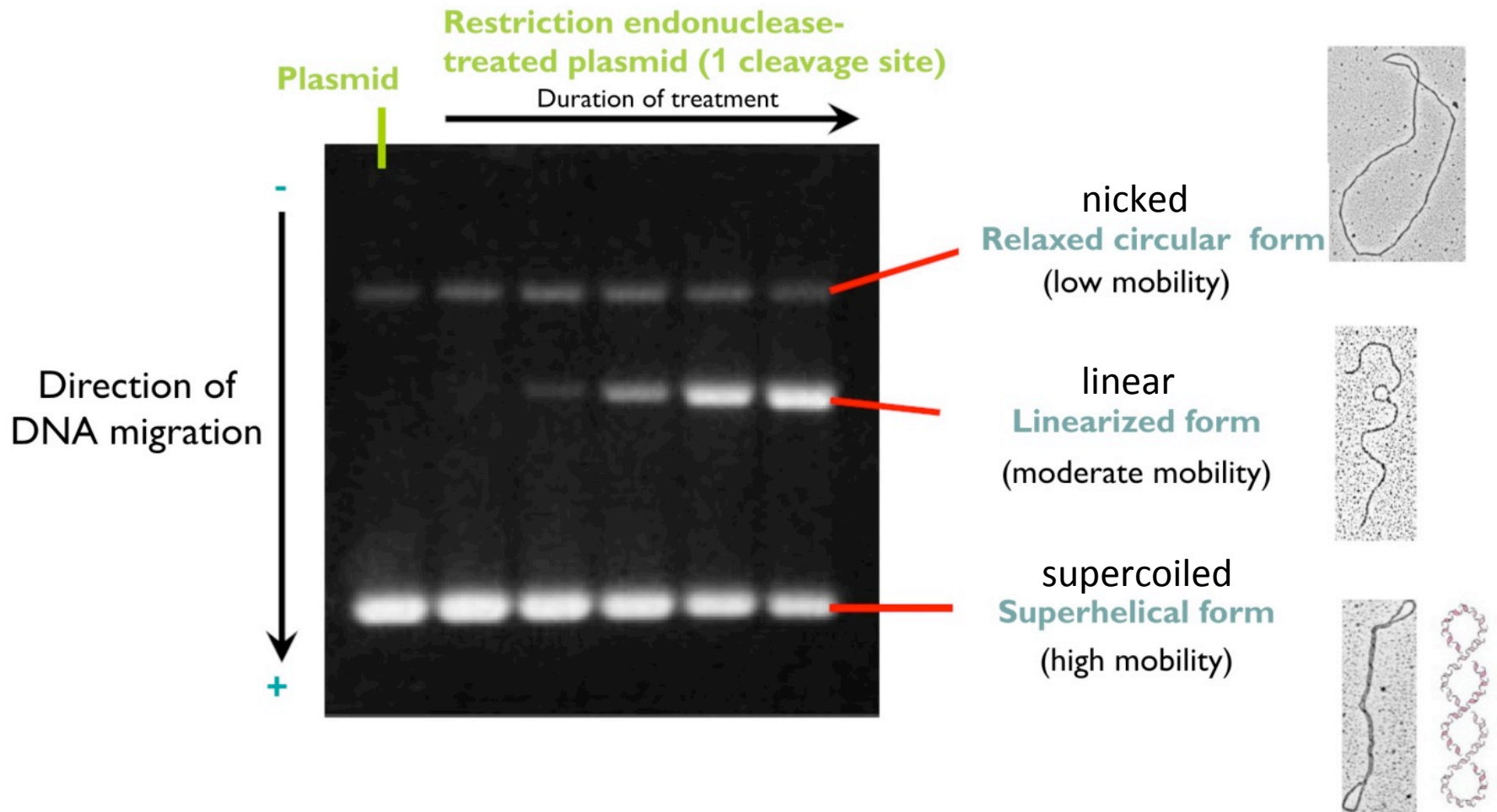


M1D3:

