M1D2:Purify induced protein

02/15/2017

1. Start lysis of 2x BL21 *E. coli* pellets (+/- IPTG)
2. Prelab discussion
3. Continue protein purification
4. Leave protein overnight in dialysis cassettes

M1 major assignments

- Data summary (15%)
  - in teams, on Stellar
  - draft due 03/10, final revision due 03/27
  - bullet points, .PPTX
- Mini-presentation (5%)
  - individual, video via Gmail
  - due 03/18
- Lab quizzes (extra credit on homework grade)
  - M1D3, M1D5, and M1D7
- Notebook (5% total)
  - one day will be collected and graded by Rob on M1D7
- Blog: http://be20109s17.blogspot.com/ (participation: 5% total)
  - by 04/03

Office hours

Noreen Lyell

- M 2-5
- in 16-317

Leslie McClain

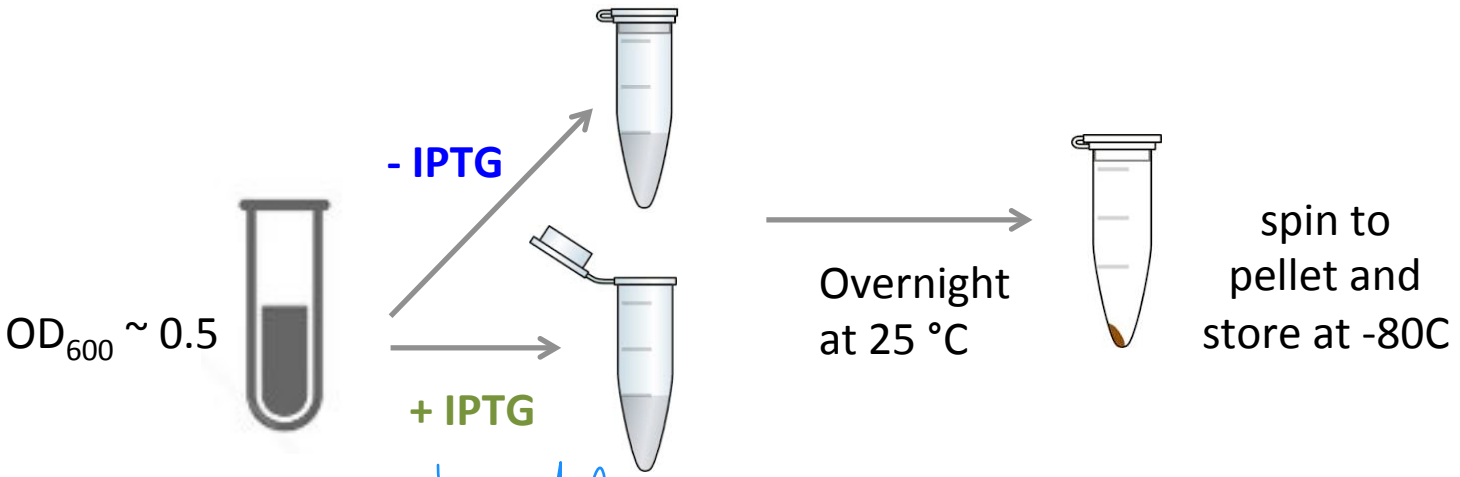
- T 9:30-11
- in 56-341c

Maxine Jonas

- R 9:30-11
- in 16-239

We are happy to meet outside these times, just email: nllyell@, lesliemm@, jonas_m@

