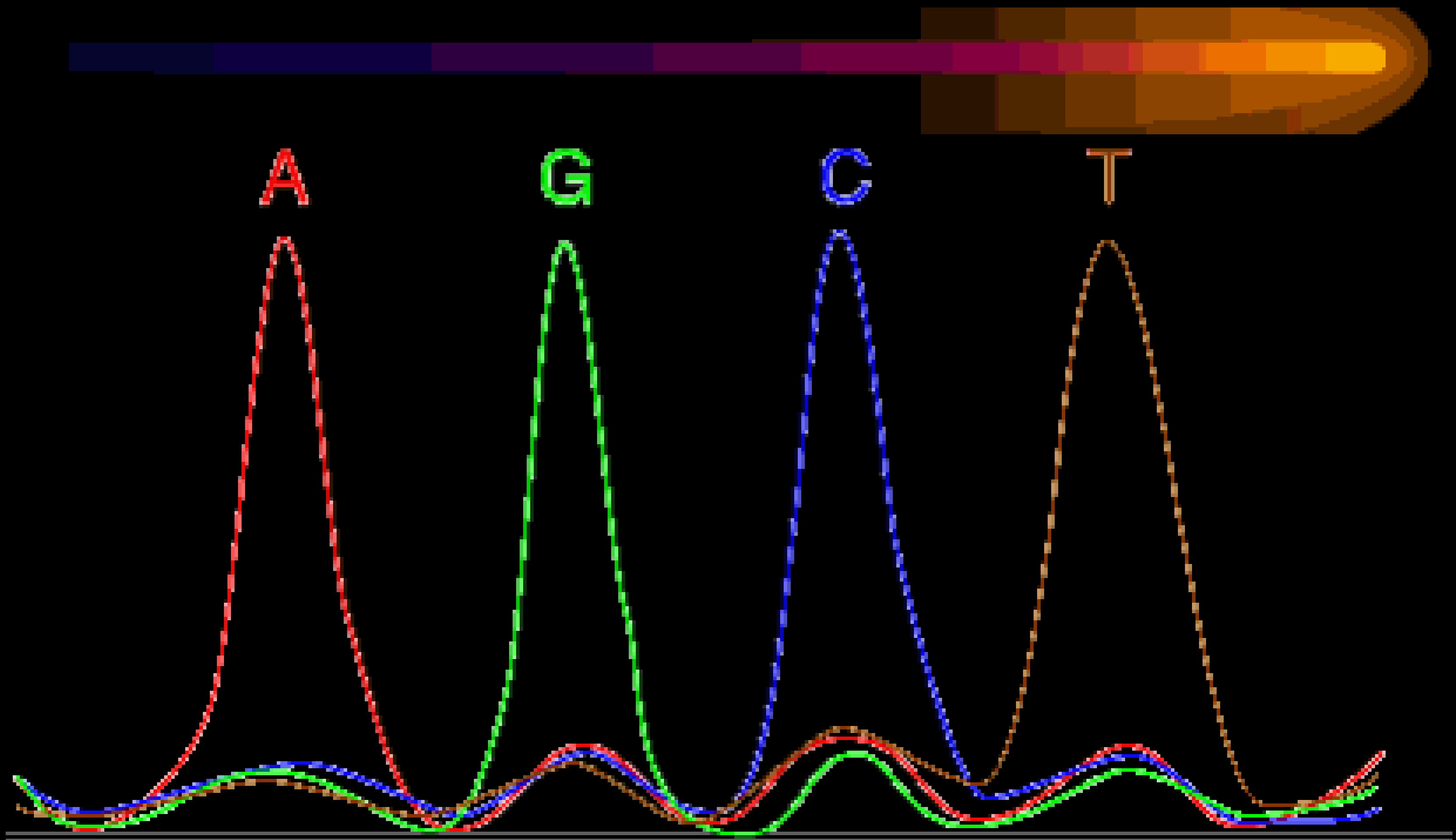
BEENG 183

Trey Ideker

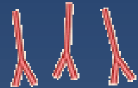


ELISA Assay

- Enzyme-Linked Immunosorbant Sandwich Assay
- Based on the principle of antibody-antibody interaction
- The ELISA is a fundamental tool of clinical immunology, e.g. used as a screen for HIV

The ELISA Method

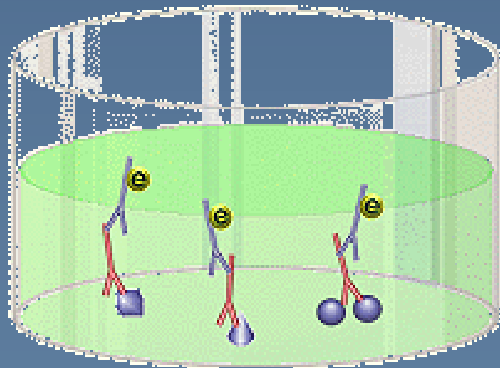
Partially purified, inactivated HIV antigens pre-coated onto an ELISA plate



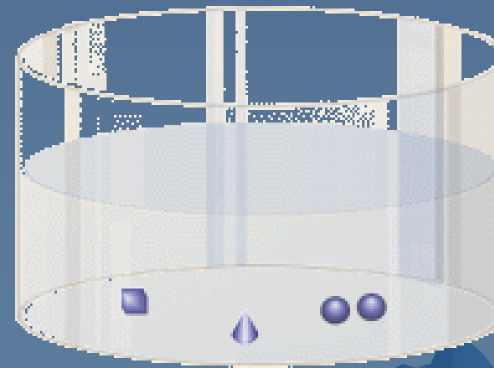
Patient serum which contains antibodies. If the patient is HIV+, then this serum will contain antibodies to HIV, and those antibodies will bind to the HIV antigens on the plate.

Anti-human immunoglobulin coupled to an enzyme. This is the second antibody, and it binds to human antibodies.

Chromogen or substrate which changes color when cleaved by the enzyme attached to the second antibody.