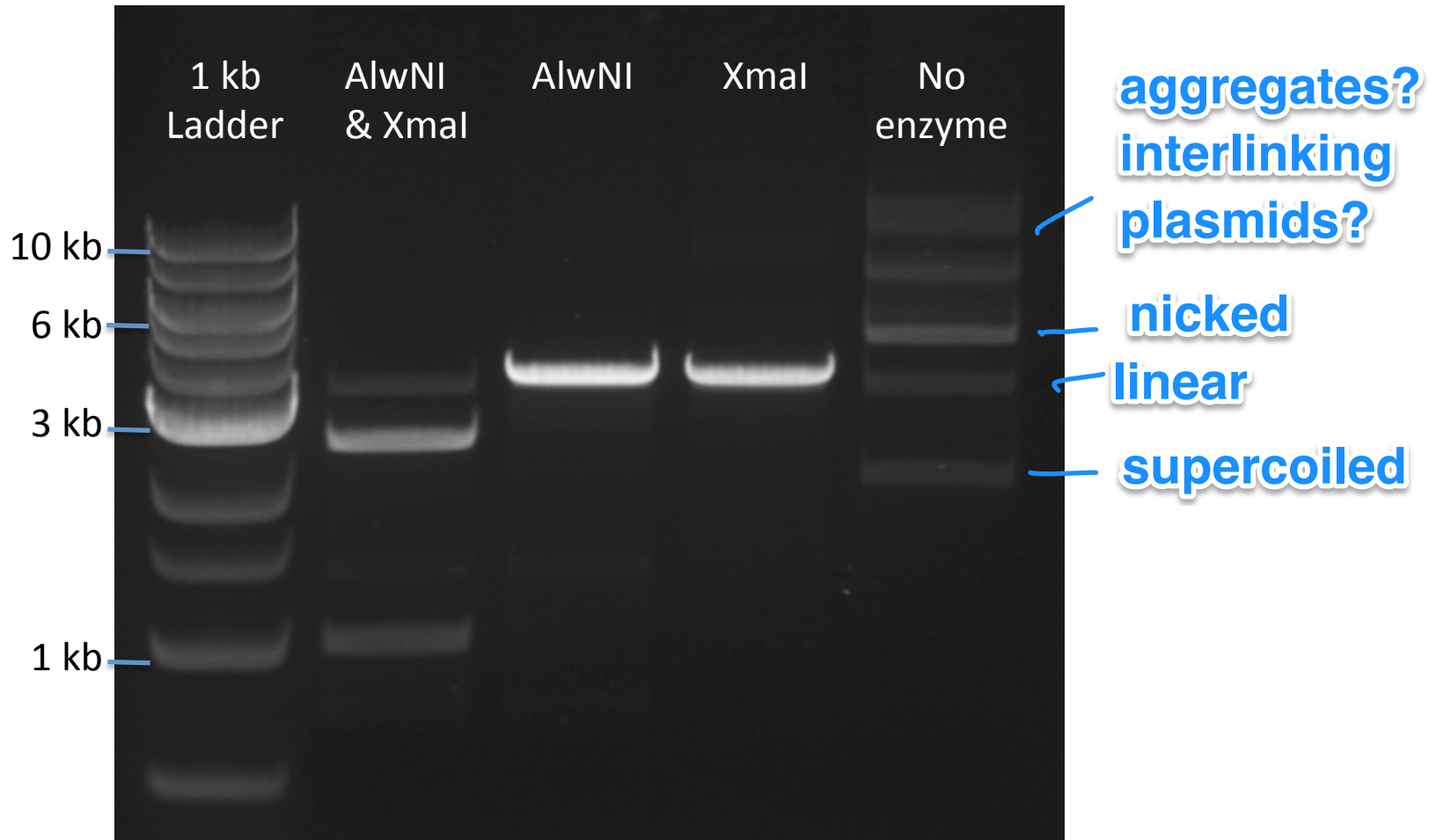
Purify protein for secondary assays

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

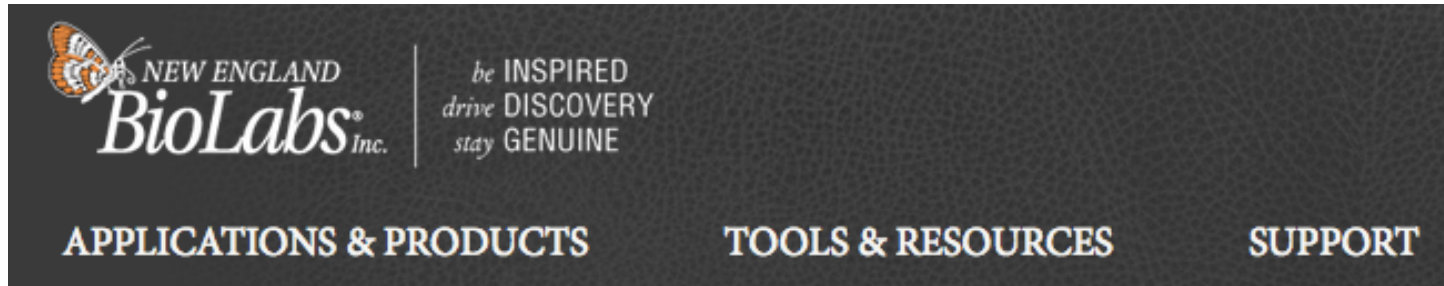
Deciphering confirmatoin digest results



Deciphering confirmatoion digest results



Other potential explanations

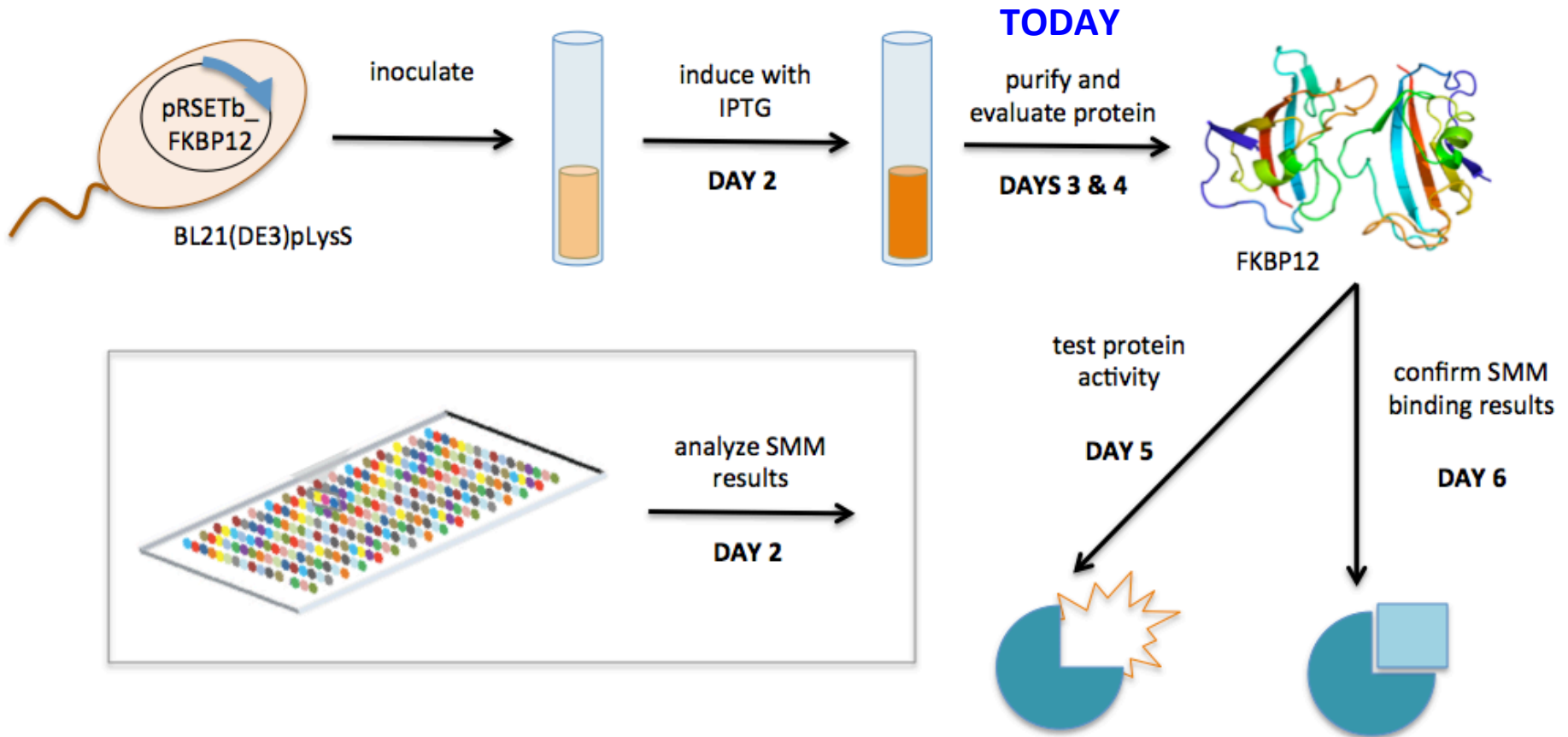


[Home](#) > [Tools & Resources](#) > [Troubleshooting Guides](#) > Restriction Enzyme Troubleshooting Guide