Since Friday…
Induction of FKBP12 protein expression in BL21 *E. coli*

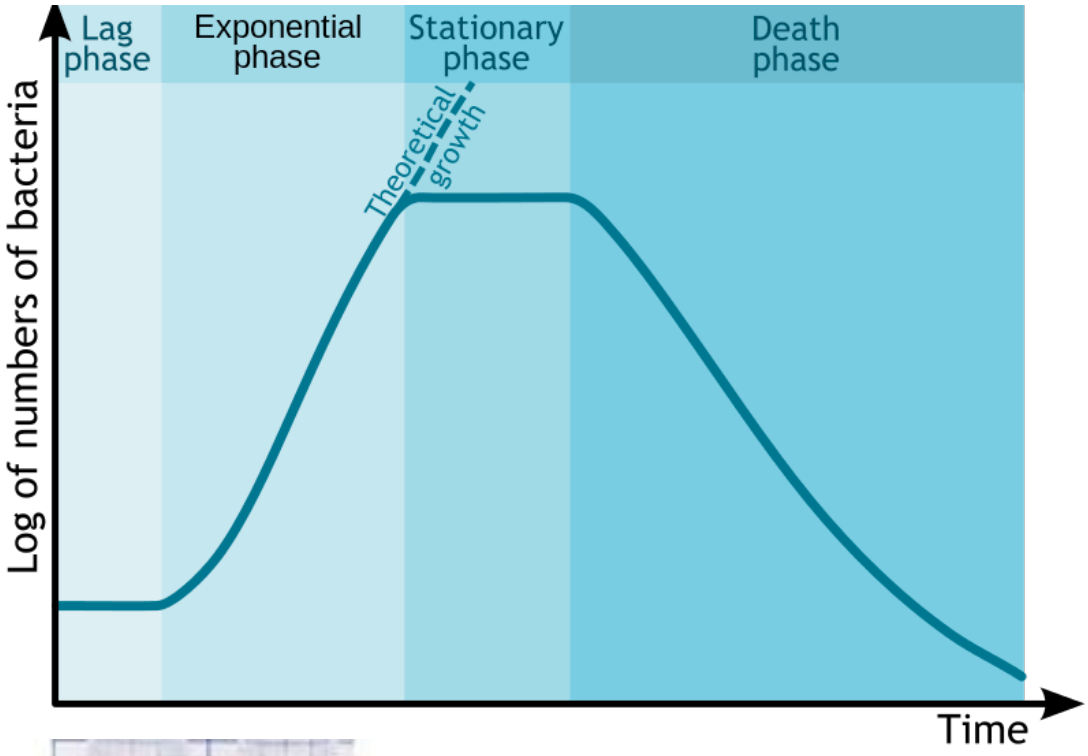


Luria
Broth
+ Ampicillin
+ Chloramphenicol

1mM

Optimal protein expression in *E. coli* during exponential phase

- Lag phase
- Exponential phase
  - binary fission
  - OD_{600} ~ 0.4 - 0.8
  - machinery ready
- Stationary phase

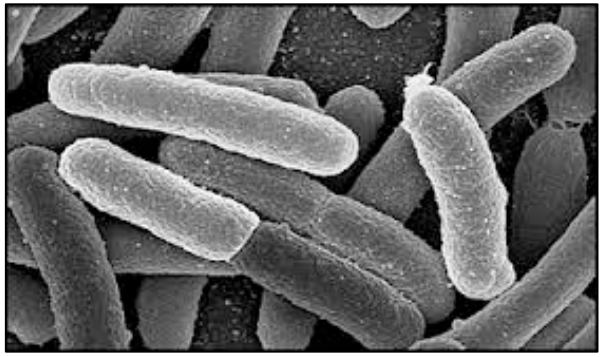


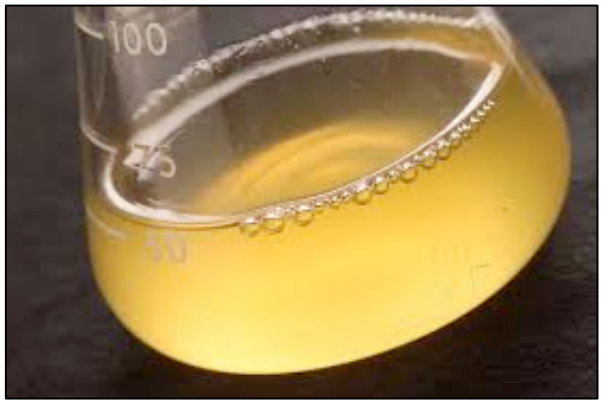
600nm

- OD ≠ absorbance
  - optical density
    - turbidity
    - **cells don't absorb light at 600nm**
    - **cells yellow**

BL21(DE3)pLysS competent cells

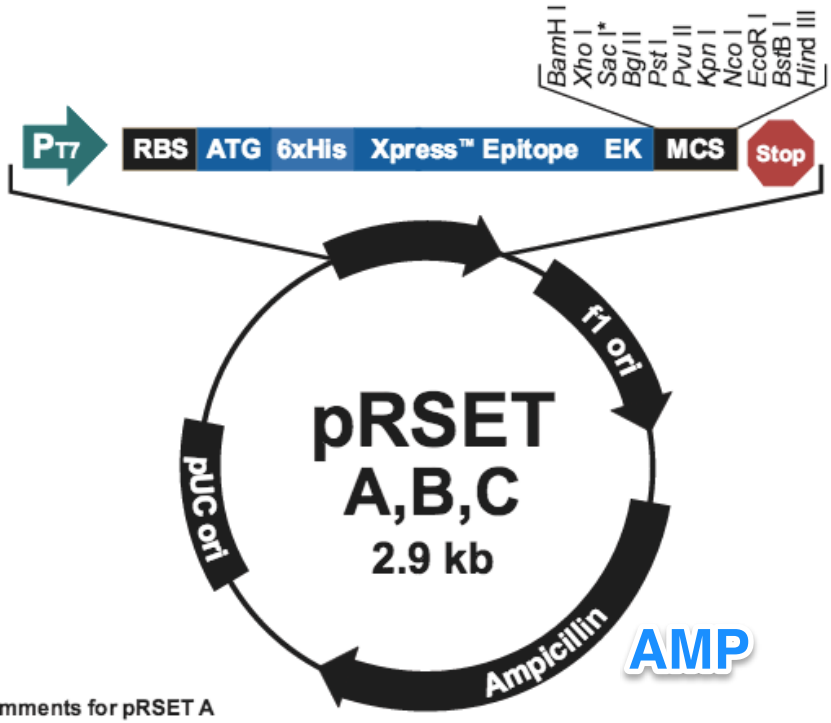
- BL21: E. coli bacterial strain
- forced expression of protein (FKBP12)
  - induction by lactose or analog: isopropyl β-D-thiogalactoside (IPTG)
- DE3: bacteriophage (virus)
  - used to integrate the *lac*/T7RNAP construct into *E. coli*
- pLysS: protein that produces
  - lysosyme, which binds to T7RNAP, reducing basal "leaky" expression
  - retained by antibiotic (chloramphenicol, Cam) selection