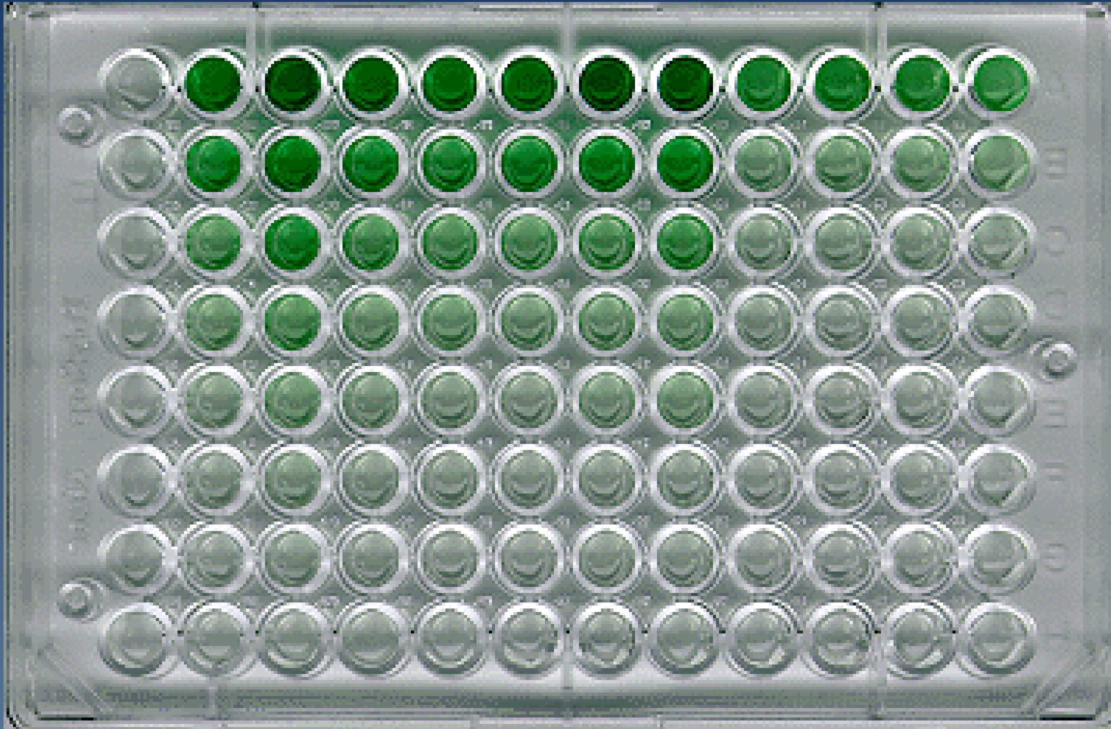


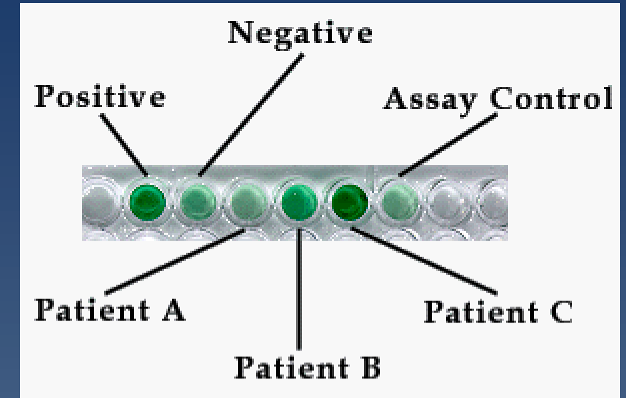
Positive ELISA Test



Negative ELISA Test

An ELISA plate





8 cm x 12 cm plastic plate which contains an 8 x 12 matrix of 96 wells, each of which are about 1 cm high and 0.7 cm in diameter.

Plate is read by measuring optical density at 450nm.

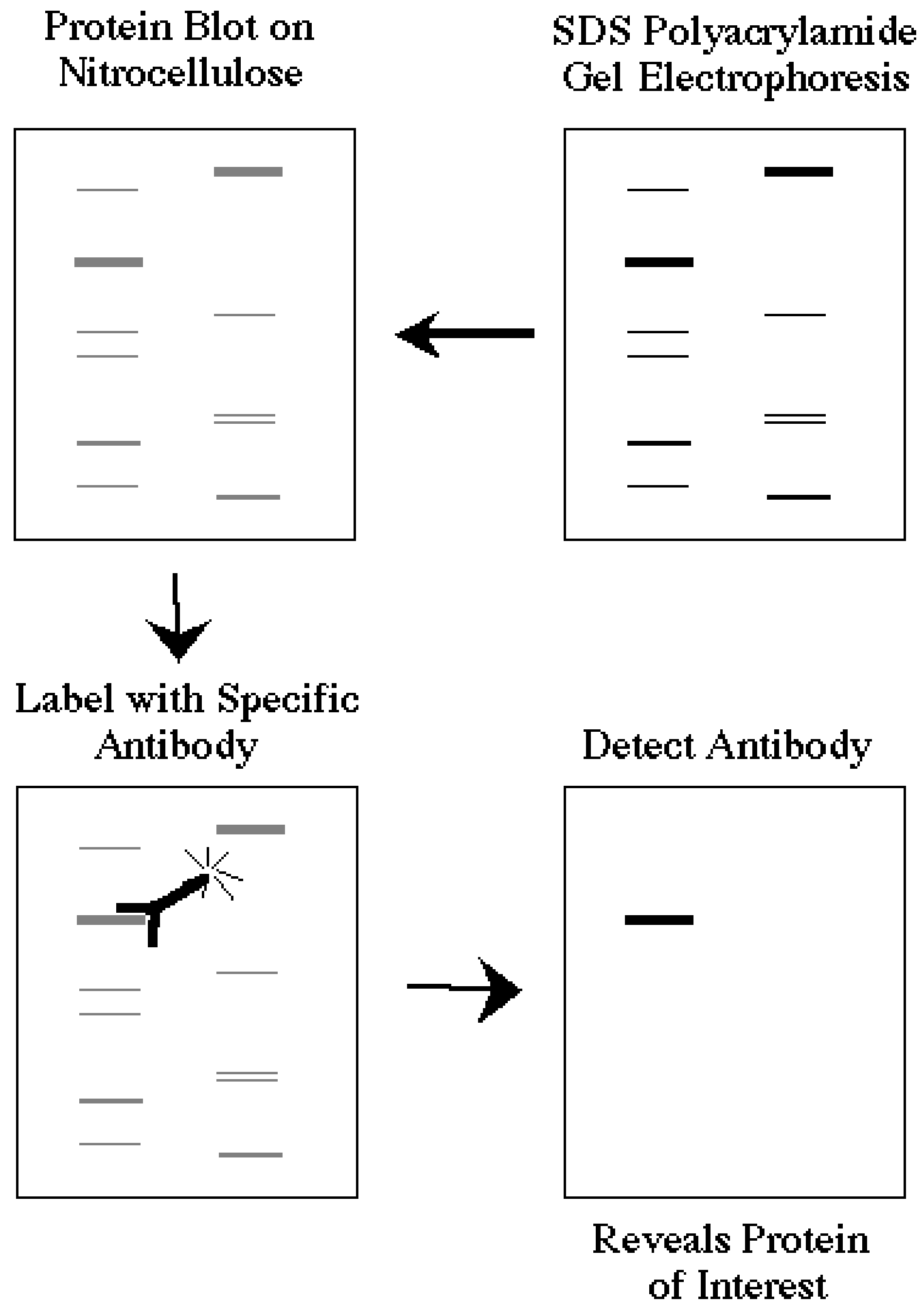
Western Blots

Determines the molecular weight of a protein and measures relative amounts of the protein present in different samples.

- Proteins are separated by gel electrophoresis, usually SDS-PAGE, and transferred to a sheet of special blotting paper called nitrocellulose.
- The blot is incubated with a generic protein (such as milk proteins) that bind any remaining sticky places on the nitrocellulose.
- An antibody is added which binds to its specific protein. The antibody has an enzyme (e.g. alkaline phosphatase or horseradish peroxidase) or dye attached.
- The location of the antibody is revealed by incubating it with a colorless substrate that the attached enzyme converts to a colored product that can be seen and photographed.

