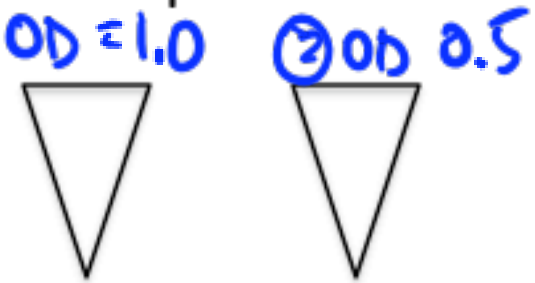
- Announcements
  - Discuss mid-term feedback
  - FNT heads up methods, 3Q (got rid of one)
  - Day 7: quiz; staggered arrivals (~1-1.5 hrs)
  - OH: 1hr. this Mon; next Sun + Tue, 1hr.; Mon, 2hr.
- Pre-lab Lecture
  - SDS-PAGE
  - Affinity purification recap
  - Today in Lab (Mod 2 Day 6)

SDS-PAGE preparation

acrylamide - toxic

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



Vmax = 15 uL

① 7.5uL + 7.5uL H_2O
② 15 uL

- Gel separates proteins based on size, shape, charge

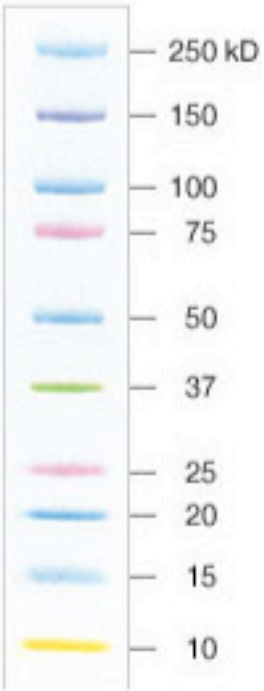
make uniform

- Sample preparation
  - SDS: coat proteins w/ (-) charge
  - β-Me: breaks S-S bonds
  - Boiling: denature higher-order structures
  - Sample Buffer has SDS, β-Me, plus glycerol, BPB dye

in hood

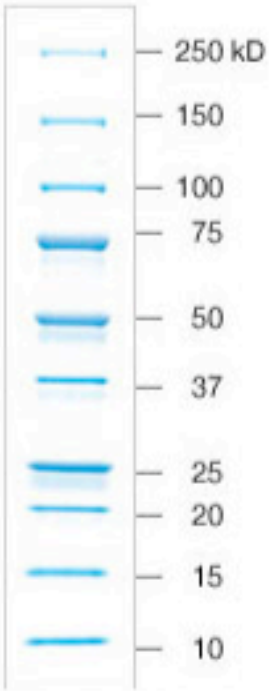
SDS-PAGE visualization, analysis

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



Kaleidoscope

in real-time, pre-stained



Unstained

respond to the stain

150 ng (@100 KDa)

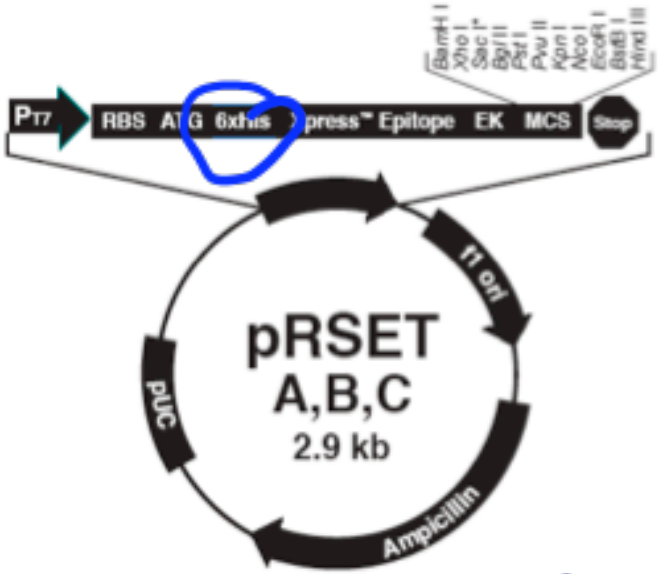
750 ng (@50 KDa)

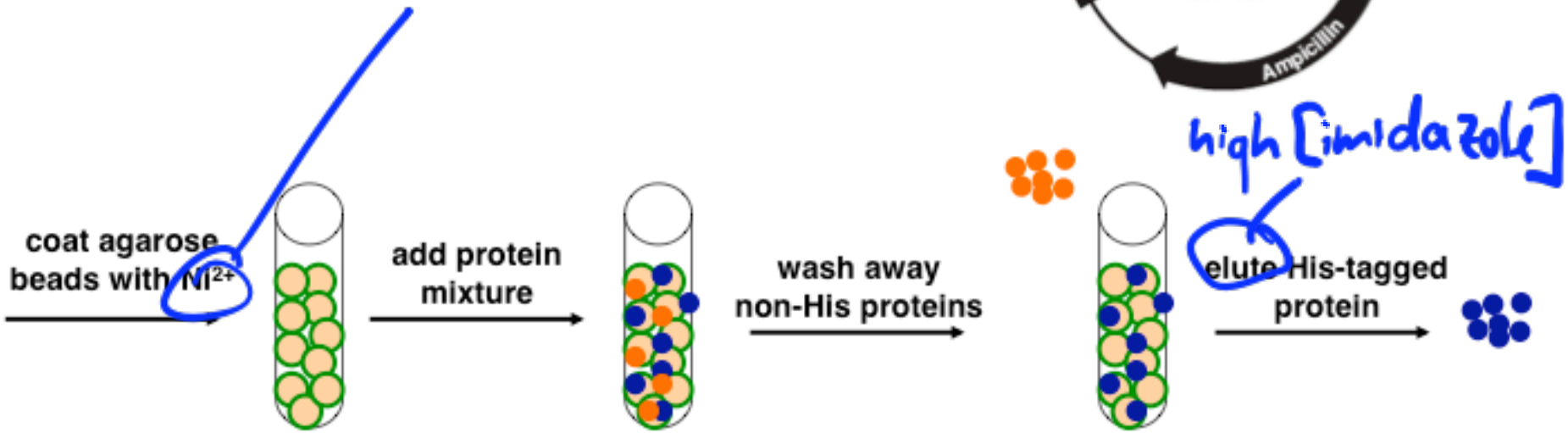
150 ng (@20 KDa)

Affinity purification

- Basis: His-tag invector

6x, binds to metals





Today in Lab

- Lyse cell pellets in BPER
  - BSA "carrier," protease inhibitors
  - Add lysis enzymes → 4 µL enzyme
- Run a 25 µL aliquot through SDS-PAGE
  - Two ladders also → boil these too
  - Stick with equal volumes if you have < 25
- Purify IPC 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