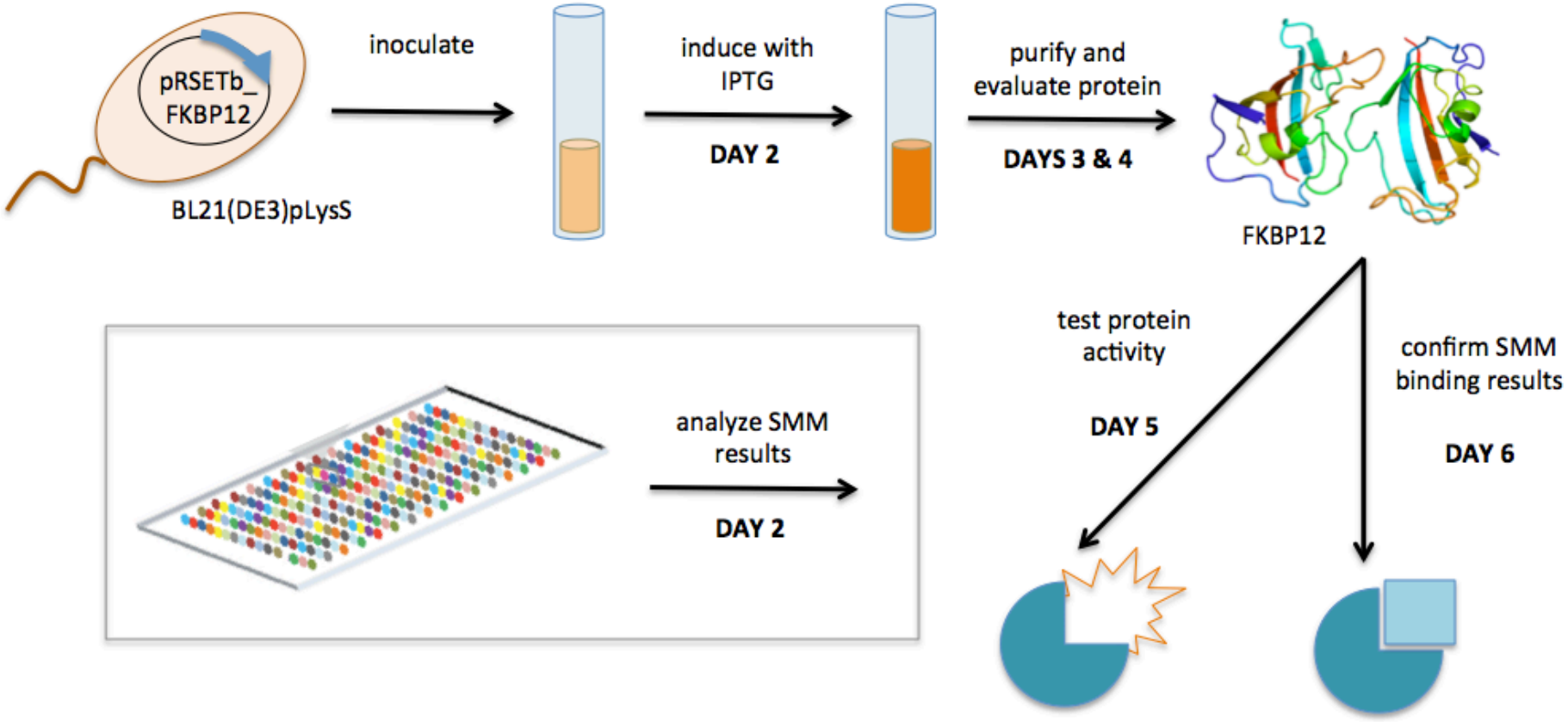
M1D3:
Purify protein for secondary assays

