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

Announcements

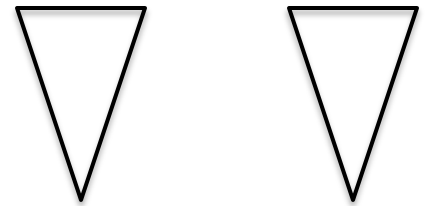
- Keeping lab running smoothly
- Understanding strain information

  Strain NB466 (genotype: MC4100 ara+ Φ(OmpC-lacZ) 10-25 ΔenvZ::KanR +pCph8 +pPL-PCBamp)
- More generally → if you don't understand something on the wiki, ask about it rather than copying it into your Methods (or other) sections
- Next journal club in 1 week! email for mtg.
- Extended OH for second report

M 2-3:30, R 2-3:30

SDS-PAGE preparation

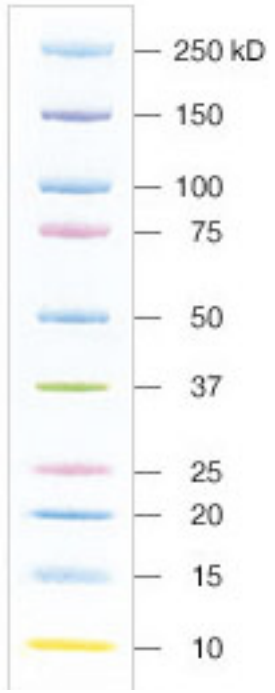
- You will make whole cell extracts with equal cell #s
  - Based on OD_{600} reading, normalize



- Gel separates proteins based on size, shape, charge
- Sample preparation reduces analysis complexity
  - SDS:
  - β-Me:
  - Boiling:
  - Sample Buffer has SDS, β-Me, plus

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

1. Retrieve the sequences
2. Compare your sequence (= query) vs pCph8 (= NB466)


```
WT  CACGCTGCTGATGGCGGGGGTAAGT-CACGACTTGCGCACGCCGCTGA 95
    ||||||||||||||||||||||||| |||||||||||||||||||||||
MUT CACGCTGCTGATGGCGGGGGTAAGTGC-CGACTTGCGCACGCCGCTGA 99
```

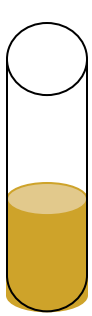
How will you interpret mismatches?

How will you understand action of amino acid changes (if you have any)?

Slide from N. Kuldell

Today in Lab (M2D6): Workflow

Check OD of cells

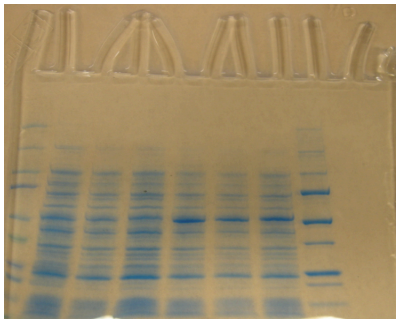


measure @ 1:10

Lyse an aliquot and load onto SDS-PAGE

- 2 hD-spin pellet
- 30 µl cells + 30 of SB

Also ladder & (+) control



Let run 1 hour

Transfer to blot – run 1 hour

Store in milk solution until next time

Meanwhile…

- ASTACHOW

Sequencing analysis (ApE or BLAST)

Mutant bacterial photograph

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