

M2D8:  
Readout: Protein

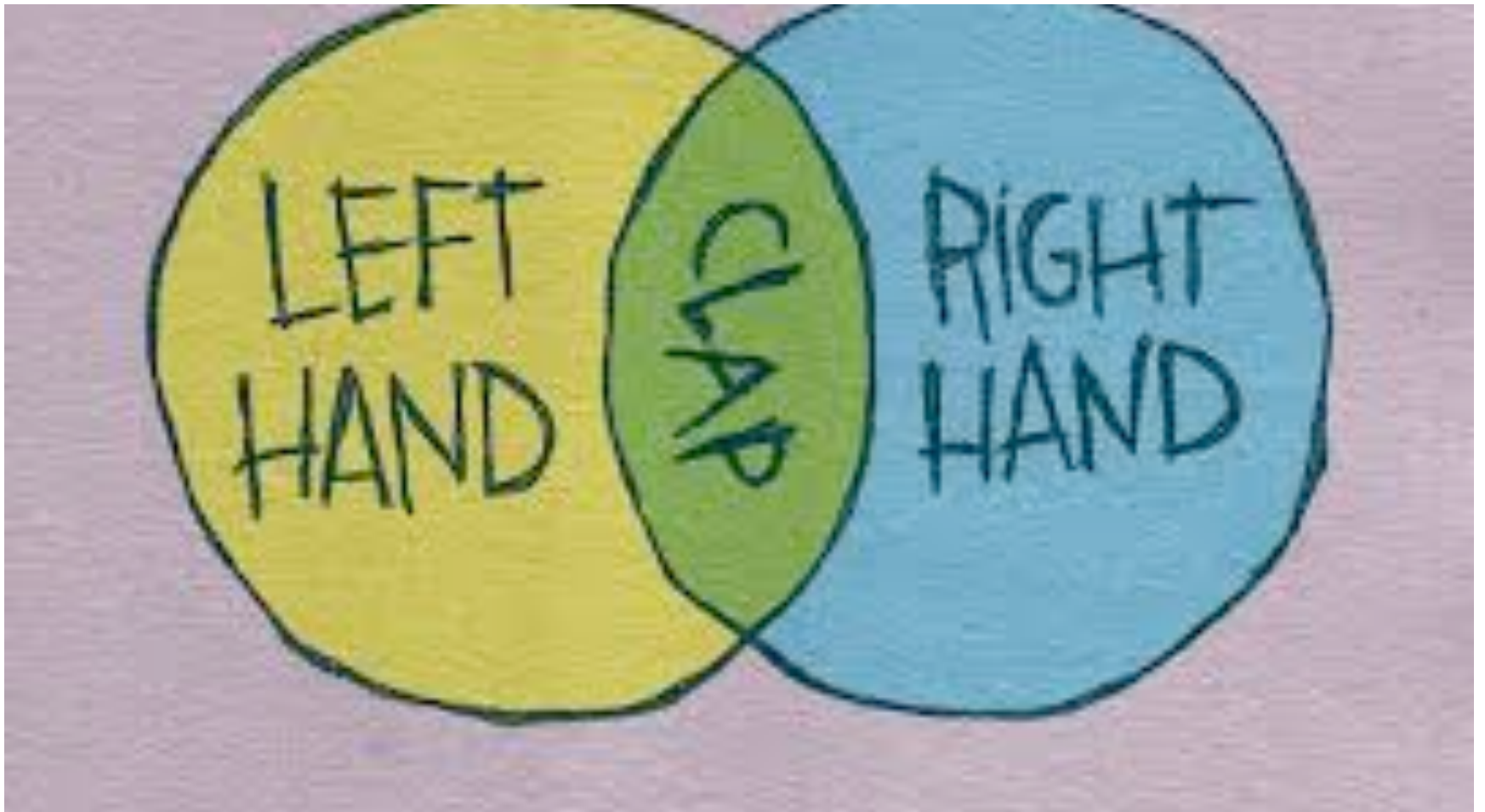
11/4/14

# Lab business



- Lab treat
- For next time...
  - Read through front page of Mod 3
- Mod 2 research report due November 12 at 12p
  - Material and methods drafts returned on R
  - Natalie: office hours by appointment
  - Agi: office hours R 4-5:30p and M TBD (check wiki)
  - Noreen and Shannon: will email
- Blog post due November 13
- Atissa will email you to review Journal Club

Thank you, Yongjin!



# Review of last time...

- Sample preparation:

SDS  $\rightarrow$   $\ominus$  charge

BME  $\rightarrow$  breaks S-S bonds

Boil  $\rightarrow$  break 2<sup>o</sup> structure

- Protein separation:

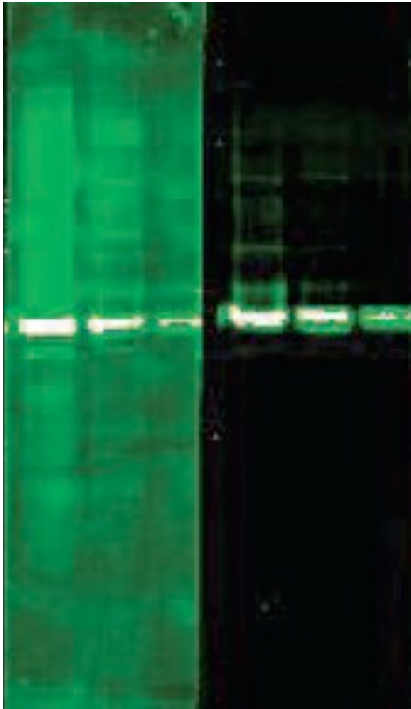
polyacrylamide  
separated by size  
↳ SDS