Western Blots

Also called a ***protein immunoblot***



SDS-PAGE

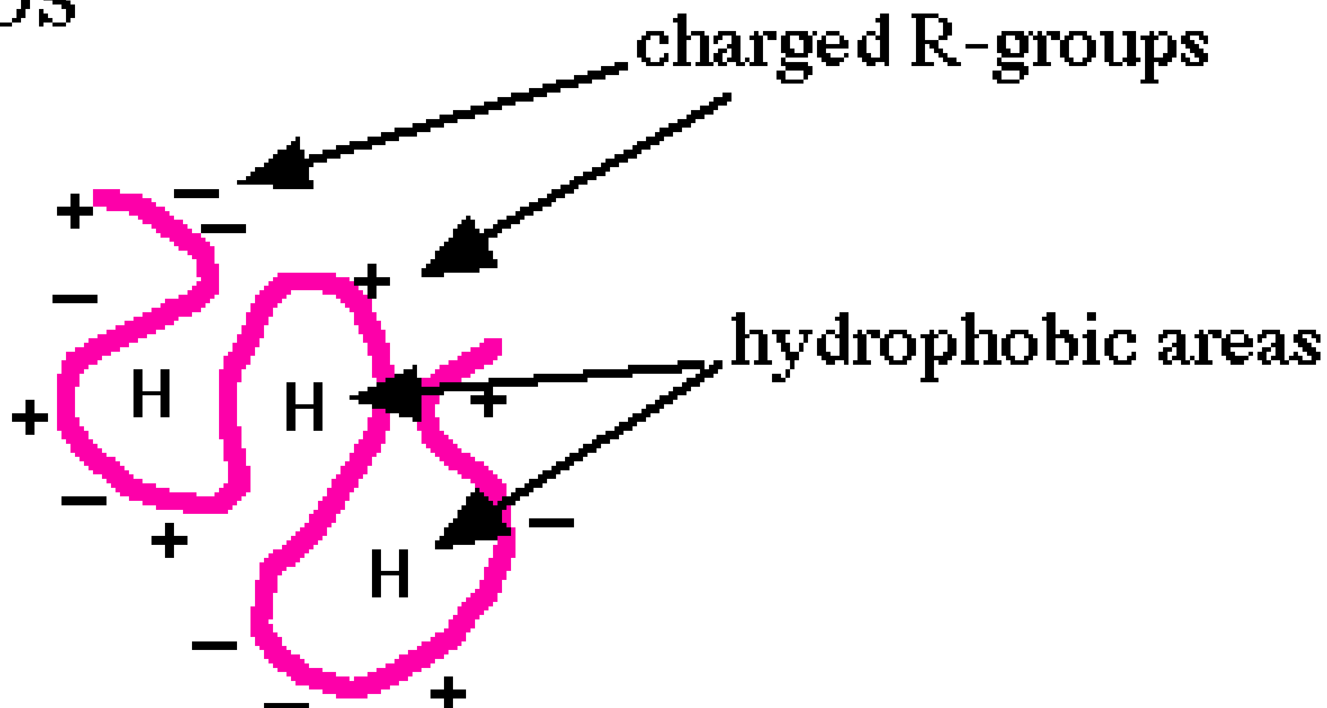
- Sodium Dodecyl Sulfate – PolyAcrylamide Gel Electrophoresis
- Separates proteins according to size
- First want protein **denatured** of structure

Consider two proteins that are each 500 amino acids long but one is shaped like a closed umbrella while the other one looks like an open umbrella. If you tried to run down the street with both of these molecules under your arms, which one would be more likely to slow you down, even though they weigh exactly the same?

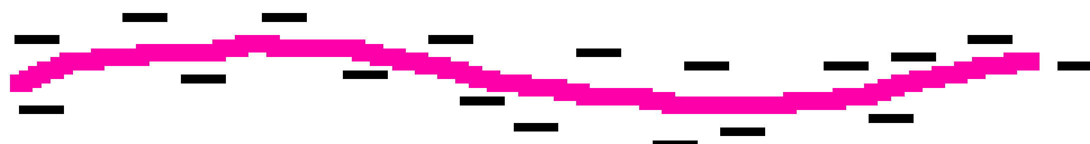
The detergent SDS

- Usually, a protein has polar R groups on the outside and non-polar R groups that collect in the core to avoid the surrounding polar H_2O
- SDS is a detergent (soap) that can dissolve hydrophobic molecules but also has a negative charge (sulfate) attached to it.
- Thus, we can use SDS to denature all proteins to the same linear shape.
- Also, all proteins have a large negative charge which means they will migrate towards the + pole in an electric field.

BEFORE SDS



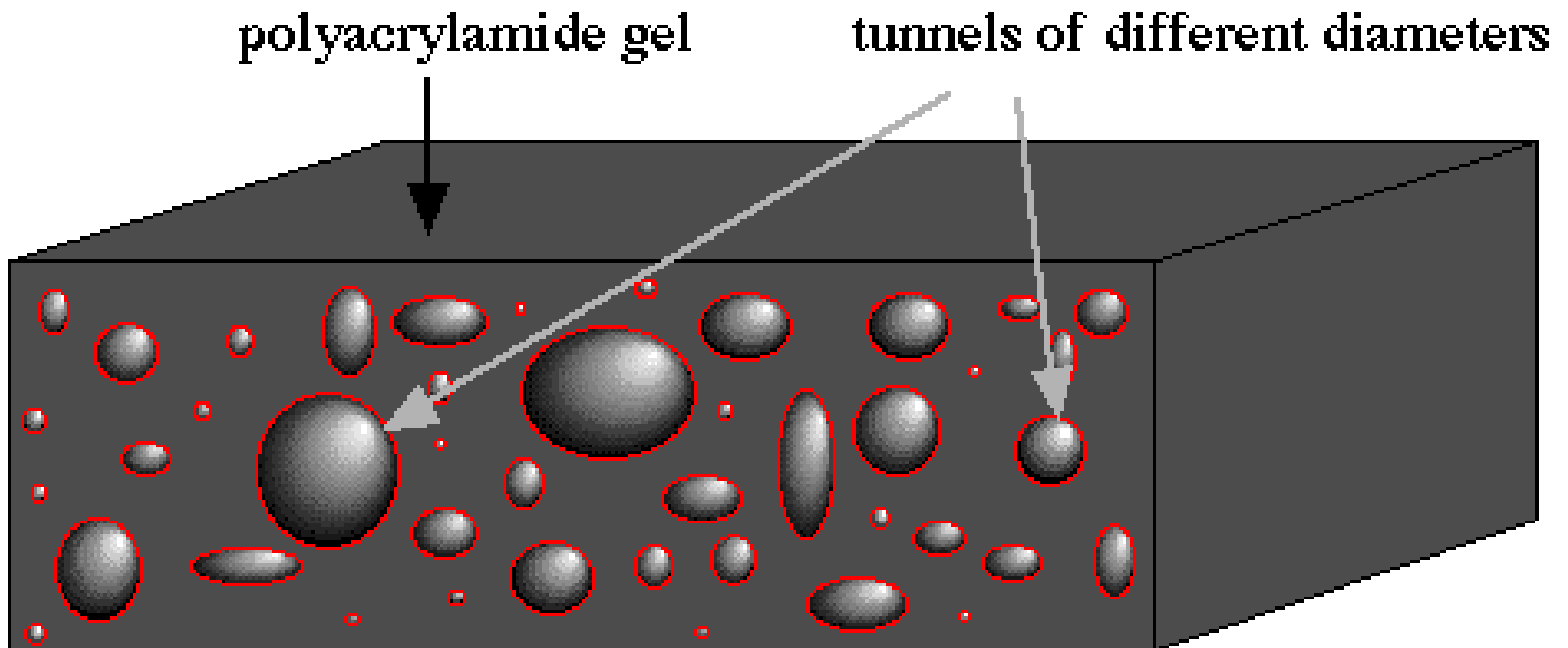
AFTER SDS



PAGE

- An environment that causes differently sized proteins to move at different rates
- PAGE uses a Polymer of acrylamide monomers consisting of a labyrinth of tunnels through a meshwork of fibers.
- In an electric force field, every molecule feels approximately the same force.
- However, small molecules maneuver through the polyacrylamide “forest” faster than big molecules
- After a certain time, we turn off the field and then stain the proteins and see how far they moved through the gel

