

- Announcements
- Lab Quiz
- Pre-lab Lecture
  - ❖ Antibodies + Western analysis
  - ❖ Today in Lab (Mod 2 Day 7)

# Announcements

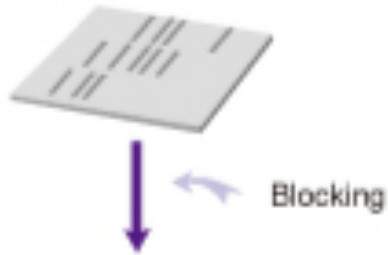
- Meet in 16-336 next W for journal club, 1 pm
- No lecture next Thu or lab next Fri, but...
- Report due following Mon, 11:12 am
  - Usual OH on Monday 11.05.12 from 2-3 pm
  - Additional OH times... ?



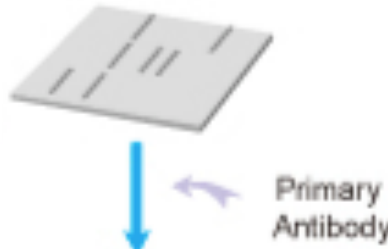
+ please post your data DS+6 Talk  
lecture Tue group mtg.

# Western Analysis

milk  $\rightarrow$  block non-specific binding sites for Ab  
\* washes TBS-T = Tween detergent



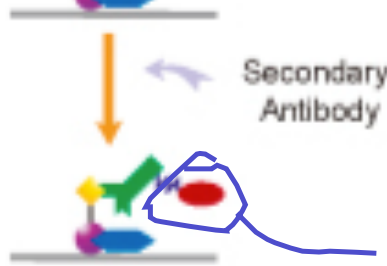
Blocking



Primary Antibody

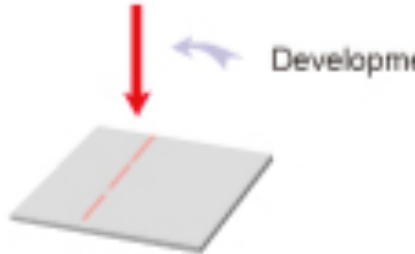
1<sup>o</sup> =  $\alpha$ -EUVZ raised in rabbits

\*  
2<sup>o</sup> =  $\alpha$ -rabbit raised in goats (Fc)



Secondary Antibody

\*  
alkaline phosphatase



Development

add substrate  $\Rightarrow$  purple precipitate

# Western Analysis--what if?

- What if you skip milk? or skip Tween?

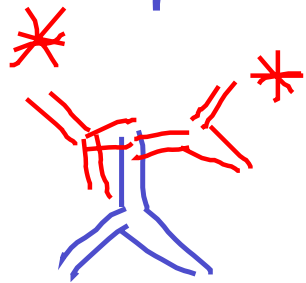
poor signal: noise

- What if protein is unstable?

absent/weak/smaller bands

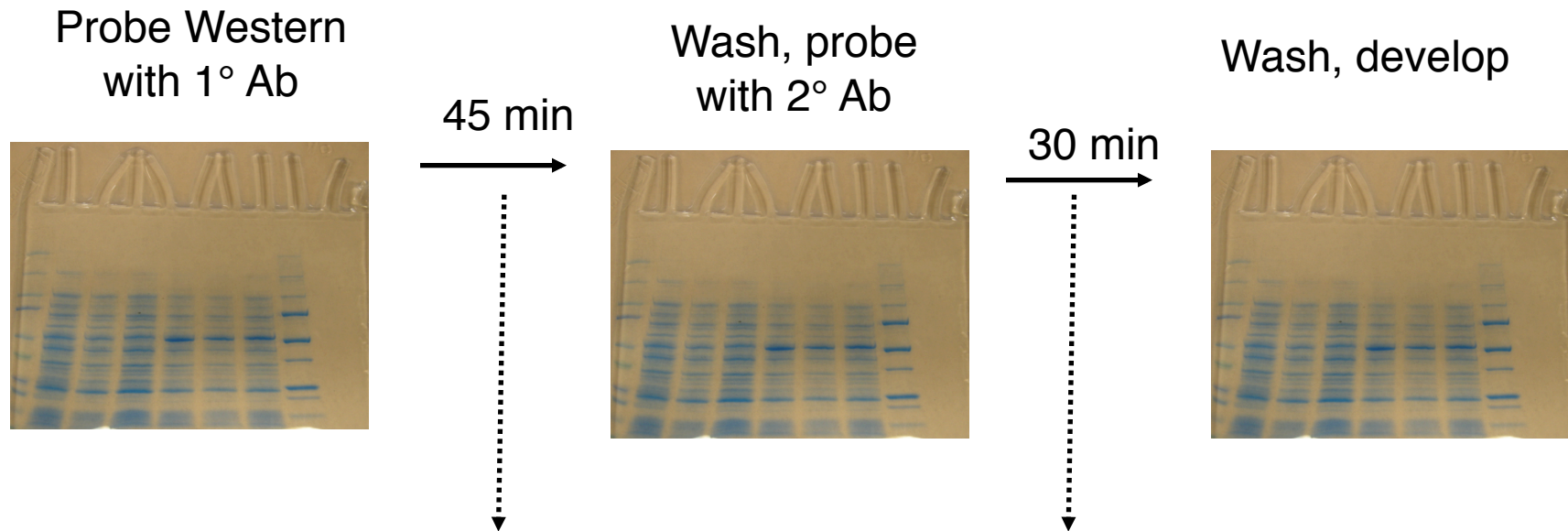
- What if 1° Ab were labeled instead of 2°?

pros of labeling 2°



- amplify ~~not~~ signal  
- efficiency (time/\$)  
→ for many 1°'s

# Today in Lab (M1D7): Workflow



Check out any plates set up last time

Repeat B-gal assay, sequencing, etc. if desired

Discuss and post your findings so far