- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Antibodies + Western analysis
  - Sequencing recap
  - Today in Lab (Mod 2 Day 6)

Announcements

- Meet in 16-336 this Fri for journal club, 1 pm
- No lab next Wed
- Report due 1 week from Fri
  - Revise for up to 1 letter grade improvement
    - Due one week after you get it back
  - OH: NK Tue 11-1 Mon 1:30-3pm (+ by appt.)
    - I'll start w/a ~15 min lecture on the different sections of a lab report (especially results vs. discussion)

Module 2: where are we/going?

Discuss goal of each phase/experiment with a partner

- Construction phase:

  Make Cph8 library (NK) and put into cells (109)

  overall: improve dynamic range of BP system using K+ or pt of EnvZ (with Cph8)

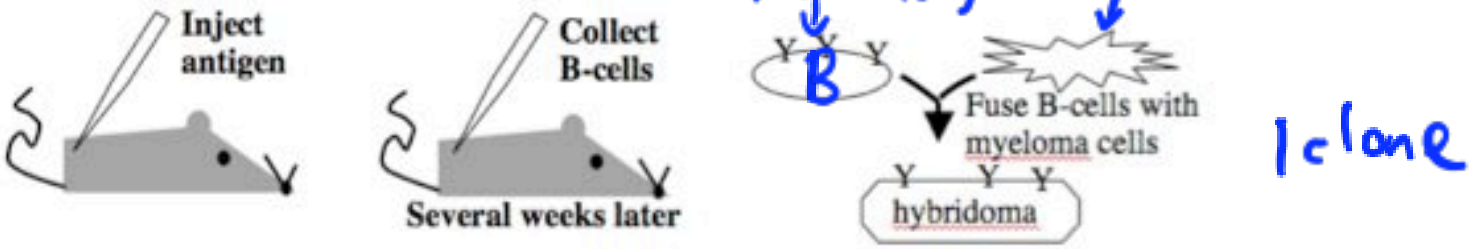
- Evaluation phase:

  DNA
  - Diagnostic Digest — Do I have a mutant?
  - Sequencing — What is the mutant?

  protein
  - β-gal assay — Does mutant affect B-gal production? under red light and/or dark
  - Western — Could change in B-gal be due to overall Cph8 expression?

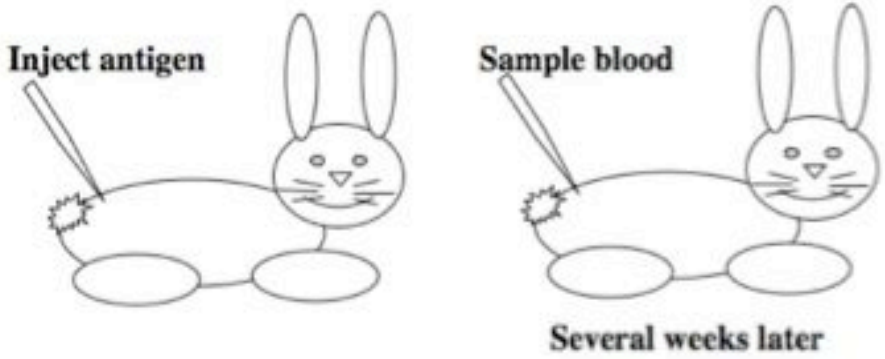
Antibody production

- Monoclonal – against 1 specific epitope on antigen

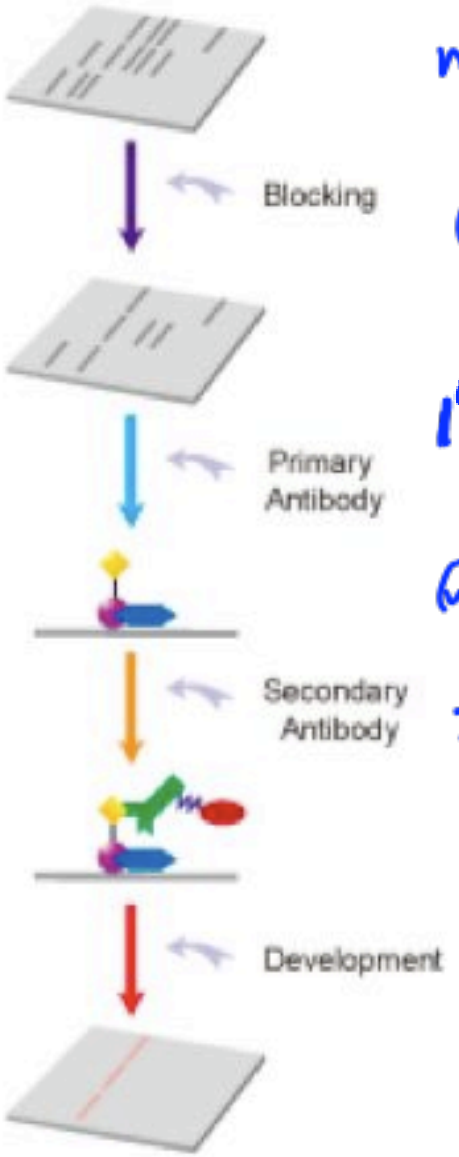


1 clone

- Polyclonal – against multiple epitopes



Western Analysis



milk (BSA) → block sites for non-specific (often hydrophobic) driven binding of Abs

⊛ washes w/detergent = Tween

1° Ab - anti-HA tag
made in mice

(wash)

