- Announcements
- Pre-lab Lecture
  - Troubleshooting catch-up
  - Recap SDS-PAGE
  - Recap sequencing
  - Sequence analysis preview
  - Today in Lab (M2D6)

Announcements

- Final M2 quiz on Wed 11/5
- Journal club next time
  - 16-336 starting at 1:15 pm sharp
  - upload slides to Stellar in advance
  - T/R guest, then your 7 talks → 8 total
- Report due Wed 11.12.12
  - best day(s) for extra OH?

main data probably 11/5

R 6 + ~~F 7~~

F 7 + M 10 ?

other combos?

other days?

Troubleshooting catch-up

- Problem(s): light/dark WT backwards; magnitude lower
- Conditions for repeat: → verifying WT ✓ mutants - not yet
  - fresh antibiotics + media
  - WT/KD: fresh re-streaks from freezer stocks ✓
  - M1/2: restreaks (from liq. cult.) on plain LB/anti-biotic plates, hope for best!
- Today's samples: Plate samples re-inoculated in fresh LB/antibiotics
- Review reasons for having signaling but no light response
  1) basal / background / leaky expression
     p(event) ↑ or ↓ not binary on/off
  2) cross-talk when cognate partner is missing
  3) reminder: light turns signaling off, not on, in our system

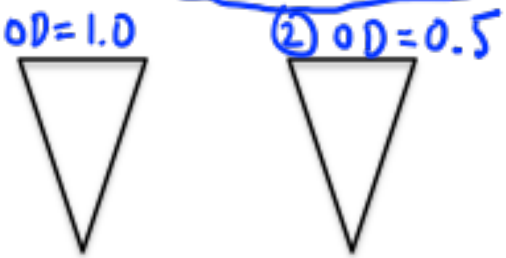
SDS-PAGE preparation

↳ acrylamide monomer is toxic → why?

