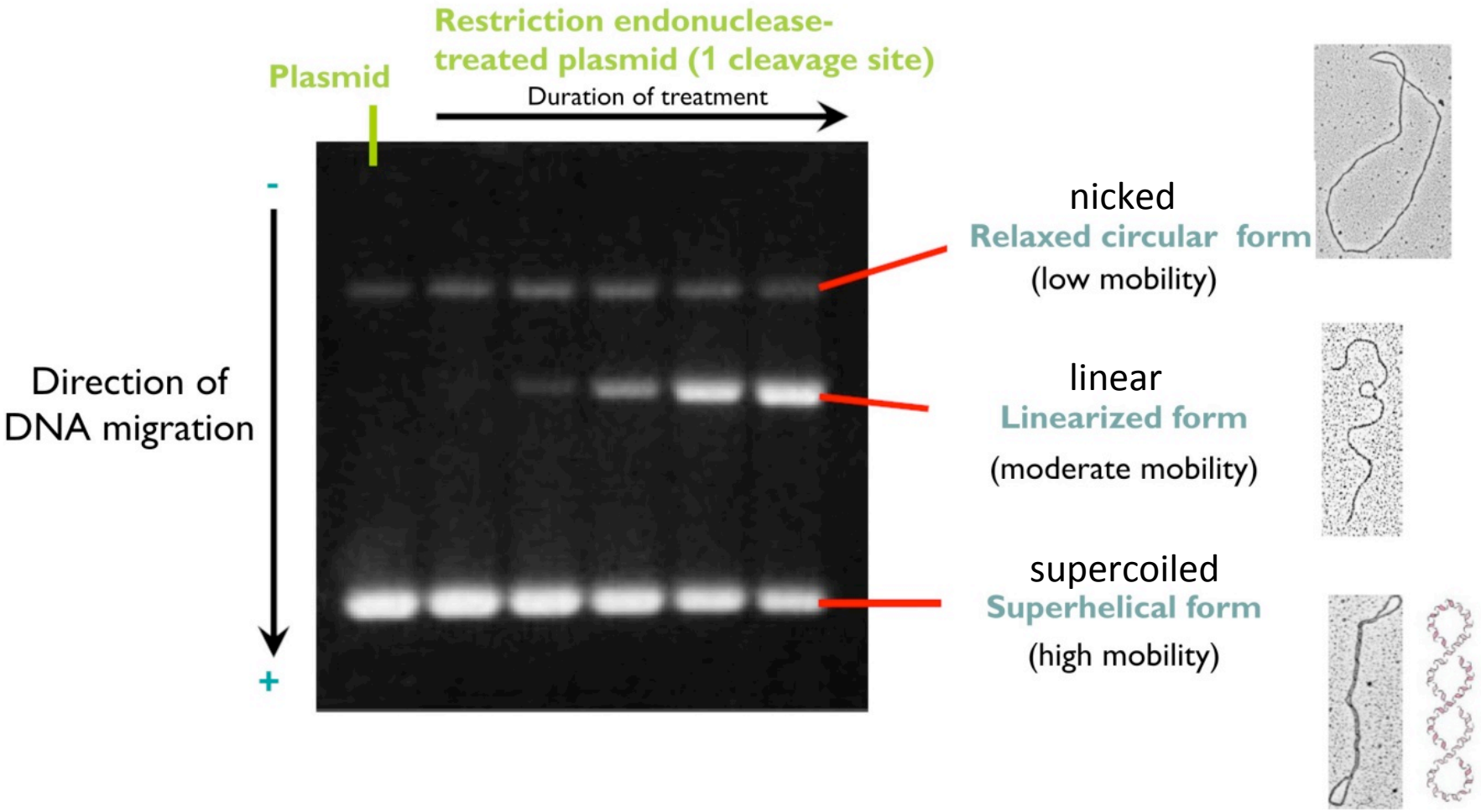
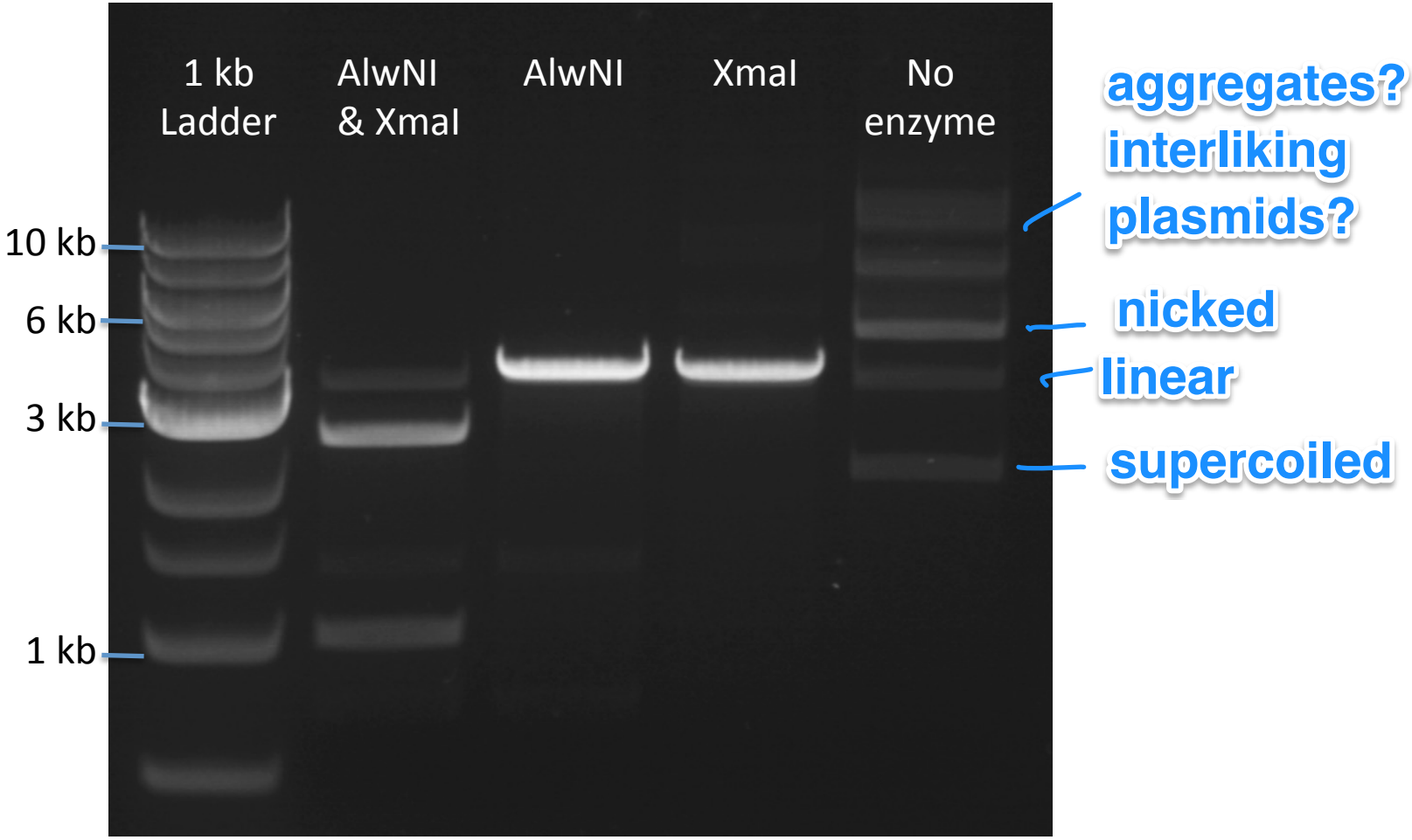
M1D3:
Purify protein for secondary assays

