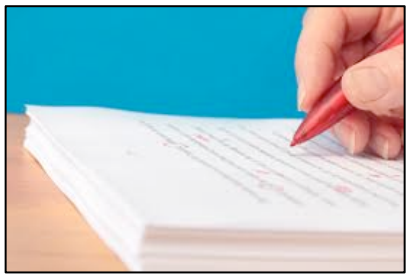
M2D4: Purify protein

10/22/2015

1. Prelab lecture
2. Lyse bacteria and prep SDS-PAGE samples
3. Purify protein
4. Measure purified protein

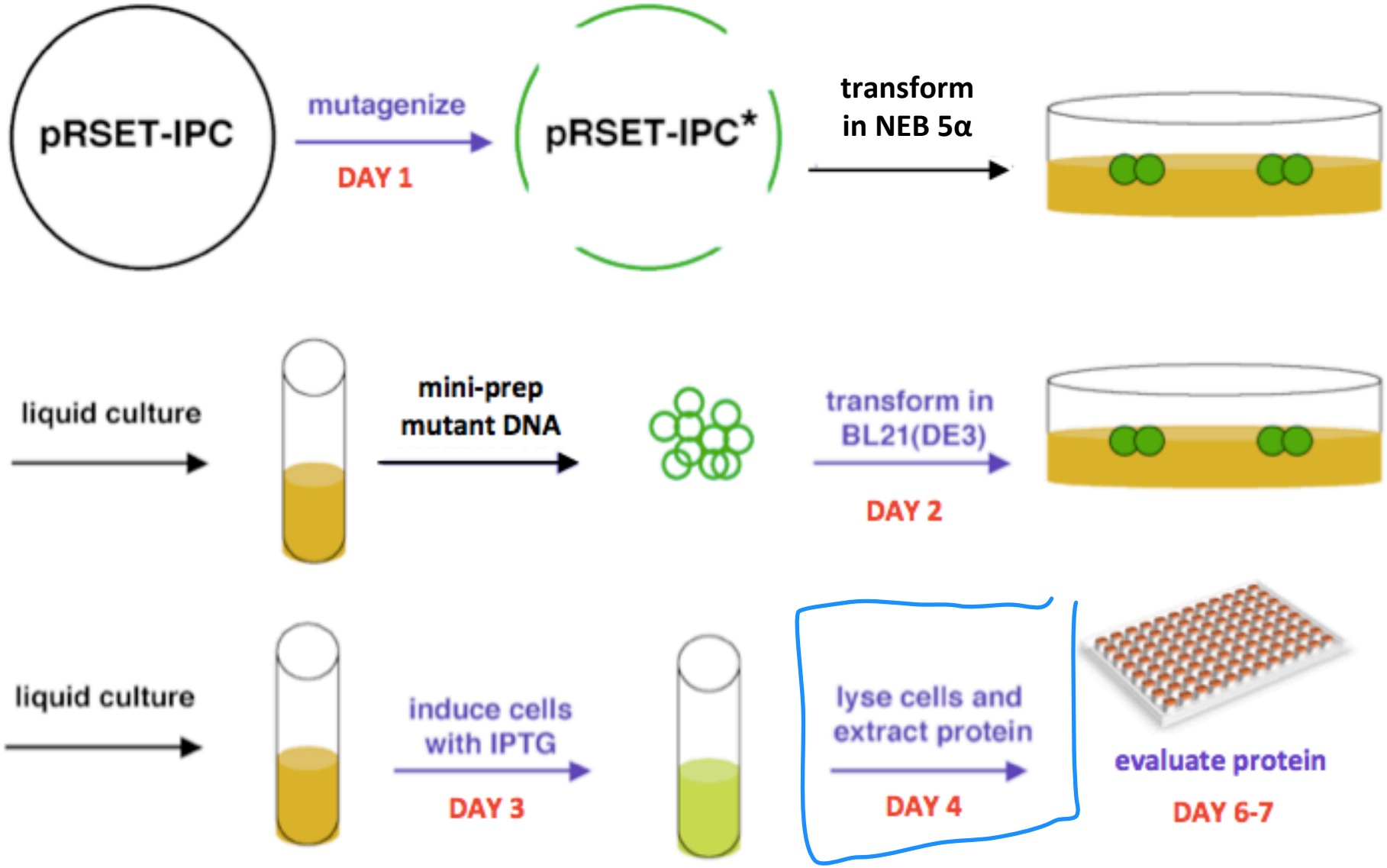
Assignments on the horizon



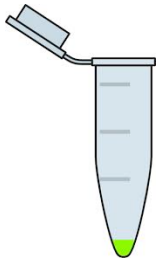
- DNA engineering summary revision
  - due by 5pm on Saturday, Oct. 24
- Blog post for M1
  - due by 5pm on Sunday, Oct. 25

