


- Announcements, Old HW
- Lab Quiz
- Pre-lab Lecture
  - ❖ Where we are/going
  - ❖ Antibodies + Western
  - ❖ RNA Isolation
  - ❖ Today in Lab, FNT

# Announcements, old HW

- This week's BE seminar is on actin assembly
- Module 2 draft of results+discussion piece
  - For this report, keep a more strict division between results as observations and discussion as interpretation.
  - Lead reader through logic of the controls (and exp).
  - Discussion should ideally go beyond *one* explanation *consistent* with the data, to...  (but not all, trivial)

evaluate multiple explanations and  
determine which is most likely

# Module 2: where are we/going?

- Construction phase:



added TAP-TRP PCR product to yeast gene by HR

- Evaluation phase:

Colony PCR - is TAP tag in genome

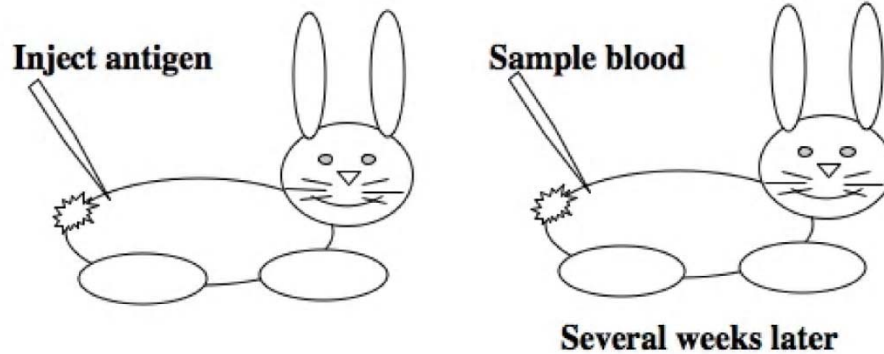
Phenotypes - does TAP tag affect sensitivity to environment (protein function)

Western - is TAP tag on the protein\* (\*right size)

Microarray - does TAP tag affect gene expression levels?

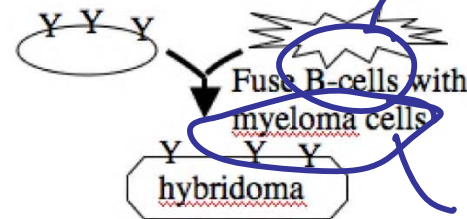
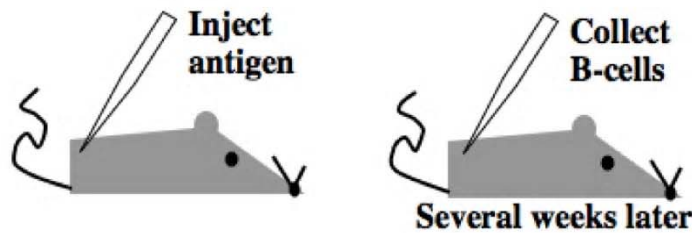
# About antibodies

- Polyclonal



serum - against multiple epitopes or same antigen

- Monoclonal - single epitope specificity make Abs



immortal

# The 1° antibody you'll use...

part of TAP tag

## P3775 Anti-Protein A antibody produced in rabbit

Sigma fractionated antiserum, lyophilized powder



MDL number [MFCD00164723](#)

[Expand/Collapse All](#)

### Price and Availability

Product Number	Your Price USD	Available to Ship	Quantity	Actions
P3775-1VL	114.50	10/20/2008 <a href="#">details...</a>	<input type="text"/>	

### Descriptions

<b>Immunogen</b>	Protein A from <i>Staphylococcus aureus</i>
<b>Reconstitution</b>	Reconstitute with 2 ml deionized water.
<b>Physical form</b>	Lyophilized from 0.01M phosphate buffered saline, pH 7.2

### Properties

<b>antibody form</b>	fractionated antiserum
<b>form</b>	lyophilized powder
<b>packaging</b>	vial of 2 mL lyophilized antiserum
<b>application(s)</b>	Ouchterlony double diffusion: 1:8
<b>storage temp.</b>	2-8°C

# The 2° antibody you'll use...

**A8025 Anti-Rabbit IgG (whole molecule) - Alkaline Phosphatase antibody produced in**  
Sigma **goat** *detection*  
affinity isolated antibody, buffered aqueous glycerol solution

MDL number [MFCD00162782](#)

[Expand/Collapse All](#)

## Price and Availability

Product Number	Your Price USD	Available to Ship	Quantity	Actions
A8025-.25ML	63.00	10/20/2008 <a href="#">details...</a>	<input type="text"/>	
A8025-.5ML	110.00	10/20/2008 <a href="#">details...</a>	<input type="text"/>	
A8025-1ML	189.50	10/20/2008 <a href="#">details...</a>	<input type="text"/>	
A8025-5X1ML	783.00	10/20/2008 <a href="#">details...</a>	<input type="text"/>	

## Descriptions

<b>Specificity</b>	Binds all rabbit Igs.
<b>Physical form</b>	Solution in 0.05 M Tris, pH 8.0, containing 1% bovine serum albumin, 1 mM MgCl <sub>2</sub> , 50% glycerol and 15 mM sodium azide.

