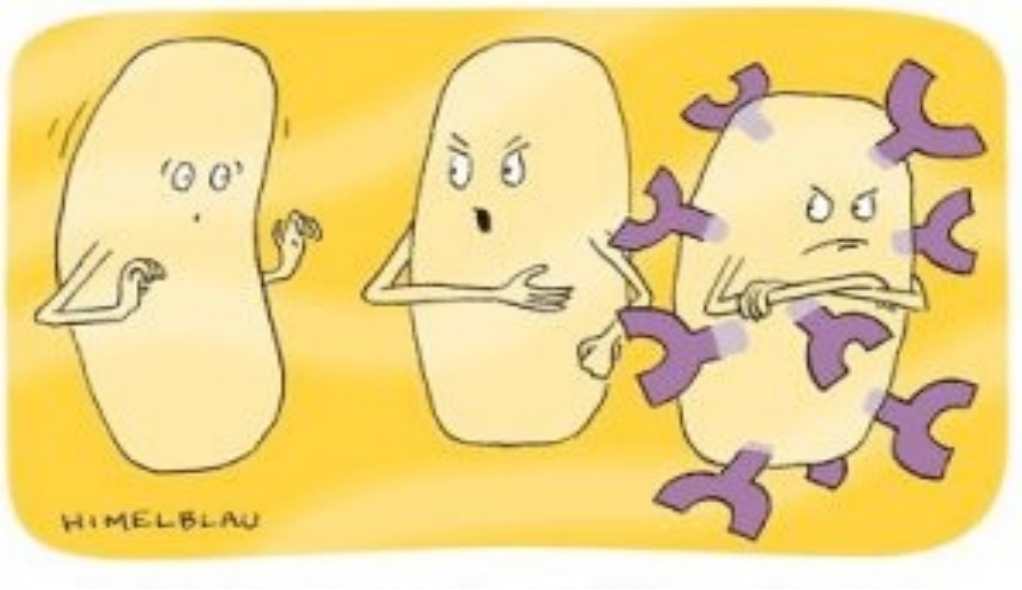
M1D3: Induce and purify TDP43 protein

1. Prelab discussion #1
2. Protein purification
3. Prelab discussion #2



"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!

Get started on protein purification

- Have a pellet of bacterial cells which express TDP43_RRM12

- **Resuspend pellet in lysis buffer** and lyse cells for 15 minutes on nutator at front bench
  - During lysis, Christine will show you how to prepare column

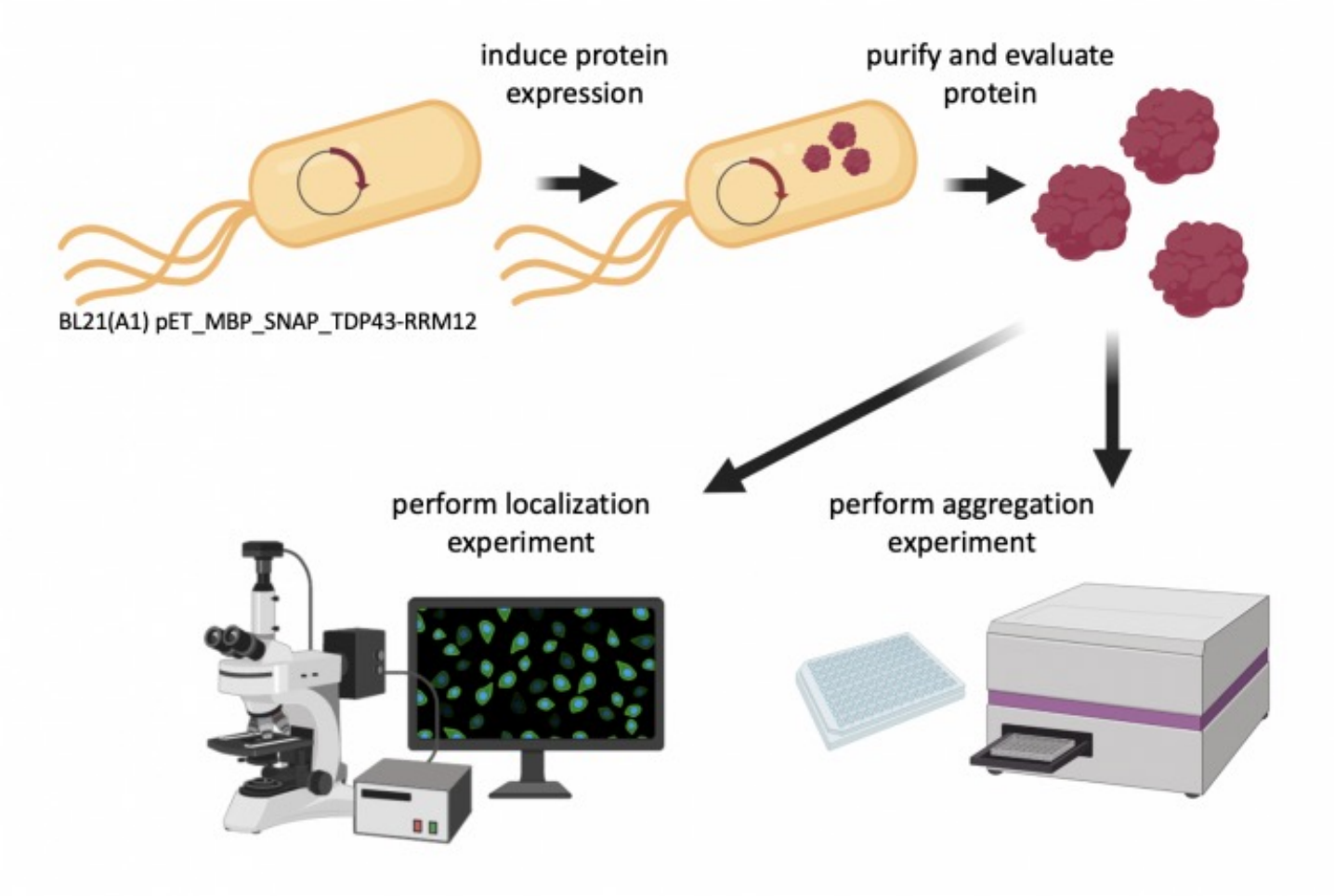
- **Divide material** from cell lysis between 2 Eppendorf tubes and bring to front bench
  - Lysed cells will be centrifuged for 30 minutes to separate soluble protein
  - During centrifugation, prepare nickel resin, wash buffer, elution buffer