- For M2D5:
  - journal club readings, meet in 16-336
- For M2D6:
  - estimate your protein concentration (from Bradford assay) in excel
  - write up Methods section

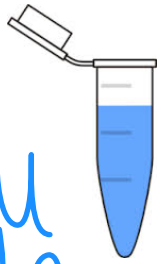
Module 2 experimental overview



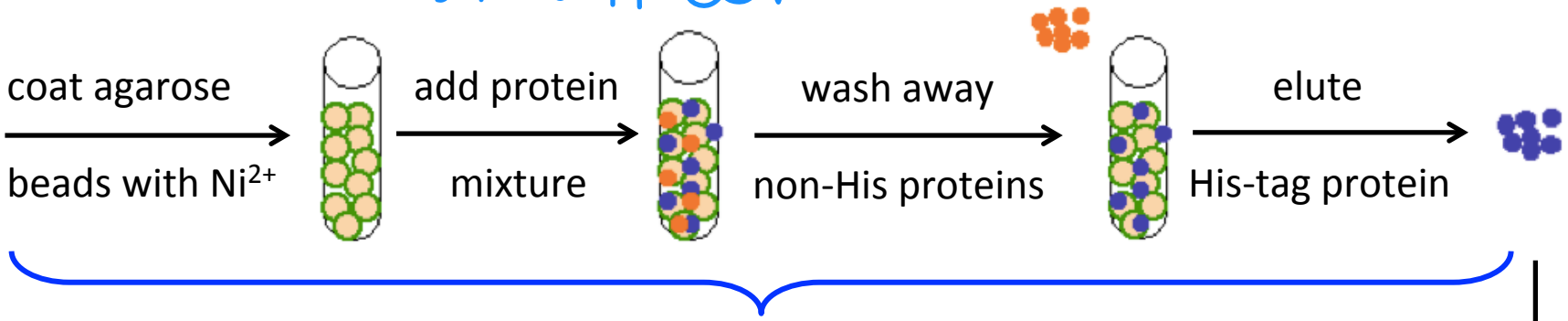
Protein purification: protocol overview



lyse with EasyLyse (and extract supernatant)

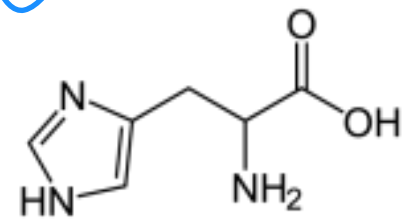


- lysozyme: damages bacterial wall
- DNAase (deoxyribonuclease): breakdown DNA
- protease inhibitor cocktail: block protein degradation
- bovine serum albumin (BSA): "carrier", stabilizer

