- Announcements
- Quiz
- Pre-lab Lecture
  - SDS-PAGE Part 1
  - Affinity purification recap
  - Today in Lab (M2D6)

Announcements

- Lab etiquette reminder
- Upcoming OH
  - Mon, 4/8, 3-4 pm
  - Wed, 4/17, by appt (no class Tue)
  - Sat or Sun Apr 20/21, c. 2:30-4:30 pm
- Revision due tomorrow at 11 AM
- Hey, what happened last time?
  - S08, S09, S10: no antibiotics during induction
  - S12: odd cell behavior (plasmid loss?), so used antibiotics, *but* spring break was after D3 not D4
  - S13: combo of week-old plates and fresh antibiotics most likely challenged cell survival+growth

+ discuss evals next time

Diagnostic digests and ODs

- What might the identities of extra bands be?

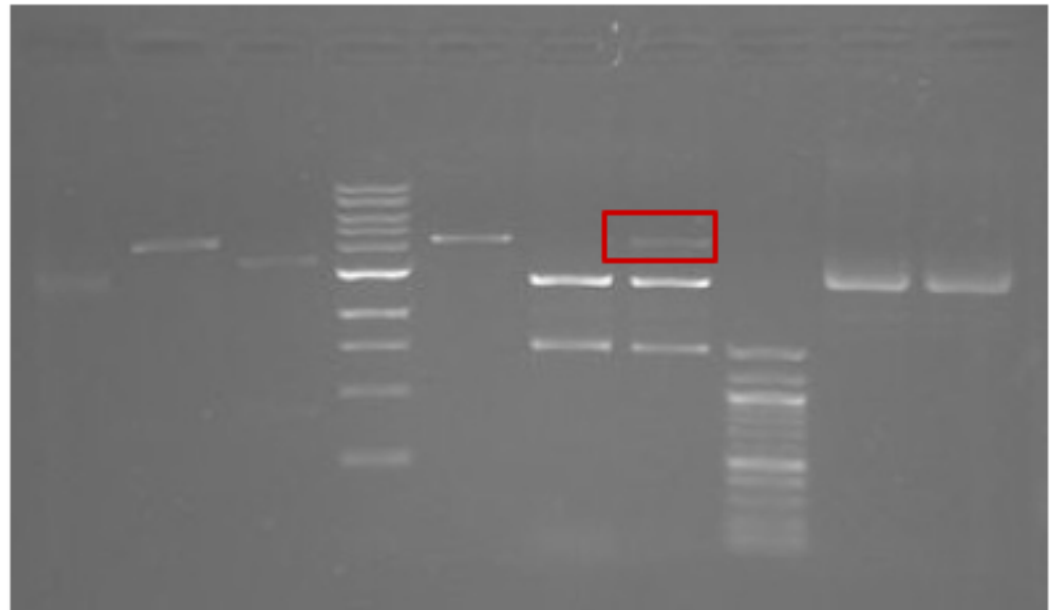
  from partial digestion (rxn pool, not binary)

- What could *unaccounted for* extra bands indicate?

  * activity; contamination; other mutation

- Besides cell normalization, why might getting -IPTG and +IPTG OD values be useful?

  mutant growth phenotypes; [protein] expectations



Courtesy T/R Platinum.
Expect: 3300/800 REF; 2700/1500 MUT

SDS-PAGE preparation

acrylamide – toxic

- You will make whole cell extracts with equal cell #s
  - Based on OD_{600} reading, normalize ① OD = 1.0 ② OD = 0.5

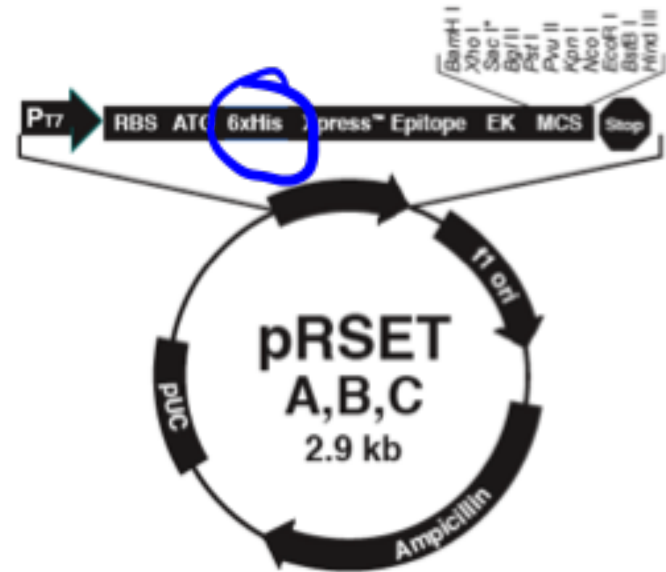
Vmax = 15 µL
② 15 µL
① 7.5 µL + 7.5 µL H_2O

