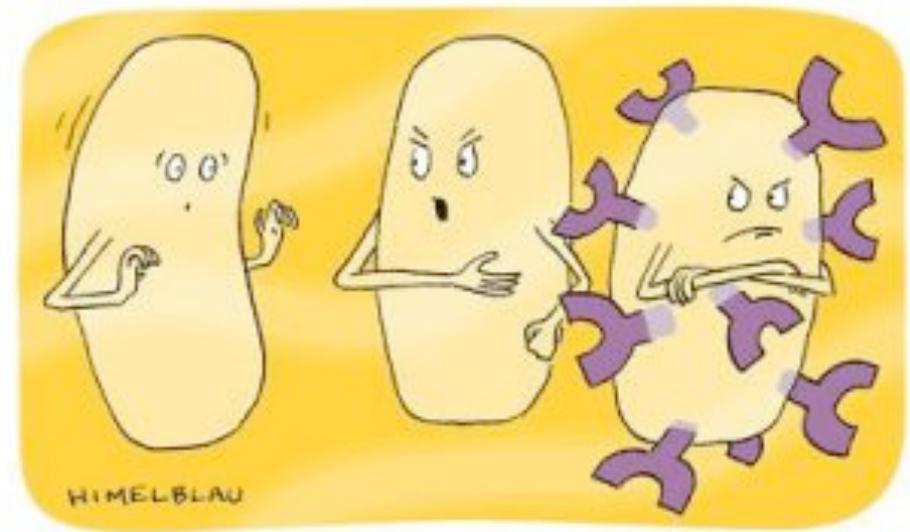


M1D2:

Purify protein for secondary assays

1. Prelab discussion
2. Gel electrophoresis confirmation digest
3. Purify FKPB12 protein

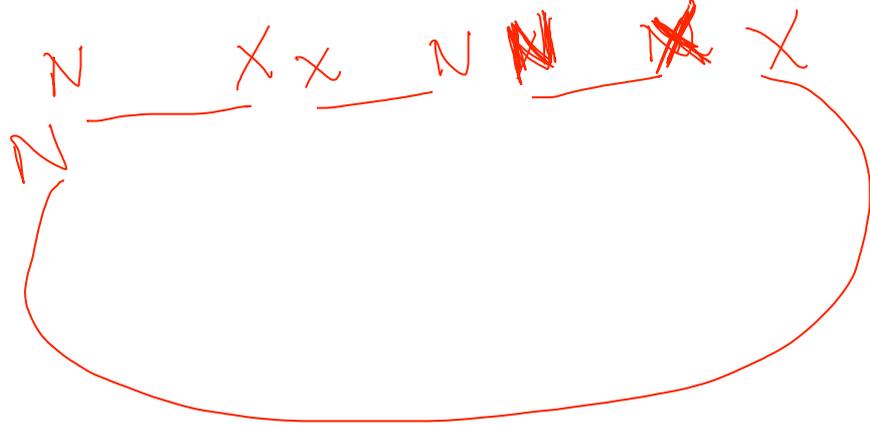


“Don’t pick it up,” I say, and he says, “It’s just a *plasmid*, what harm could it do?” Well just look at him now...who knows *what* protein he’s expressing!

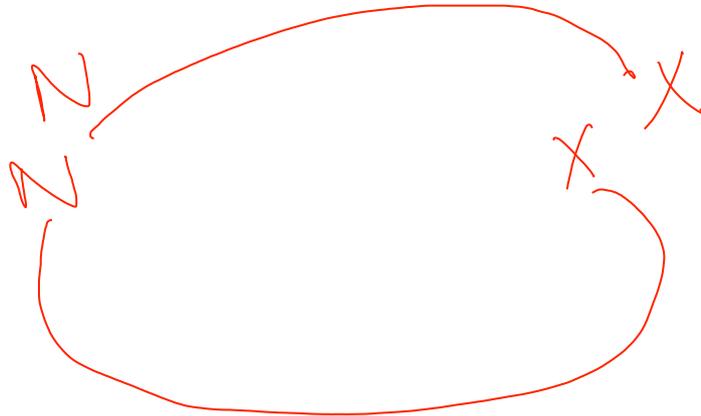
Ideally, 4:1 **molar** ratio of insert:backbone

Why perform confirmation digests?