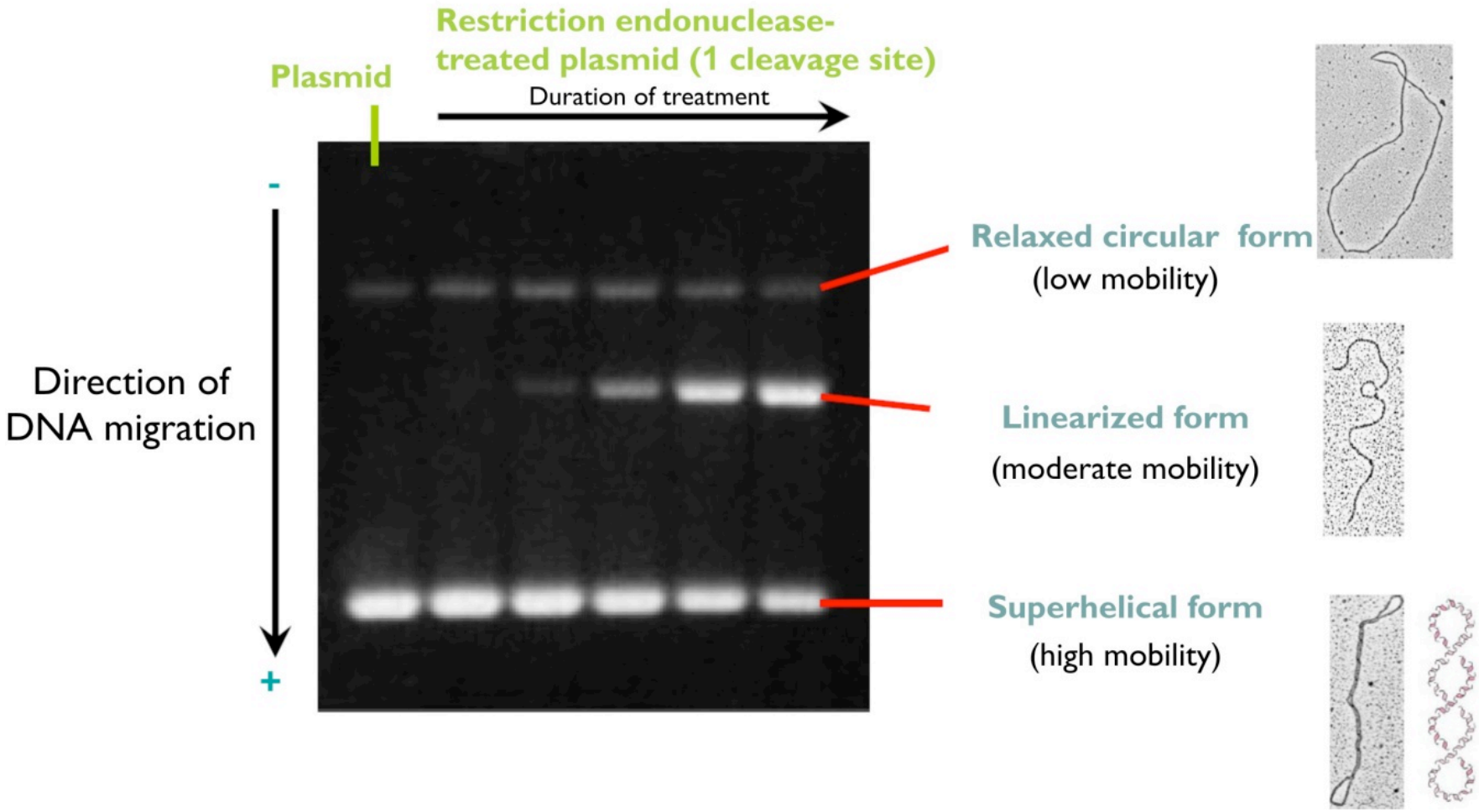
1. Pre-lab discussion
2. Purify FKBP12 protein
3. Select ligands from identified SMM hits