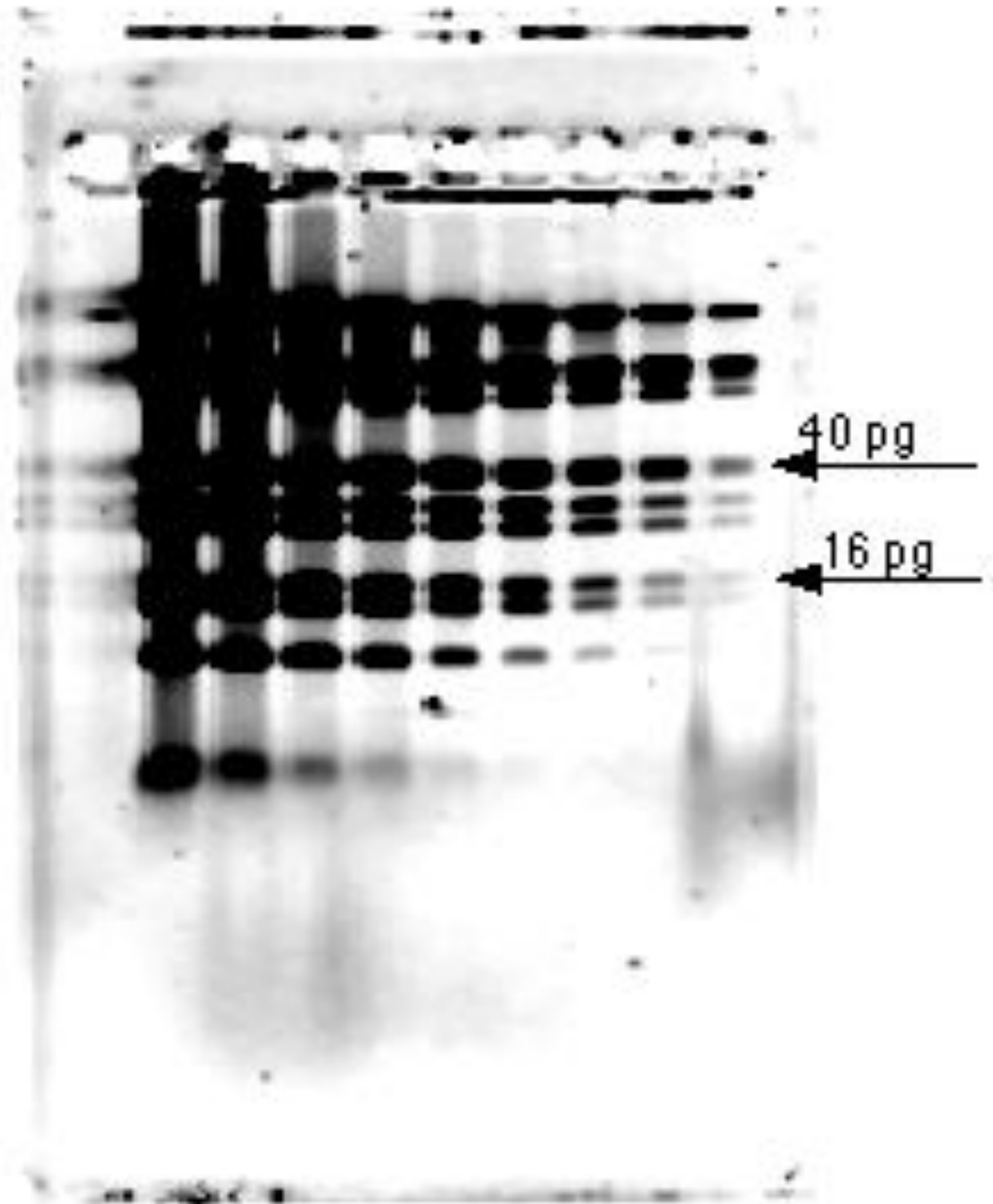
PAGE (continued)



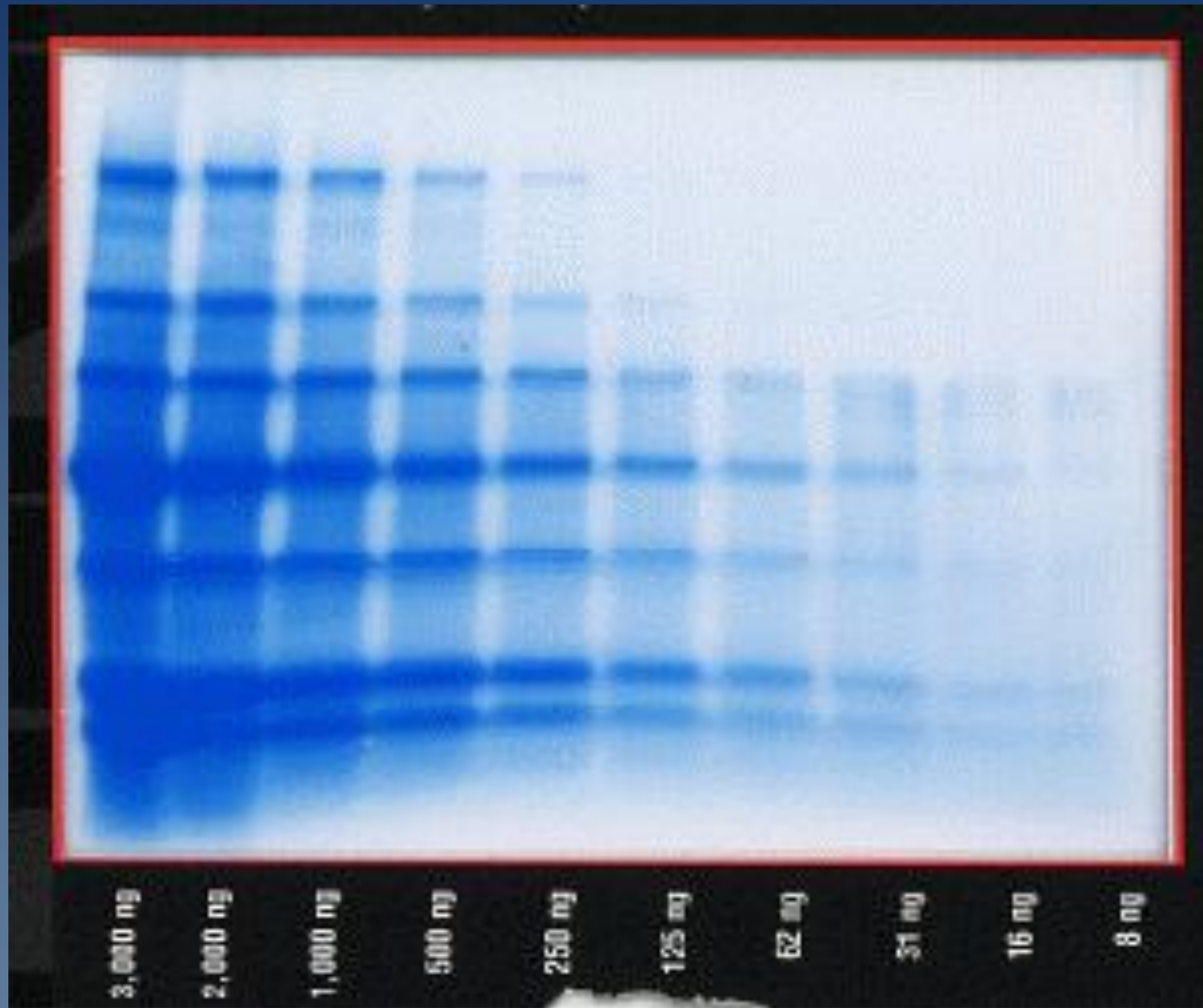
An example SDS-PAGE

How many proteins are in a band?

Protein stains:
Silver
Copper
Coomassie Blue



A gel stained with Coomassie Blue



This figure has from 3,000 ng (far left lane) to 8 ng (far right lane) of total protein loaded in the lanes.

