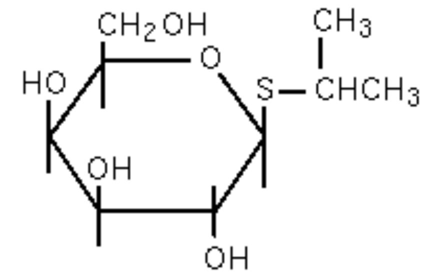
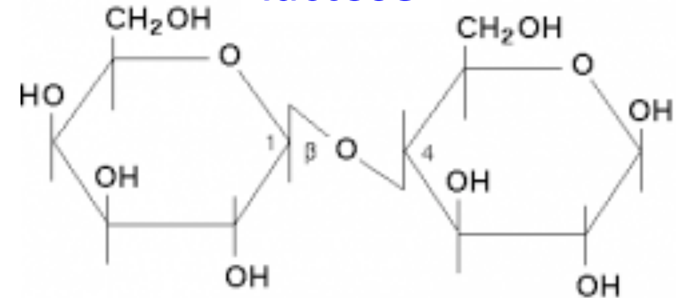
M2D4:
Purify protein

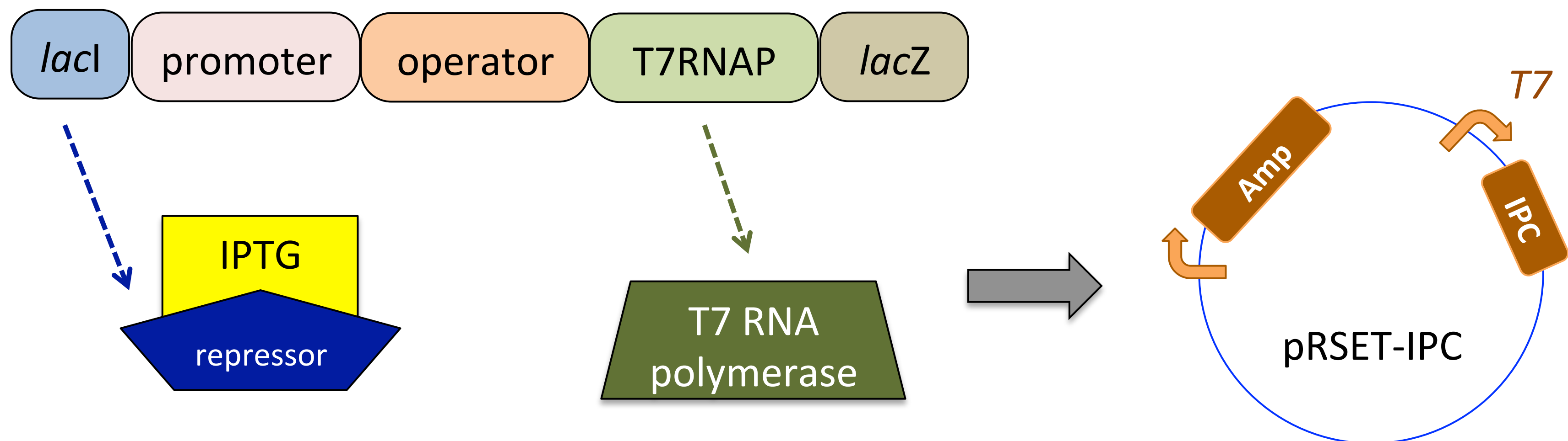
10/22/2015

IPTG is a lactose analogue

- isopropyl β-D-1-thiogalactoside
- structural mimic of lactose
- unlike lactose, **IPTG is not cleaved by β-galactosidase and so will not be broken down or used by the cell ➔ [IPTG] constant**