- Too much insert

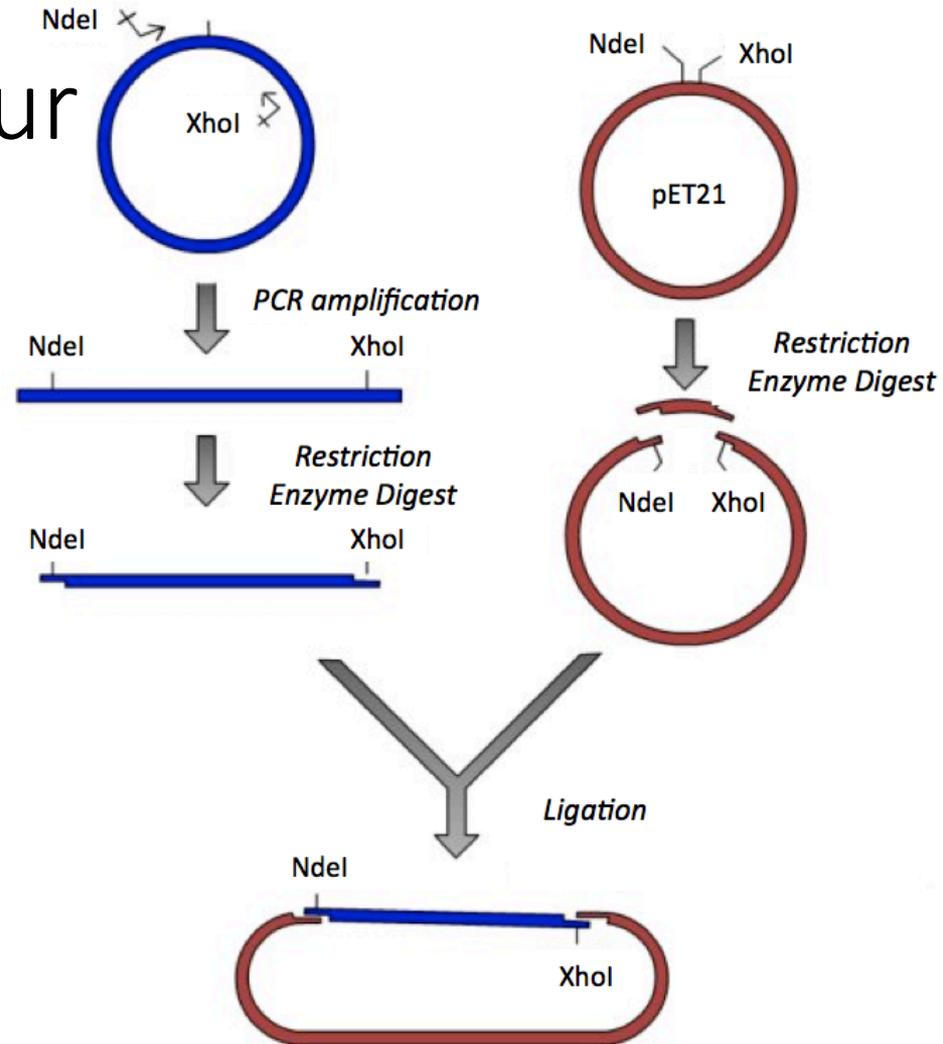


- Too much backbone



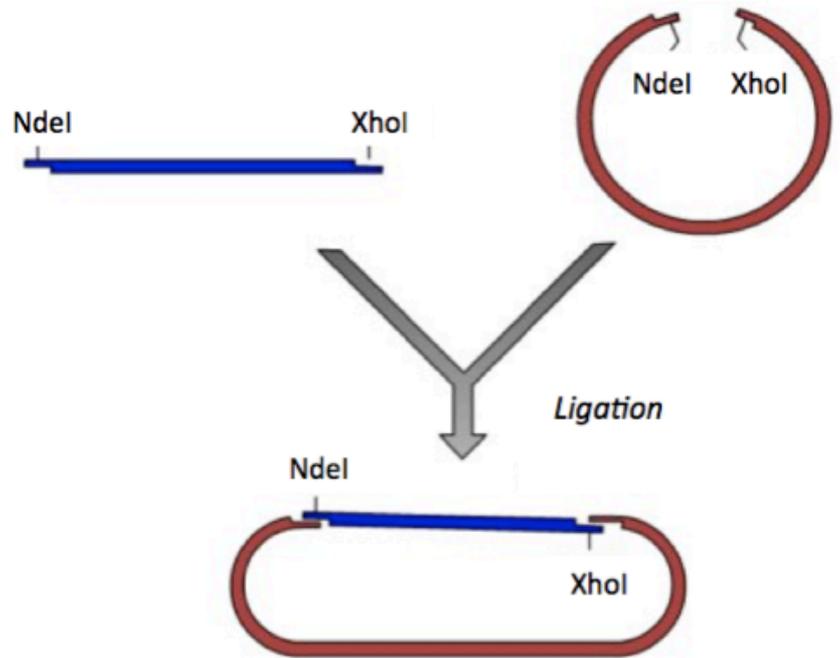
How did we clone our expression vector?

- Amplification
- Digestion
- Ligation



How did we check our product?