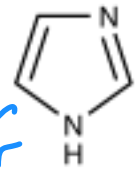


(2) 1 wt IPC 1 mut IPC

Protein purification: a few notes

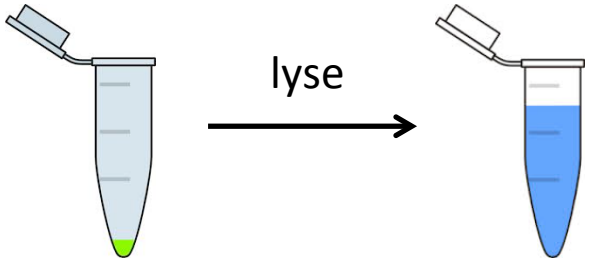


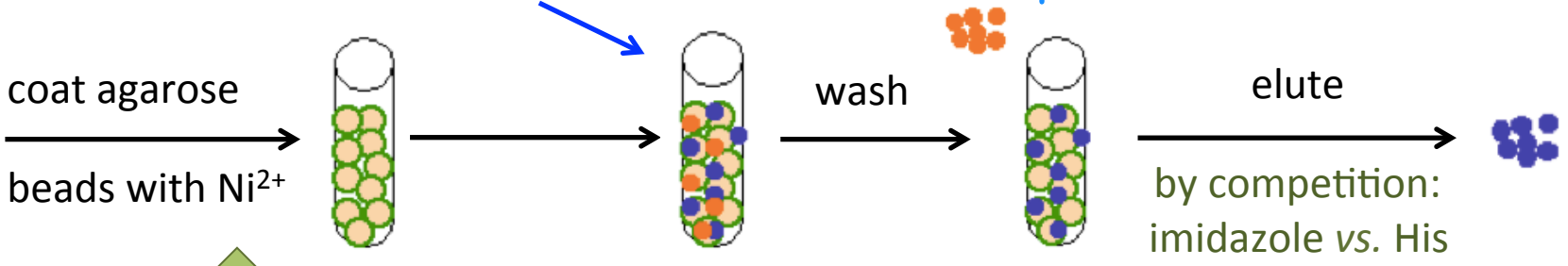
histidine



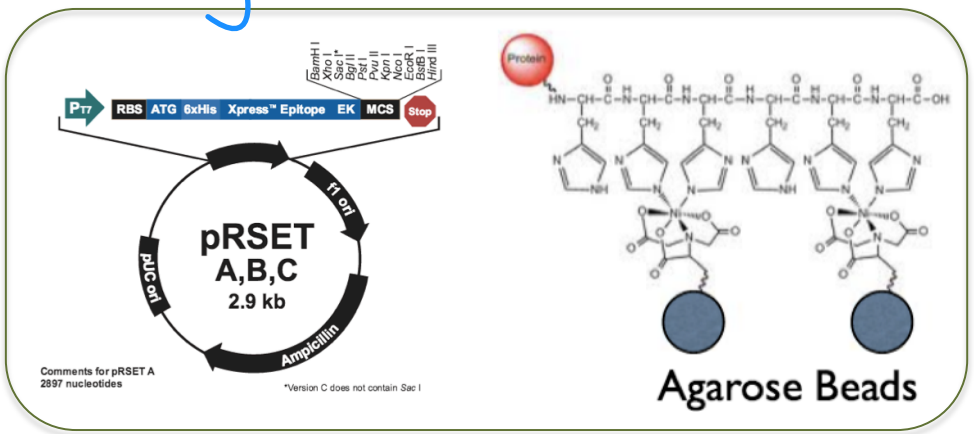
imidazole

(4) 15ml for SDS PAGE -IPTG/+IPTG





His tag binds to metals



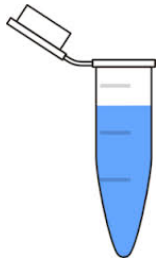
desalt

remove salts + imidazole

IPC - Ca^{2+} interferes

evaluate protein M2D6-M2D7

Prepare samples for SDS-PAGE



- Set aside whole cell extracts
  - equal number of cells based on OD_{600} (from M2D3)

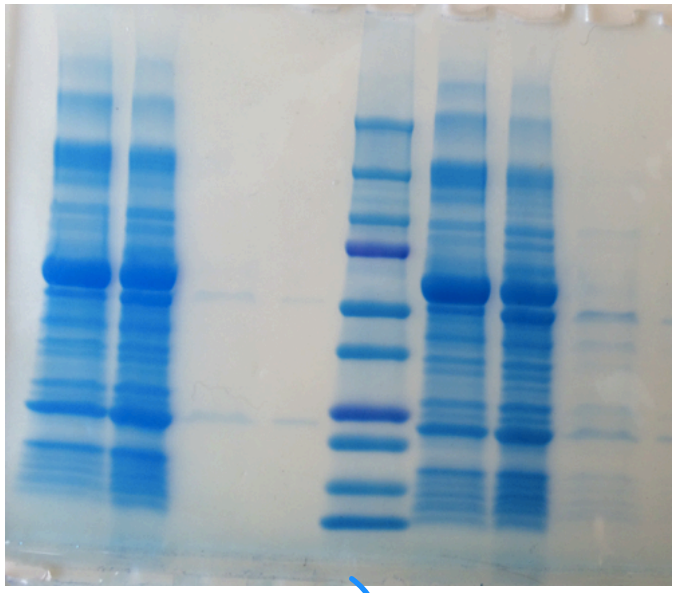
1 2 3 4

sample	example		wt IPC		selected X#Z	
	- IPTG	+ IPTG	- IPTG	+ IPTG	- IPTG	+ IPTG
OD600	lowest: 0.5	0.75				
sample volume (µL)	15	15*0.5/0.75 → 10				
water volume(µL)	0	5				
total volume (µL)	15	15	15	15	15	15
add 6x buffer (µL)	3	3	3	3	3	3