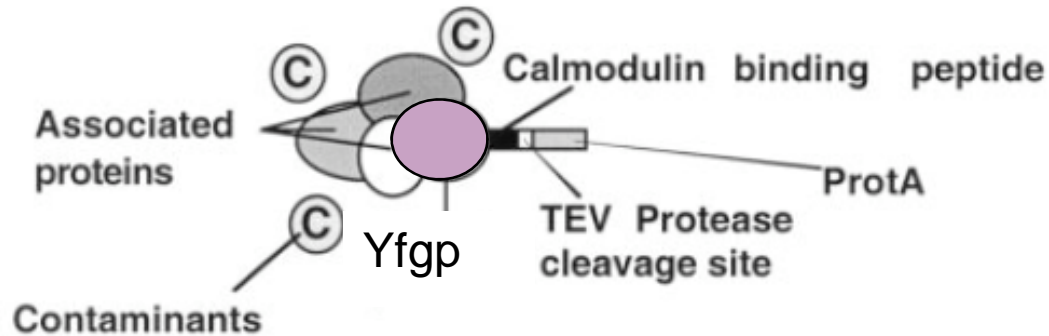
## Properties

<b>antibody form</b>	affinity isolated antibody
<b>form</b>	buffered aqueous glycerol solution
<b>application(s)</b>	direct ELISA: 1:7,000
<b>shipped in</b>	wet ice
<b>storage temp.</b>	2-8°C

# Western Analysis

why 2°?

- amplify signal
- flexibility
- specificity



1° Ab =  $\alpha$ -ProtA  
(wash)

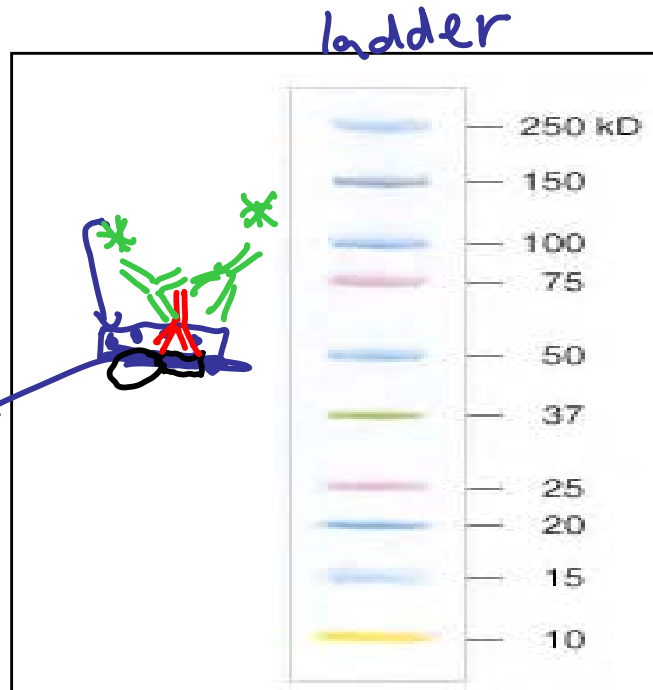
2° Ab =  $\alpha$ -rabbit  
\* AP

Substrate (2-part)