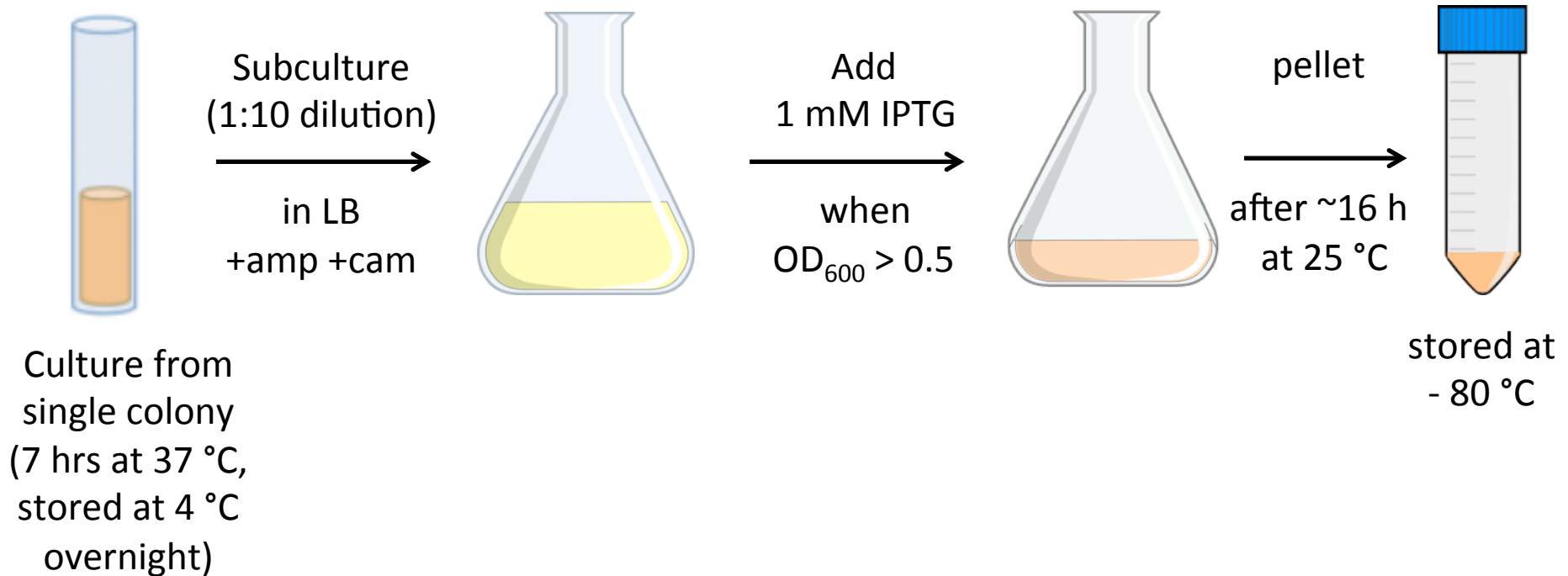
Restriction Enzyme Troubleshooting Guide

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

Overview of Mod1 experiments



How did we induce protein expression?



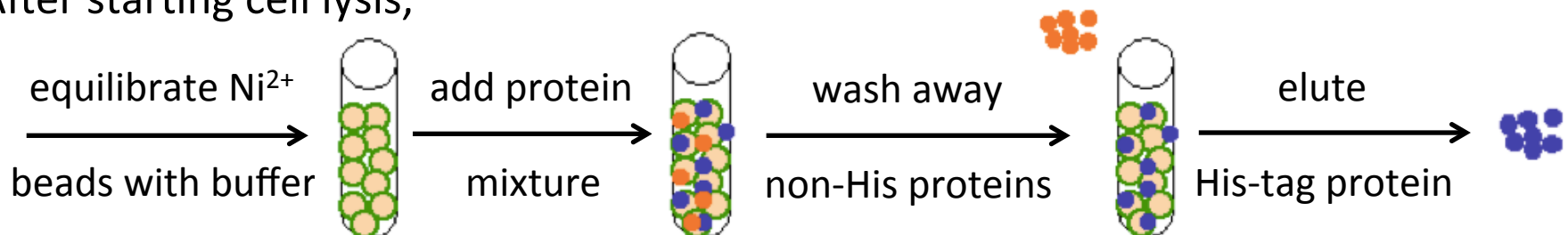
In addition to your induced sample, you will also examine an un-induced sample for FKBP12 expression

How will we retrieve our protein?