= compounds
= binders
= pinacs of magic dust
= small molecules

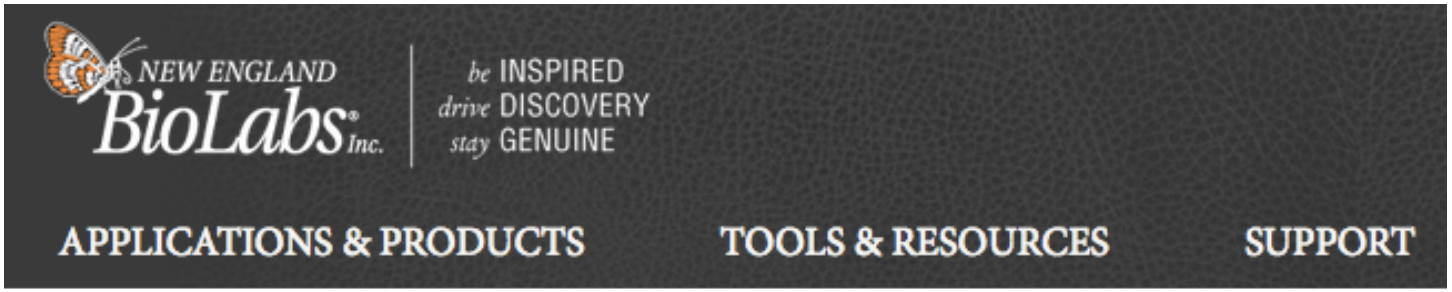
Overview of Mod1 experiments



But first, deciphering your digest results



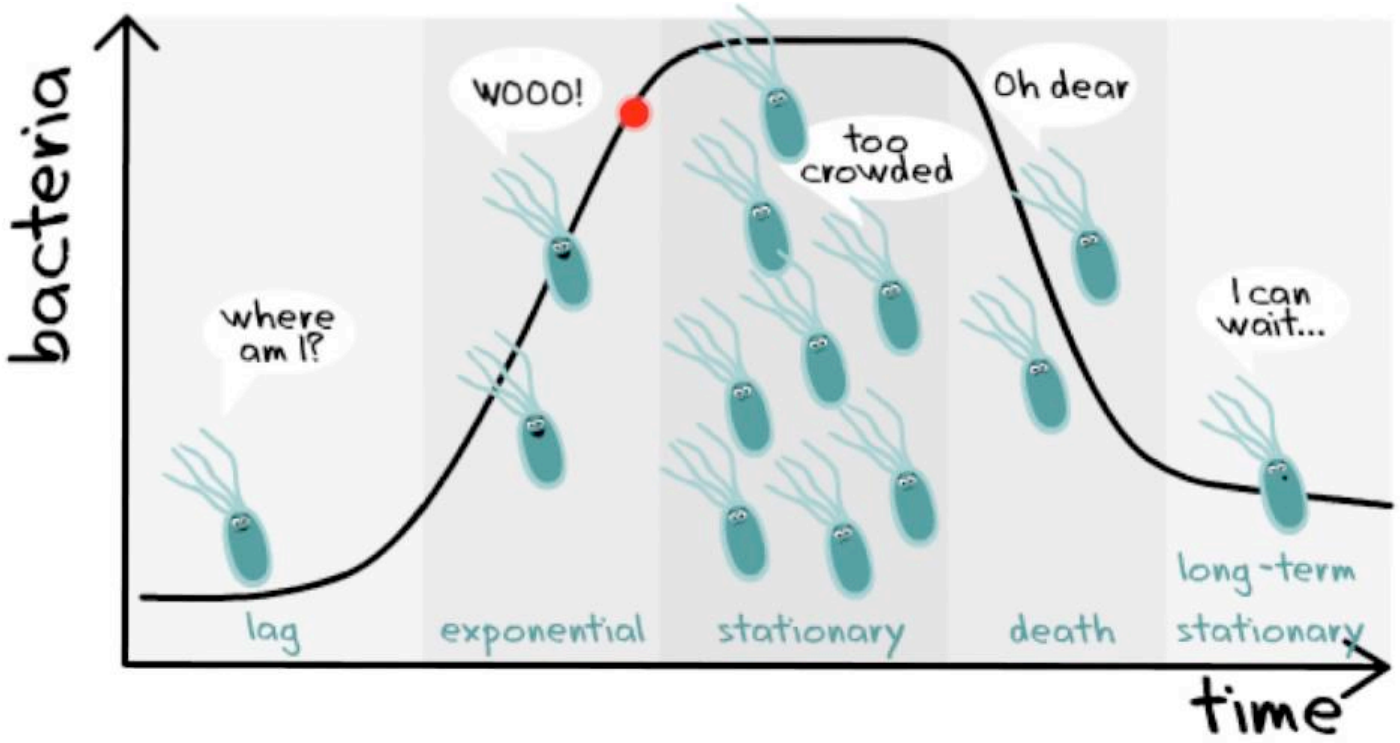
Other potential explanations



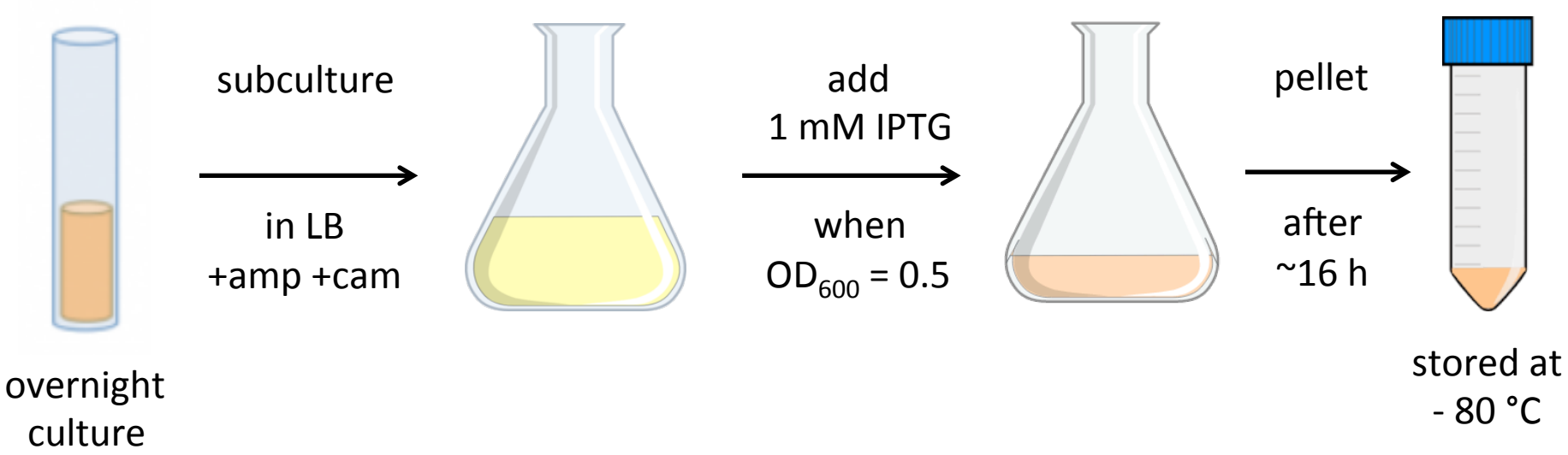
- Smear?
- Incomplete and / or no cutting?
- Extra bands?

Why do we induce at OD_{600}?