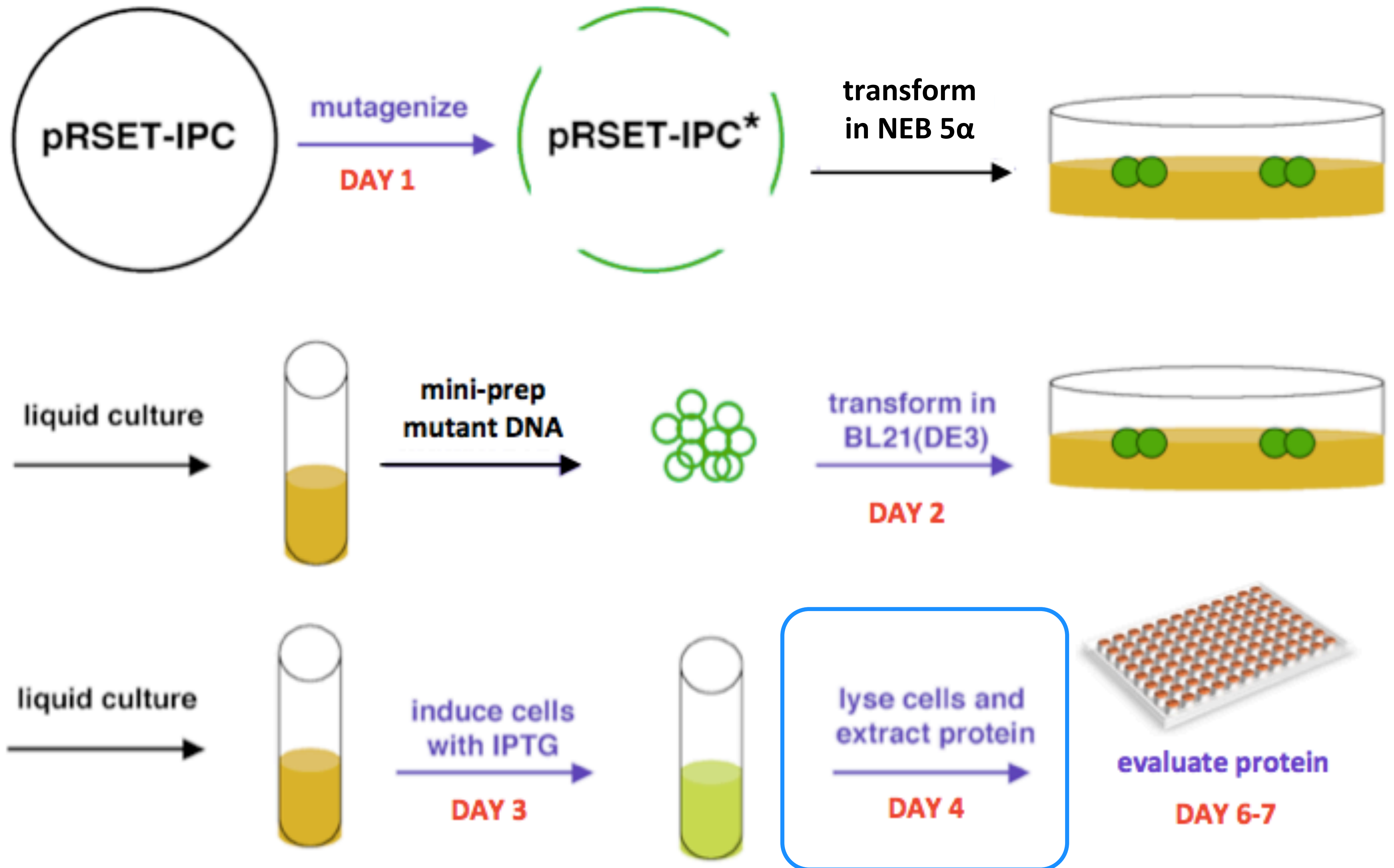




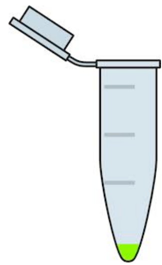
Assignments on the horizon

- 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
- For M2D6:
  - estimate your protein concentration (from Bradford assay)
  - write up Methods section