- Gel separates proteins based on size, shape, charge (make uniform)
- Sample preparation (finish next time)
  - SDS: coat proteins w/(–) charge
  - β-Me: break S-S bonds (in hood)
  - Boiling: denaturing higher-order structures
  - Sample Buffer has SDS, β-Me, plus glycerol, BPB dye

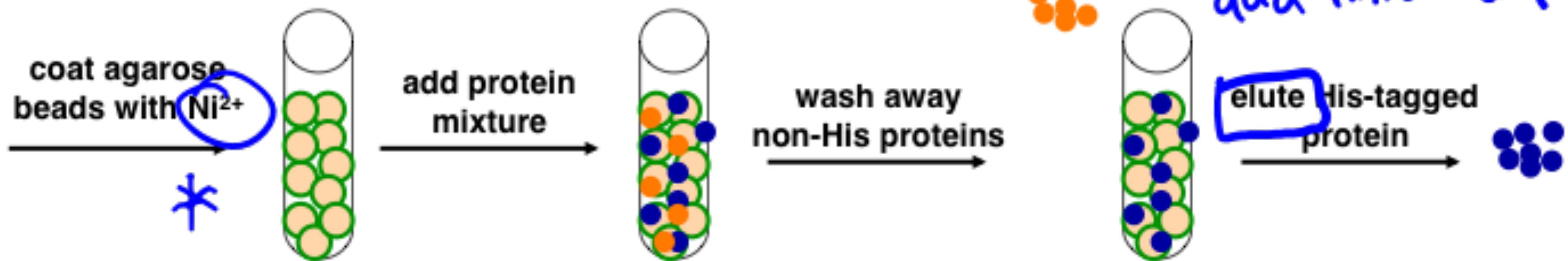
Affinity purification

- Basis: His tag (6x) binds to metals *

elute by competition



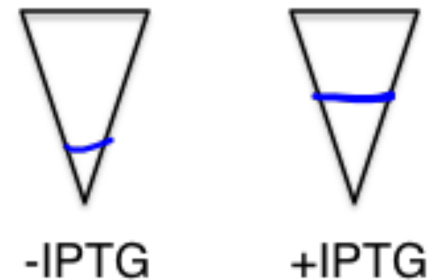
add imidazole

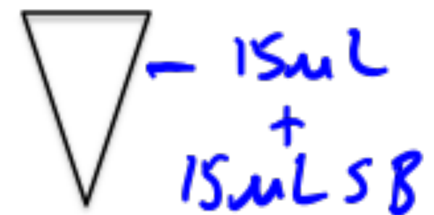


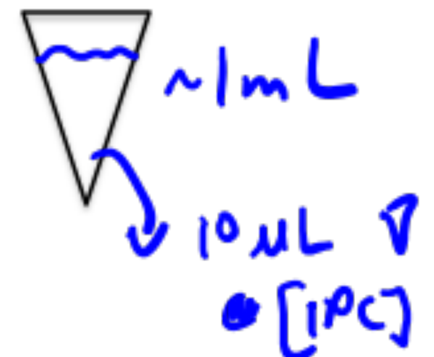
⊕ desalting → bye-bye ¡¡imidazole!

Today in Lab (M2D6)

- Lyse cell pellets in BPER
  - BSA "carrier," protease inhibitors
  - Add lysis enzymes at 1:2000
- Prep an aliquot for SDS-PAGE
- Purify IPC protein from the rest (long!)
  - Two steps: affinity purification, desalting
  - Immediately take 10 µL aliquot and measure concentration
  - The rest is stabilized w/BSA, to be titrated against calcium next time







* low [IPC] may still give high enough fluorescence (sensitivity)