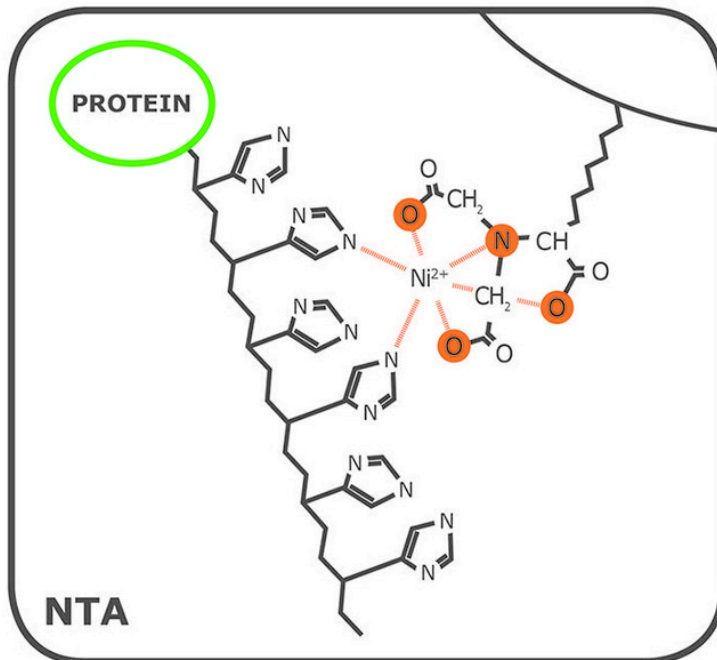
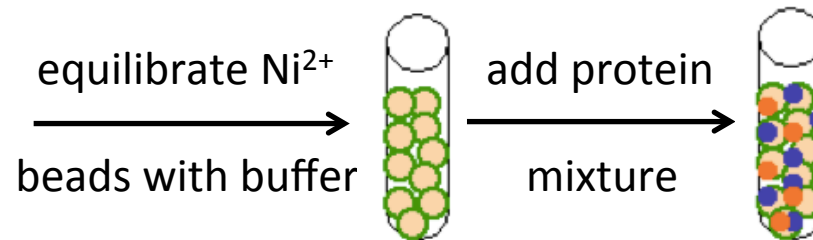
Cell lysis buffer components:

- 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,



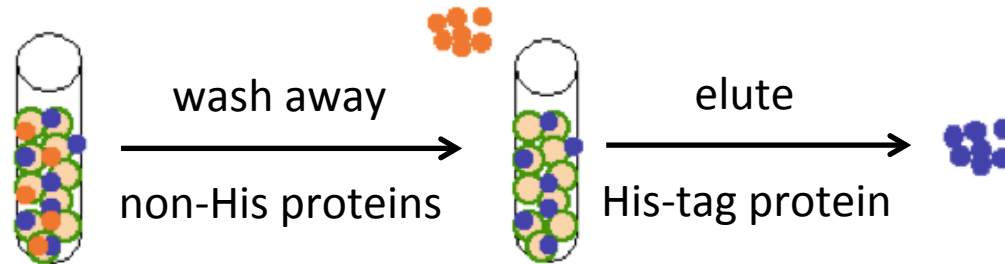
6x His residues enable binding to Ni^{2+}



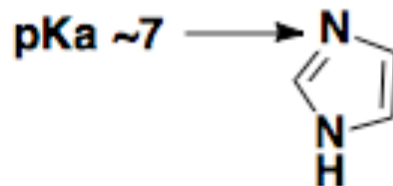
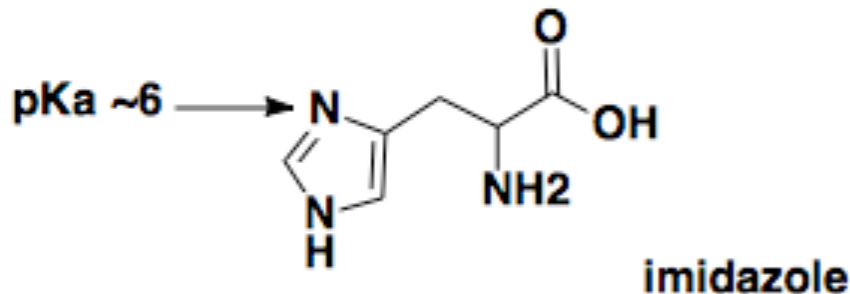
- Ni^{2+} chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand

Wash and elute protein:

Imidazole competes for binding to Ni^{2+}



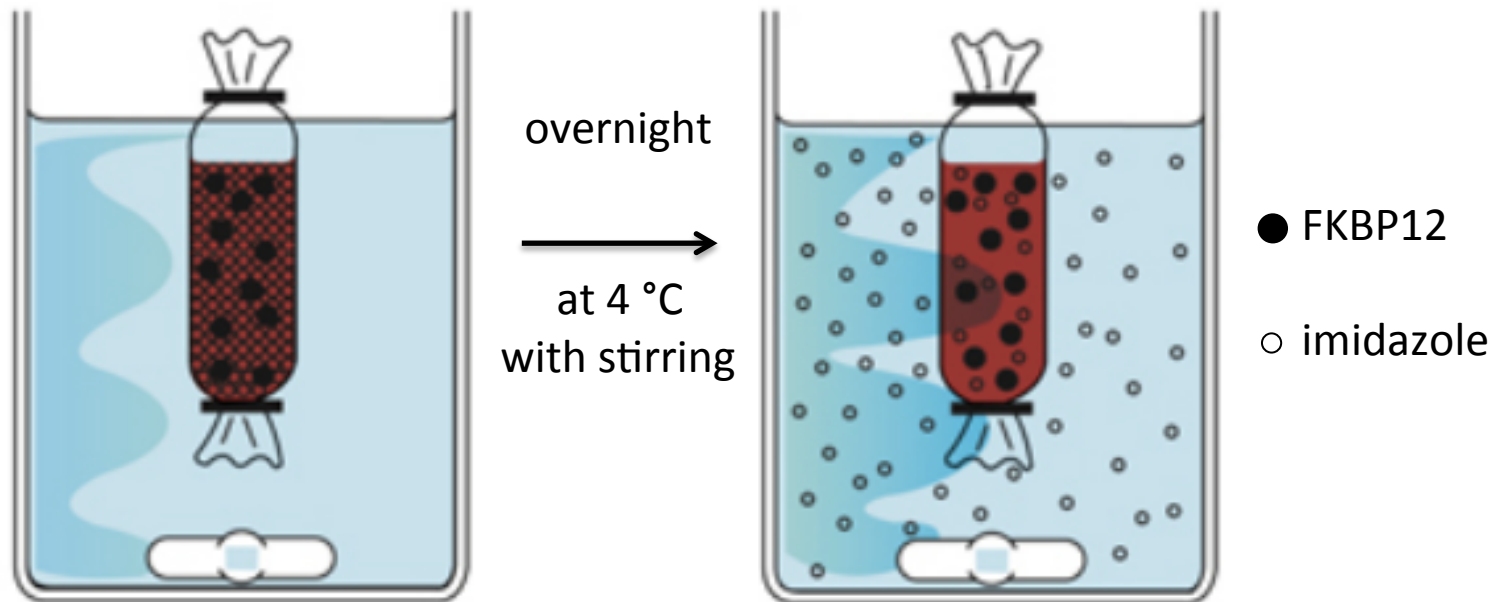
histidine



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

Dialyze to remove imidazole

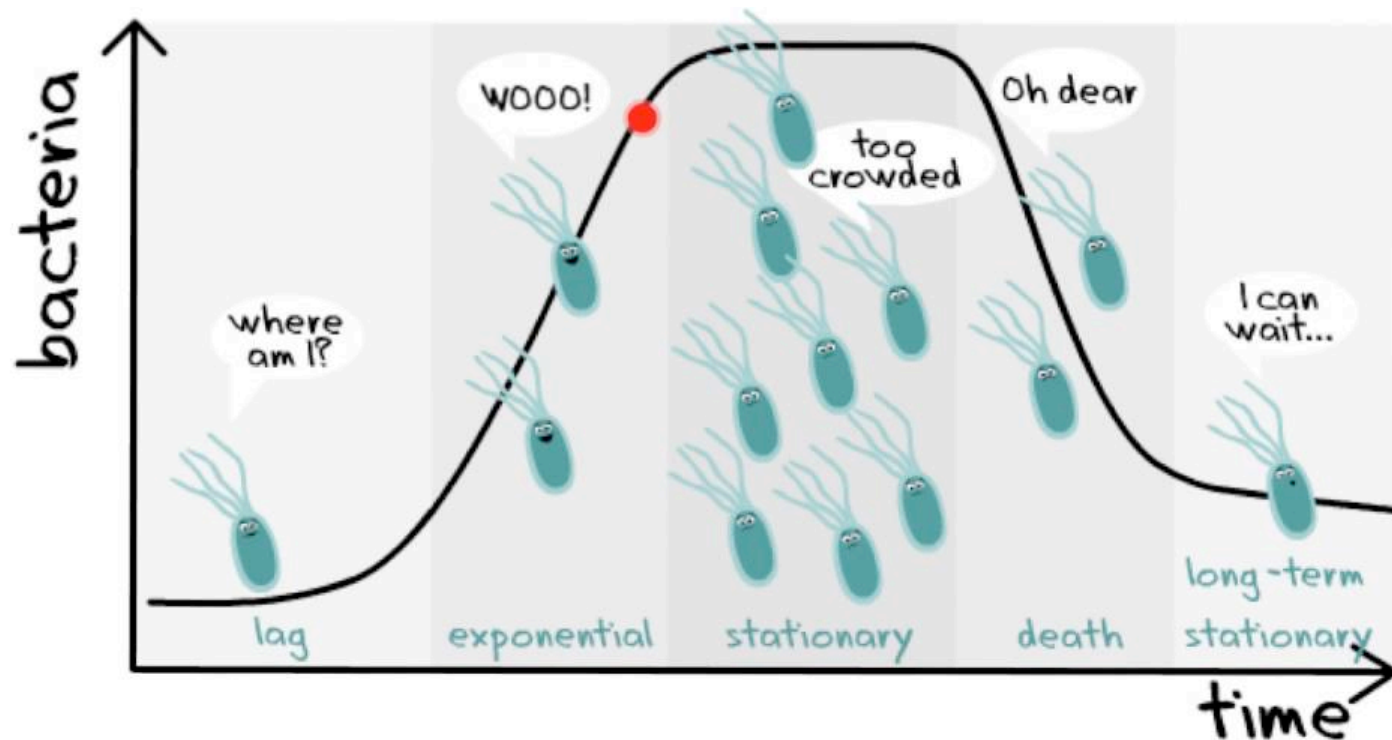
- Dialyze ('dilute out') imidazole with semi-permeable membrane of cross-linked polymers
- Molecular weight cutoff = 2000 Da
 - FKBP12-6His ~15 kDa, imidazole ~68 Da