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

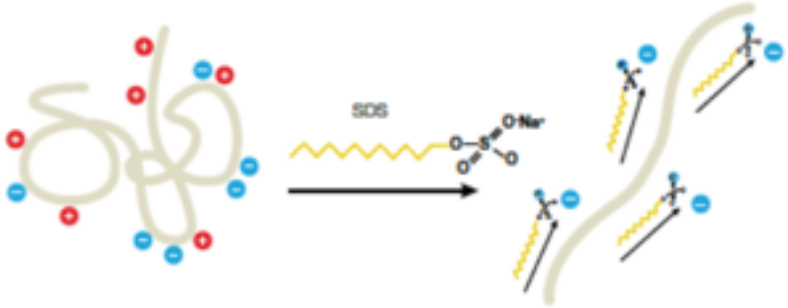
goal: 2 OD per sample

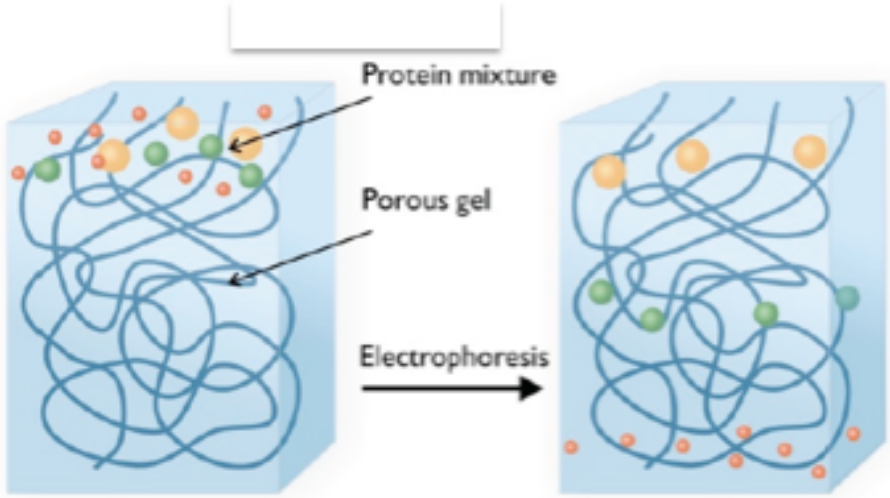
sample ① 2 mL
② 4 mL

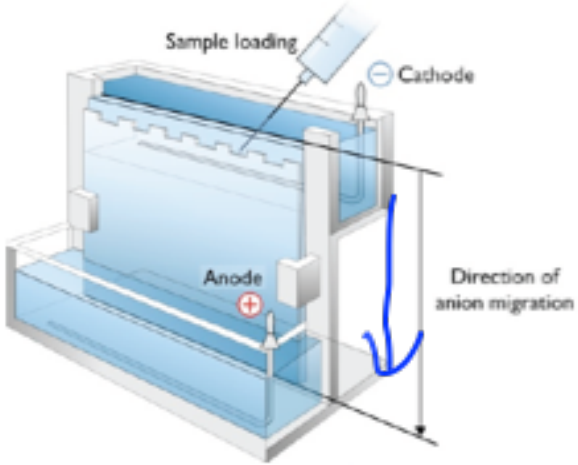


- Gel separates proteins based on size, shape, charge (mass → make uniform)
- Sample preparation reduces analysis complexity
  - SDS: coat proteins w/ (-) charge
  - β-Me: breaks S-S bonds ⇒ tips in hood to air out
  - Boiling: denature higher-order structures
  - Sample Buffer has SDS, β-Me, plus glycerol, BPB dye

SDS-PAGE separation





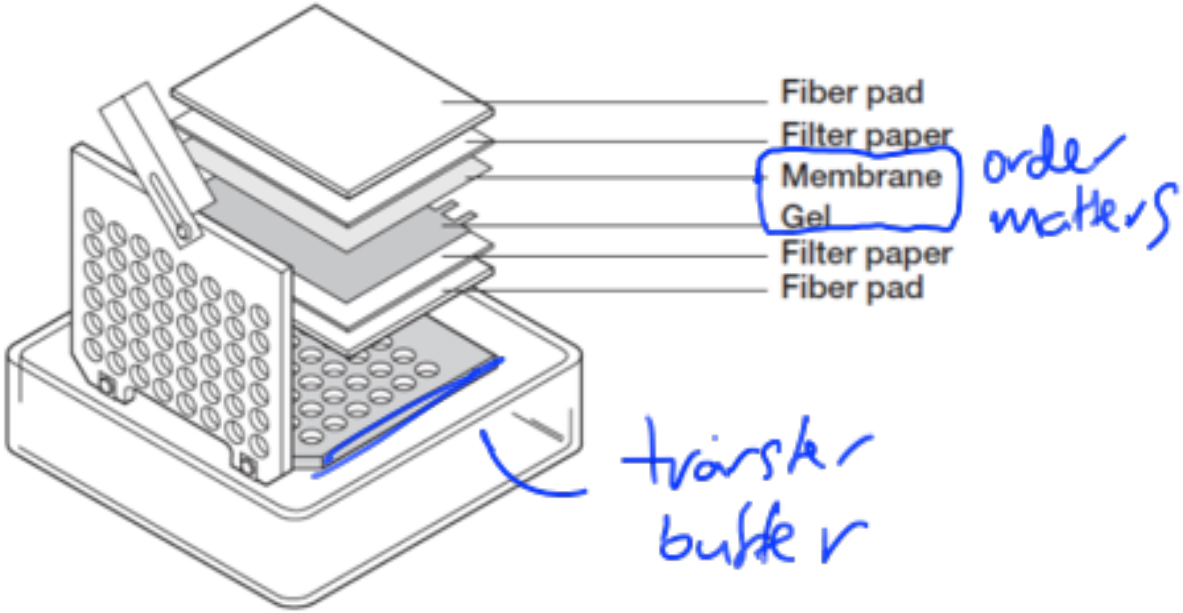


TAG SDS
buffer

Slide adapted
from Noreen L

SDS-PAGE blotting

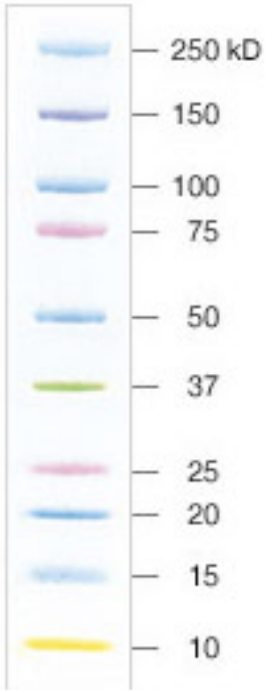
- Transfers proteins from polyacrylamide gel onto a nitrocellulose membrane
- Notes:
  - Eliminate air bubbles
  - Do not touch nitrocellulose



