- 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 in DS+6 Talk
lecture Tue, group mtg.
Western Analysis

1. Block non-specific binding sites for Ab
2. Washes TBS-T = Tween detergent
3. α-Env² raised in rabbits
4. α-rabbit raised in goats (Fc)
5. Alkaline phosphatase
   Add substrate = purple precipitate

Image from genscript.com
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 signal
  - efficiency (time/eff)  
    → for many 1°s
Today in Lab (M1D7): Workflow

- **Probe Western with 1° Ab**
  - 45 min

- **Wash, probe with 2° Ab**
  - 30 min

- **Wash, develop**

Check out any plates set up last time

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

Discuss and post your findings so far