- **Remove 30ul of supernatant** and place in fresh Eppendorf tube

- **Add remaining supernatant to nickel resin** and incubate for 2 hours
  - Prelab and Assignment lectures during this time

Overview of Mod1 experiments

Research goal: Use functional assays to characterize ligands identified as binders to TDP43 from SMM technology



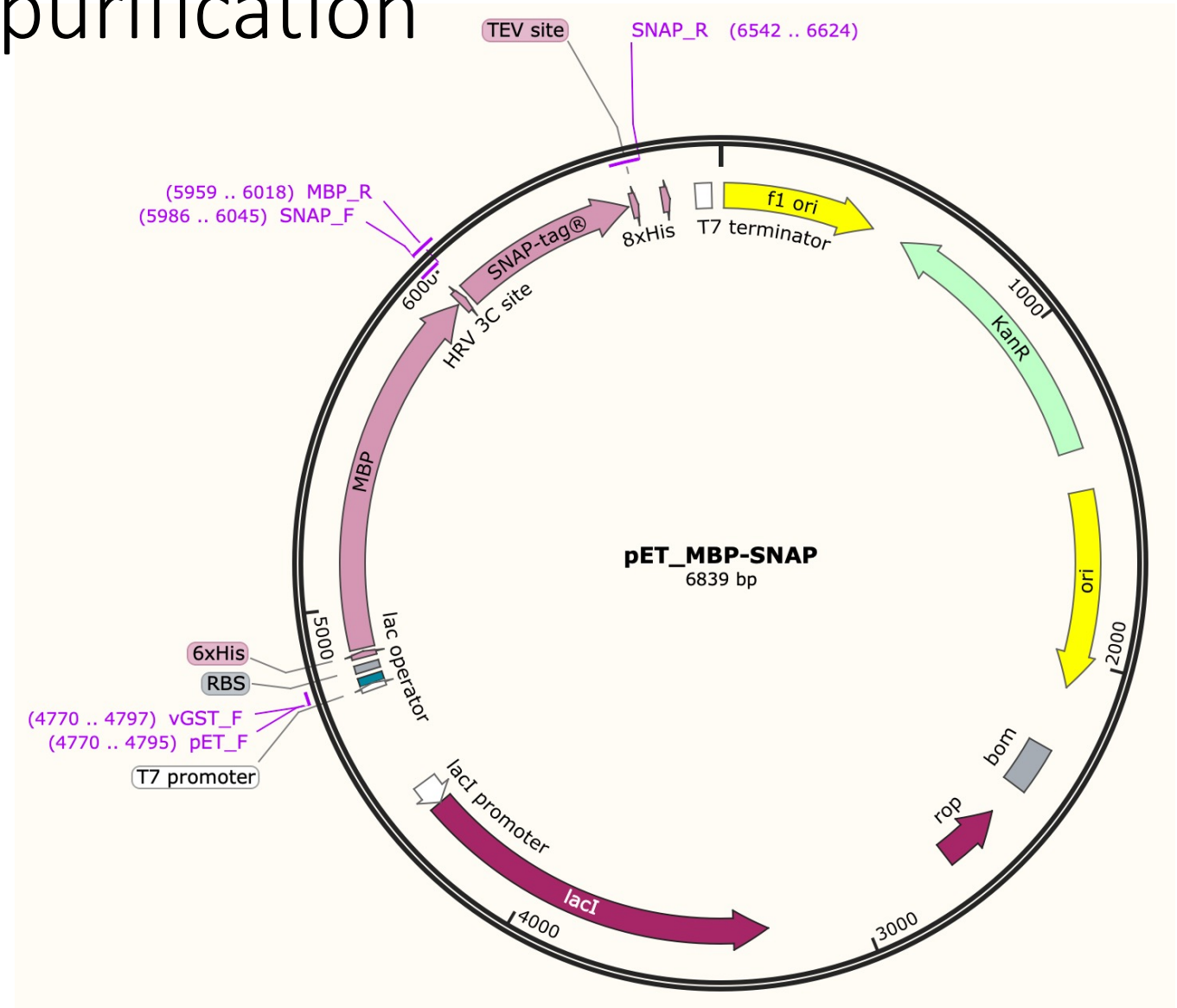
Use genetic features of the plasmid to control protein expression and purification

