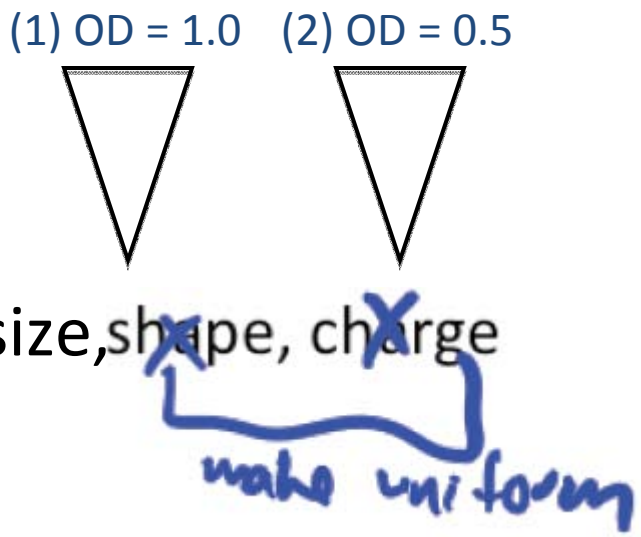
- Announcements
  - Discuss mid-term feedback
  - FNT heads up: methods, 3Q (got rid of one)
  - Day 7: quiz; staggered arrivals (~1 – 1.5hrs)
  - Office hours
- Pre-lab Lecture
  - SDS-PAGE
  - Affinity purification recap
  - Today in Lab (Mod 2 Day 6)

SDS-PAGE preparation

Acrylamide - toxic

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

    Vmax = 15mL
    (1)7.5mL + 7.5mL H_20
    (2)15mL

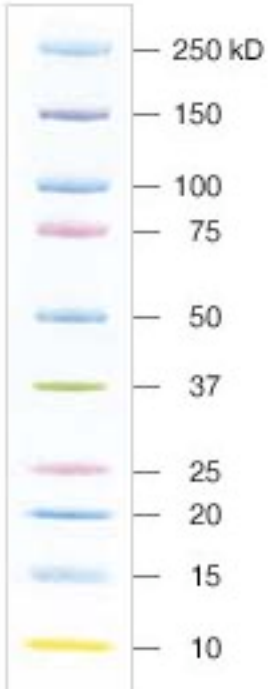


- Gel separates proteins based on size, shape, charge
- Sample preparation
  - SDS: coat proteins with negative charge

in hood
  - β-Me: breaks S-S bonds
  - Boiling: denature higher-order structures
  - Sample Buffer has SDS, β-Me, plus glycerol, BPB dye

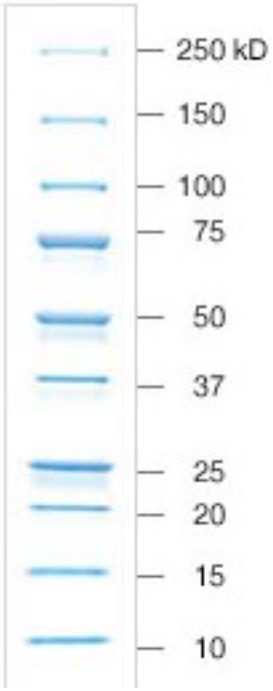
SDS-PAGE visualization, analysis

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



in real-time, pre-stained

Kaleidoscope



respond to the stain

150 ng (@100 KDa)

750 ng (@50 KDa)

150 ng (@20 KDa)

