Digression

costume day

just pretty much
always awesome day

- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Recap SDS-PAGE
  - Sequence analysis preview
  - Today in Lab (M2D6)

Announcements

- Next time I'll need to leave early and Shannon (T/R section leader for Mods 2 and 3) will come help out
- Please email us your experimental plans as you develop them
- Next journal club in 1 week
- Report due M 11.12.12 → fill out survey for extended OH

Mutant Miller assay data

Mutant 1 B-Gal Average (Dark)	Mutant 1 B-Gal Average (Light)	Mutant 1 T541X; X =	Mutant 2 B-Gal Average (Dark)	Mutant 2 B-Gal Average (Light)	Mutant 2 T541X; X =
2328.25	1928.66	1.2	--	--	
1417.88	1409.76	1.0	1450.07	454.78	3.2

Wild Type System B-Gal (Dark)	Wild Type System B-Gal (Light)	H243A Mutant B-Gal (Dark)	H243A Mutant B-Gal (Light)
1099.13	414.85	54.54	45.27
1099.13	414.85	54.54	45.27

Comparisons to make?
Representing data?

Go, Team Pinkle!

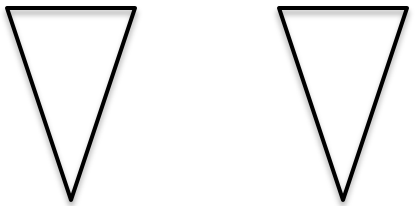
SDS-PAGE preparation

acrylamide monomer neurotoxic

- You will make whole cell extracts with equal cell #s
  - Based on OD_{600} reading, normalize

Goal: 2 OD per sample

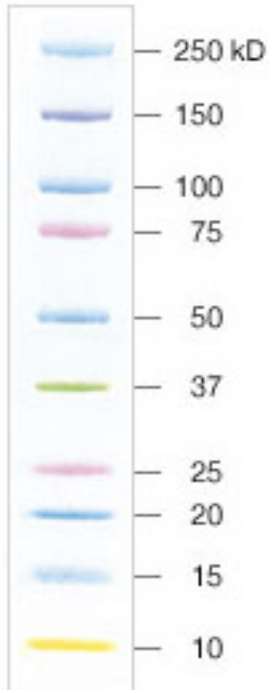
① 2mL
② 4mL



- Gel separates proteins based on size, shape, charge
- Sample preparation reduces analysis complexity
  - SDS: coat proteins w/(-) charge
  - β-Me: breaks SS bonds → tips in load
  - Boiling: denature higher order
  - Sample Buffer has SDS, β-Me, plus glycerol, BPB dye

SDS-PAGE visualization, analysis

- Determine size in comparison to ladder (pre-stained)
- Visualize specific protein amount with antibody and staining
  - antibody conjugated to enzyme
  - enzyme catalyzes colorimetric rxn
  - more detail next time
- Goal: determine if mutation affected Cph8 production



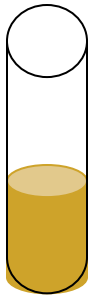
Kaleidoscope

Sequencing food for thought

- Where in the sequence do I expect a mutation?
- How do I interpret mutations occurring elsewhere?
- How do I interpret gaps (vs mismatches)?
- What resources may help me form a hypothesis about the behavior of a particular mutant?

Today in Lab (M2D6): Workflow

Check OD of cells

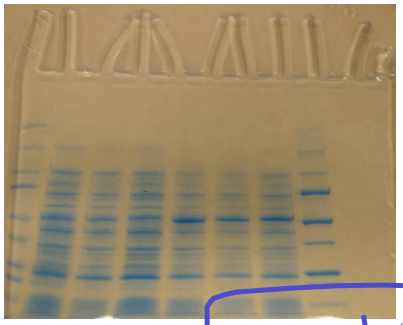


measure @ 1:10 for rearrange

Lyse an aliquot and load onto SDS-PAGE

spin 20P, resus. 100µL 30µL + 30µL SB

Also ladder & (+) control



goal: 2:30

Let run 1 hour

usually 40'

Transfer to blot – run 1 hour

Meanwhile…

Store in milk solution until next time

Sequencing analysis (ApE or BLAST)

Mutant bacterial photograph maybe

Ideas/prep for experiments next time… from seq/β-gal repeats to ?your idea here?