1. Pre-lab discussion part 1
2. Purify FKBP12 protein
3. During 1 hour incubation (2pm):
   - Pre-lab discussion part 2
   - Select ligands (a.k.a. compounds) from identified SMM hits
4. Continue with FKBP12 purification & ligand selection

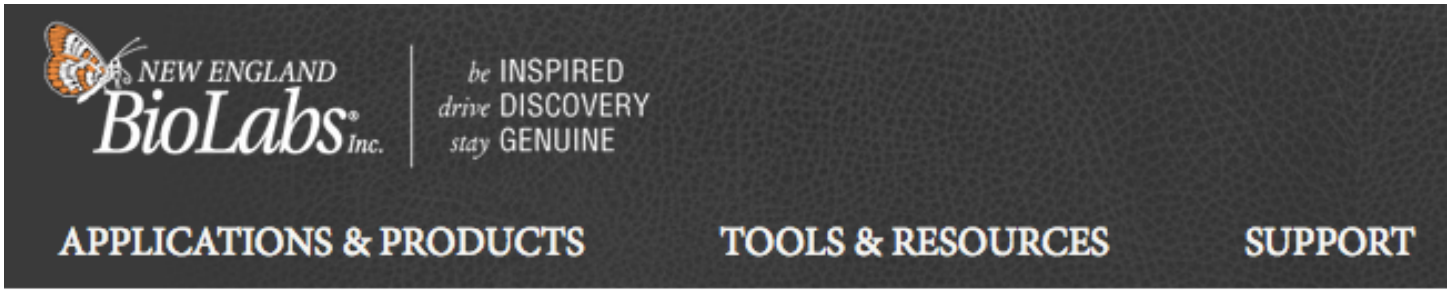
Deciphering confirmatoin digest results



Deciphering confirmatoin digest results



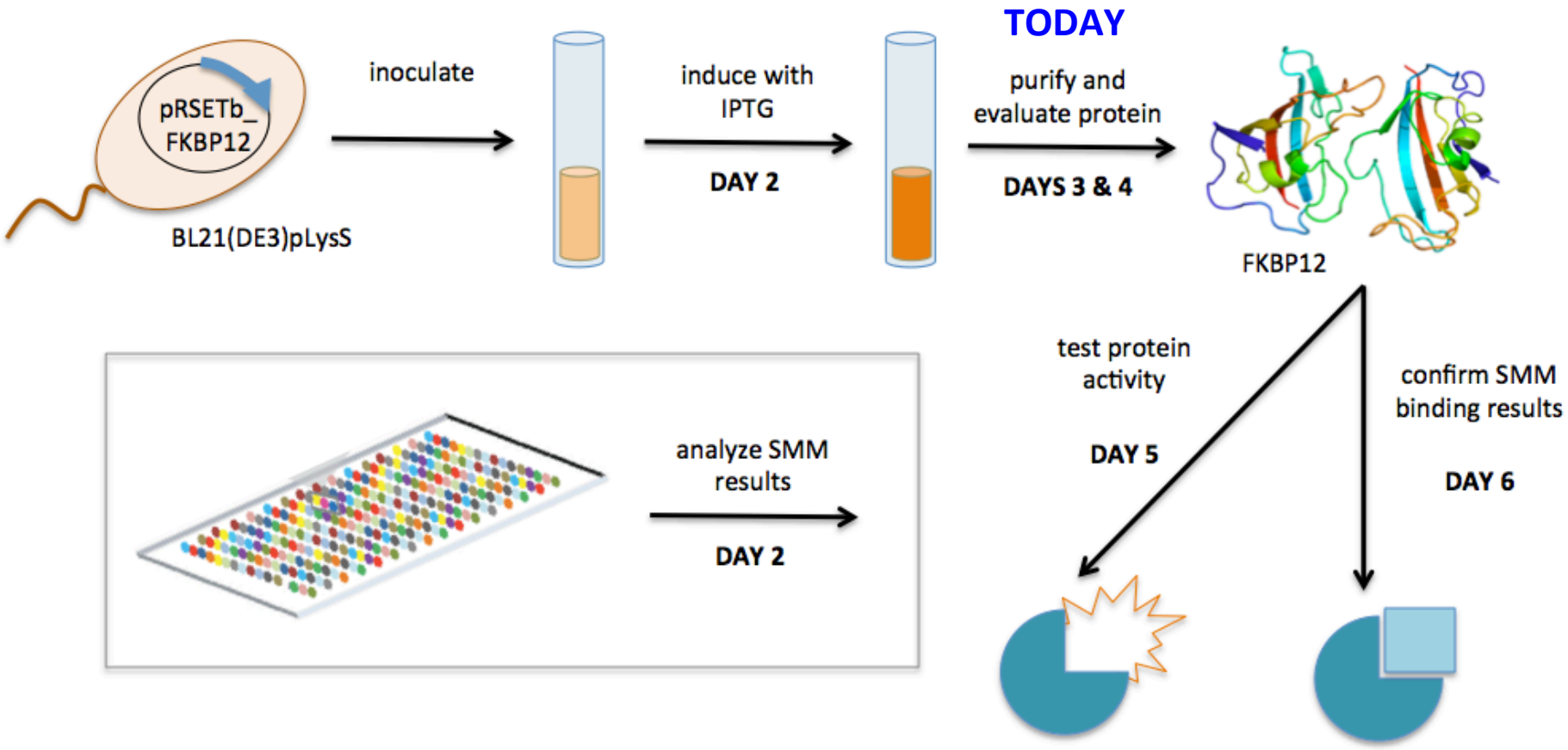
Other potential explanations



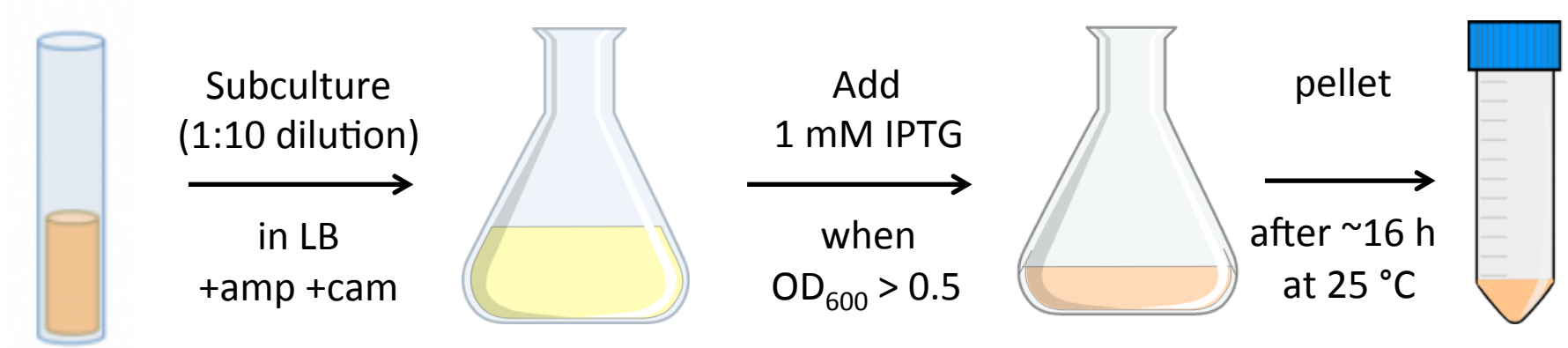