DNA->RNA->protein

Let's take a closer look at the pRSETb vector



Comments for pRSET A
2897 nucleotides

*Version C does not contain *Sac* I

T7 promoter: bases 20-39
6xHis tag: bases 112-129
T7 gene 10 leader: bases 133-162
Xpress™ epitope: bases 169-192
Multiple cloning site: bases 202-248
T7 reverse priming site: bases 295-314
T7 transcription terminator: bases 256-385
f1 origin: bases 456-911
bla promoter: bases 943-1047
Ampicillin (*bla*) resistance gene (ORF): bases 1042-1902
pUC origin: bases 2047-2720 (C)

- P_{T7} T7 promoter
- RBS ribosomal binding site

- ATG start codon
- 6xHis tag for purification
- Xpress epitope
  antibody recognition
- EK (enterokinase)
  protein cleavage site
- MCS
  multiple cloning site
- Stop
  end transcription
  UAG UGA UAA

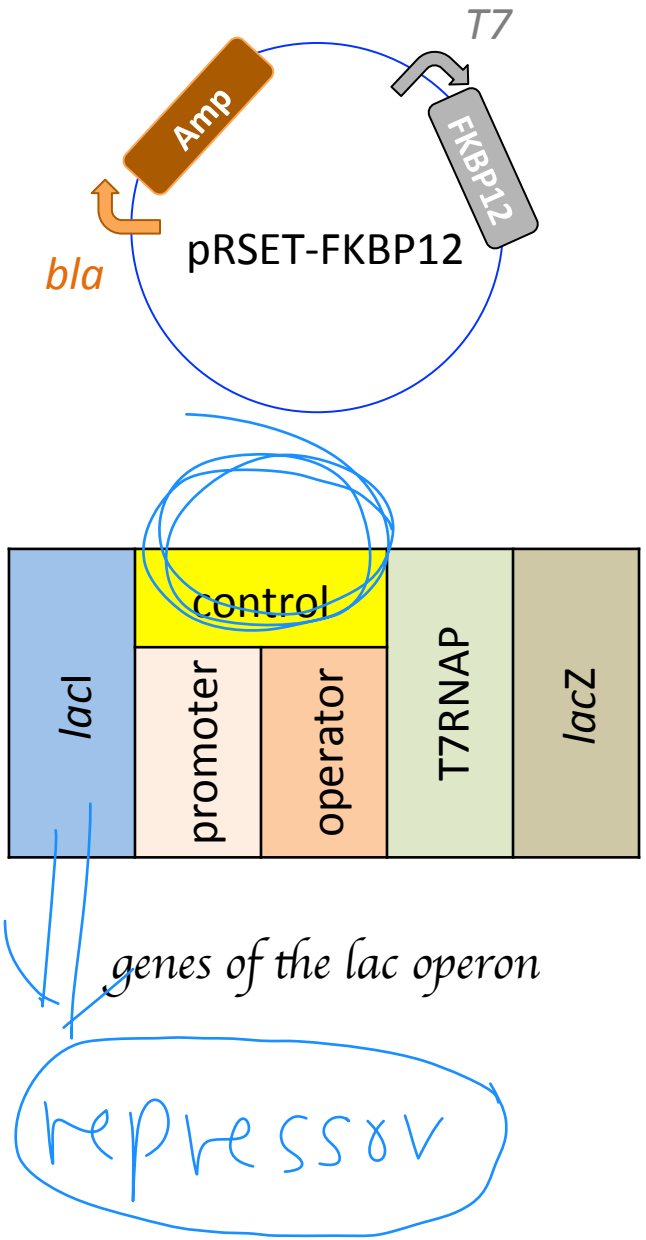
Let's piece together this "protein induction" story

① in the pRSET plasmid

- BLA promoter is constitutively *on*
- T7 promoter is turned *on* in the presence of T7 RNA polymerase

② in BL21(DE3)pLysS

- T7RNAP gene engineered in DE3 cells under a modified *lac* operon control
- *lac*I encodes a repressor that binds to control, thereby turning it *off*
- in addition, T7 lysosyme inactivates T7 polymerase