Start lysis now

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

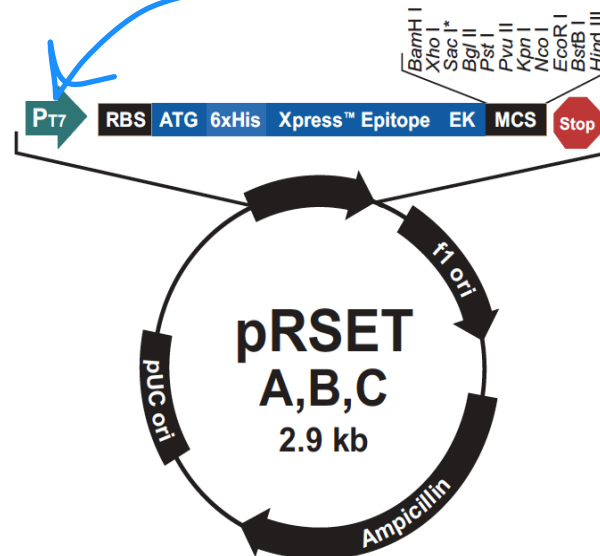
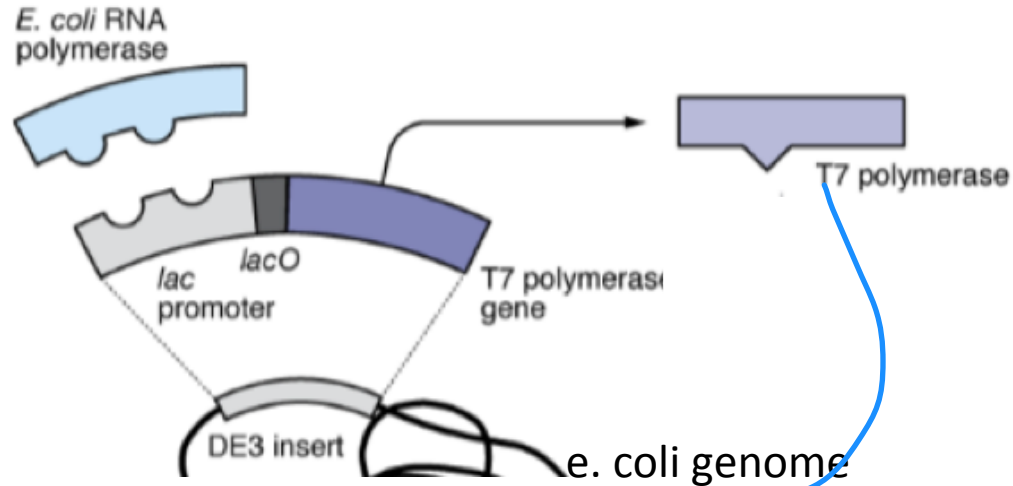
- Optical density (OD) is a measure of light scattering
 - *E. coli* scatter 600 nm light
- OD measures turbidity rather than absorption
 - Indicates # of cells present in culture
 - OD_{600} of 1 $\approx 8 \times 10^8$ cells / mL



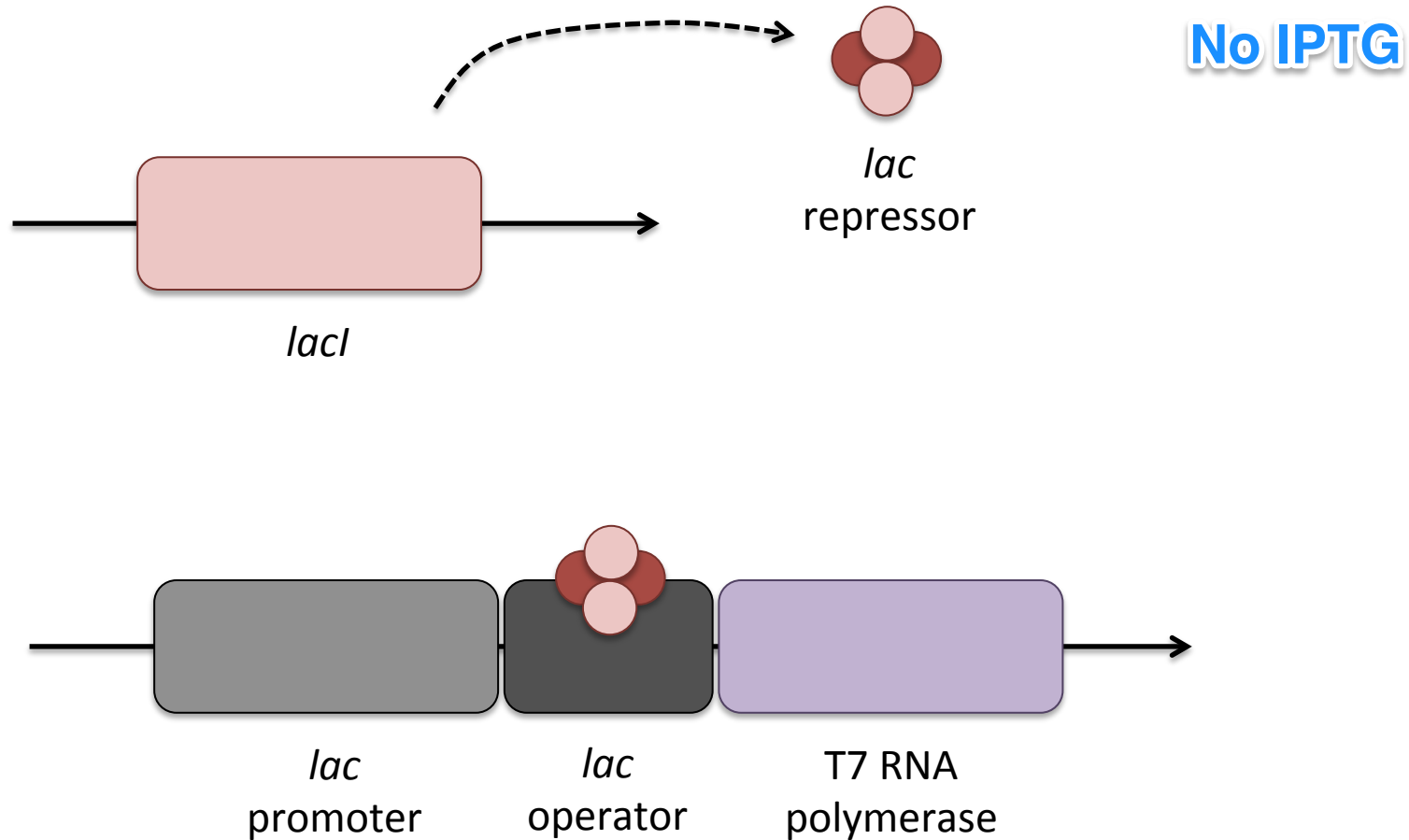
BL21(DE3)pLysS used for protein expression