If... YFG = 25 kD

+ TAP tag = ~20 kD

Colony  
precipitate



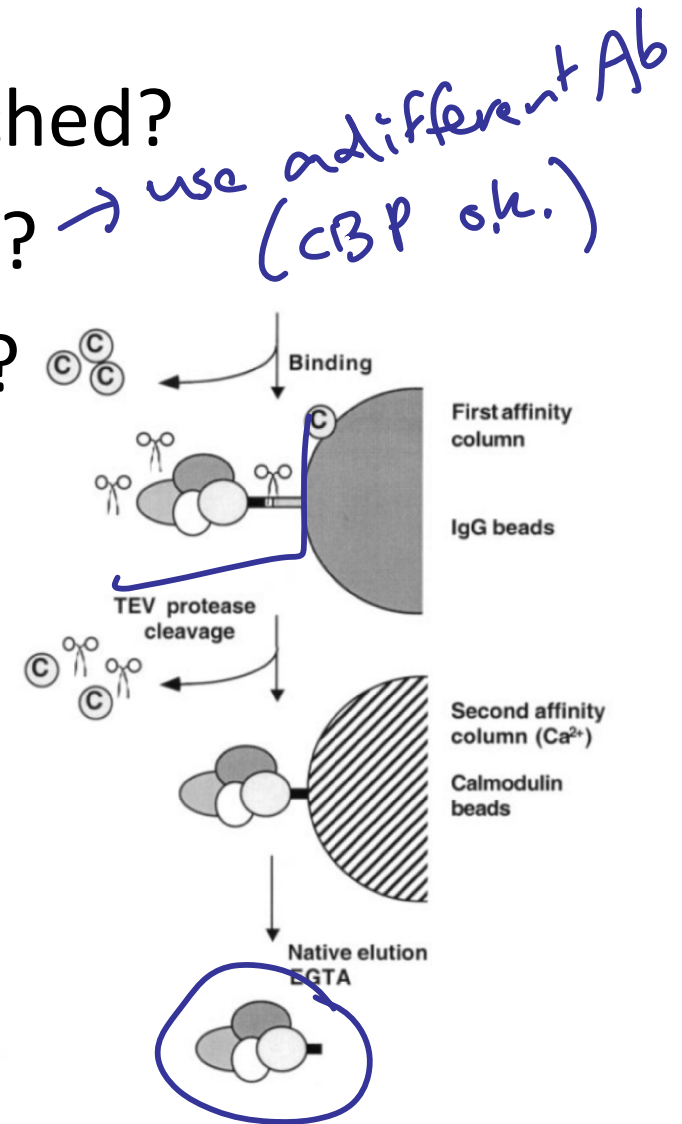
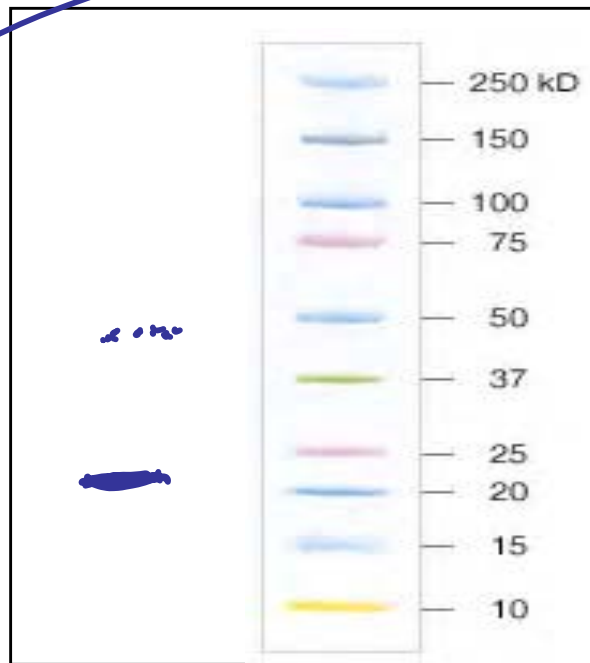
# Western Analysis--what if?

- What if TAP tag is unstably attached?
- What if you run purified protein? → use a different Ab (CBP o.k.)
- What if you skip milk, or Tween? → prevent non-specific binding

prevent non-specific binding

milk - block

Tween - kids loosely bound





# Samples for RNA Isolation

Which samples will you choose?

control - NY 420 (TRP<sup>+</sup> version of parent)

exp. - A or B or both

2 on array, but 2-3 for now. - .

# RNA Isolation Workflow

For each sample...

1. Calculate volume for  $1 \times 10^8$  cells  
 $5 \text{ OD}_{600} = 1 \times 10^8 \text{ cells/ml}$
2. Lyse cells with glass beads/vortex/PCI
3. Load lysate on column + wash
4. Elute with warm elution buffer  $\sim 100^\circ \text{C}$
5. Measure concentration of 1:100 dil'n RNAs  
 $1 \text{ A}_{260} = 40 \mu\text{g/ml}$  of RNA

→ equal #'s cells  
↓  
= total RNA

# RNA Isolation Caveats

1. RNA is sensitive to RNAses that are everywhere...

wear gloves early + often

2. Phenol- $\text{CHCl}_3$ -I will burn you if it gets on your skin, eyes, clothes...

hood, wear PPE (eyes, skin/clothes)  
MSDS upfront

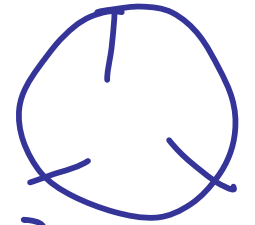
3. Do NOT mix up the samples you load on the column...

30<sup>+</sup>-step protocol label

4. The quartz cuvettes are fragile/expensive and the only ones we have...

6 yrs. strong  
+ bottle-neck

# Today in Lab



- Set up Western. During incubation steps,
  - RNA isolation of parent plus 1 or both candidates
  - Phenotype evaluation record
- Scan or photograph your spot test petri dishes which were pulled after 4 days at given temp.
- We will scan your Westerns and post the data to the talk page for today's wiki

# FNT

- Lecture is optional trip to Abcam in Kendall Square, meet 11AM 66-168
- Lab will meet in 16-336 for journal club

M2D5 FNT (due Friday if not presenting)

1. Figure of spot tests
2. Figure for Westerns
3. Calculation of volume = 2 ug RNA

4. Mid-term evaluations (anon)

→ everybody on F

M2D6 FNT (if you're presenting) 2+2

and above due Saturday by 5PM

\* email your presentation

2pt.