Let's piece together this "protein induction" story

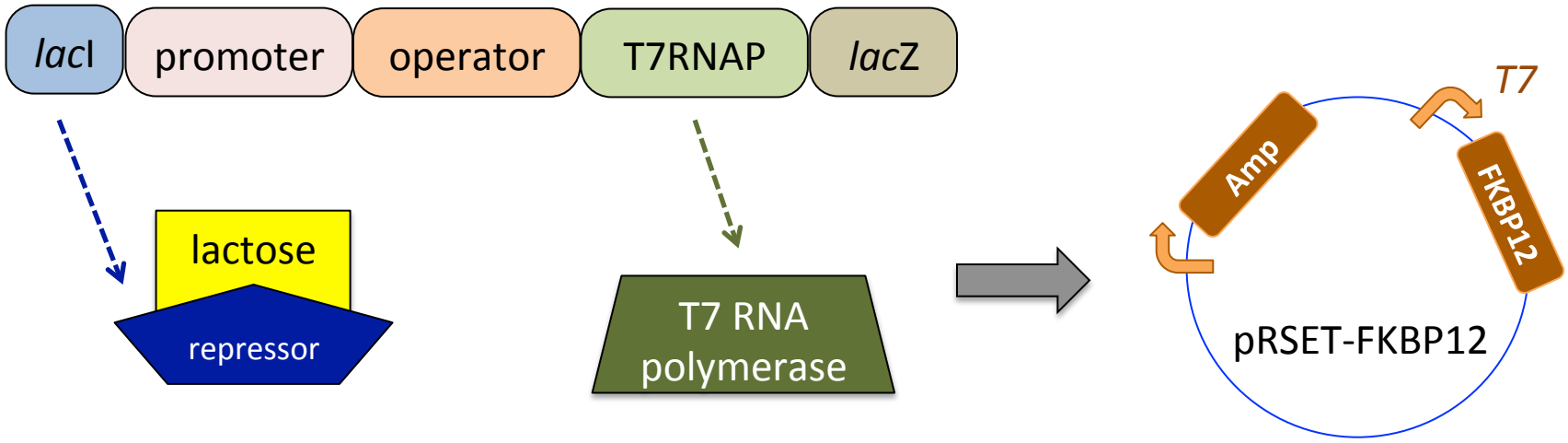
① in the pRSET plasmid, T7 promoter *on* only if T7RNAP present

② in BL21(DE3)pLysS, *lac*I => repressor binds control area => T7RNAP turned *off*

③ if lactose is present

– lactose binds to repressor and makes it **inactive** , thus turning **ON** expression of T7RNAP

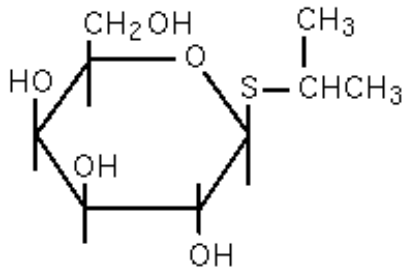
– with T7RNAP present, the T7 promoter is **ON**, and FKBP12 expressed



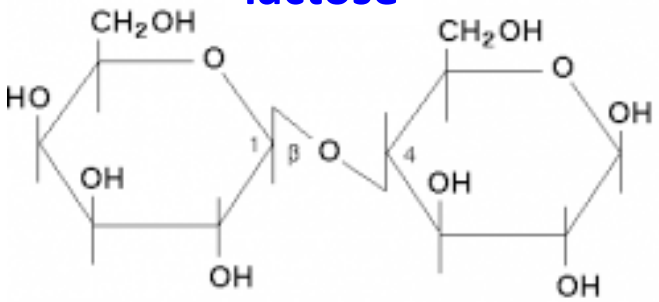
IPTG is a lactose analogue

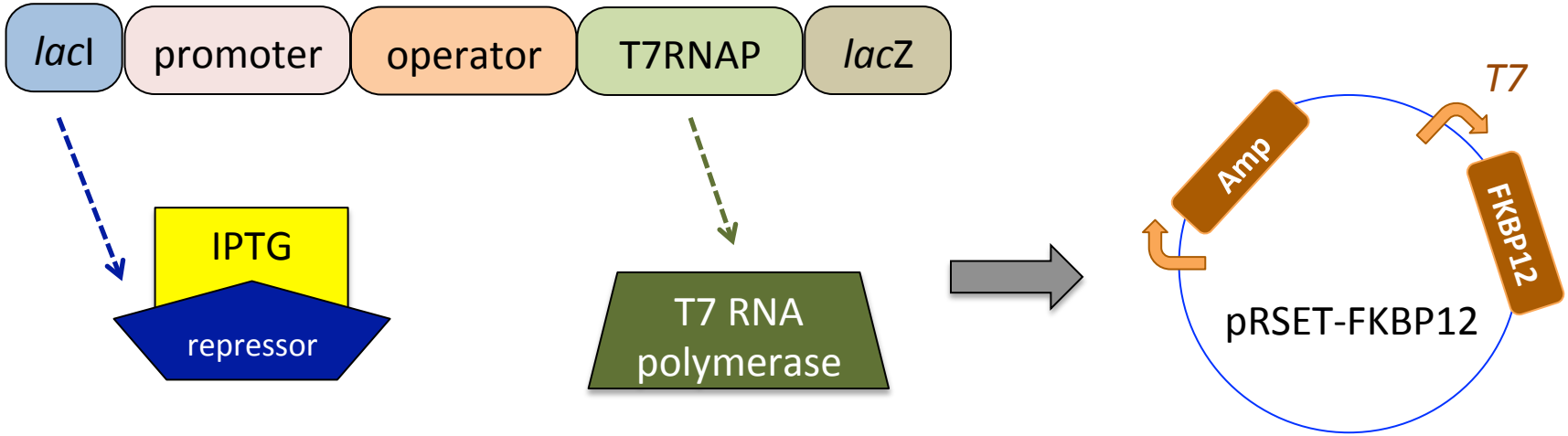
- isopropyl β-D-1-thiogalactoside
- structural mimic of lactose
- unlike lactose, IPTG is not cleaved by β-galactosidase and so will not used by the cell
  ➔ [IPTG] constant