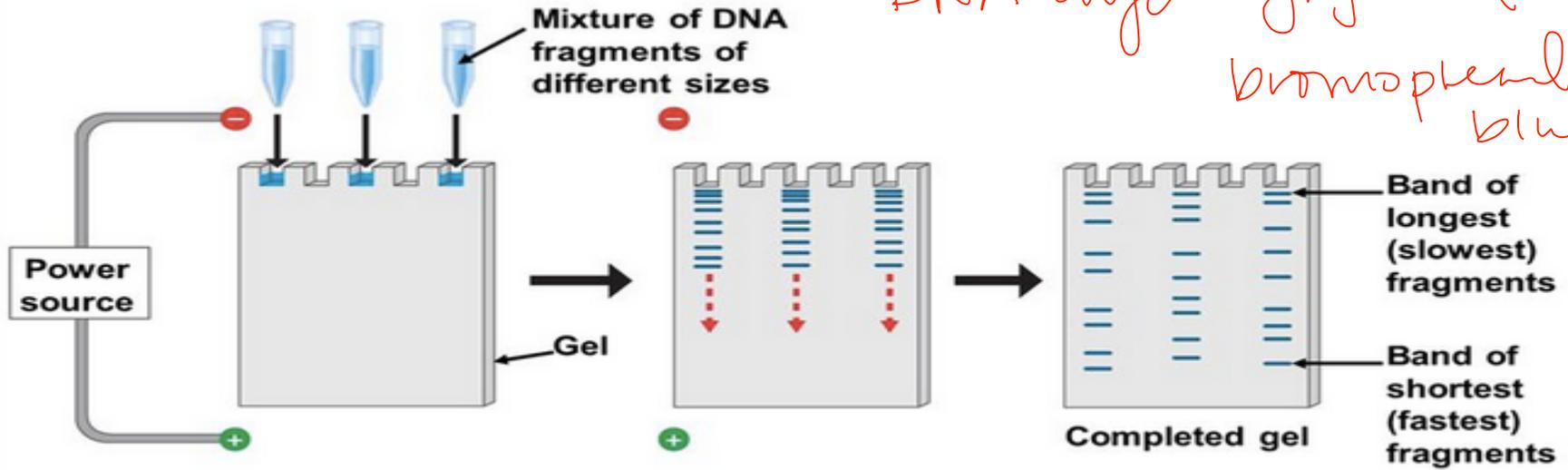
- Transformation
- Purification
- Digestion



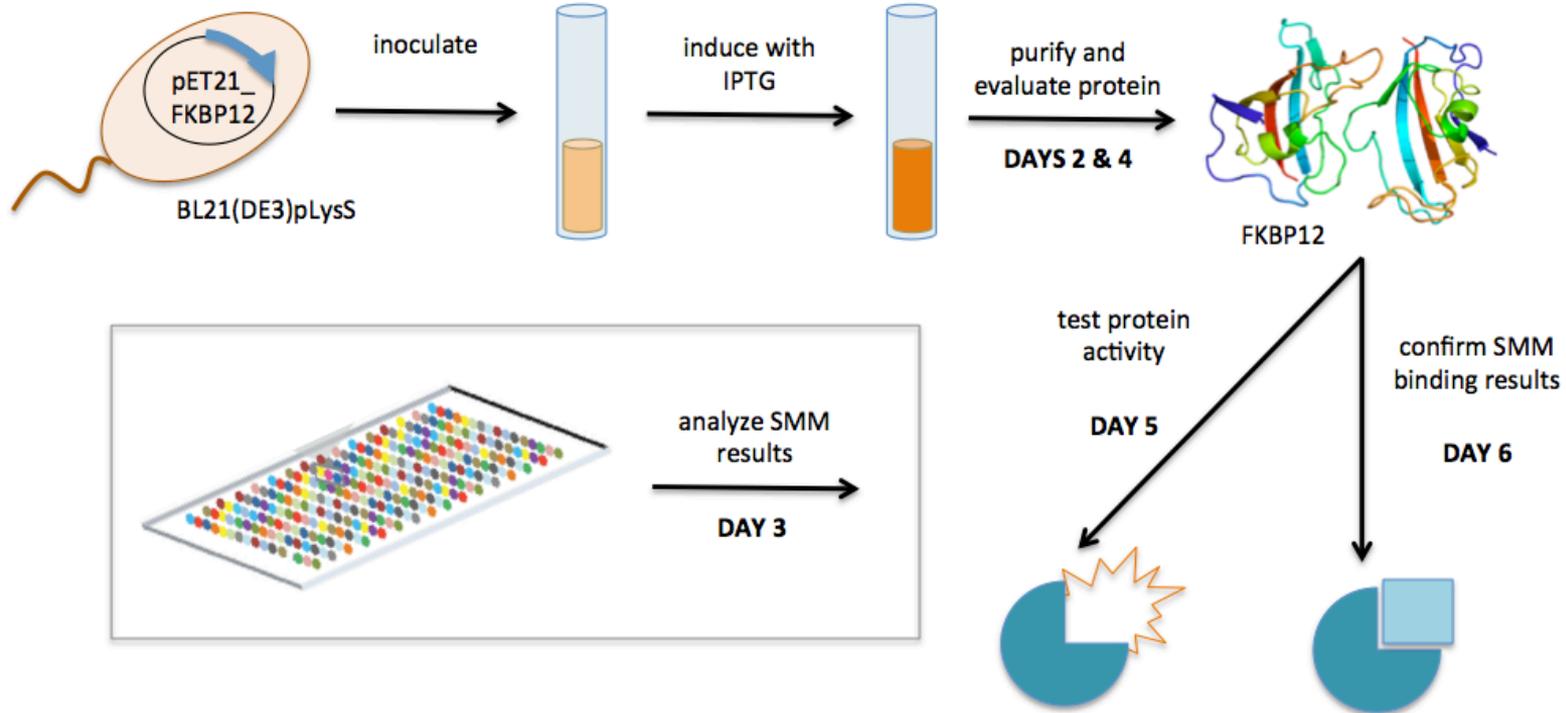
How will we visualize gel results?