Induction

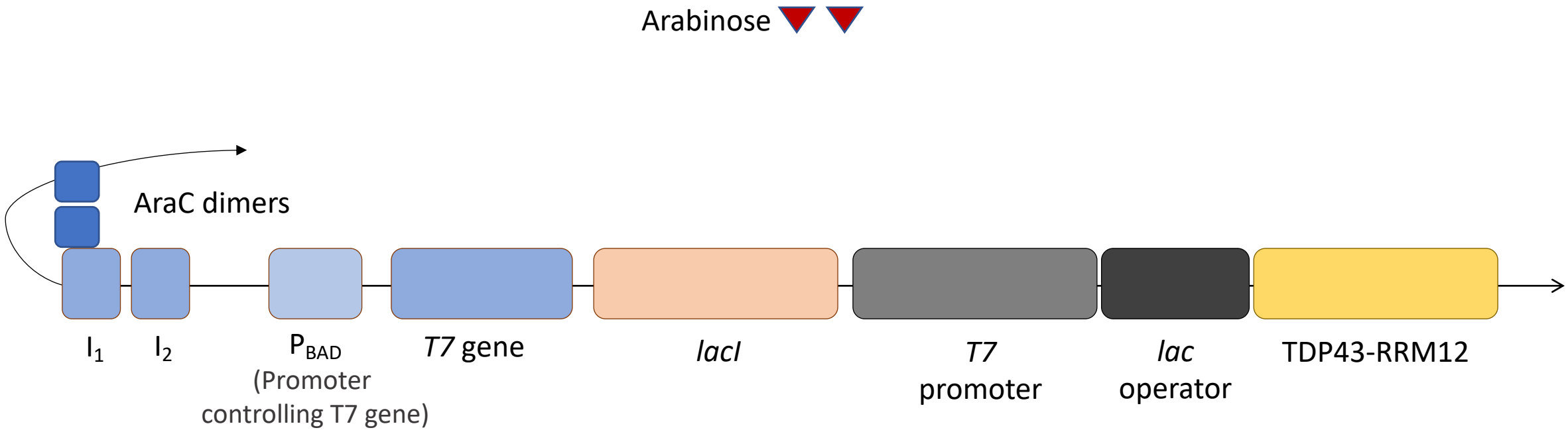
- T7 promoter
- Lac operator
- Kanamycin

