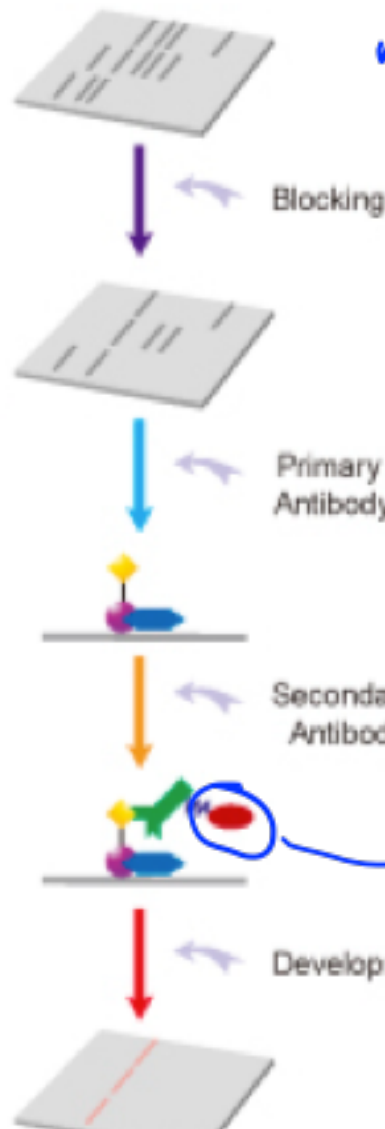


- **Announcements**
- **Lab Quiz**
- **Pre-lab Lecture**
 - ❖ **Antibodies + Western analysis**
 - ❖ **Today in Lab (Mod 2 Day 7/8)**

Announcements

- Thank you, Yongjin!
- We start Mod 3 next time
- No lecture next Tue or lab next Wed, but... *do NOT focus so much on troubleshooting*
- Report due Wed 11/12 at 12 pm – **read the handout!**
- Mod2 extra OH
 - Agi: Thu 11/6, 4-5:30 AND ~~Mon 11/10~~, *Tue 11/11 tentatively ~~3:30-5~~ 3:30-5*
 - Noreen: Fri 11/7 1-3 AND Mon 11/10, 10-12 } *16-4296*
 - Shannon: usual Mon 11/10 OH from 3-4+
 - ★ – Natalie: by appt *email NKULDELL*
- Blog post due 11/13
- Sign up for jclub meeting w/Atissa by Doodle

Western Analysis



milk \rightarrow block sites for non-specific binding of the antibody (Ab, α)
 * washes w/ TBS-T = Tween detergent

Primary Antibody $1^{\circ} = \alpha$ -Envt(His) raised in rabbits

*

Secondary Antibody $2^{\circ} = \alpha$ -rabbit raised in goats
 \hookrightarrow (Fc)

*

alkaline phosphatase (AP) conjugation
 add substrate \Rightarrow purple precipitate

Visualizing Cph8 abundance

- What is the expected size of EnvZ? Cph8?
- Lanes:

1. Ladder
2. H6-EnvZ
3. WT Cph8
4. M1 Cph8
5. M2 Cph8



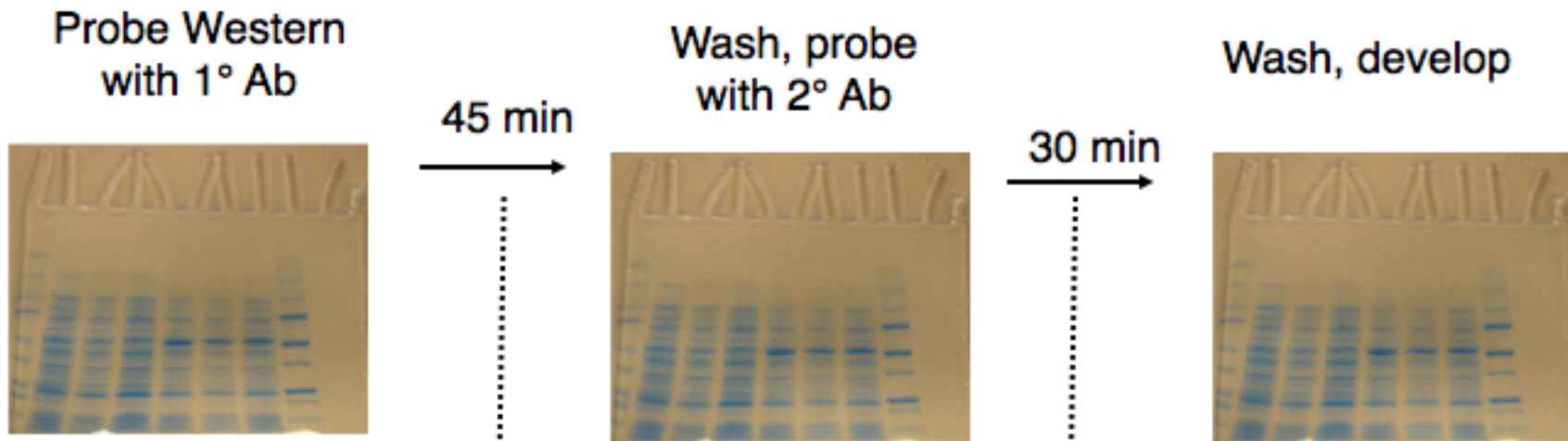
Cph8 see F, NW
EnvZ smaller
(check aa)

Western Analysis--what if?

- What if you skip milk? or skip Tween?
terrible or kinda bad, respectively, 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/\$)
⇒ use with many 1's



Today in Lab (M2D7/8): Workflow



Perform B-gal assay with 2 known mutants

Ditto bacterial photograph

Discuss and post your findings so far

Finish lab notebook – due today!

VORTEX

tech or bio?
↓
?
↓
2

List of available bacteria

- NB466 = WT
- 468 = kinase dead
- 486 = T541R
- 487 = T541Y
- 488 = T541P
- 491 = T541L