DNA fragments resolved using 1% agarose gel

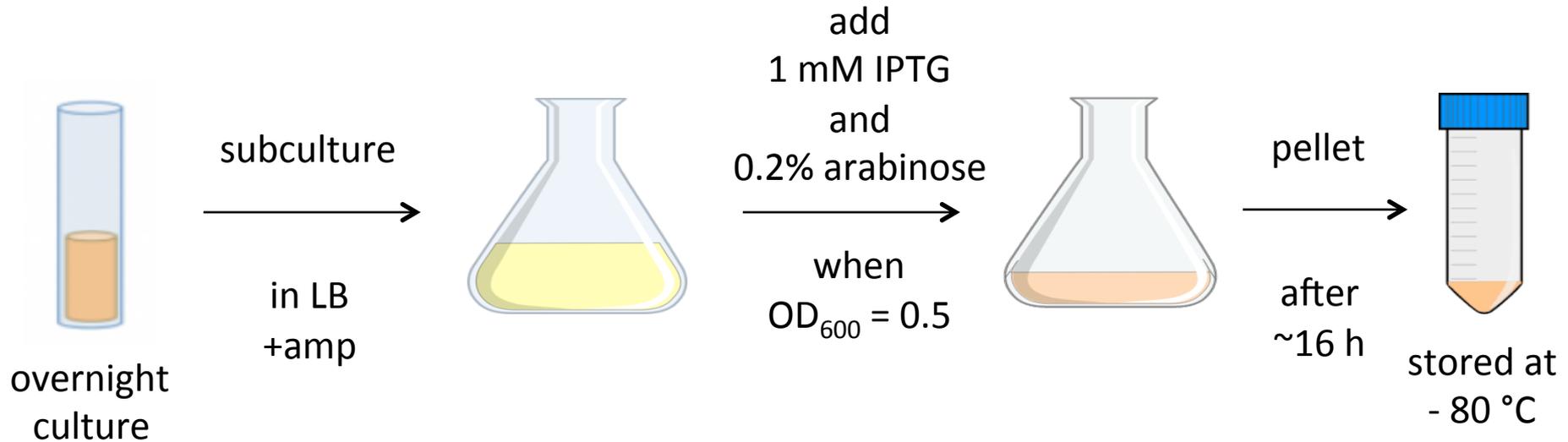
*DNA dye : glycerol
bromophenol
blue*



Overview of Mod 1 experiments



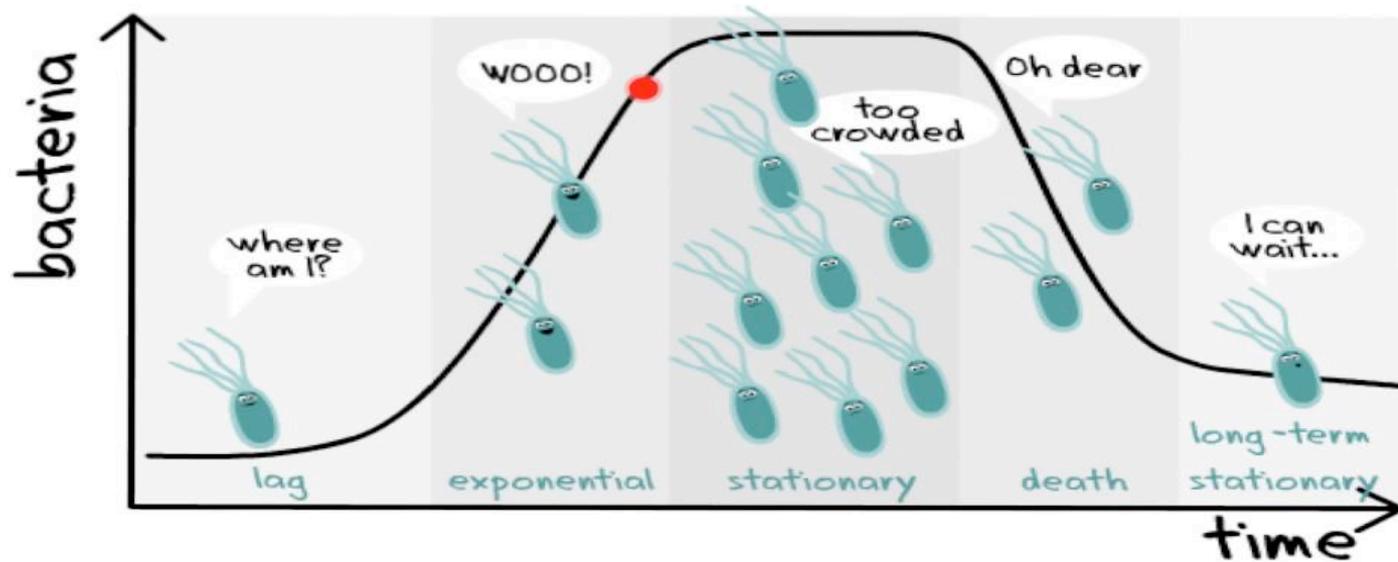
How did we induce protein expression?