e. coli

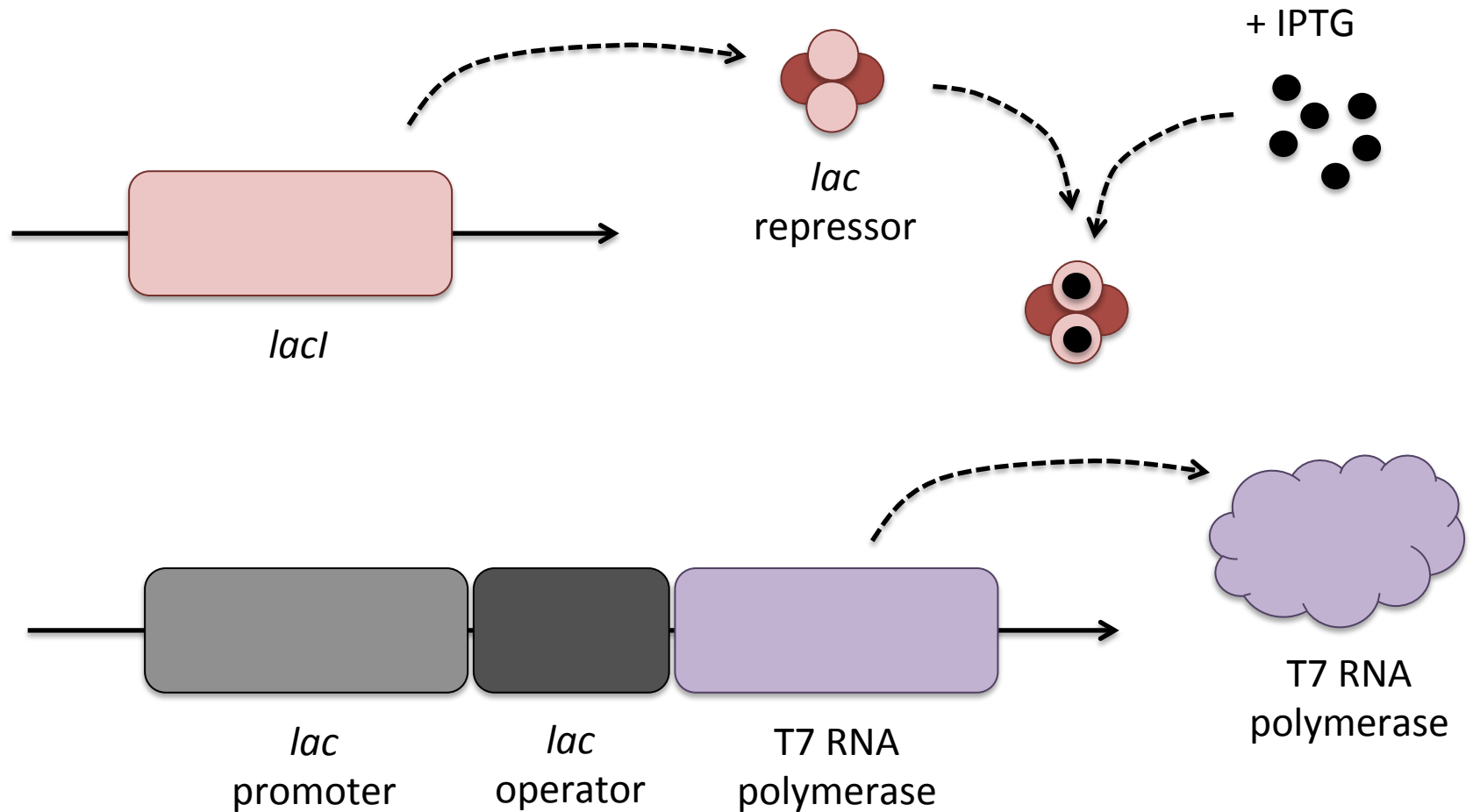
- DE3



DE3: Lac promoter controls expression of T7 RNA polymerase



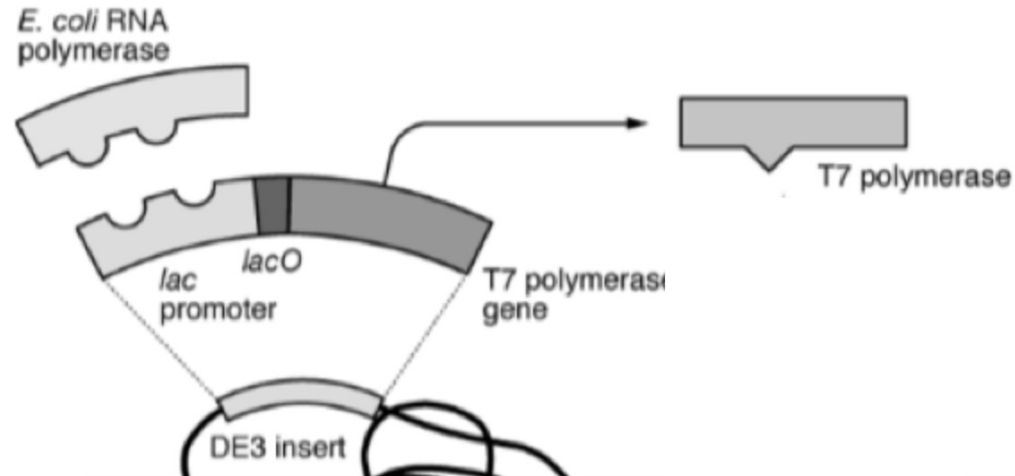
IPTG 'induces' protein expression



derepression

BL21(DE3)pLysS used for protein expression

- DE3