Purification

- His-tag

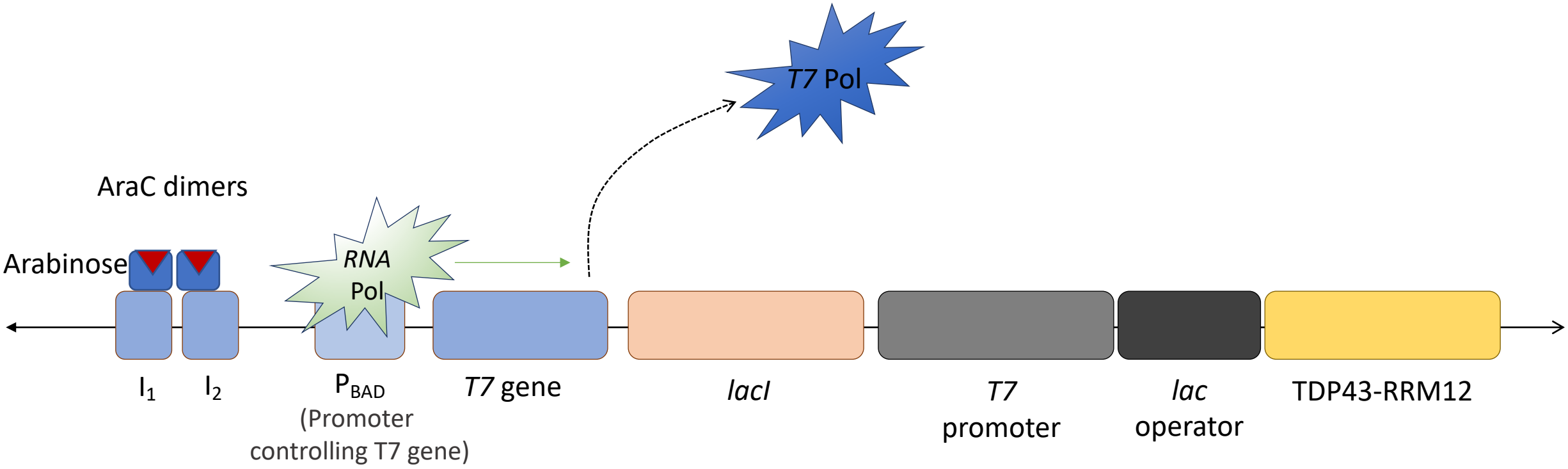


Bacterial induction: How it begins…



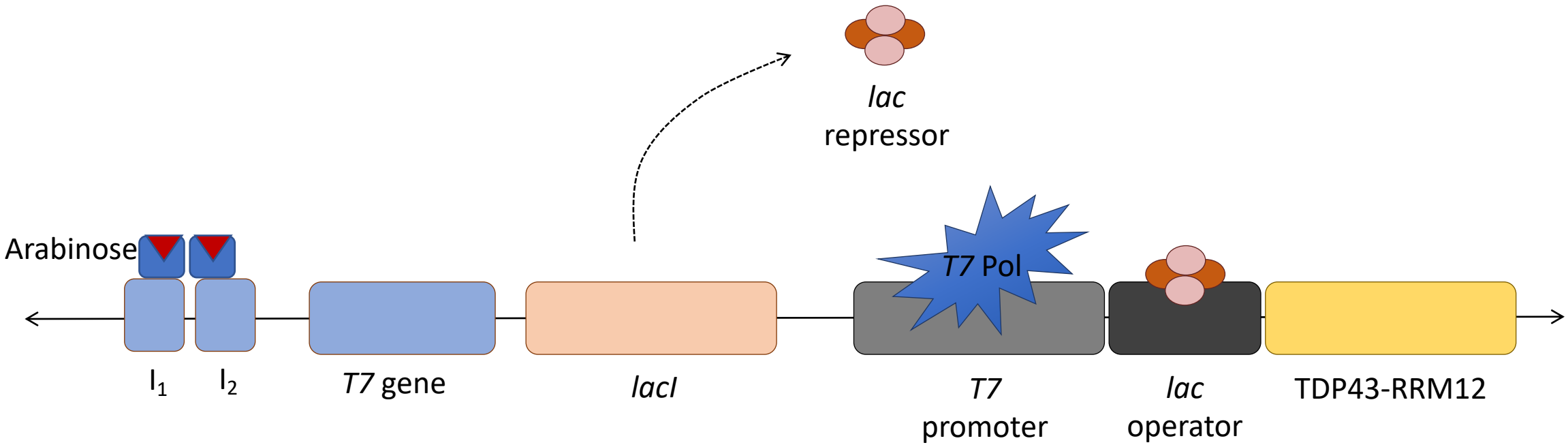
pET_MBP_SNAP_TDP43-RRM12

Bacterial induction: Arabinose controls T7 expression



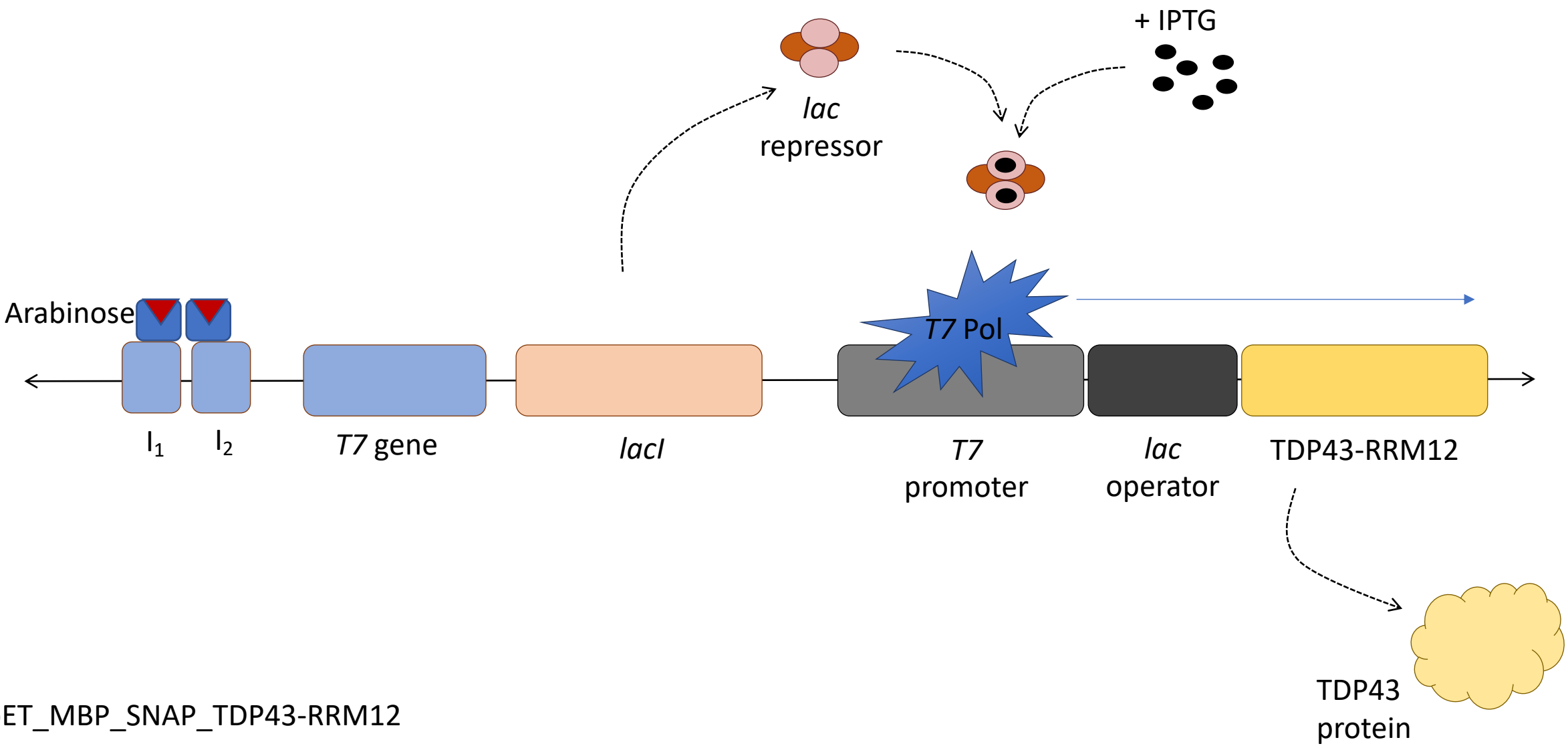
pET_MBP_SNAP_TDP43-RRM12