2D-PAGE

a)

b)

c)

d)

Dimension 1: Isoelectric focusing gel

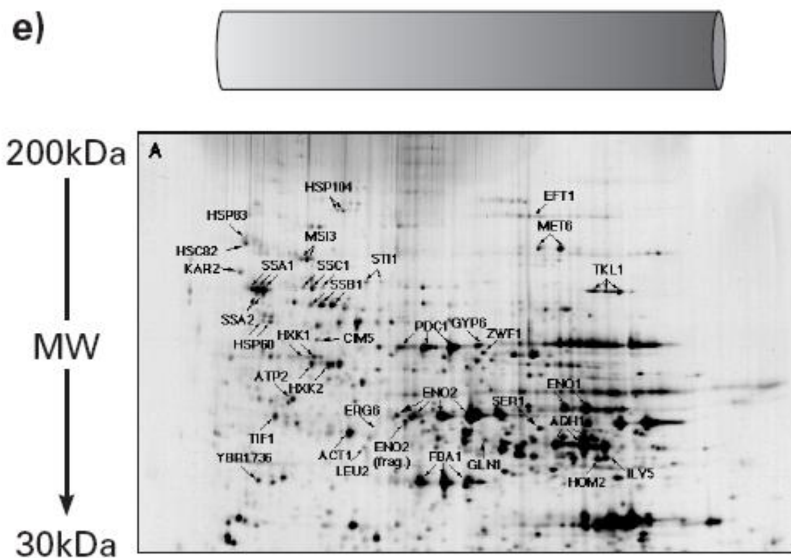
Dimension 2: size

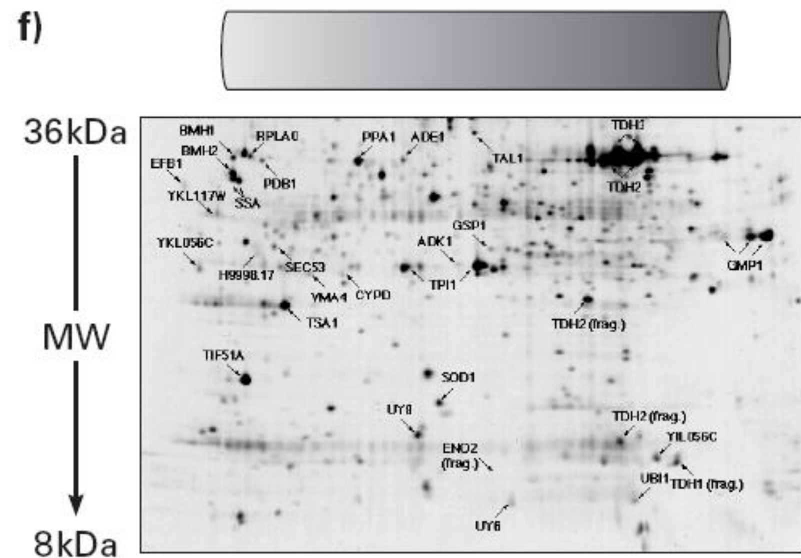
−

+

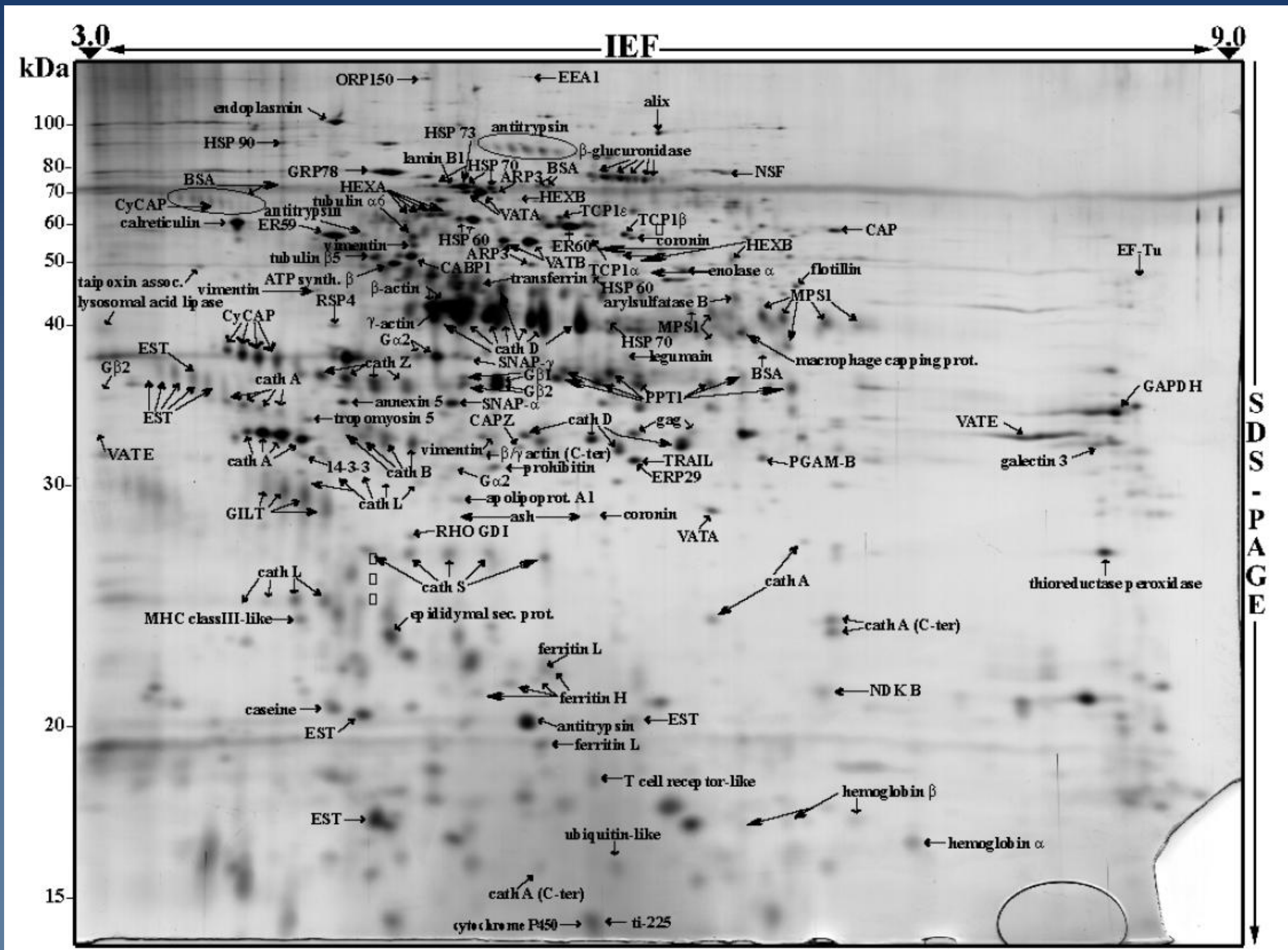
−

+





2D gel from macrophage phagosomes



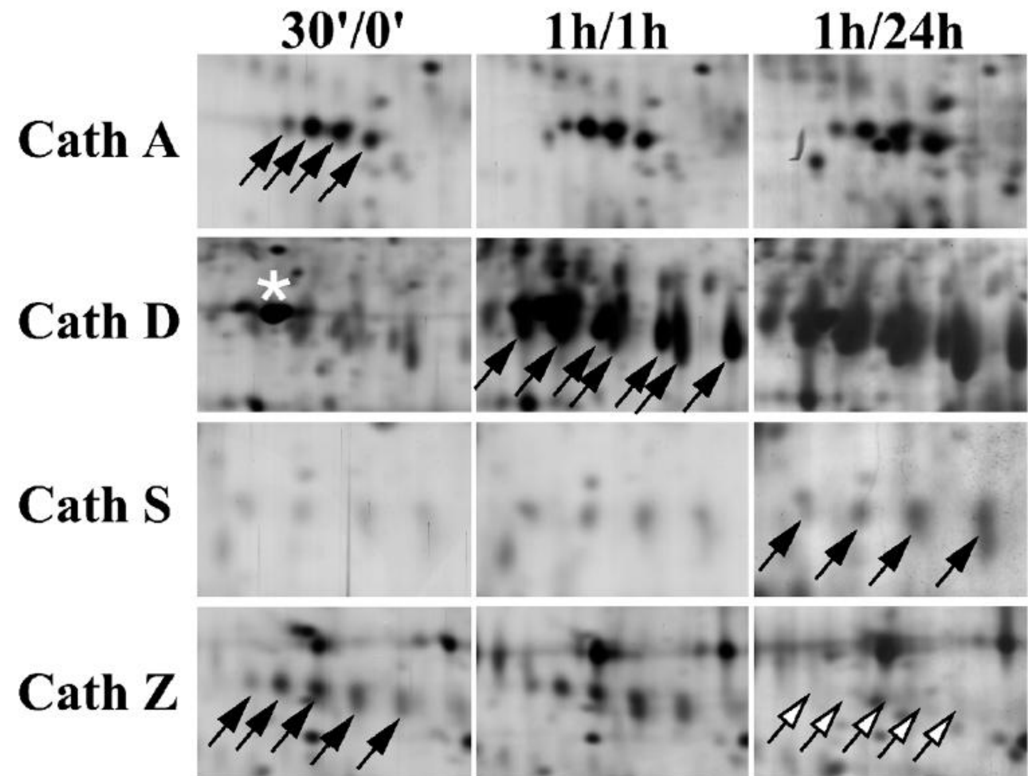
2D gel from macrophage phagosomes

Phagosomes isolated at 30 min, 1 hr, or 24 hr after treatment with latex beads

