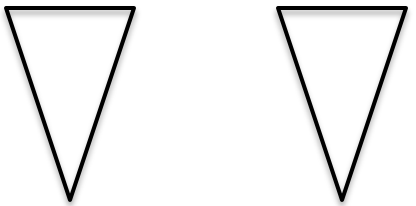
- Announcements
  - Discuss mid-term feedback
  - Lab etiquette
  - FNT extra credit
  - OH: T, W 12:45-4P ; Sun 1:30-3P (Sat? instead)
- Pre-lab Lecture
  - SDS-PAGE Part 1
  - Affinity purification recap
  - Today in Lab (M2D6)

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



V max = 15uL
① 7.5uL + 7.5uL H_2O
② 15uL

- Gel separates proteins based on size, ~~shape~~, ~~charge~~

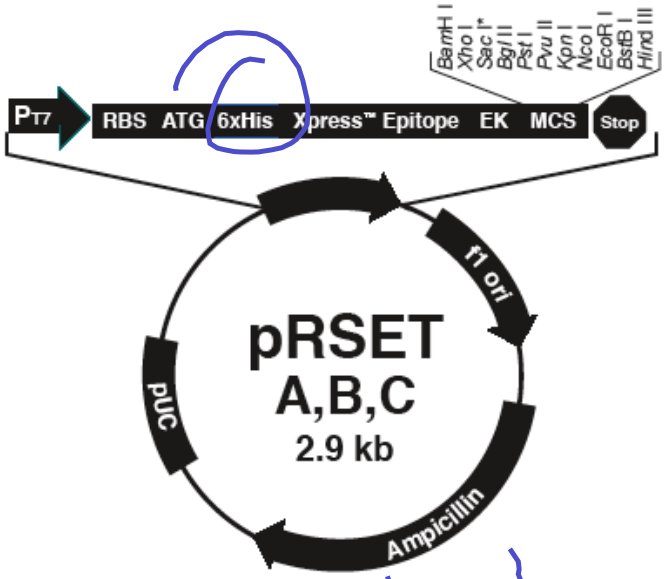
make uniform

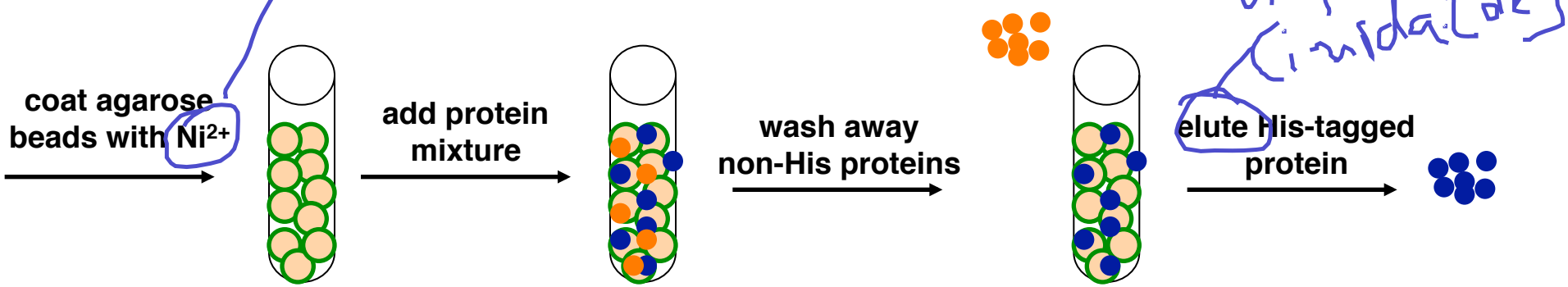
- Sample preparation (finish next time)
  - SDS: coat proteins w/ charge
  - β-Me: breaks S-S bonds
  - Boiling: denature higher-order structures
  - Sample Buffer has SDS, β-Me, plus glycerol, BPB

in hood
tips
pipets

Affinity purification

- Basis: His tag (6x) bind to metals





Today in Lab (M2D6)

- Lyse cell pellets in BPER
  - BSA "carrier," protease inhibitors
  - Add lysis enzymes
- 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