Bacterial induction: Lac repressor



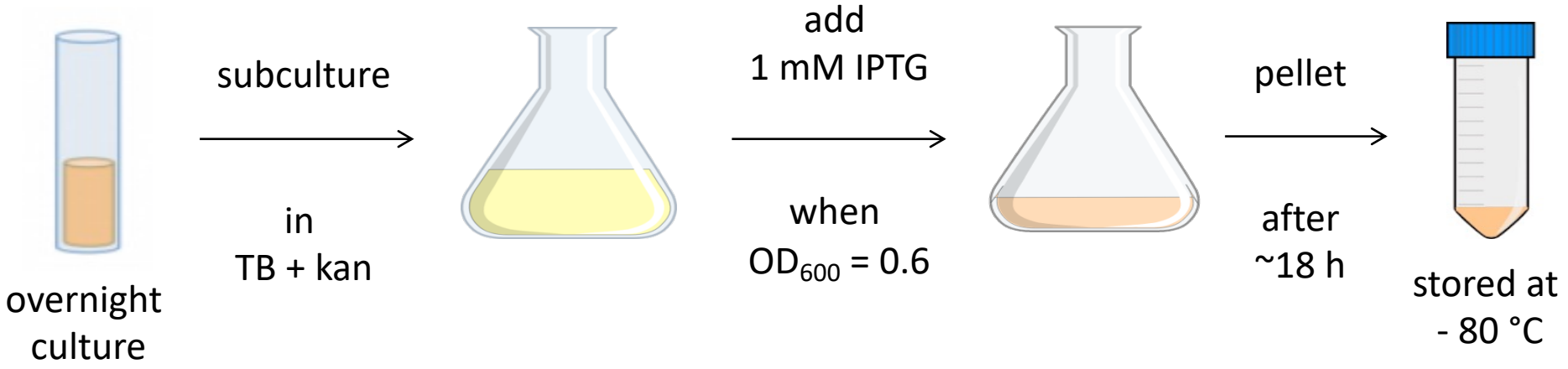
pET_MBP_SNAP_TDP43-RRM12

Bacterial induction: IPTG removes lac repression

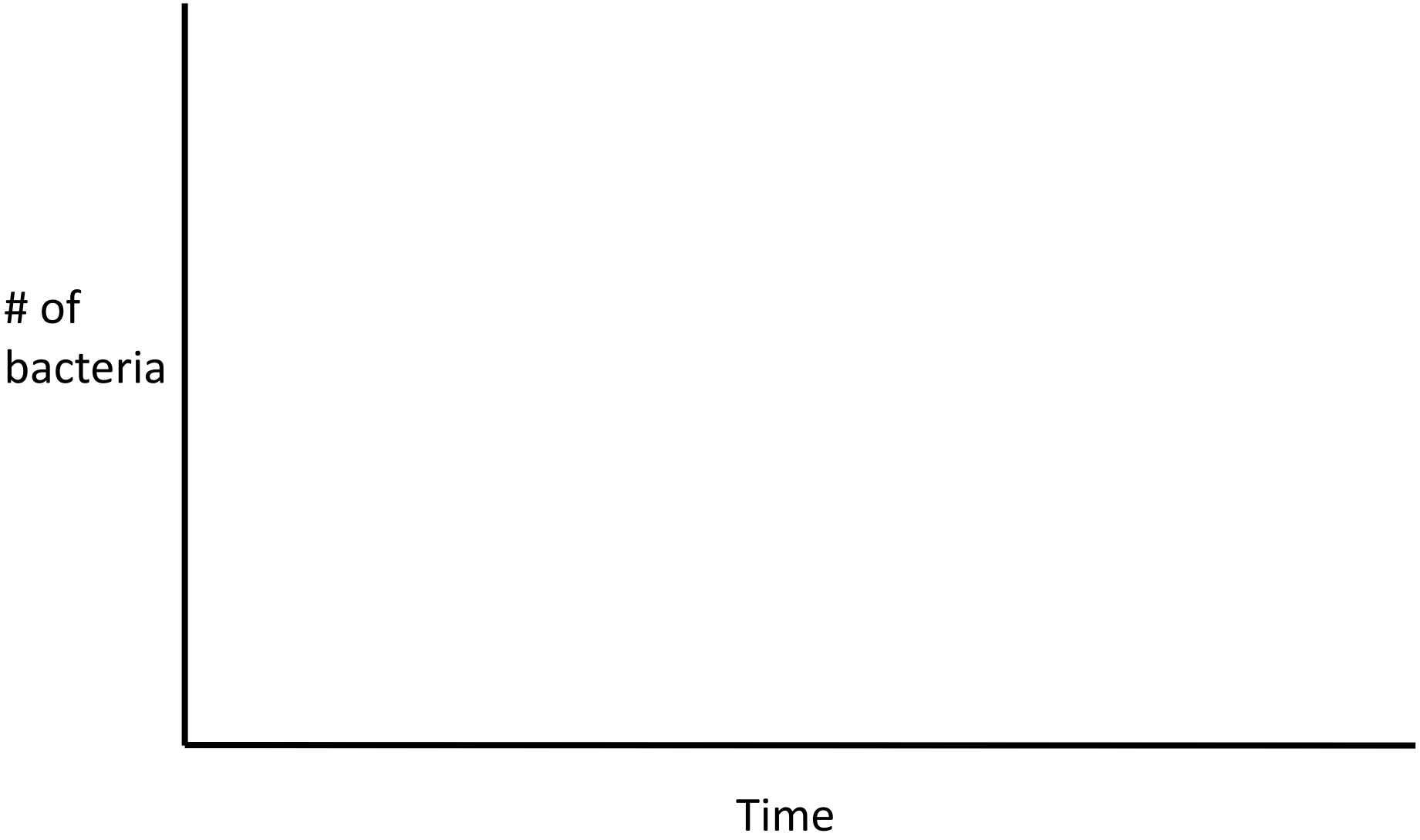


pET_MBP_SNAP_TDP43-RRM12

How do we induce protein expression?



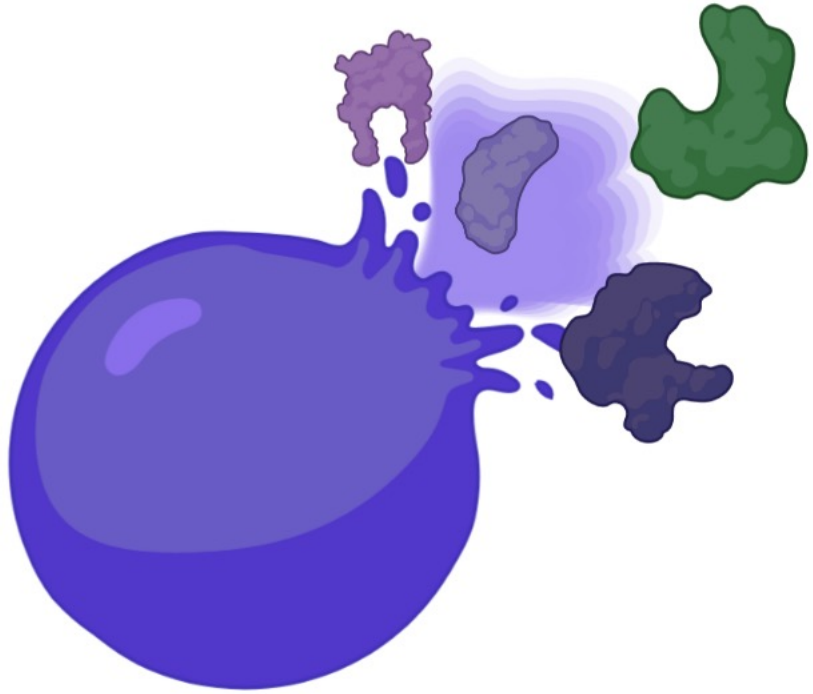