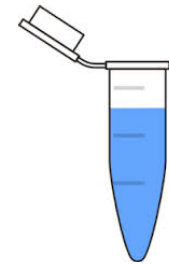
Module 2 experimental overview



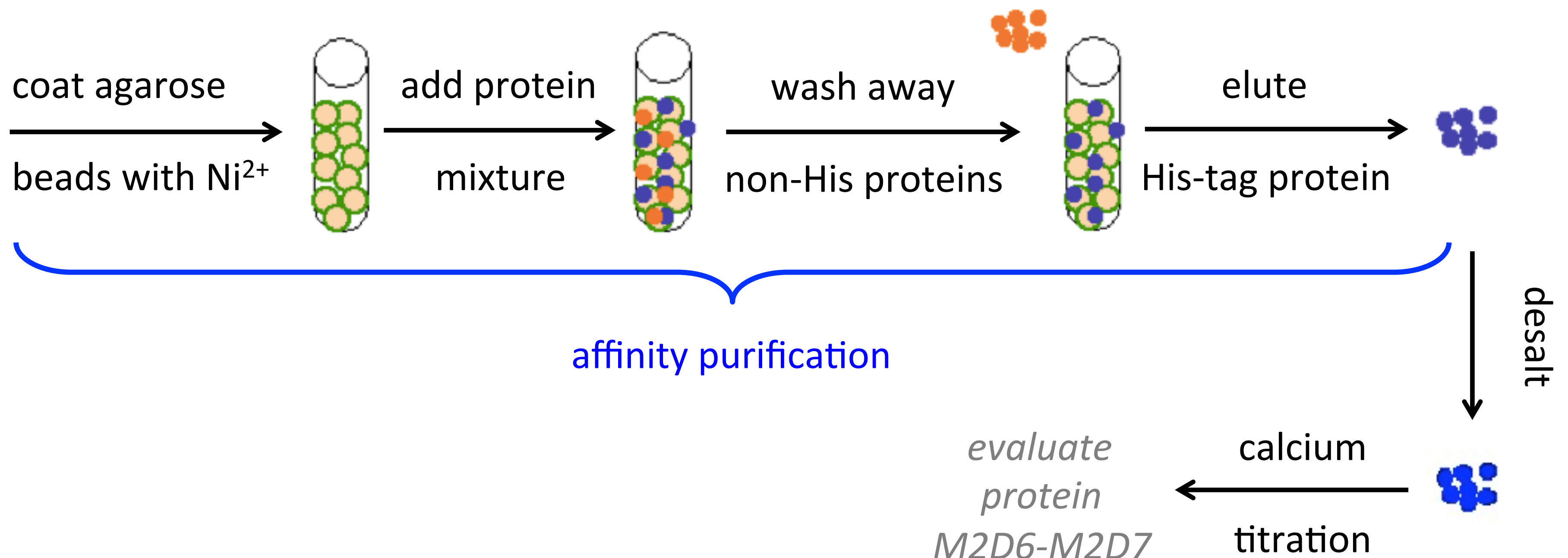
Protein purification: protocol overview



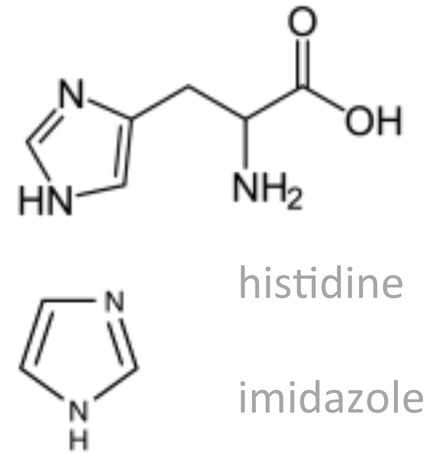
lyse with EasyLyse (and extract supernatant)