Slide from Noreen L

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 → Western
- Goal: determine if mutation affected Cph8 production



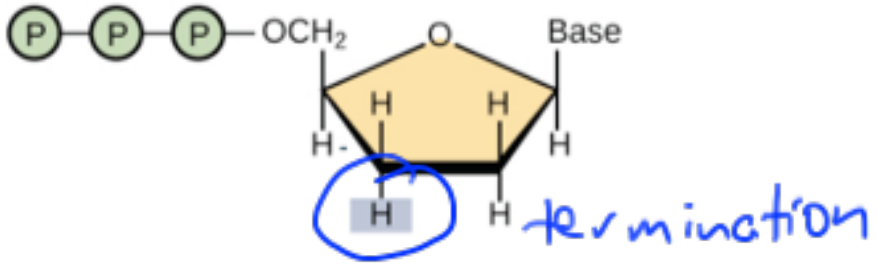
Kaleidoscope

Sequencing food for thought

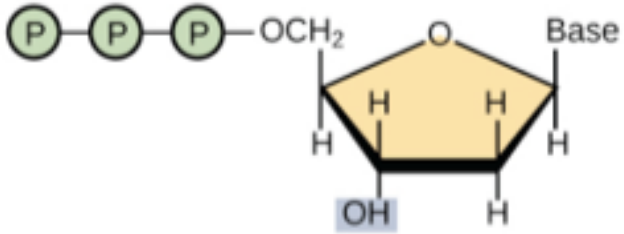
- Where in the sequence do I expect a mutation? T541X
- How do I interpret mutations occurring elsewhere?
- How do I interpret gaps (vs mismatches)?
- What resources may help me form a hypothesis about the behavior of a particular mutant?

Sanger sequencing approach

- Dye labeled dideoxynucleotides added
- Chain terminating reaction



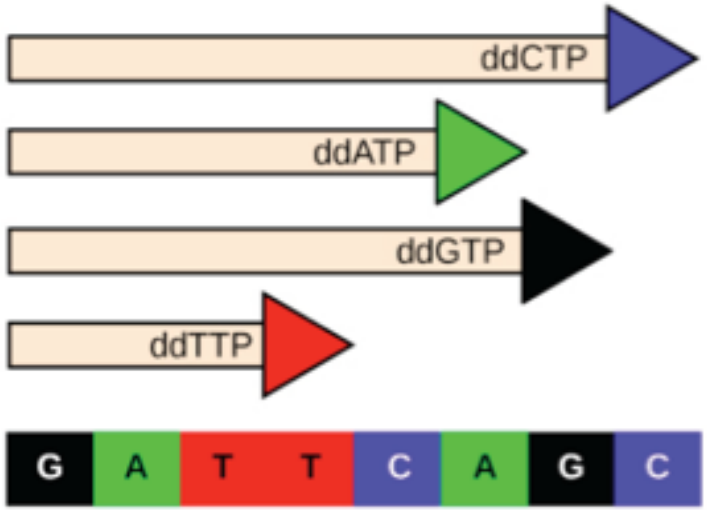
Dideoxynucleotide (ddNTP)