- Smear?
- Incomplete and / or no cutting?
- Extra bands?

https://www.neb.com/tools-and-resources/troubleshooting-guides/restriction-enzyme-troubleshooting-guide

Overview of Mod1 experiments



How did we induce protein expression?



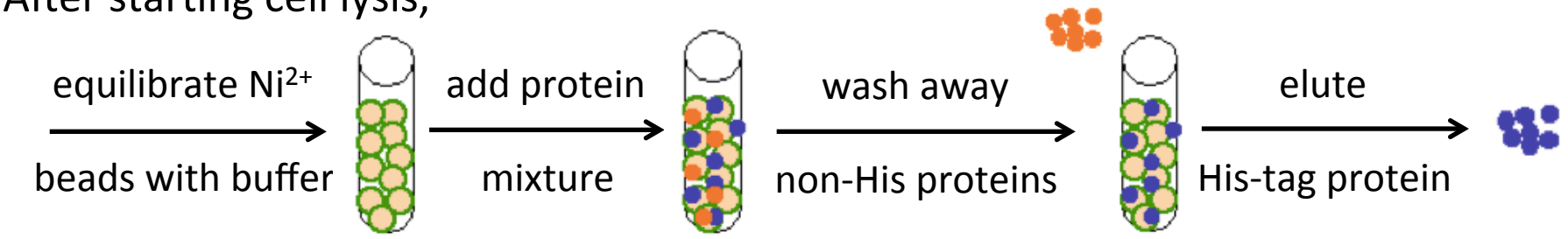
In addition to your induced sample, you will also examine and un-induced sample for FKBP12 expression

How will we retrieve our protein?

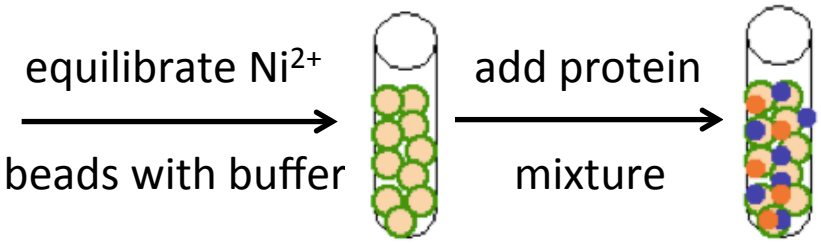
Cell lysis buffer components:

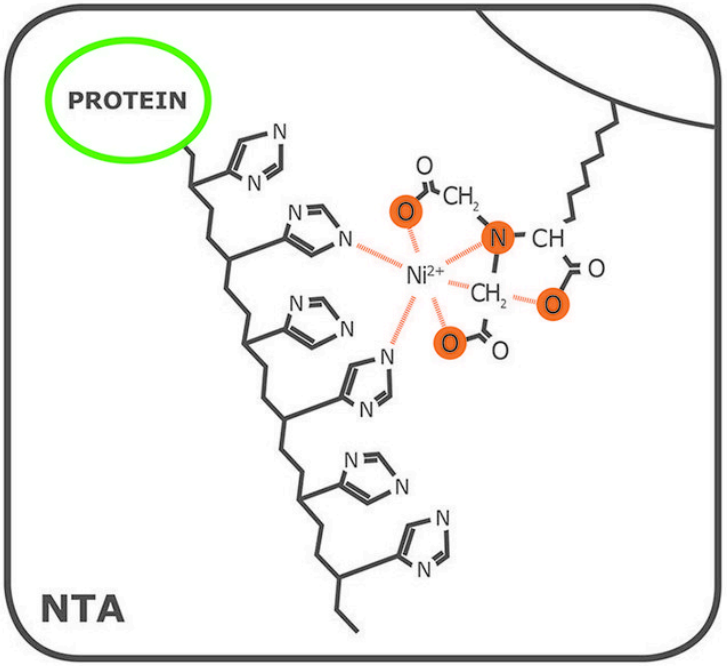
- protease inhibitor (AEBSF) — eliminates proteases
- deoxyribonuclease (DNase) — degrades DNA
- tris / salts buffer — maintain pH & osmotic pressure
- dithiothreitol (DTT) — reducing agent
- glycerol — stabilizer
- lysozyme — degrades bacterial cell wall

After starting cell lysis,



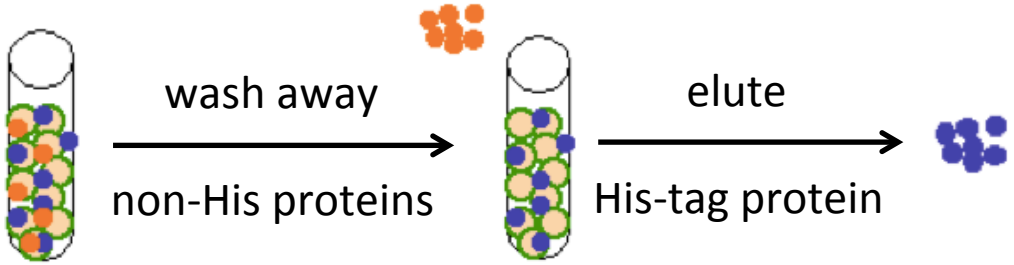
6x His residues enable binding to Ni^{2+}

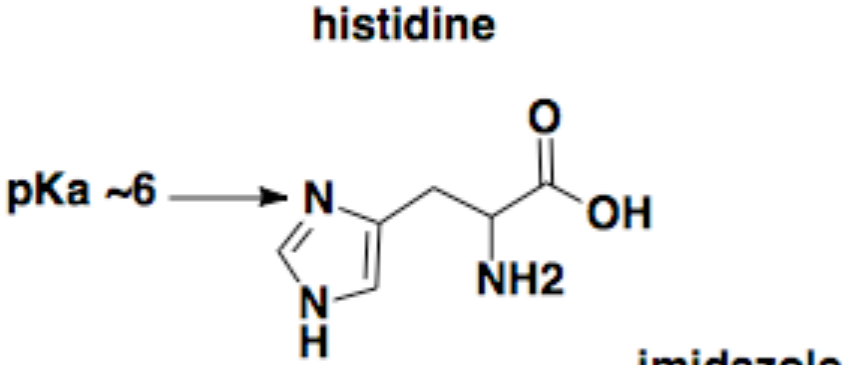


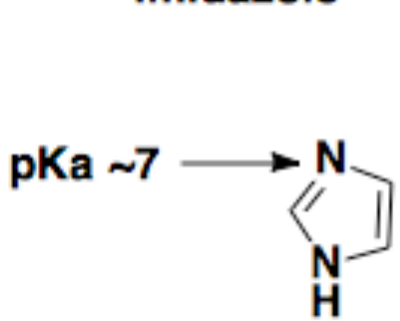


- Ni^{2+} chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand

Wash and elute protein:
Imidazole competes for binding to Ni^{2+}



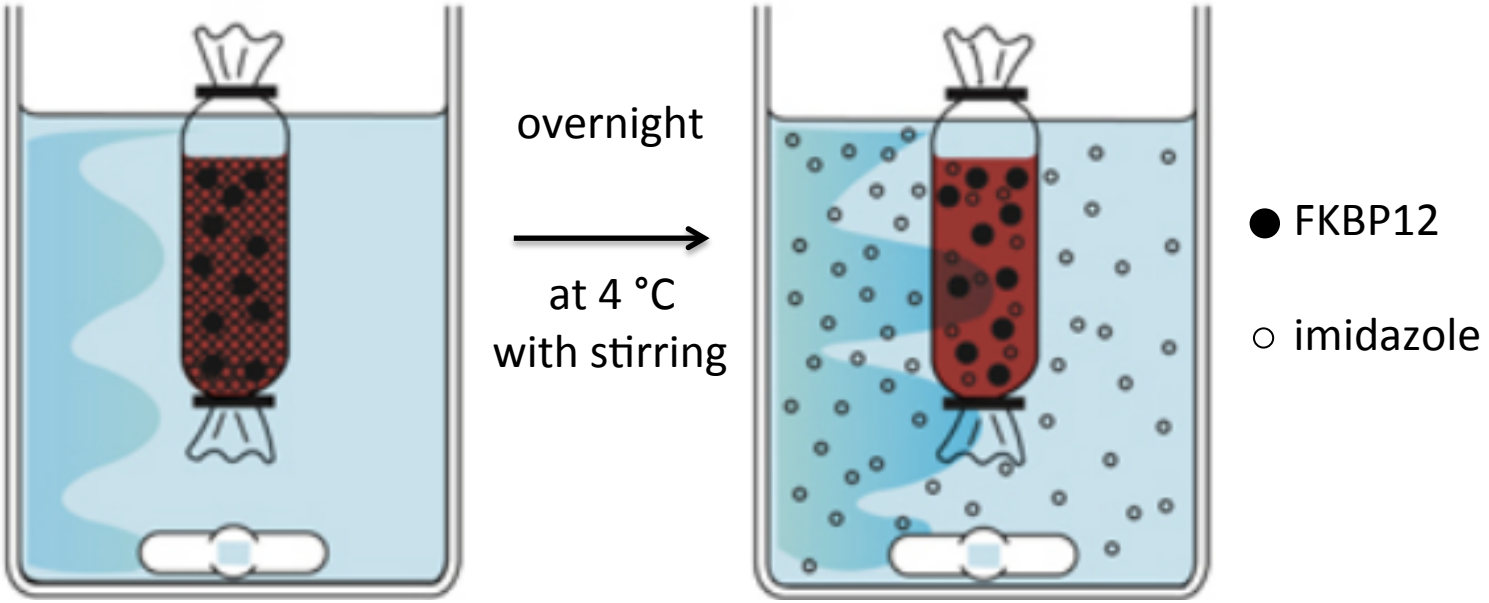




- Low concentration of imidazole included in wash buffer
- Increased 25-fold in elution buffer