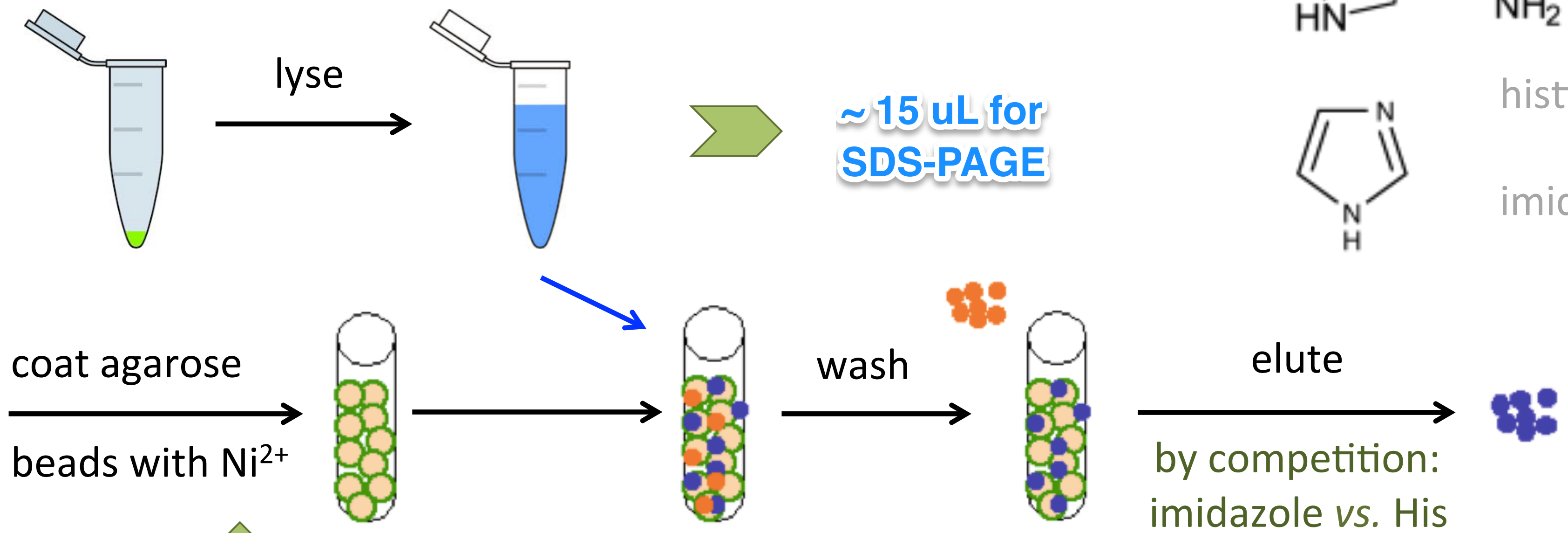


- lysozyme: **damages bacterial walls, opens up E. coli BL21**
- DNAase (deoxyribonuclease): **chews up DNA**
- protease inhibitor cocktail: **against protein degradation**
- bovine serum albumin (BSA): “carrier”, **stabilizer**