- Indicative of how many cells are present in the culture, OD_{600} of 1 ≅ 8 x 10^8 cells / mL



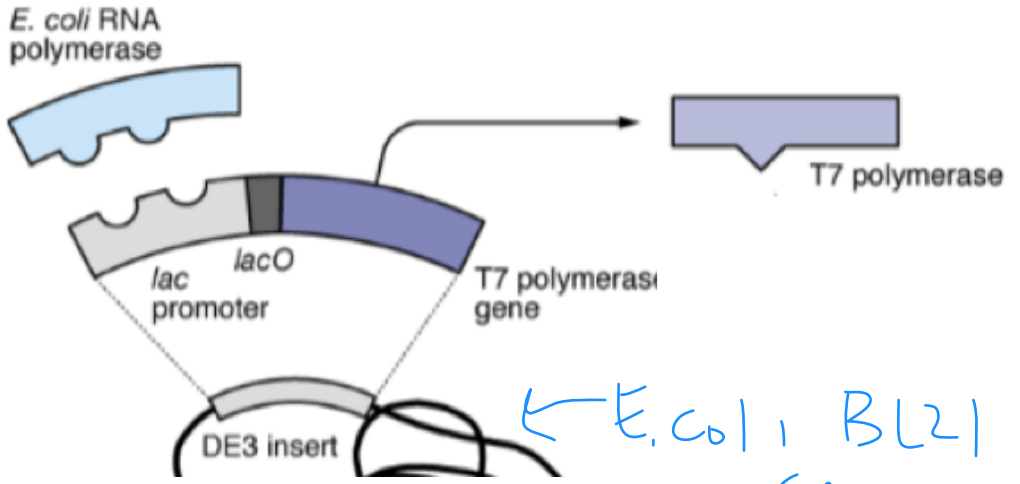
How did we induce protein expression?



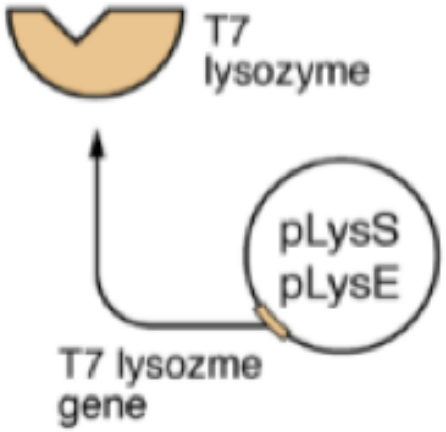
- In addition to your induced sample, you will also examine and un-induced sample for FKBP12 expression