IPTG

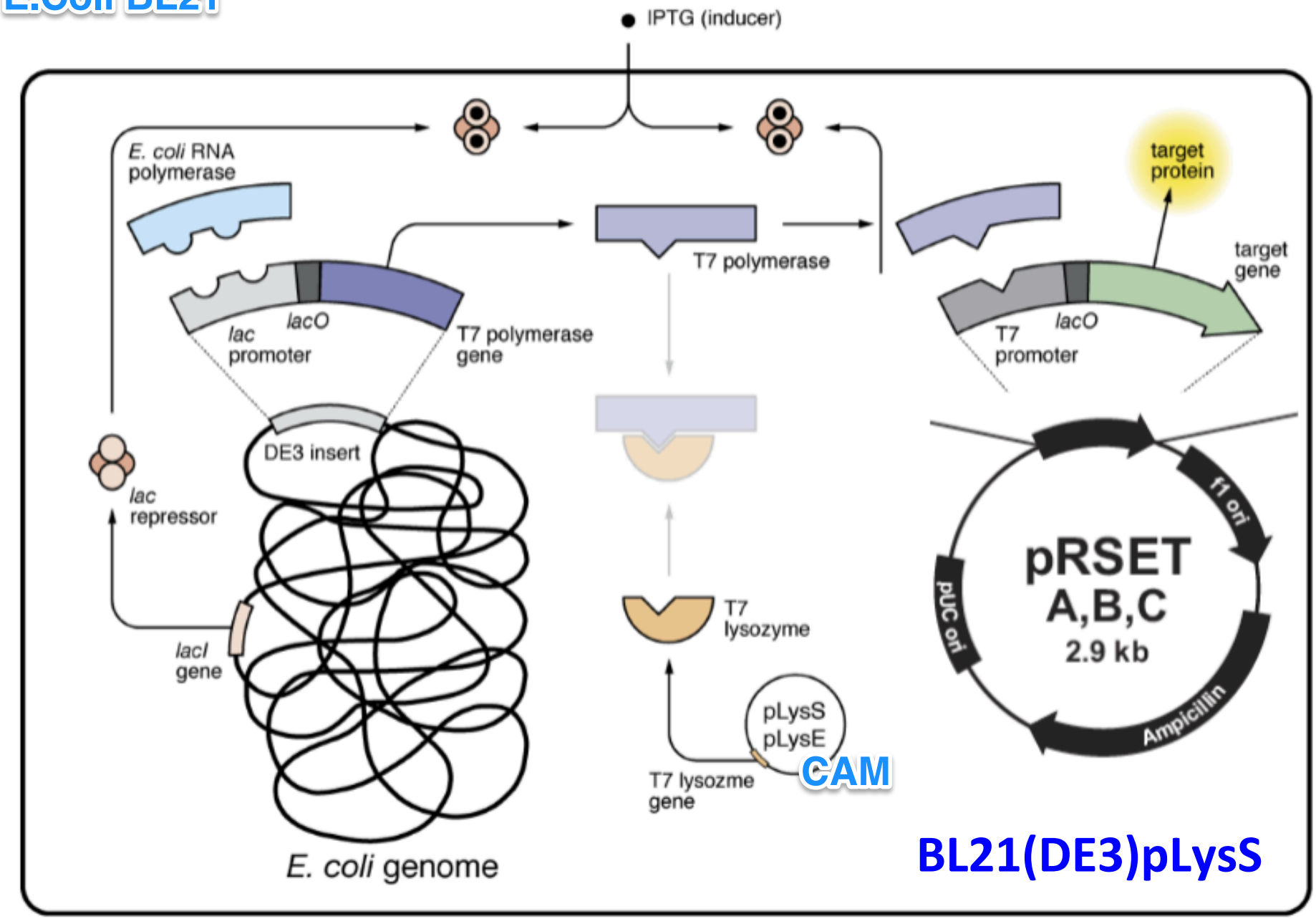


lactose





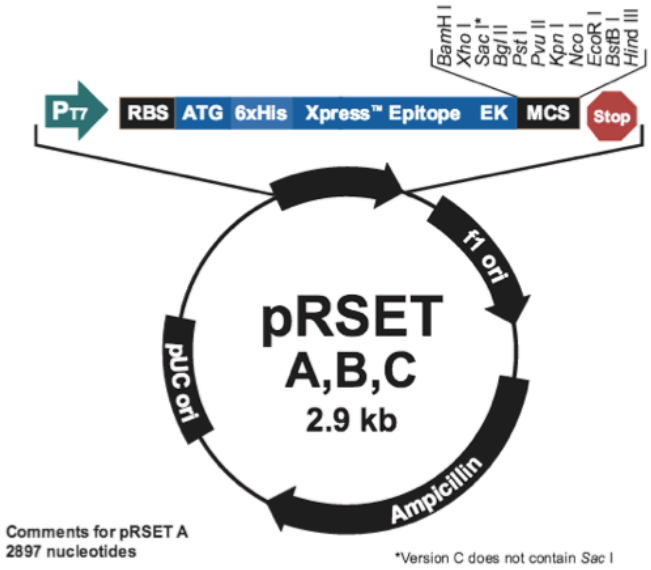
E.Coli BL21

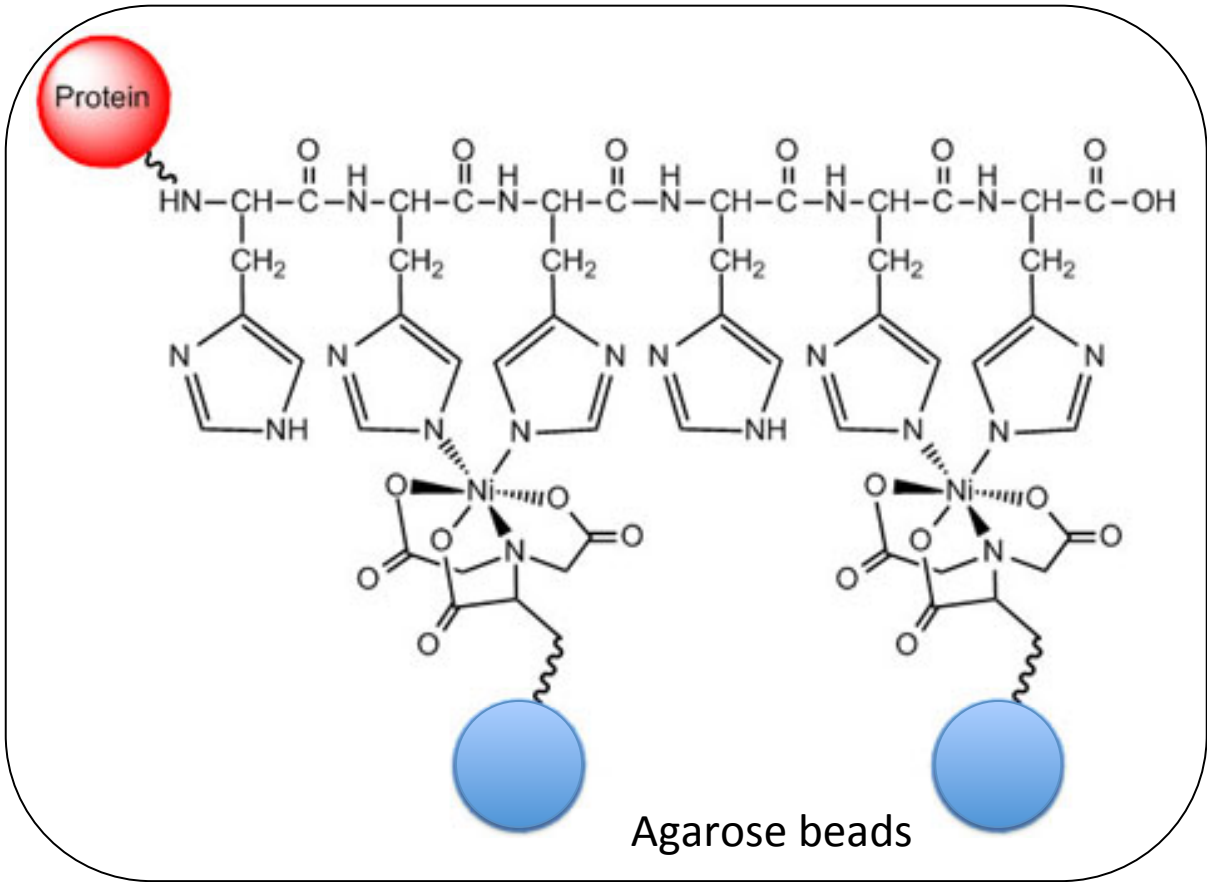


BL21(DE3)pLysS

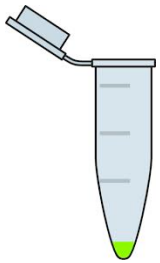
The polyhistidine (6XHis) tag binds nickel