2° Ab* - anti-mouse (against Abs, Fc)
made in goats

* alkaline phosphatase label

develop - add substrate → colored 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?

if cut

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

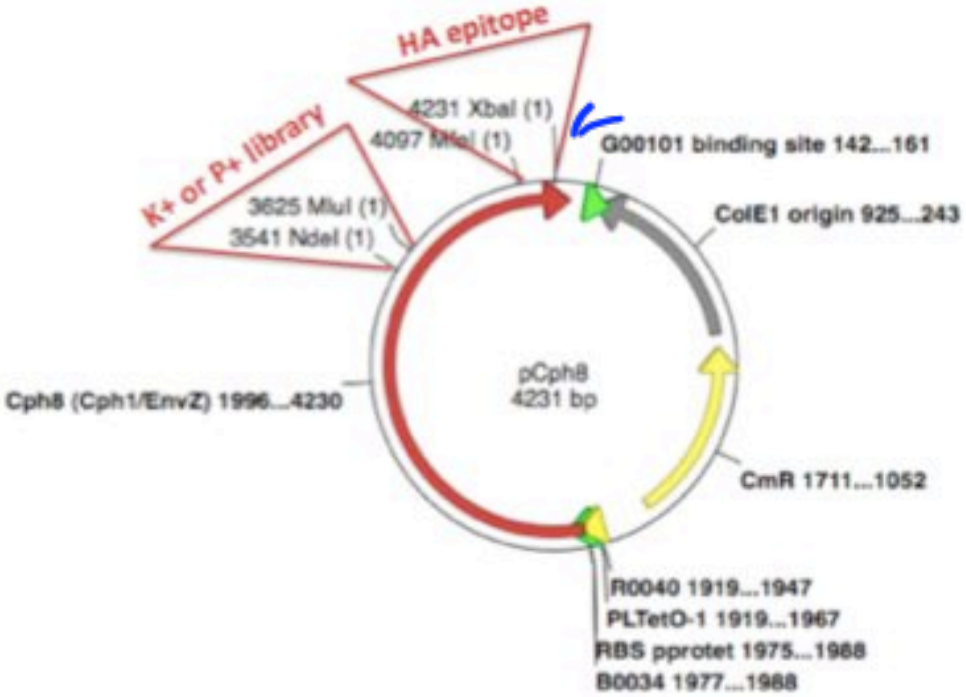
pros of labeling 2°: – amplify signal

– flexibility (use 2° on/any mouse 1°)

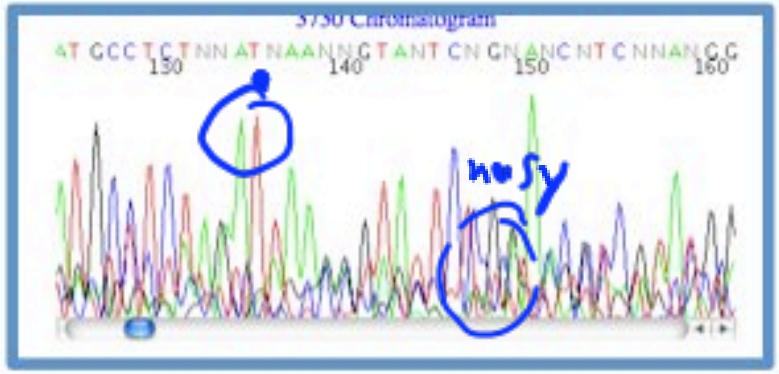
↳ time/cost

but often more cross-reactivity.

Sequence analysis (slide adapted from N. Kuldell)



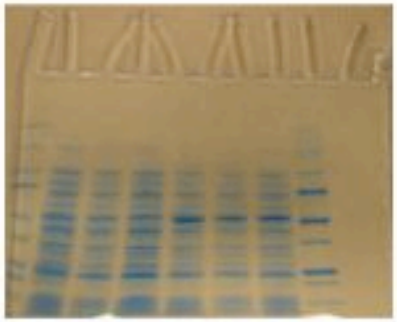
1. Retrieve the sequences
2. Find reverse complement
3. BLAST
   your sequence (= query)
   vs pCph8 (= subject)



Will pCph8 have HA tag?
What will you *do* if you find residue changes?

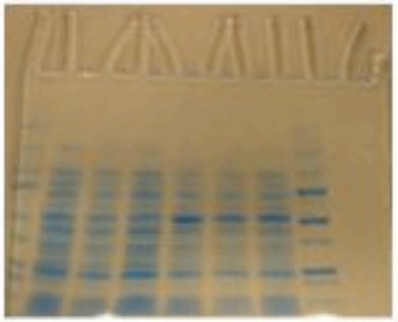
Today in Lab: Workflow

Probe Western with 1° Ab



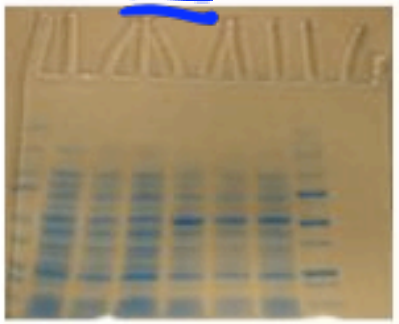
45 min

Wash, probe with 2° Ab



30 min

Wash, develop (O/N)



Run β-gal assay

and identity of samples?

M1, M2, 366 (374) (4D) #A1

2-3 reps. (16-24 hr)

Analyze sequencing data (or do at home)

Finish β-gal assay if needed

Optional: take photo w/mutant cells