Dialyze to remove imidazole

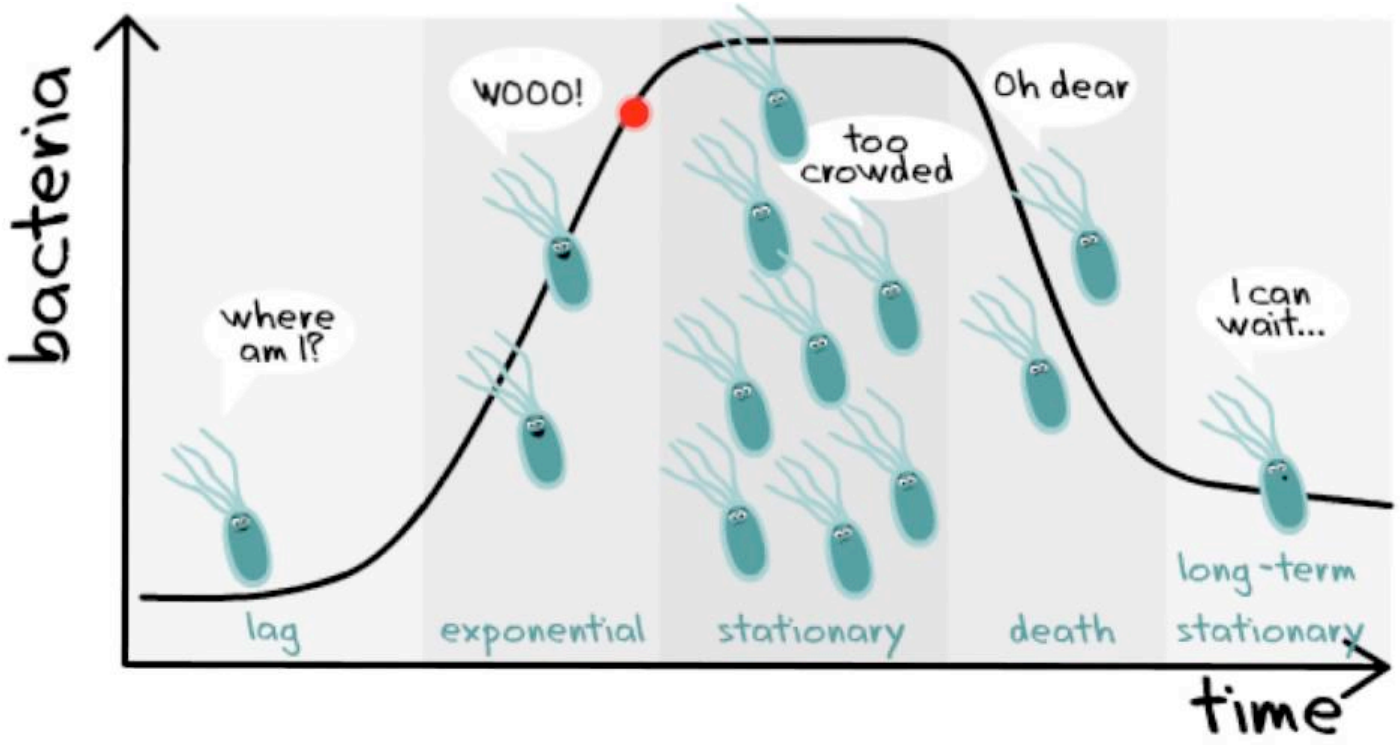
- Dialyze ('dilute out') imidazole with semi-permeable membrane of cross-linked polymers
- Molecular weight cutoff = 2000 Da
  - FKBP12-6His ~15 kDa, imidazole ~68 Da



Start lysis now

Why do we induce at OD_{600} = 0.5-0.8?