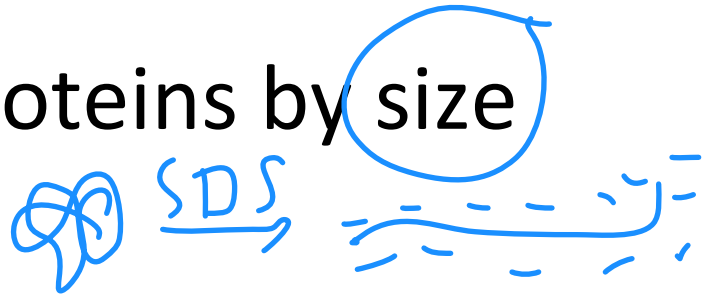
18 18

- SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel
  - separation by size ? shape ? charge ?

SDS-PAGE separates proteins by size



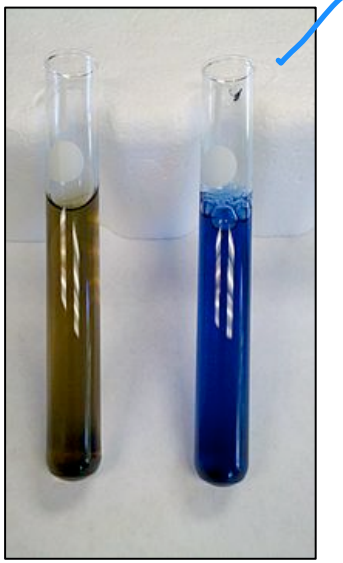
coomassie

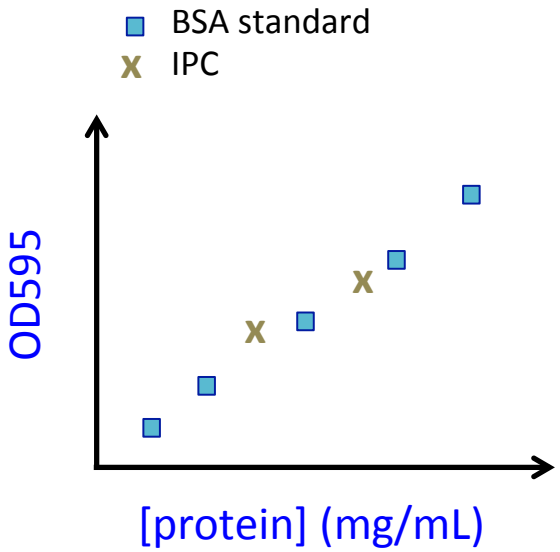


- Laemmli sample buffer:
  - + SDS: surfactant / detergent
    denatures proteins, coats them with negative charge
  - + β-mercaptoethanol
    reduces disulfide bonds
  - + bromophenol blue → visualize smallest migration
  - + glycerol
- boiling denatures higher-order structures

The Bradford colorimetric assay measures protein concentration

blue, absorbs orange ~595nm





- Coomassie brilliant blue G-250 dye
  - red if unbound (cationic form)
  - blue if bound to protein (anionic)
  - Van der Waals & hydrophobic interactions
  - arginine residues in particular
  - monitor OD_{595} absorption
- calibration with BSA
  - 0.1 – 1.0 mg/mL BSA
  - relative (not absolute) estimate of [IPC]: $[Arg]_{BSA} \neq [Arg]_{IPC}$
- work fast! (5-20min ideal)

Today in lab

- Lyse 4 cell pellets (wt IPC -/+ IPTG and "good" mutant -/+ IPTG)
- Set aside aliquots for SDS-PAGE (M2D6) ~15µl
  - add 6X sample buffer (Laemmli buffer) to each
- Purify protein (1 wt IPC + 1 mutant) -IPTG +IPTG
  - long!
  - 2 steps: affinity purification + desalting
- Immediately aliquot 10 µL for Bradford assay and 15µL for SDS-PAGE
- Stabilize rest of purified protein with BSA
  - ~ 1 mL protein + 10 µL of 10% BSA
  - to be titrated against Ca^{2+} on M2D6