Histidine polar, positive
His forms coordination bond with nickel agarose beads

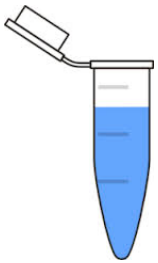




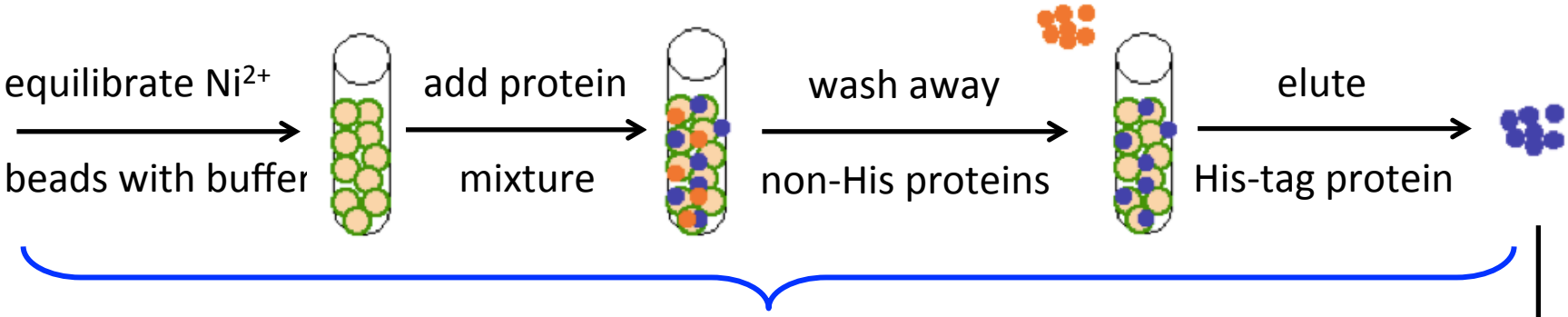
Protein purification: protocol overview



lyse (and extract supernatant)