Why do we induce protein expression at OD_{600} = 0.6?

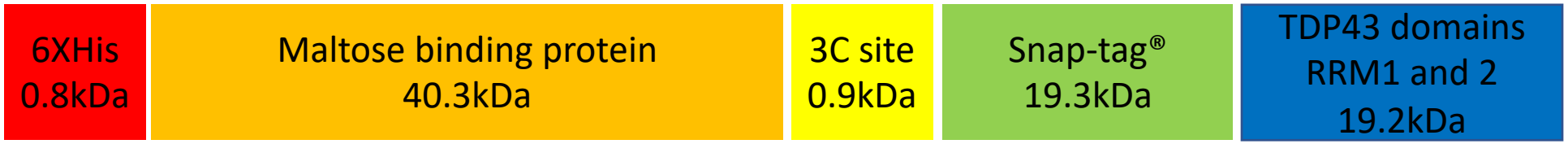

# of bacteria
Time

How will you purify TDP43_RRM12?

First, need to lyse cells to release proteins:

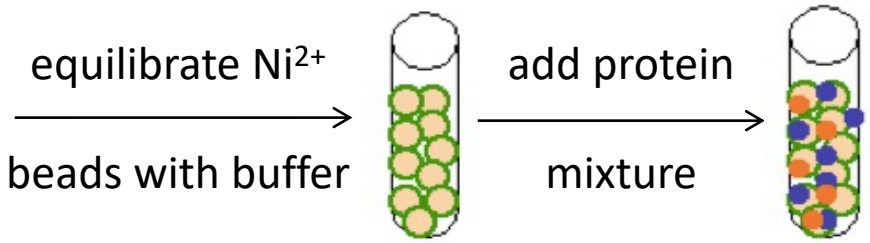
- B-PER bacterial extraction reagent
- Lysonase
- Protease Inhibitor Cocktail