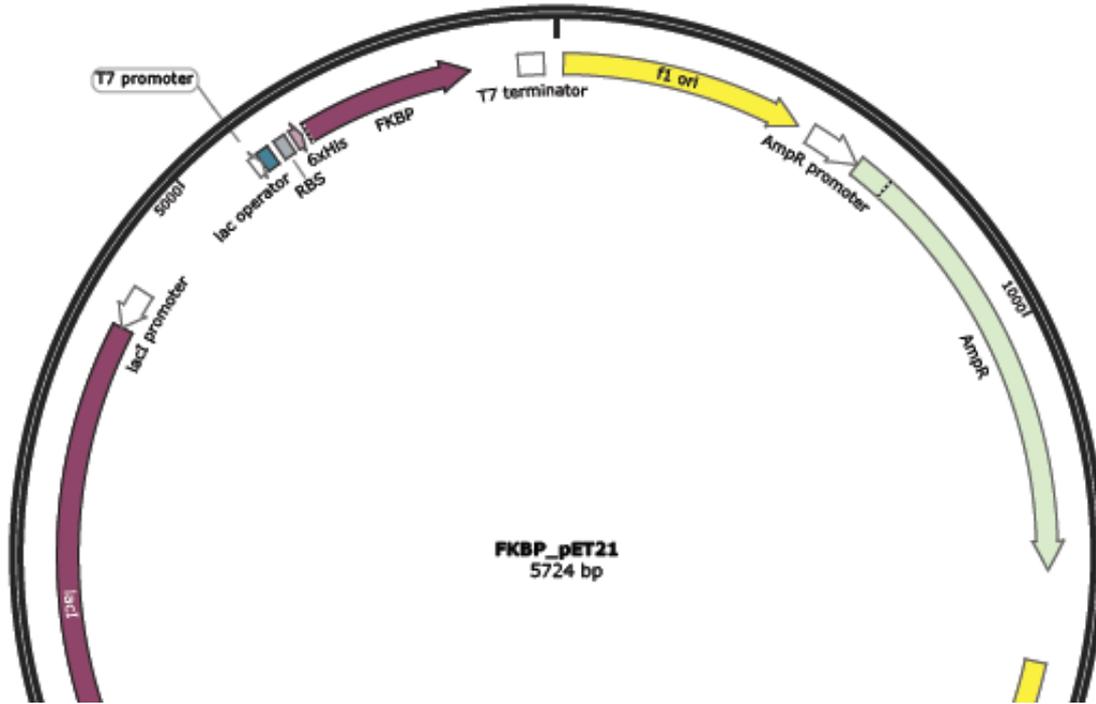
In addition to your induced sample, you will also examine an un-induced sample for FKBP12 expression

Why do we induce at OD₆₀₀?

Indicative of how many cells are present in the culture,
OD₆₀₀ of 1 \cong 8×10^8 cells / mL