BL21(DE3)pLysS used in protein expression

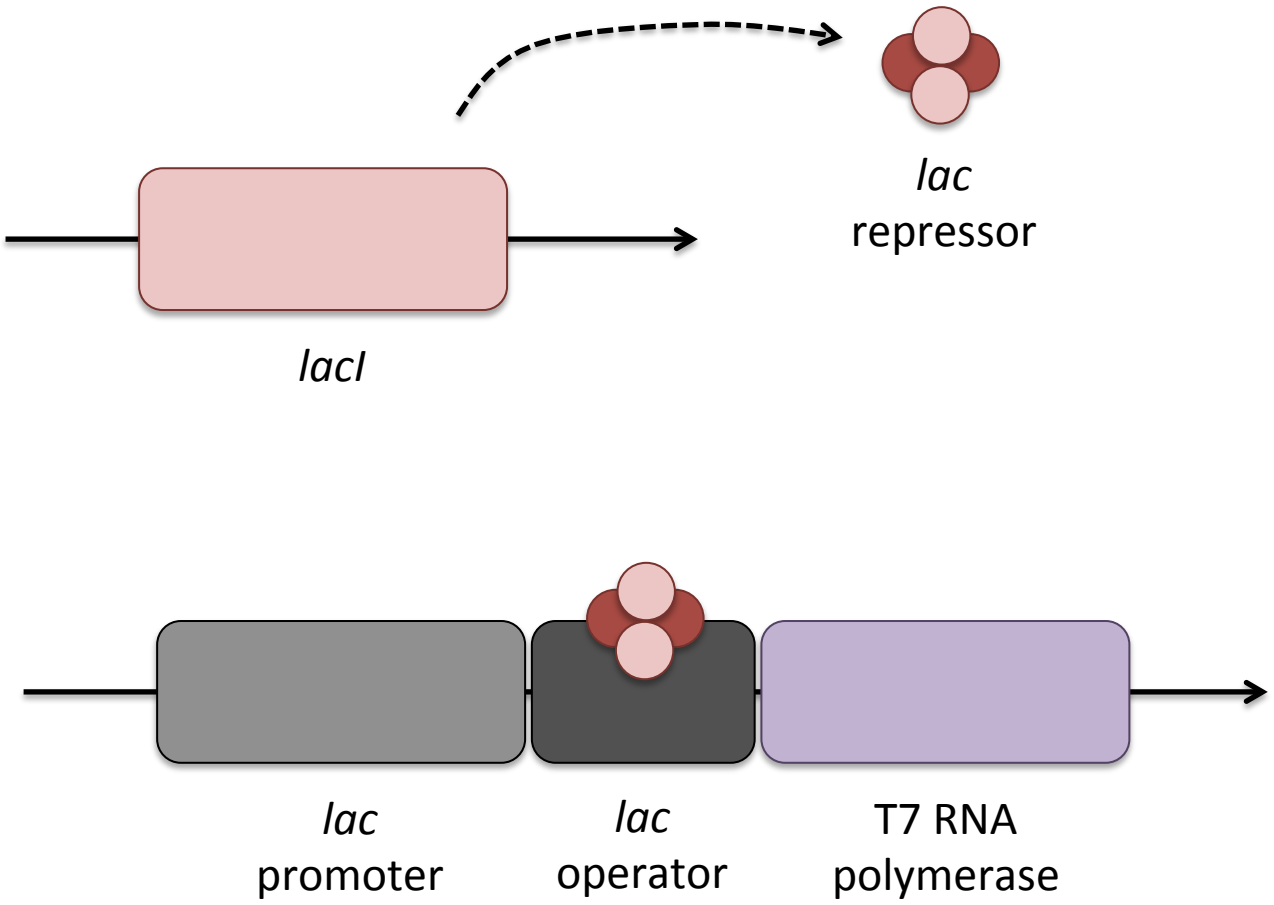
- DE3



- pLysS



IPTG ‘induces’ protein expression



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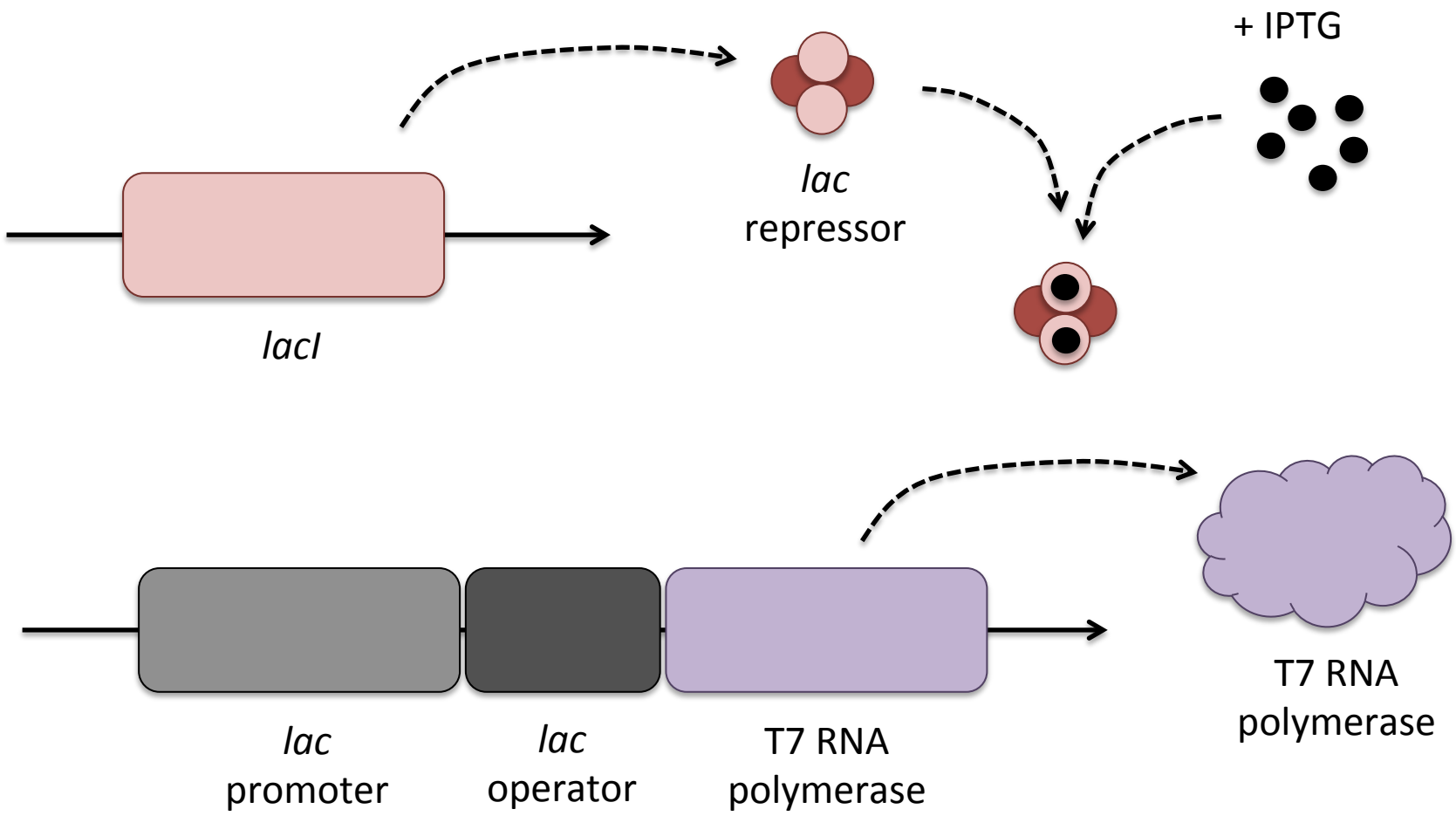
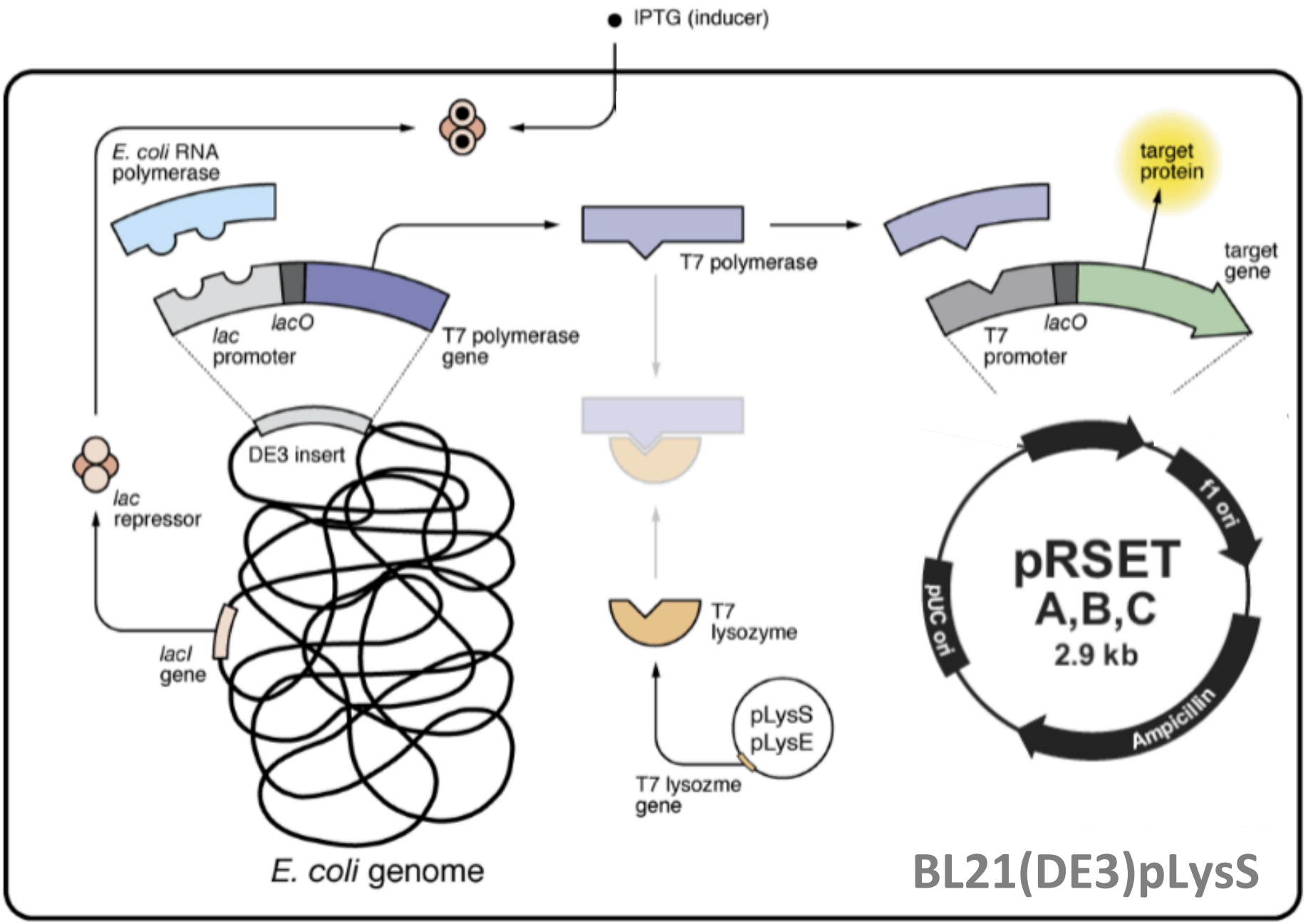


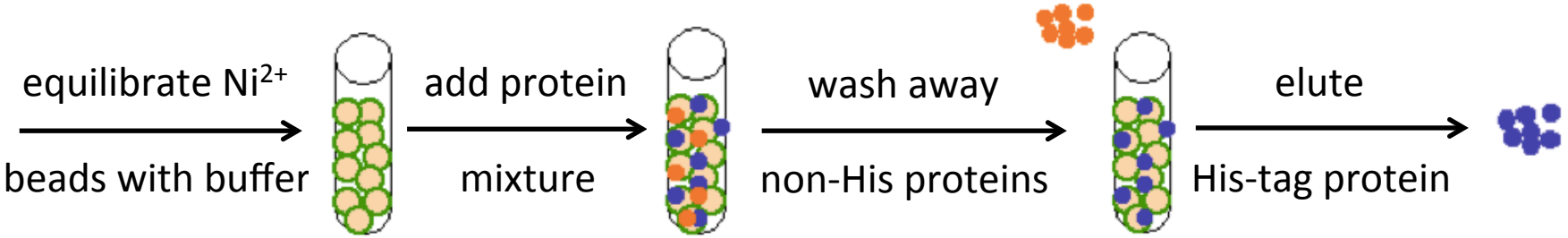

IPTG (inducer)
E. coli RNA polymerase
lac promoter
lacO
T7 polymerase gene
DE3 insert
lac repressor
lacI gene
E. coli genome
T7 polymerase
T7 lysozyme
pLysS pLysE
T7 lysozme gene
target protein
target gene
T7 promoter
lacO
pRSET A,B,C
2.9 kb
f1 ori
pUC ori
Ampicillin
BL21(DE3)pLysS