Open arrows indicate loss of signal

Multiple arrows point to multiple spots for each cathepsin

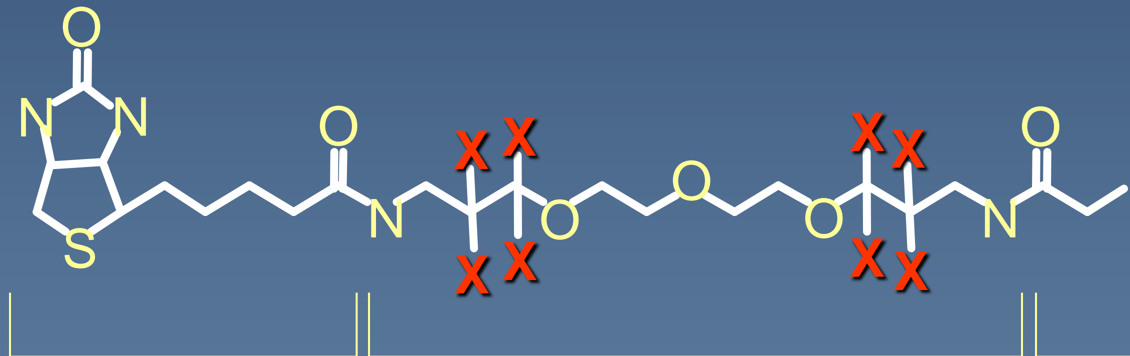
The * indicates a precursor of cathepsin D



Isotope Coded Affinity Tags (ICAT)

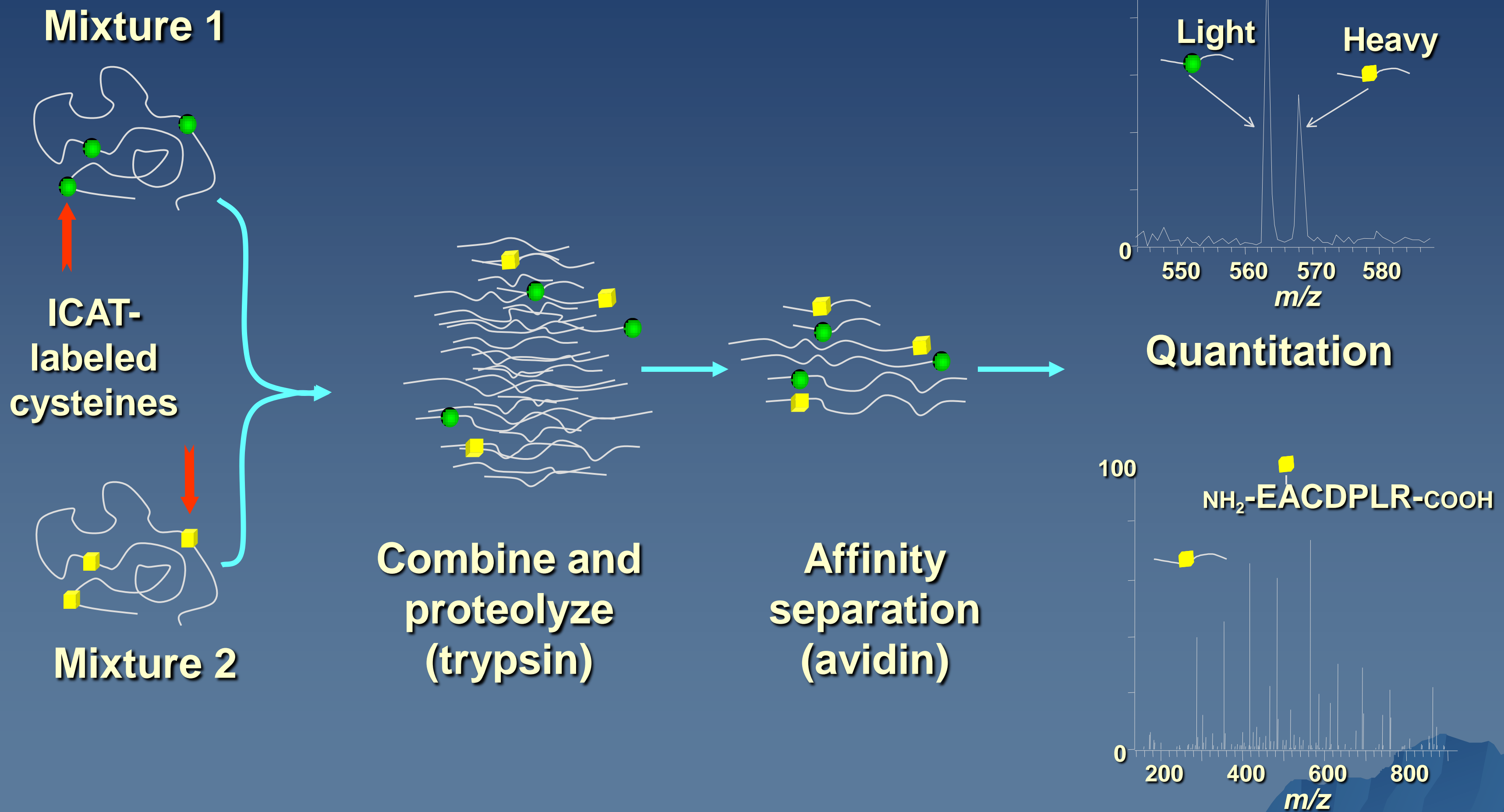
Mass spec based method for measuring relative protein abundances between two samples

ICAT Reagents: **Heavy reagent: d8-ICAT (X=deuterium)**
Normal reagent: d0-ICAT (X=hydrogen)



