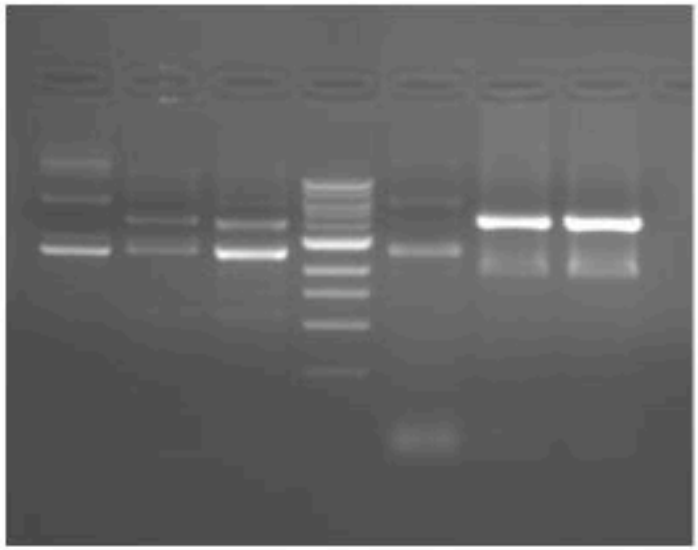
- Announcements → quiz next time, staggered arrivals
- Pre-lab Lecture
  - Briefly, data from last time
  - SDS-PAGE
  - Affinity purification recap
  - Today in Lab (Mod 1 Day 6)

Diagnostic digests, etc.

- What might really large bands be?
- Why might really small bands not show up?
- What might the identities of extra bands be?
- What could *unaccounted for* extra bands indicate?



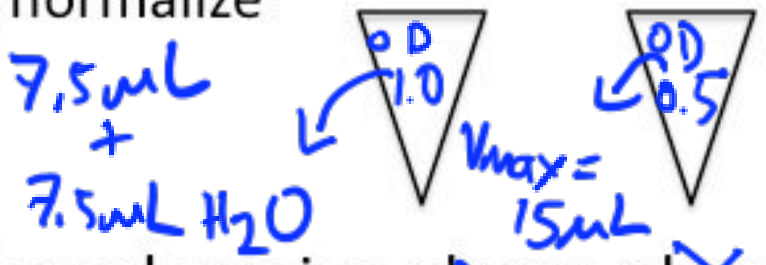
- Besides cell normalization purposes, why might getting –IPTG and +IPTG OD values be useful?

growth phenotype

SDS-PAGE preparation

acrylamide - toxic

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

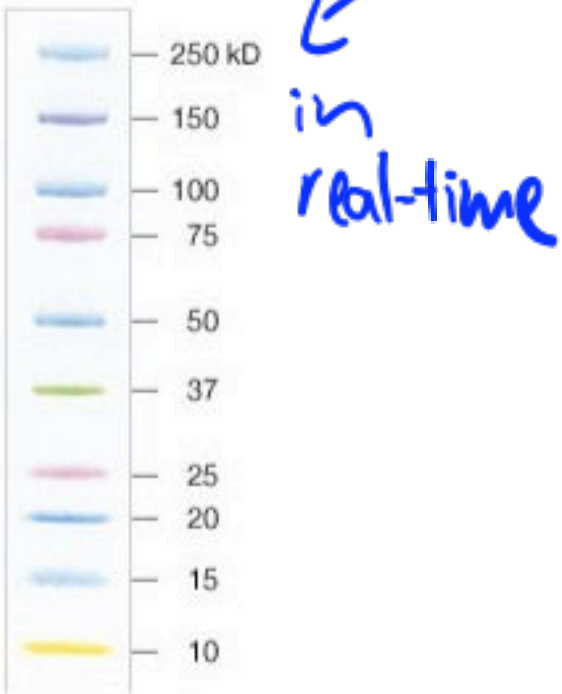


- Gel separates proteins based on size, ~~shape~~, ~~charge~~
- Sample preparation
  - SDS: coats proteins w/uniform (−) charge
  - β-Me: breaks S-S bonds
  - Boiling: denature → use locks!
  - Sample Buffer has SDS, β-Me, plus glycerol, dye (BPB)