More about our expression vector

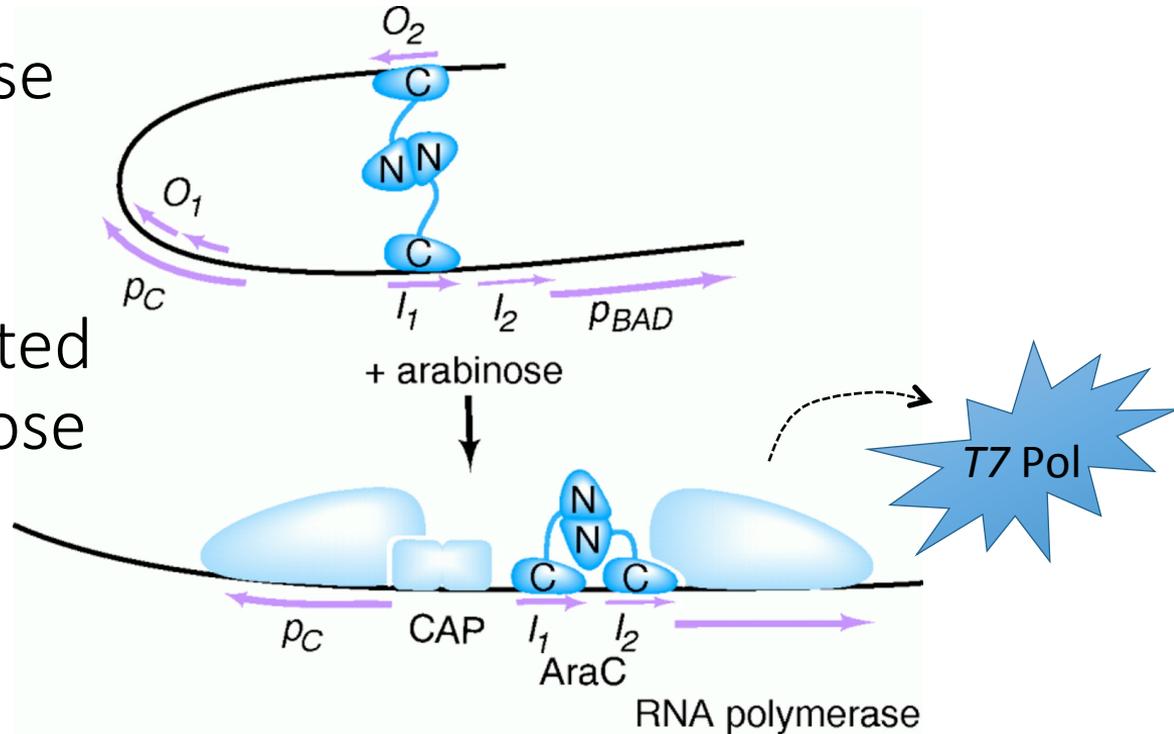


FKBP_pET21
5724 bp

- P_{T7} *expression of FKBP12*
- RBS *needed for translation*
- 6xHis *purification*
- Lac system *regulates FKBP12 expression*
- AmpR *selection marker*

BL21-A1 cells used in protein expression

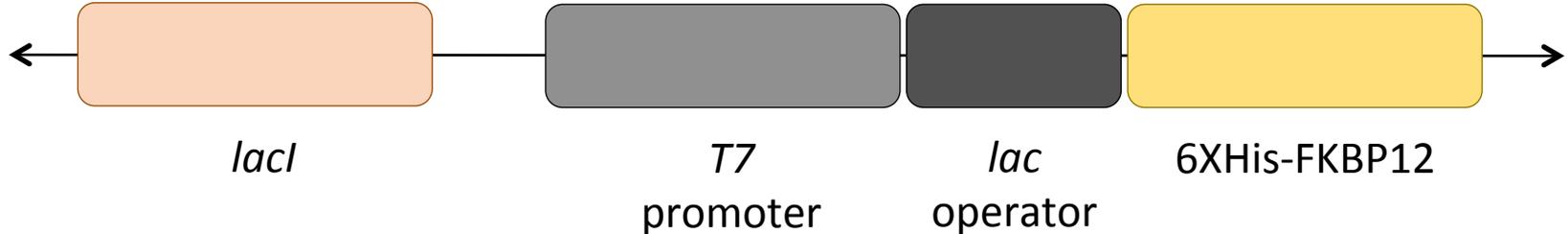
- T7 RNA polymerase expressed from BL21-A1 genome
- Expression regulated by P_{BAD} via arabinose induction