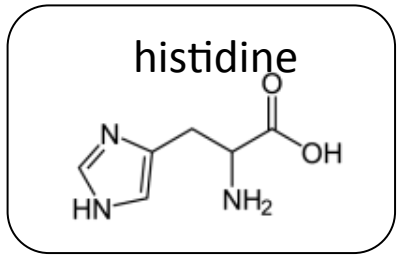


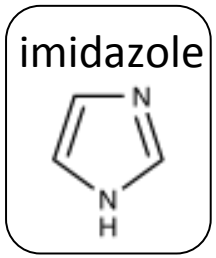
- protease inhibitor: **AEBSF, protein intact**
- DNAase (deoxyribonuclease): **degrade DNA**
- Tris buffer: **pH~7**
- salts: **osmotic pressure, folding**
- DTT (dithiothreitol): **reducing agent**
- glycerol: **stabilizer**
- lysozyme: **damages E. coli cell wall**

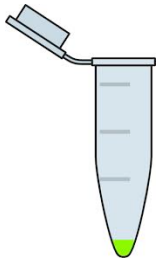


SMM screening + *Evaluate protein*

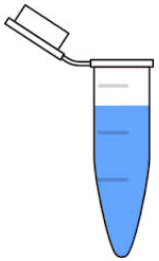
Protein purification: a few notes







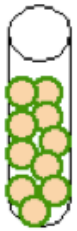
lyse

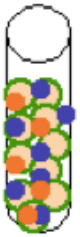


set aside lysed bacteria for SDS-PAGE

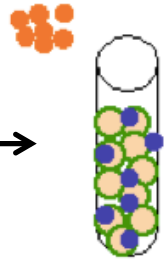
equilibrate Ni^{2+}

beads with buffer





wash



elute

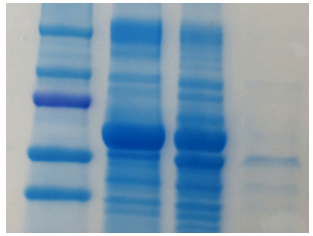
by competition:
imidazole *vs.* His

dialysis

imidazole interfere with assay

Evaluate [Protein]