What's happening in words…

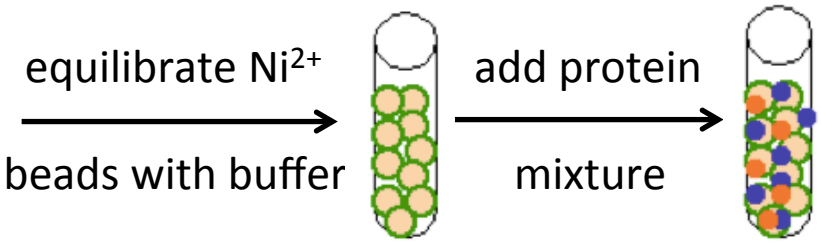
- In absence of IPTG:
  - LacI repressor binds *lac* operator; represses transcription of T7 RNA polymerase
  - Leaky expression of T7 RNA polymerase corrected for by T7 lysozyme
- In presence of IPTG:
  - IPTG binds LacI; prevents binding to *lac* operator
  - T7 RNA polymerase transcribed and binds at P_{T7}; initiates transcription of *Fkbp12*

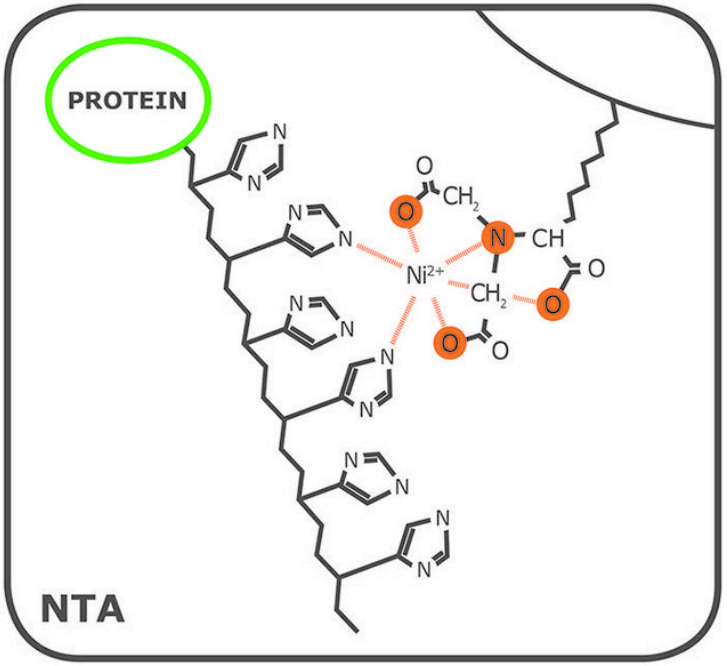
How will we retrieve our protein?

- Cell lysis buffer components:
  - protease inhibitor (AEBSF) eliminates proteases
  - deoxyribonuclease (DNase) degrades DNA
  - tris / salts buffer maintain pH, maintain osmotic pressure
  - lysozyme degrades cell wall
  - dithiothreitol (DTT) reducer, reduce oxidative damage
  - glycerol stabilizer



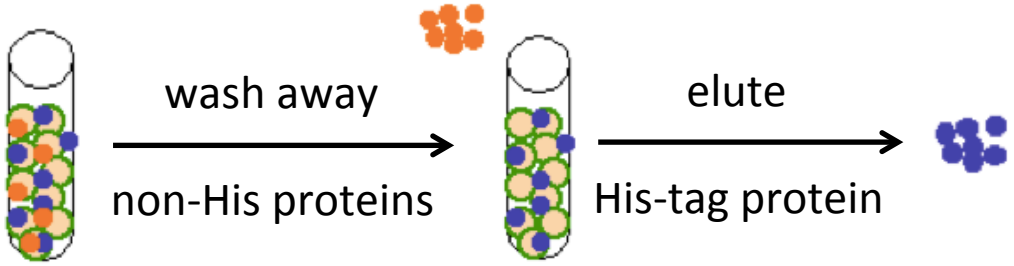
6x His residues enable binding to Ni^{2+}