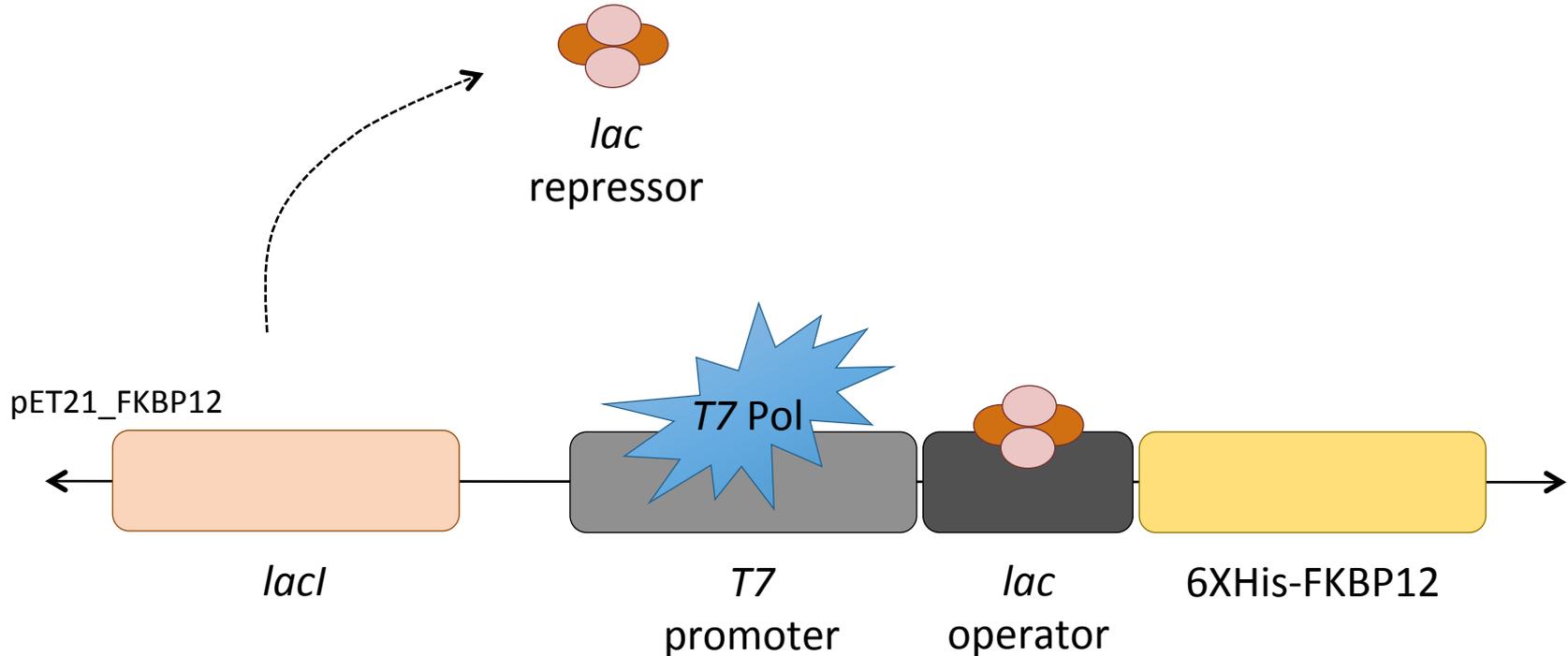
Induction system in pET21_FKBP12



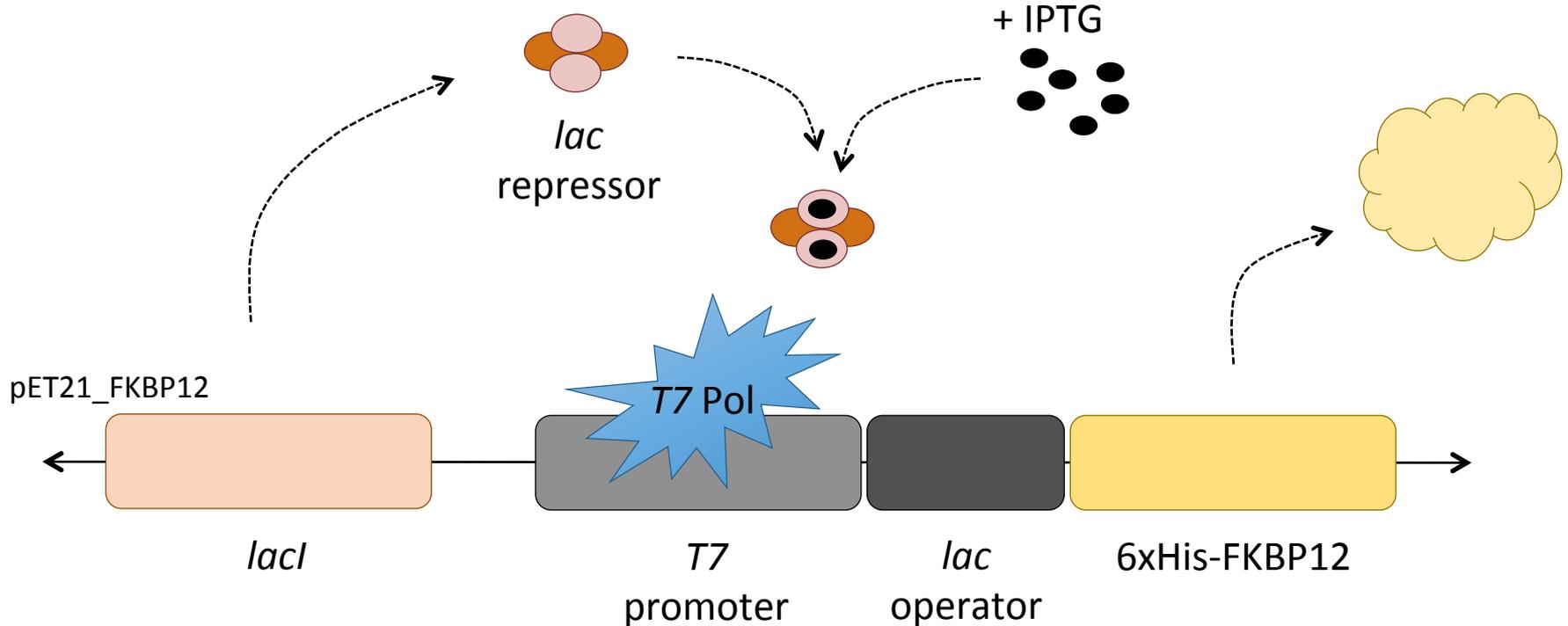
pET21_FKBP12



LacI repressor blocks transcription



IPTG 'induces' protein expression



Quick review of induction system...

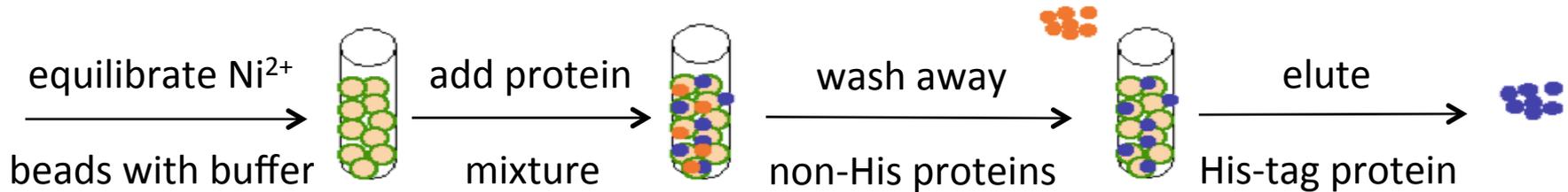
- When is T7 RNAP transcribed?
- When is FKBP12 transcribed?