Biotin tag | **Linker (d0 or d8)** | **Thiol specific reactive group**

Protein Quantification & Identification *via* ICAT Strategy

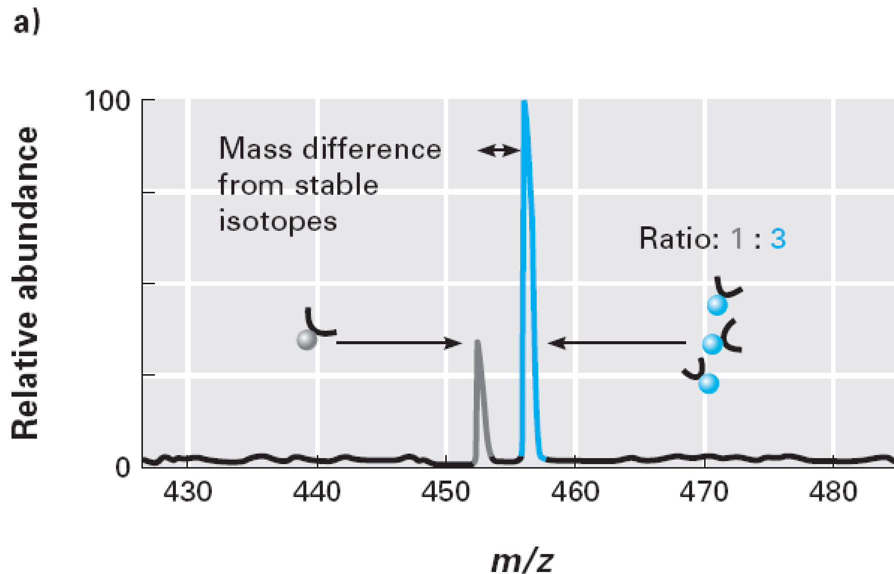


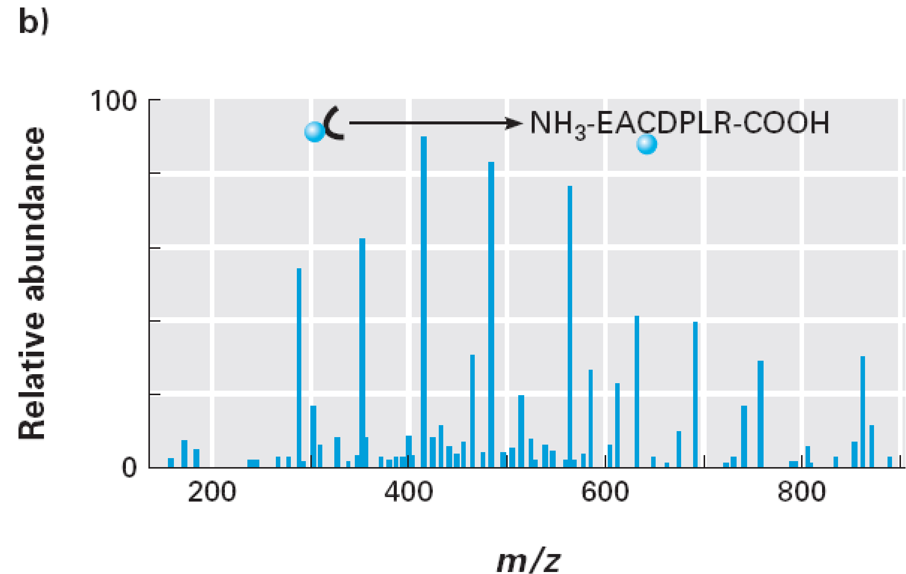
ICAT Flash animation:

http://occawlonline.pearsoned.com/bookbind/pubbooks/bc_mcampbell_genomics_1/medialib/method/ICAT/ICAT.html

ICAT continued

- The heavy (blue) and light (gray) peptides are separated and quantified to produce a ratio for each peptide – here, a single peptide ratio is shown
- Each peptide is subjected to CID fragmentation in the second MS stage in order to identify it