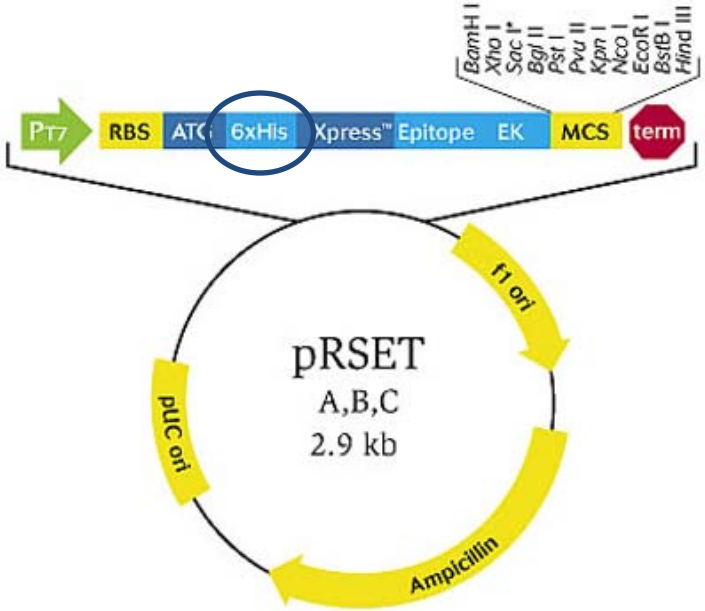
Unstained

Affinity purification

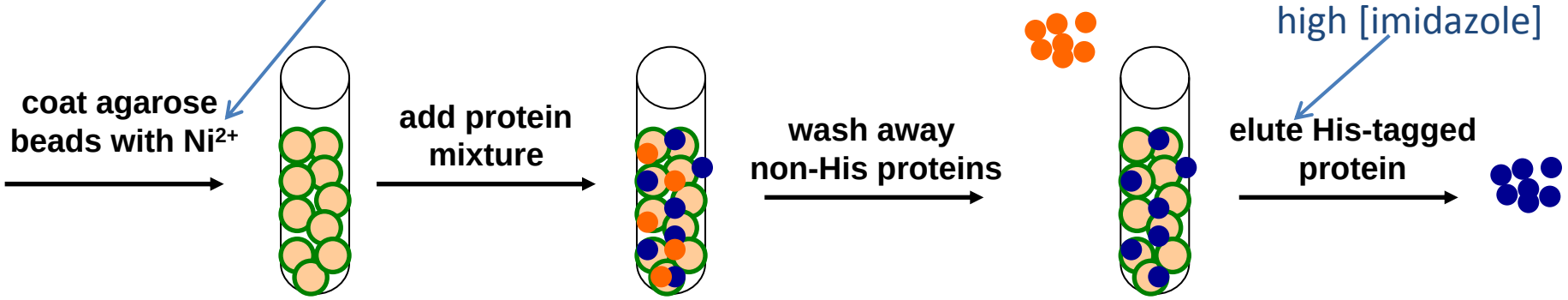
- Basis:

His-tag in vector

6x, binds to metals

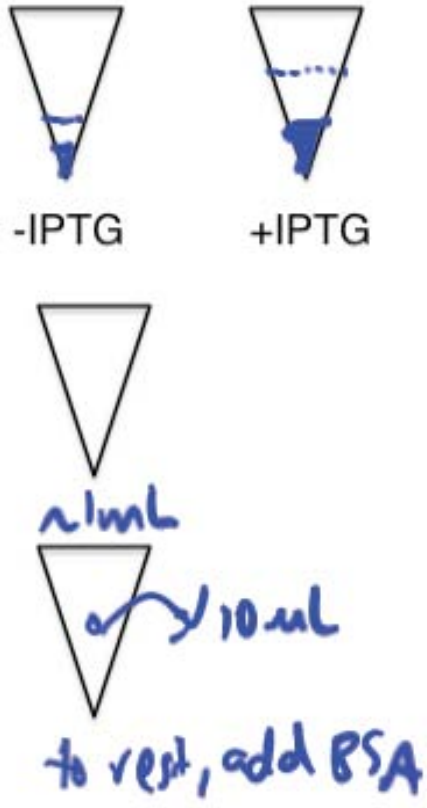


Courtesy of Life Technologies, Carlsbad, CA. Used with permission.



Today in Lab

- Lyse cell pellets in BPER
  - BSA “carrier,” protease inhibitors
  - Add 4 mL lysis enzymes
- 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



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