	- arabinose	+ arabinose
- IPTG	- RNAP - FKBP12	+ RNAP - FKBP12
+ IPTG	- RNAP - FKBP12	

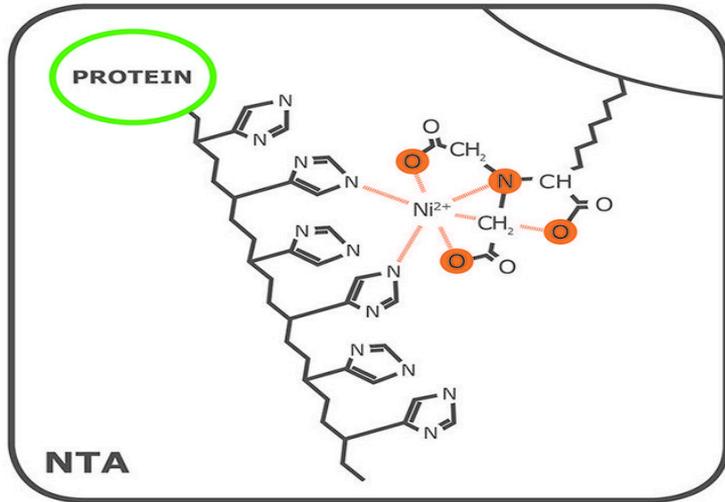
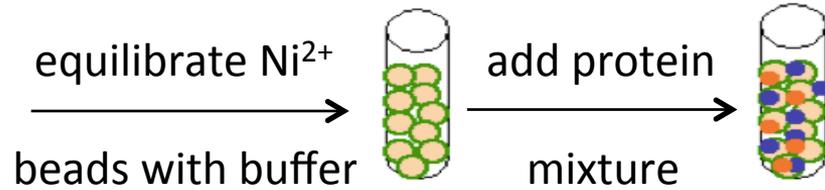
How will we retrieve our protein?

Cell lysis buffer components:

- protease inhibitor (AEBSF)
- deoxyribonuclease (DNase)
- tris / salts buffer
- lysozyme
- dithiothreitol (DTT)
- glycerol

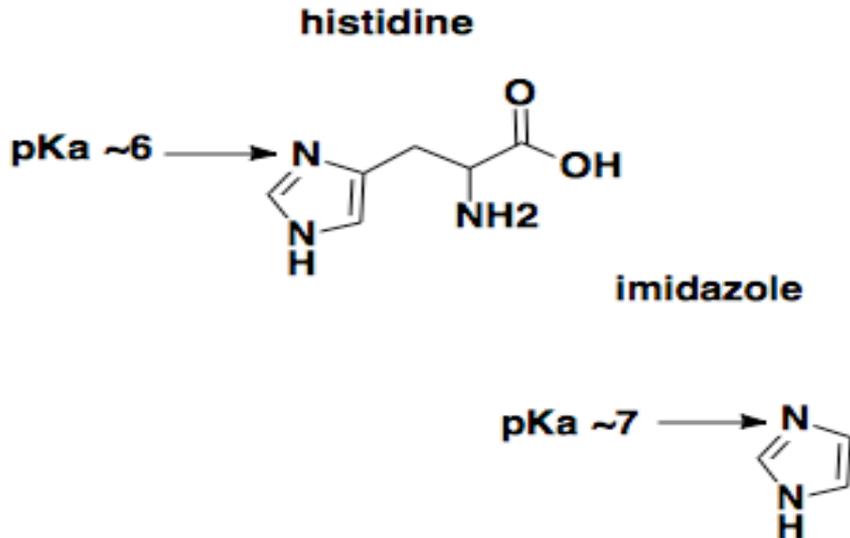
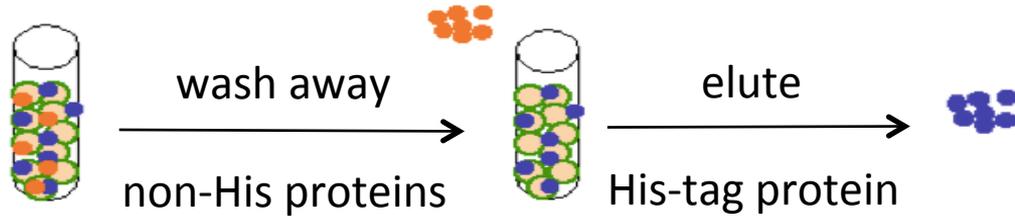


6xHis tag enables binding to Ni^{2+}



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

Imidazole competes for binding to Ni²⁺



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

For today...

- Remember to wipe your bench with 70% EtOH
- ② • Complete gel electrophoresis for confirmation digests
- ① • Purify FKPB12 protein

For M1D3...

- Draft a figure of your confirmation digest results for your Data summary
 - INCLUDE a title and caption

Notes on figure making:

- Image **should not be** the entire page
 - Only needs to be large enough to be clear
- Title **should be** conclusive
 - Don't include what you did, rather include what you found
- Caption **should not** detail the methods
 - Define abbreviations, symbols, etc.