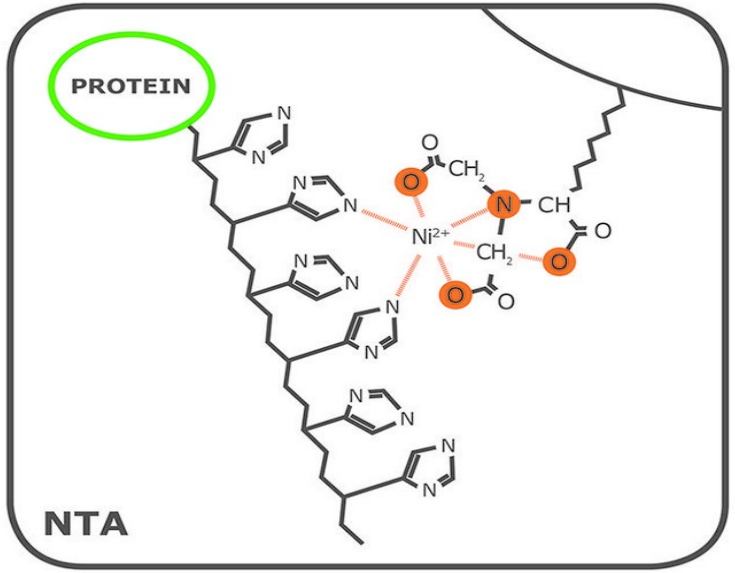




Recombinant protein of TDP43_RRM12

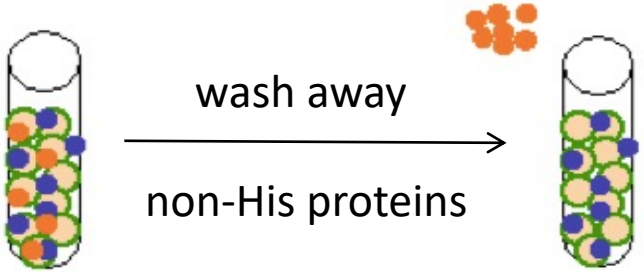
6xHis tag binds to Ni^{2+} resin / column to allow purification of protein of interest

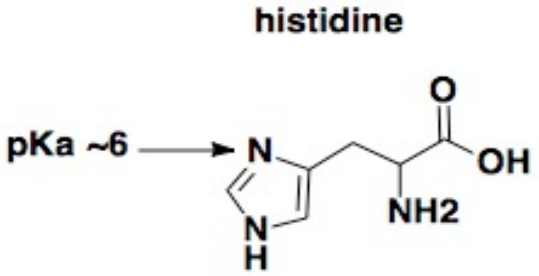


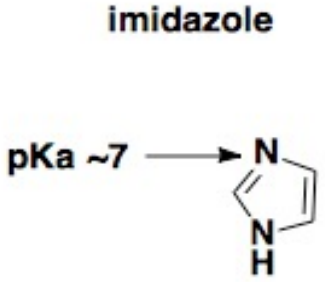


- Ni^{2+} chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand
- His tag chelates to Ni^{2+} causing protein to ‘stick’ to resin / column

Non-specific binders washed from Ni^{2+} resin / column using a low concentration of imidazole