- Protein blotting:

onto nitrocellulose

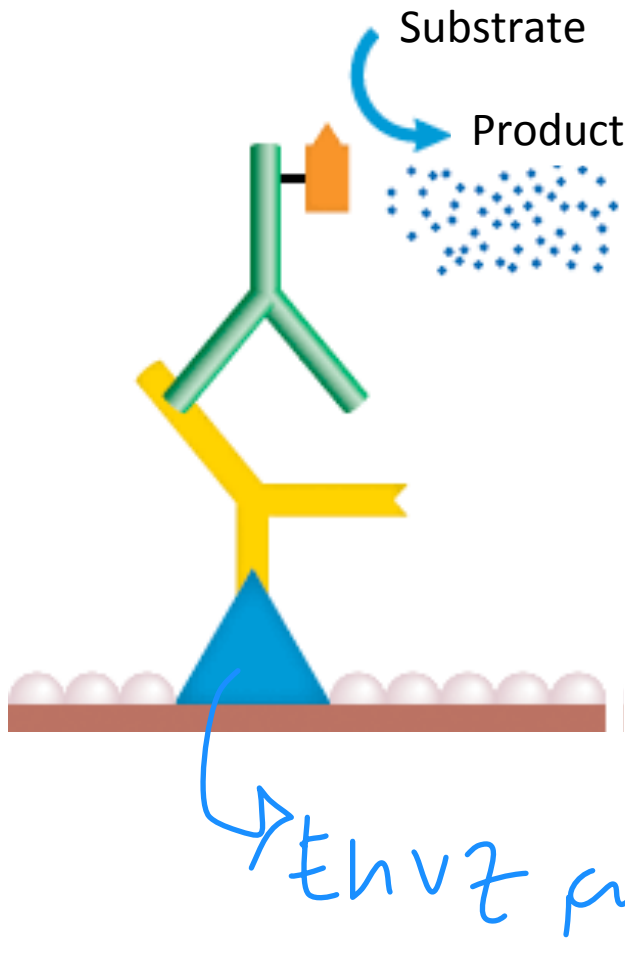
# While you were away...

- Nitrocellulose membrane incubated in blocking solution (5% milk)



milk → casein

# Probing for Cph8 protein



- 1° antibody = binds EnvZ  
bunny
- 2° antibody = binds 1°  
goat alkaline phosphatase
- Substrate =  
BCIP/NBT
- Product = purple prec.

# Visualizing Cph8 abundance

- What is the expected size of EnvZ? Cph8?

- Lanes:

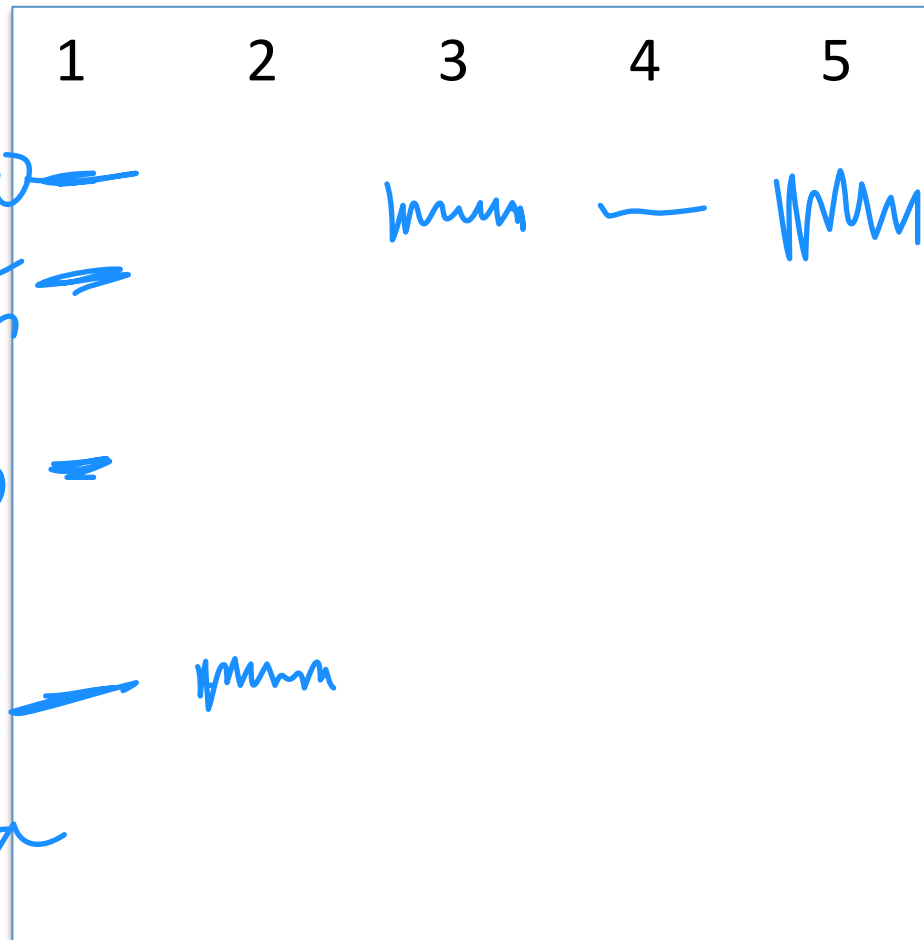
1. Ladder 100 —

2. H6-EnvZ 75 —

3. WT Cph8

4. M1 Cph8 50 —

5. M2 Cph8



cph8 = 74 46p 25 —  
 83000  
 EnvZ = 22 96p 25600  
 25600

# Today

1. Finish Western blot analysis
2. Use previously isolated mutants to generate data for Mod 2 report (Topic 3)
  - Complete beta-galactosidase assay
  - Setup bacterial photographs

notebook entry due. M2D2  
466 WT  
468 KD