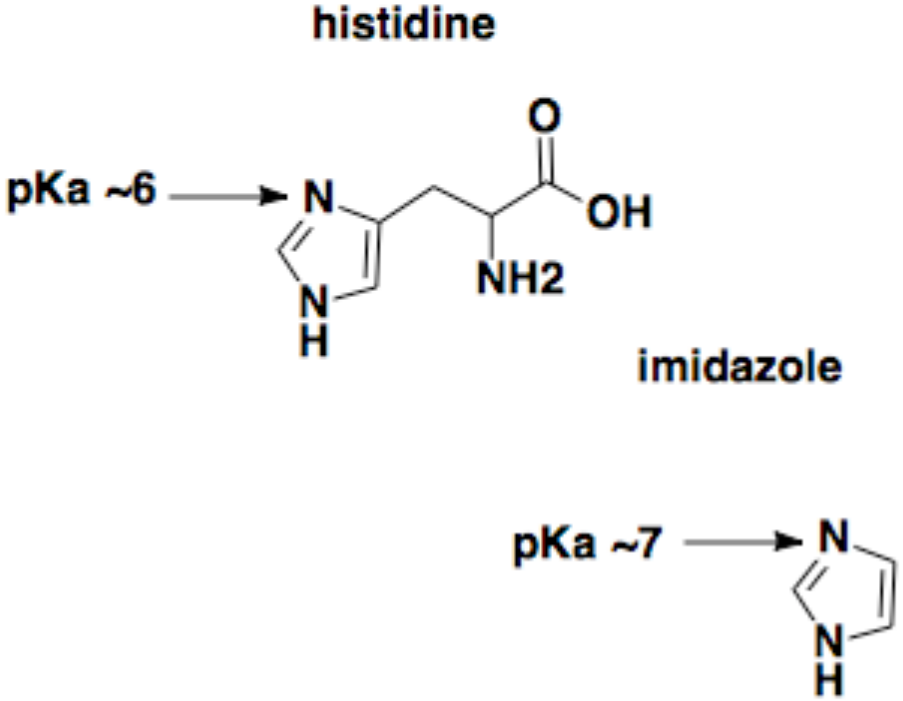




- Ni^{2+} chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand

Imidazole competes for binding to Ni^{2+}

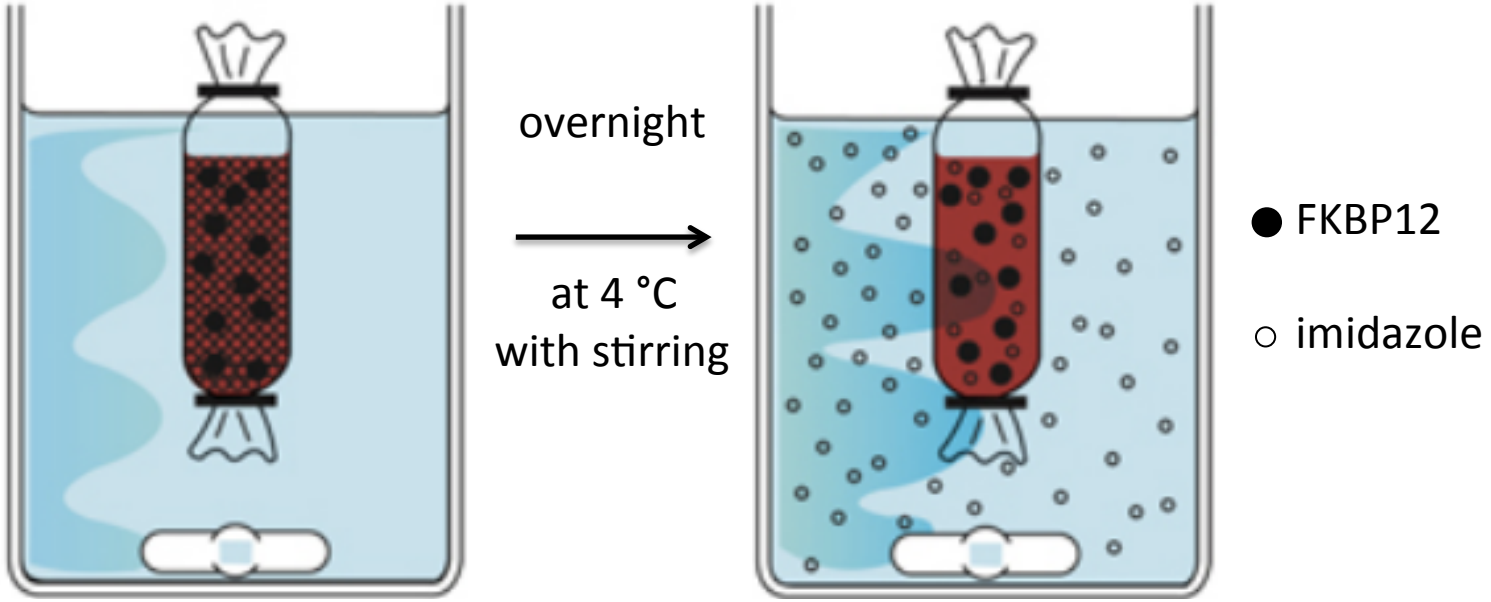




- Low concentration of imidazole included in wash buffer
- Increased 25-fold in elution buffer

How do we 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



In lab today…

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

For next time…

- 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