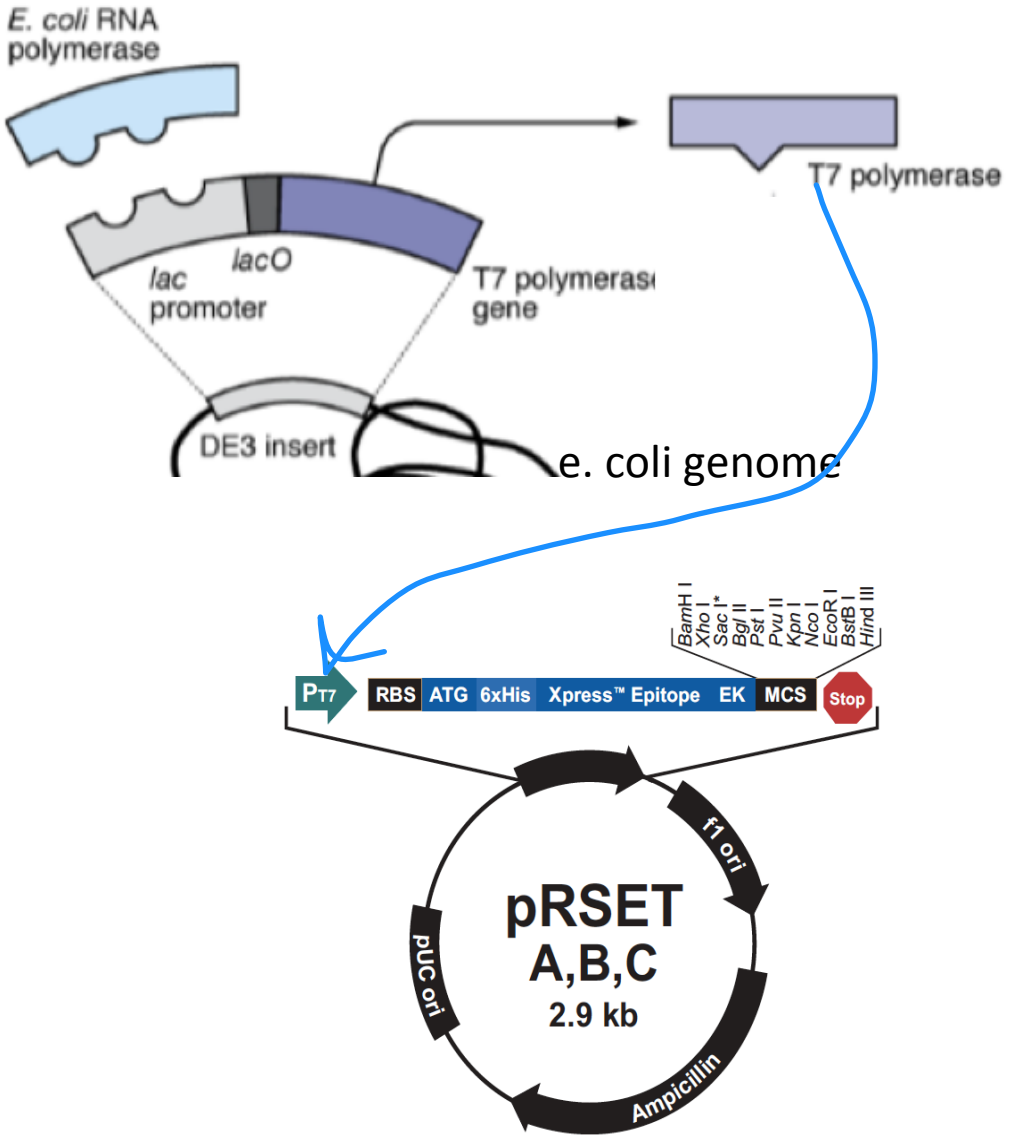
- Optical density (OD) is a measure of light scattering
  - *E. coli* scatter 600 nm light
- OD measures turbidity rather than absorption
  - Indicates # of cells present in culture
  - OD_{600} of 1 ≅ 8 x 10^8 cells / mL



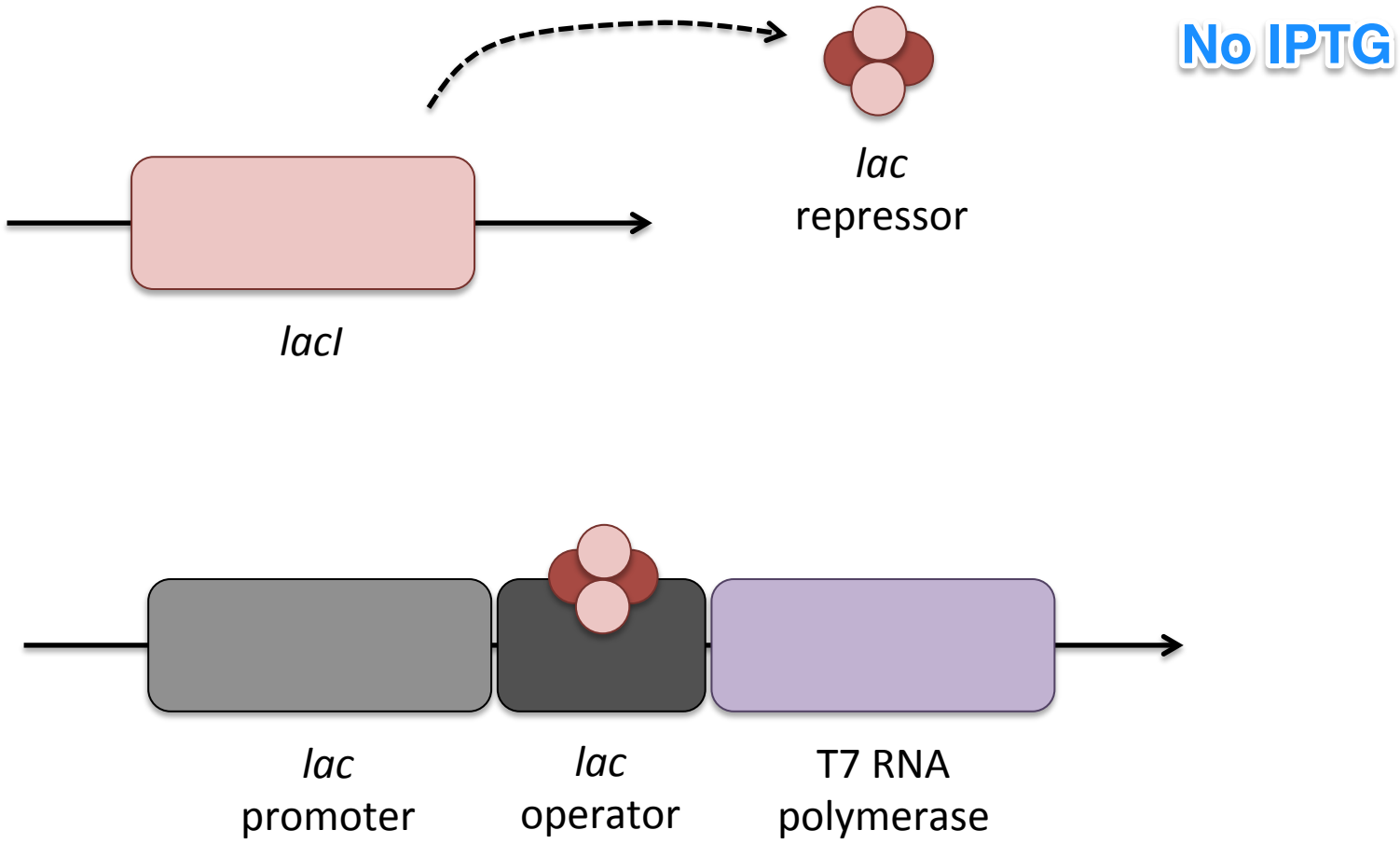
BL21(DE3)pLysS used for protein expression