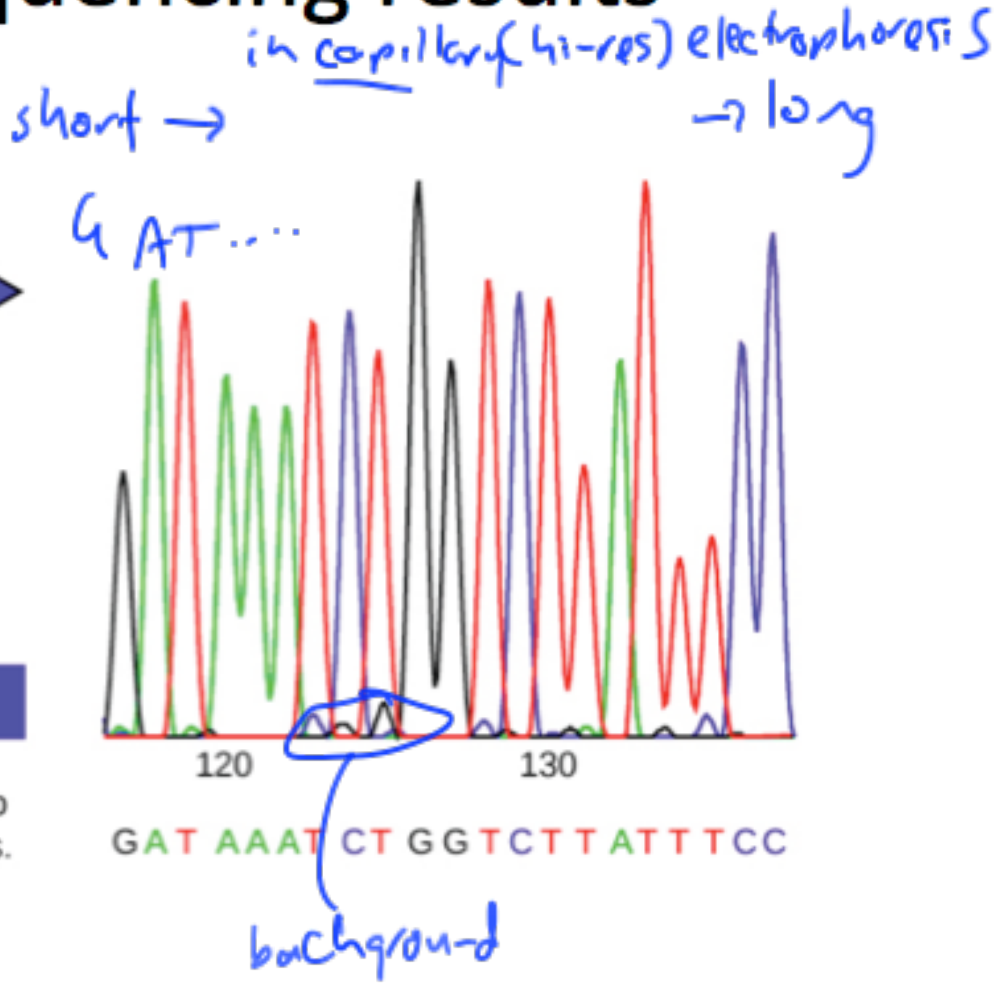
Deoxynucleotide (dNTP)

Slide from Noreen L

Sanger sequencing results



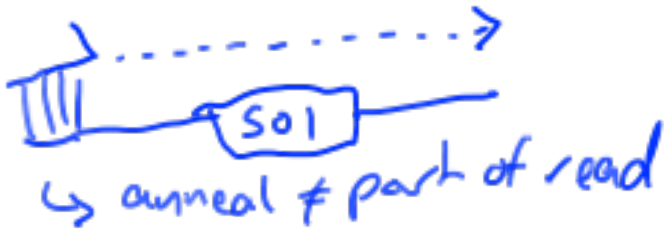
Dye–labeled dideoxynucleotides are used to generate DNA fragments of different lengths.



Slide from Noreen L

Sanger sequencing limitations

- Note: primer needs to anneal upstream of SOI
  - SOI = sequence of interest
- Max of ~ 1000 bp read lengths
- Sequence at beginning, end, is unreliable
  - start: need to establish baseline fluorescence
  - end: decreasing resolution in capillaries as DNA size ↑
  - both: less probability to form those products, more noise
- Note: it's okay to put "Ns" in DNA software



+ less signal

10 vs. 11 bp
10^{11} difference in length

100 vs. 101
100^{11}

etc...

Today in Lab (M2D6): Workflow

Check OD of cells

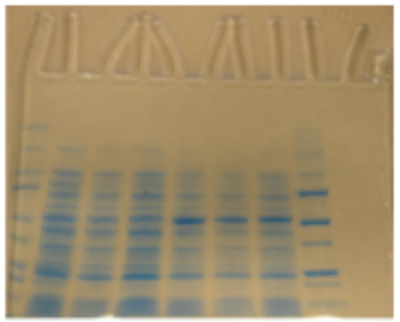
measure at a 1:X that gives linear range on spec. (e.g., X=10)

Meanwhile…

Lyse an aliquot and load onto SDS-PAGE

spin 2.0 OD, resuspend in 100 µL → take 30 µL, and add 30 µL of 2X SB.

Also ladder & (+) control



Let run 1 hour → often more like 40'

Transfer to blot – run 1 hour

Store in milk solution until next time

Wash incubations

Sequencing analysis (ApE or BLAST)

Mutant bacterial photograph – NOT YET!

Ideas/prep for Day 8 experiments… from seq/β-gal repeats to ?your idea here?