in hood

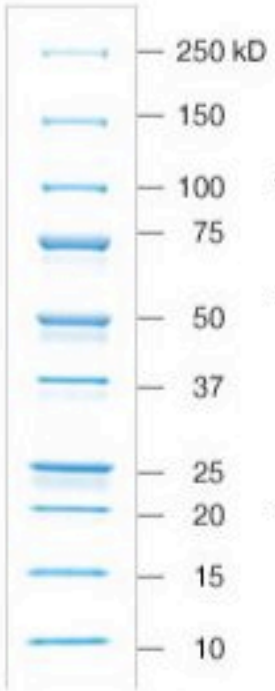
SDS-PAGE visualization, analysis

- Visualization: Coomassie stain (binds certain AA)
- Two ladders: visualization, quantification



in real-time

Kaleidoscope



respond to stain

150 ng (@100 KDa)

750 ng (@50 KDa)

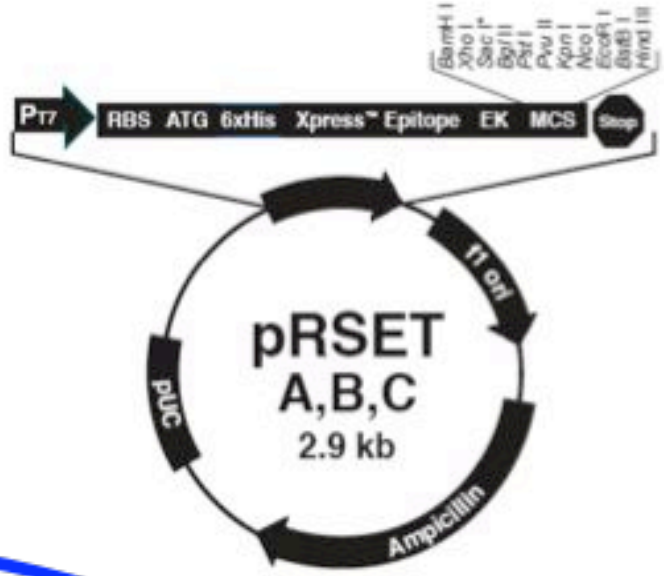
150 ng (@20 KDa)

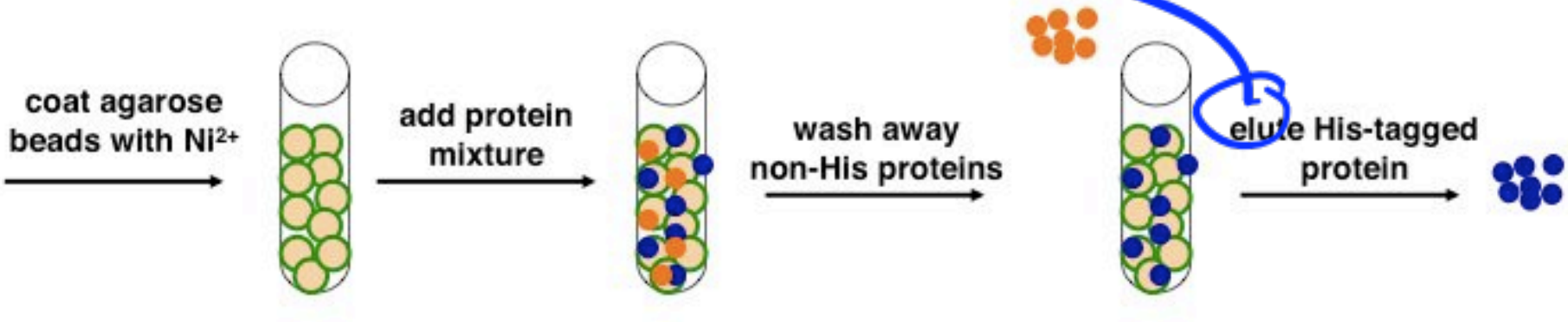
Unstained

Affinity purification

- Basis: 6x His tag in vector
  ↓
  bind metals

high [imidazole]





Today in Lab

- Lyse cell pellets in BPER
  - BSA "carrier", protease inhibitors
- Run a 15 µL aliquot through SDS-PAGE
  - Two ladders also → 15 µL
- Purify protein from the rest (long!)
  - Immediately take 10 µL aliquot and measure concentration
  - The rest is stabilized w/BSA, to be titrated against calcium next time