Evaluate [Protein] and purity



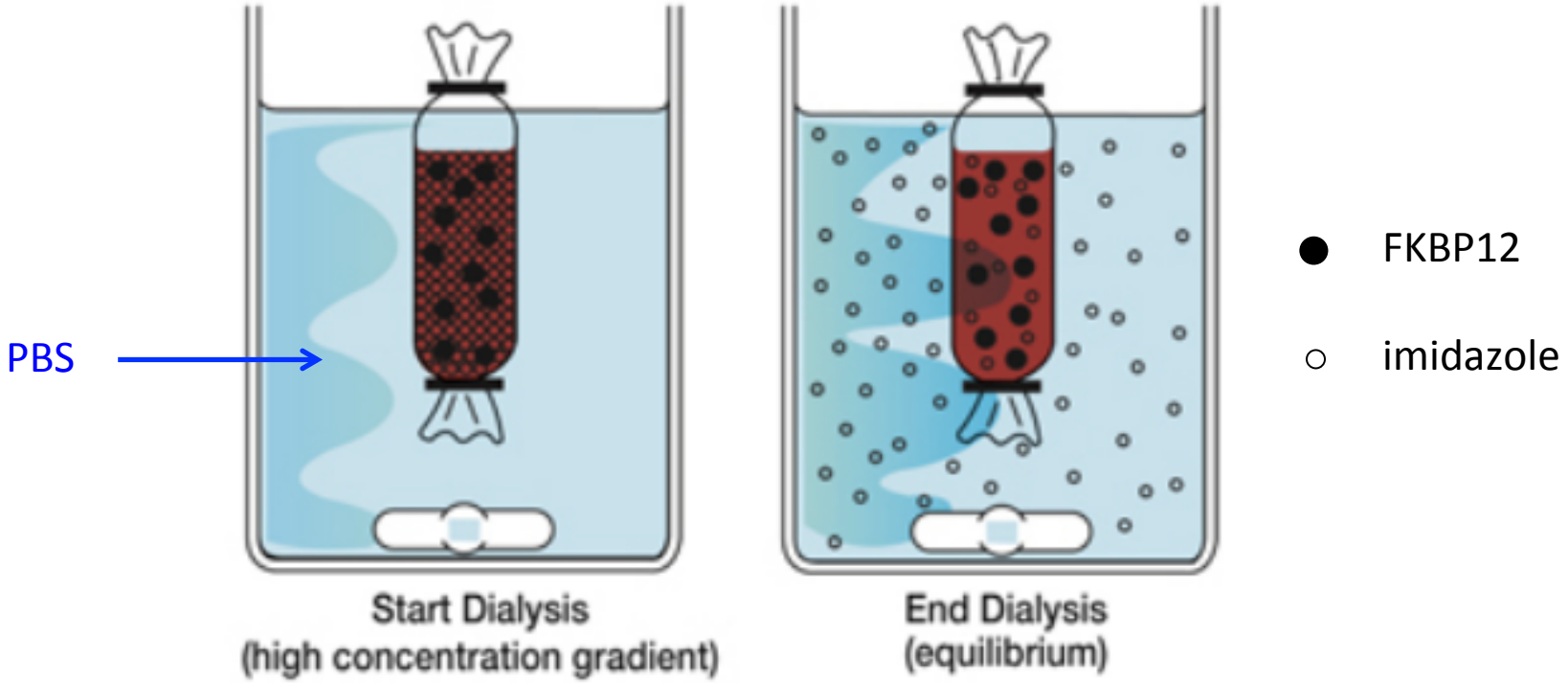
SMM ligand screening

binds to metals

binds poly-His tags

Dialysis: separation based on size rejection

- semipermeable membrane of crosslinked polymers on side of cassette
- molecular weight cut-off (MWCO): solute size retained > 90%
- 2000Da is our membrane's MWCO
  - FKBP12-6His ~ 15 kDa (15,000Da) -- imidazole ~ 68 Da



HW due M1D3: Create a schematic figure that outlines protein purification

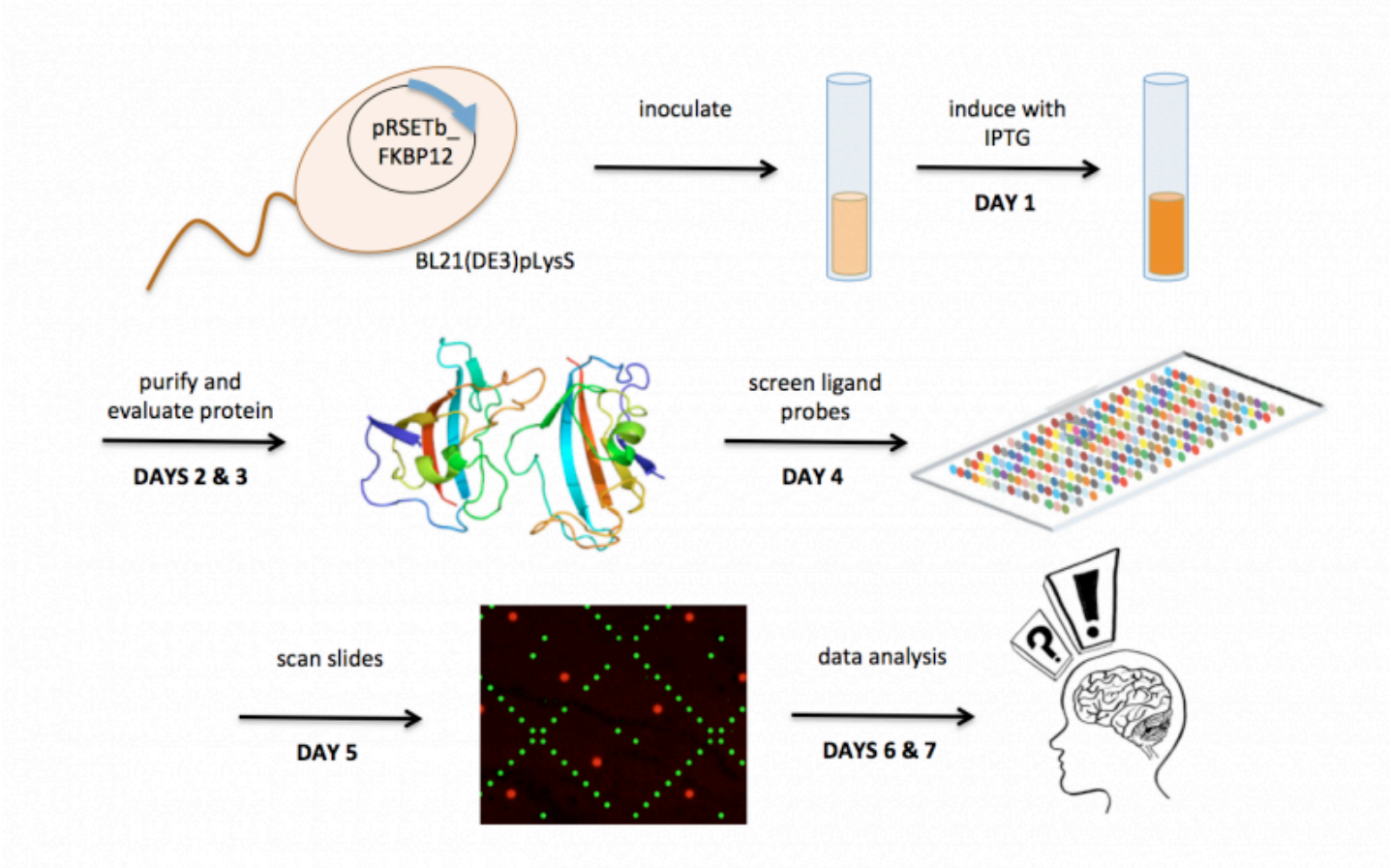


Figure 1. Overview of Module 1 High-throughput ligand screening. Continue with figure caption immediately after figure title.

Today in lab:

1. Complete FKBP12 protein purification
2. Leave purified protein to dialyze overnight in cold room

- Homework due Friday, M1D3
  - Schematic of protein purification(+ title & caption)
  - Before M1D4 (next Friday) visit BE Comm Lab, instructions on wiki
  - Quiz on M1D3, covers lecture and prelab material