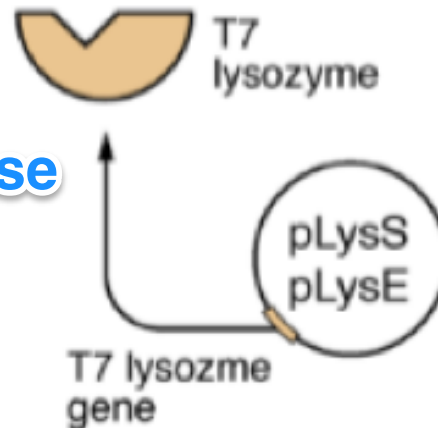


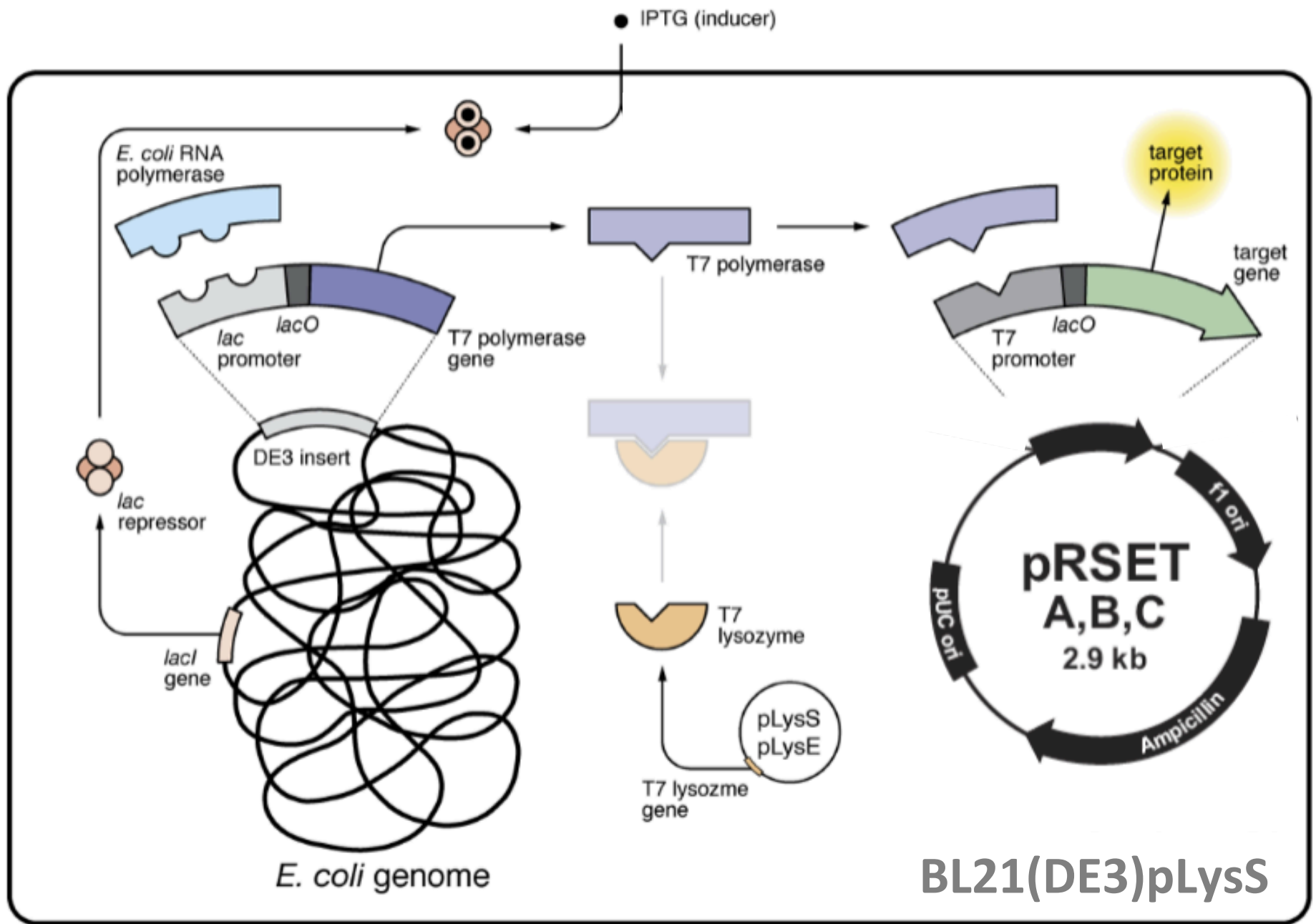
- pLysS

T7 lysozyme binds T7 polymerase and degrades it

controls for leaky expression

maintained by chloramphenicol (cam)





IPTG 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