e. coli

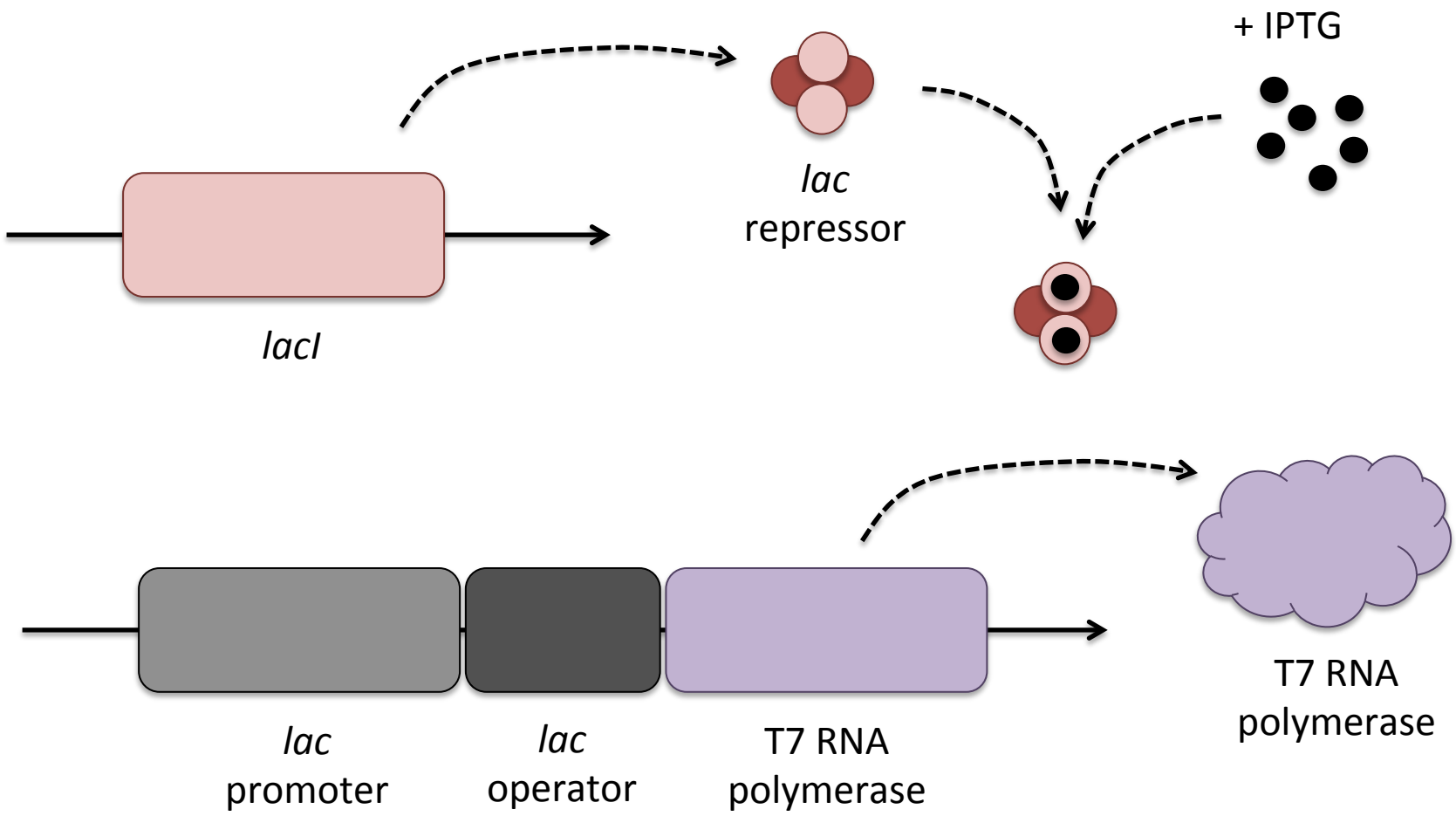
- DE3



DE3: Lac promoter controls expression of T7 RNA polymerase



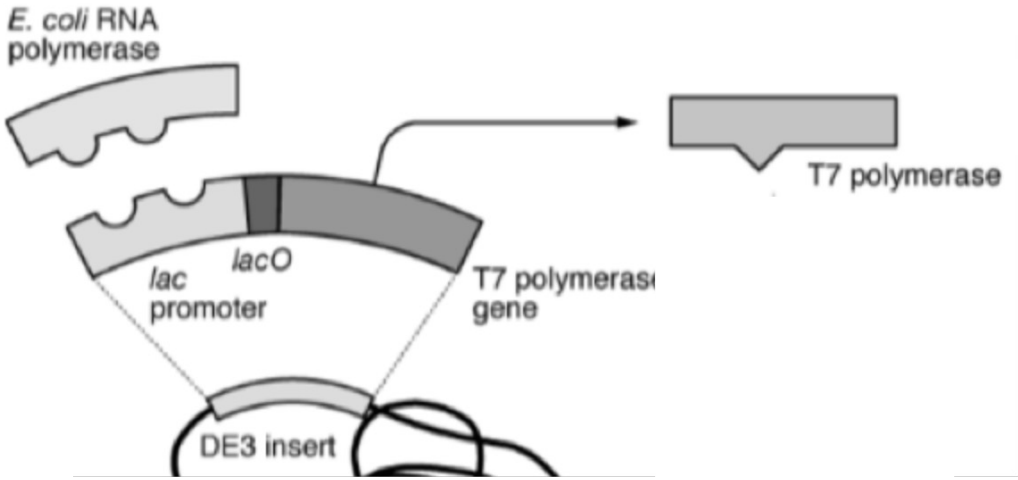
IPTG ‘induces’ protein expression



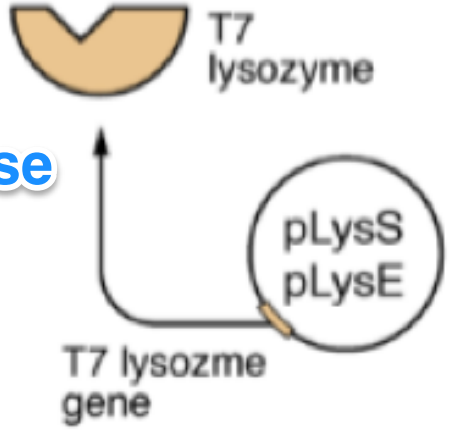
derepression

BL21(DE3)pLysS used for protein expression

- DE3



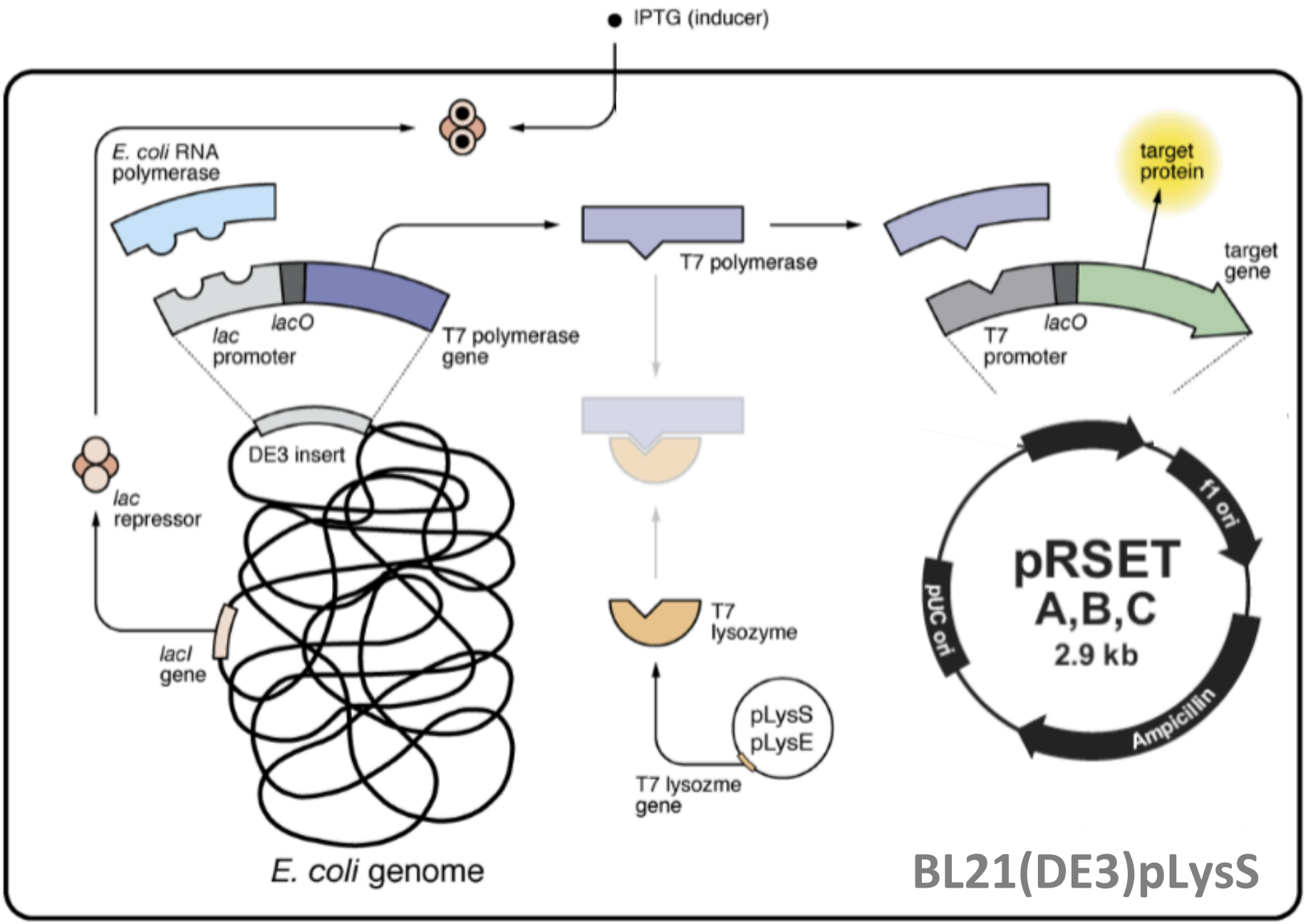
- pLysS



T7 lysozyme binds T7 polymerase and degrades it

controls for leaky expression

maintained by chloramphenicol (cam)


IPTG (inducer)
E. coli RNA polymerase
lac promoter
lacO
T7 polymerase gene
DE3 insert
lac repressor
lacI gene
E. coli genome
T7 polymerase
T7 lysozyme
pLysS pLysE
T7 lysozme gene
target protein
target gene
T7 promoter
lacO
pRSET A,B,C
2.9 kb
f1 ori
pUC ori
Ampicillin
BL21(DE3)pLysS

IPTG induction of FKBP12 expression in words…

- In absence of IPTG:
  - LacI repressor binds *lac* operator;
    represses/activates transcription of T7 RNA polymerase
  - Leaky expression of T7 RNA polymerase corrected for by T7 lysozyme
- In presence of IPTG:
  - IPTG binds LacI; enables/prevents binding to *lac* operator
  - T7 RNA polymerase transcribed and binds at P_{T7}; initiates transcription of FKBP12

In lab today…

- Sign-up for compounds at the front laboratory bench
  - Each group will test **two** compounds

For next time…

- There will be a Comm Lab workshop in 56-614
- Draft schematic (image, title, caption)
- Outline introduction using topic sentences
  - Include reference information!!

Notes on topic sentences:

- Used to introduce each paragraph

- Should 'funnel' from big picture topic to your specific research project
- All claims should be supported by trusted sources