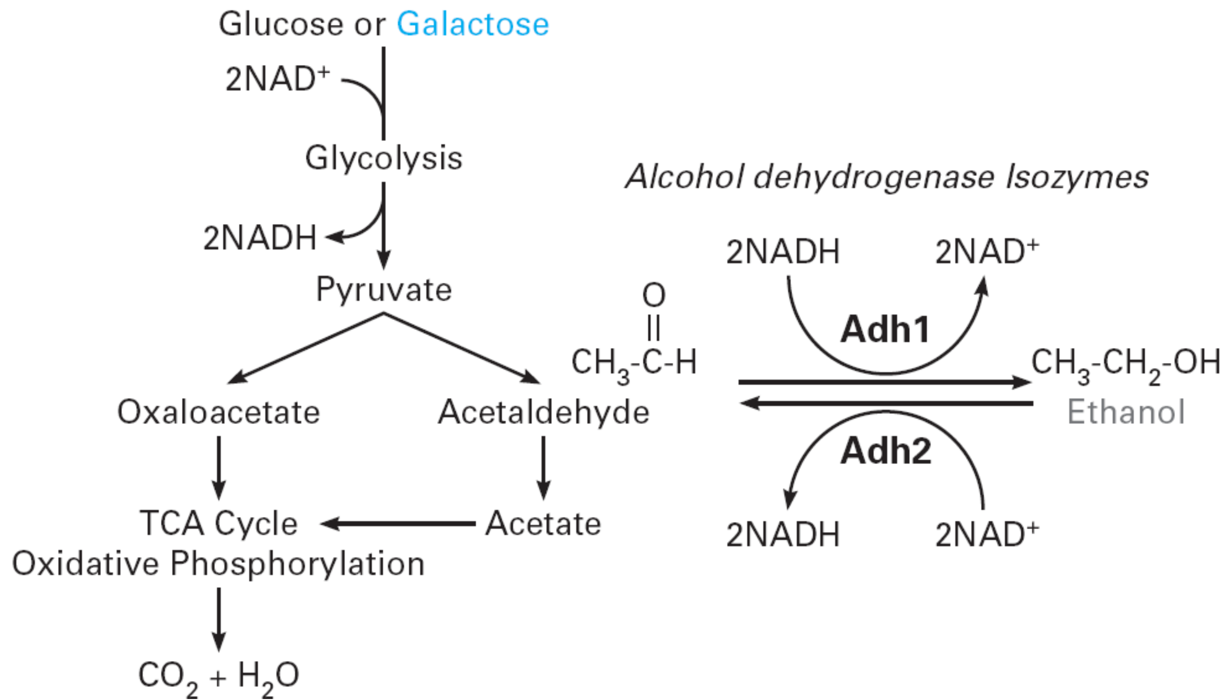


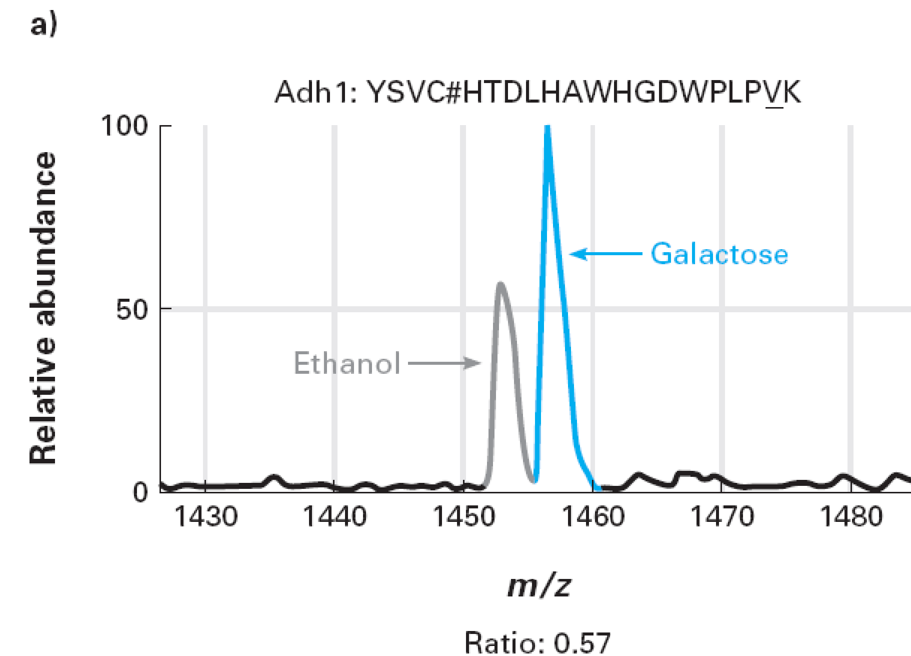


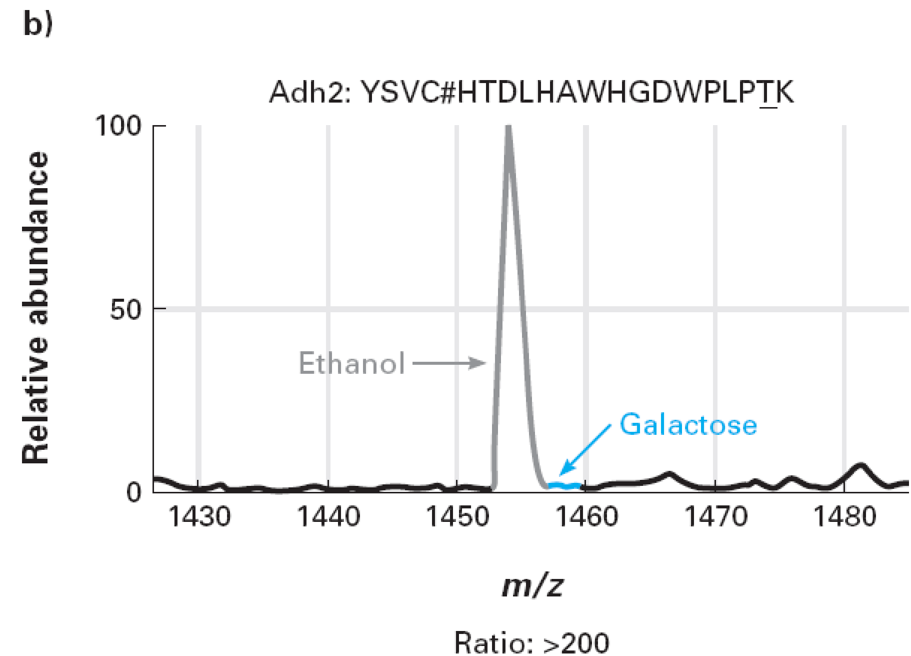
An Example

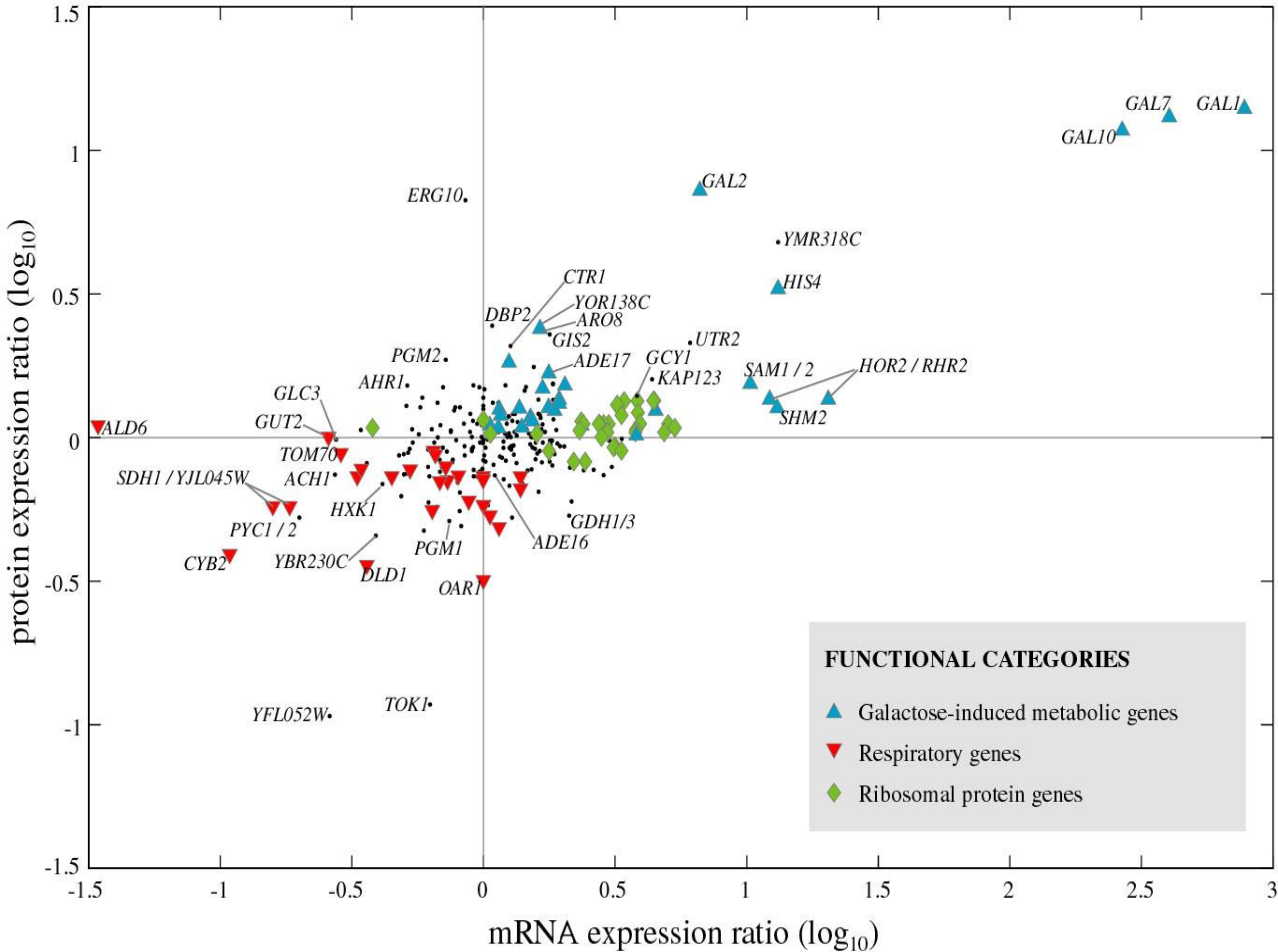
Yeast grown in ethanol vs galactose media were monitored with ICAT

Adh1 vs. Adh2 ratios are shown below…









protein expression ratio (log_{10})
mRNA expression ratio (log_{10})
1.5
1
0.5
0
-0.5
-1
-1.5
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3
GAL7
GAL1
GAL10
GAL2
ERG10
YMR318C
CTR1
HIS4
YOR138C
DBP2
ARO8
GIS2
UTR2
PGM2
ADE17
GCY1
HOR2 / RHR2
SAM1 / 2
KAP123
AHR1
GLC3
SHM2
ALD6
GUT2
TOM70
SDH1 / YJL045W
ACH1
HXK1
GDH1/3
PYC1 / 2
ADE16
PGM1
CYB2
YBR230C
DLD1
OAR1
FUNCTIONAL CATEGORIES
Galactose-induced metabolic genes
Respiratory genes
Ribosomal protein genes
TOK1
YFL052W