Protein purification: a few notes





His-tag binds to metals

pRSET A,B,C 2.9 kb

RBS ATG 6xHis Xpress™ Epitope EK MCS Stop

f1 ori

pUC ori

Ampicillin

Comments for pRSET A 2897 nucleotides

*Version C does not contain Sac I

Protein

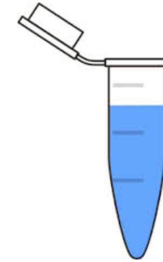
Agarose Beads

get rid of imidazole because it interfers with IPC-calcium binding

desalt

evaluate protein M2D6-M2D7

Prepare samples for SDS-PAGE

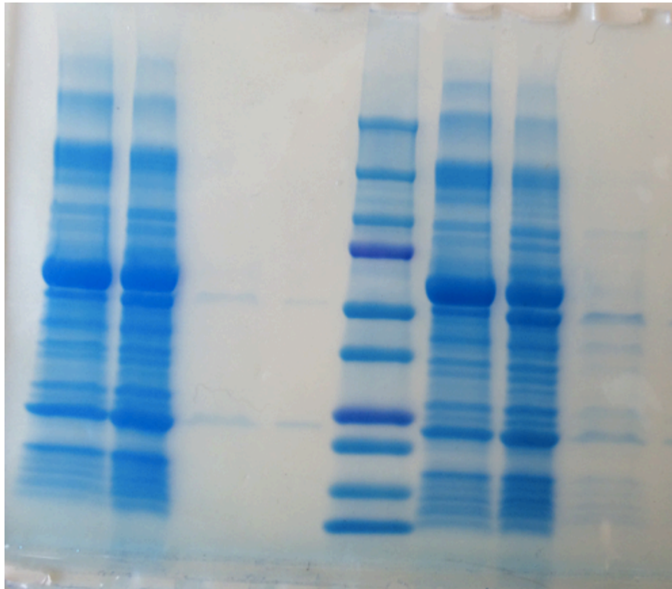


- Set aside whole cell extracts
  - equal number of cells based on OD_{600} (from M2D3)

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				
water volume(µL)	0	5				
total volume (µL)	15	15	15	15	15	15
add 6x buffer (µL)	3	3	3	3	3	3

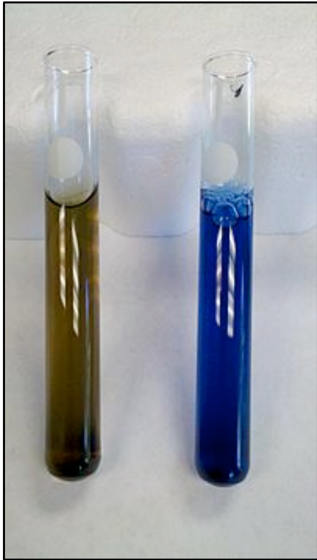
- SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel
  - separation by size ? shape ? charge ?

SDS-PAGE separates proteins by size



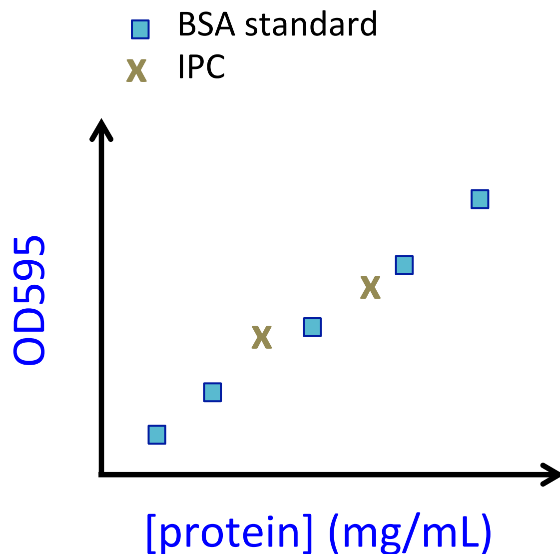
- Laemmli sample buffer:
  + SDS: surfactant / detergent
    denatures proteins, coats them with negative charge
  + β-mercaptoethanol
    reduces disulfide bonds
  + bromophenol blue
  + glycerol

- boiling denatures higher-order structures



The Bradford colorimetric assay measures protein concentration

- 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
  - relative (not absolute) estimate of [IPC]: $[Arg]_{BSA} \neq [Arg]_{IPC}$
- work fast!



Today in lab

- Lyse 4 cell pellets (wt IPC -/+ IPTG and "good" mutant -/+ IPTG)
- Set aside aliquots for SDS-PAGE (M2D6)
  - add Laemmli buffer to each
- Purify protein (1 wt IPC + 1 mutant)
  - long!
  - 2 steps: affinity purification + desalting
- Immediately aliquot 10 µL for Bradford assay
  + 15 uL for SDS-PAGE
- Stabilize rest of purified protein with BSA
  - ~ 1 mL protein + 10 µL of 10% BSA (0.1% BSA total in samples)
  - to be titrated against Ca^{2+} on M2D6