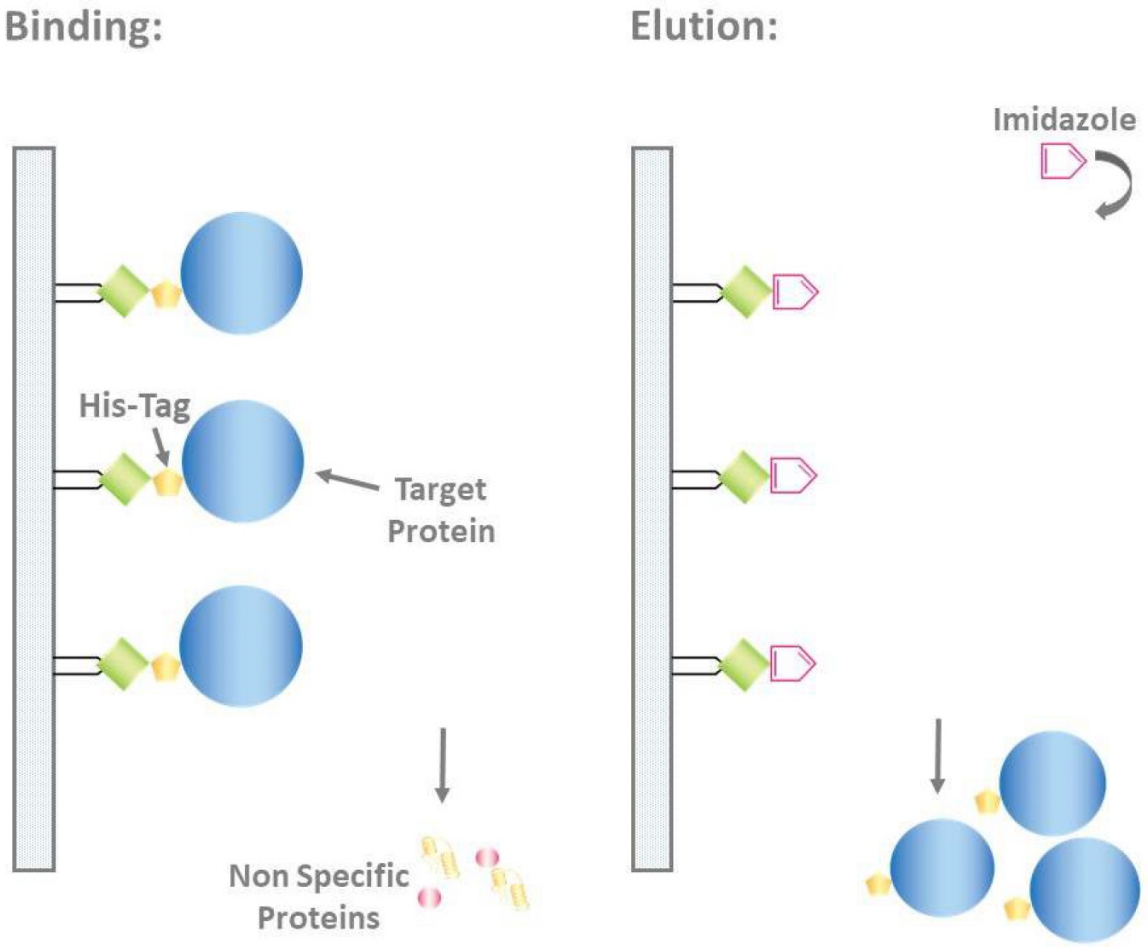






- Low concentration of imidazole included in wash buffer
- Imidazole competes for binding to Ni^{2+} resin
  - Low affinity binders / non-specific binders are outcompeted and released from the resin

High concentration of imidazole is used to elute the protein from the Ni^{2+} resin / column

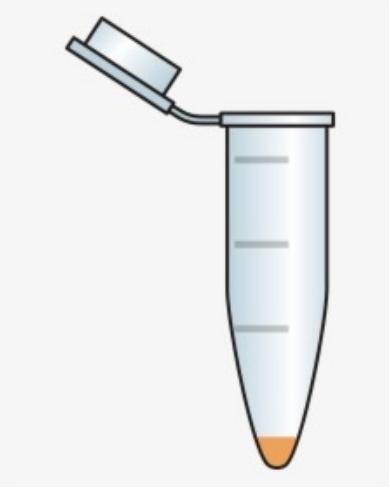


- Similar concept to wash
  - Wash uses 10mM imidazole
  - Elution used 250mM imidazole
- Instead of competing away non-specific binding, we can now out-compete the His Tag

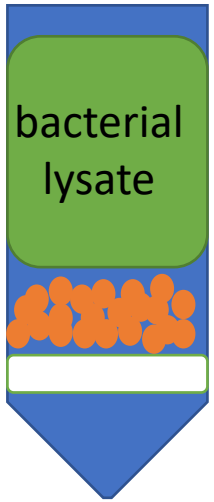
GoldBio

Purification process (and where you will save samples)

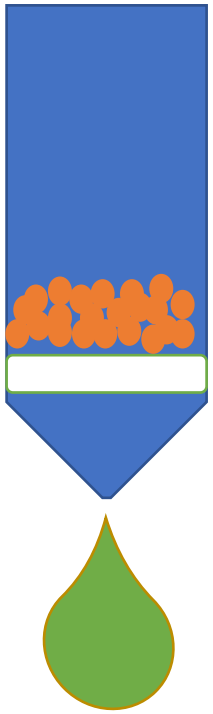
Pellet



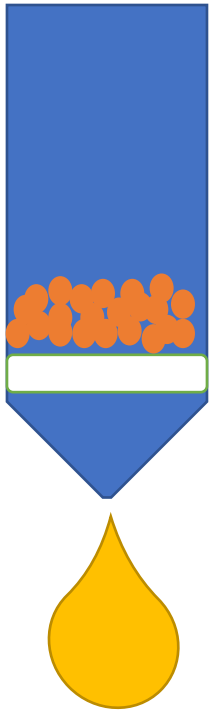
Lysate



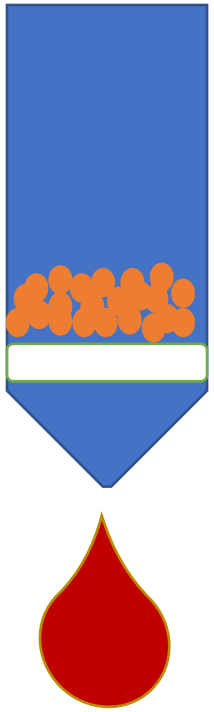
Flowthrough



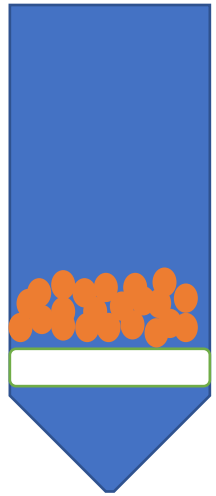
Wash



Elution



Slurry



For today…

- Discuss Background and Motivation with Noreen
- Complete protein purification
  - Deliver all purification samples and final elution to instructors by end of lab!

For M1D4…

- Work with your lab partner to write a methods section for the protein purification protocol
  - Checklist on the wiki provides useful guidance
- Visit Comm lab before M1D5

Pro tips for writing a methods section

Include enough information to replicate the experiment

- List manufacturer's name (Company)
- Be **concise and clear** in your description

Use subsections with descriptive titles

- Put in logical order, rather than chronological order
- Begin with topic sentence to introduce purpose / goal of each experimental procedure

Use clear and concise full sentences

- NO tables or lists, all information should be provided in full sentences and paragraphs
- Write in passive voice and use past tense

Use the most flexible units

- Write concentrations (when known) rather than volumes

Eliminate 20.109 specific details

- Example "labeled Row A, Row B…"
- Do not include details about tubes and water!
- Assume reader has some biology experience
- Include parts of the protocol that the teaching faculty completed, but do not say "completed by teaching faculty."

How can you improve this example?

"Cells were grown in 12 mL of RMPI supplemented with FBS. We spun down the cells and counted them with a hemocytometer. Flasks were incubated in 37 C incubator."