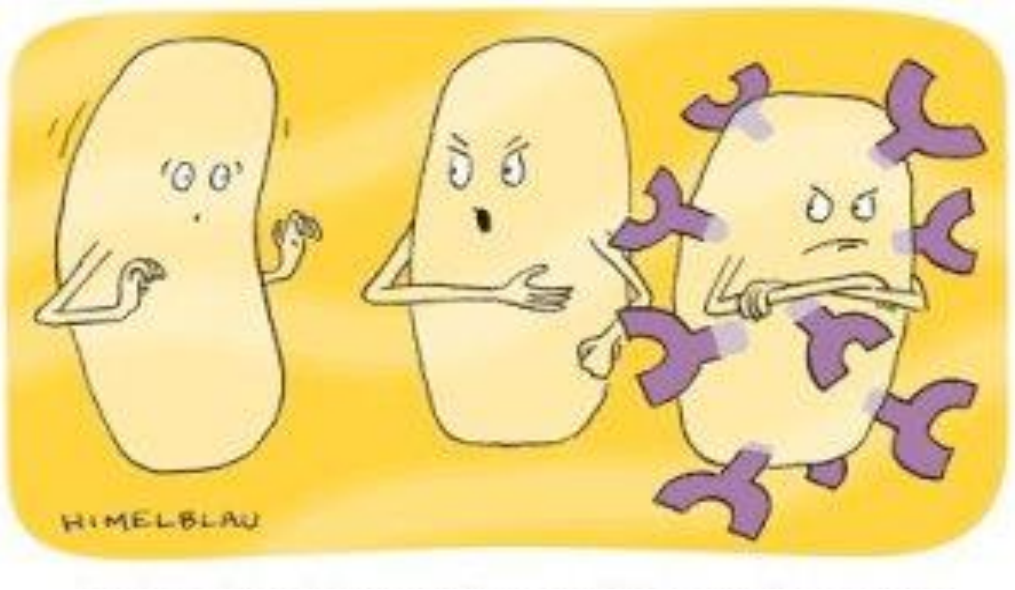
M1D3: Induce and purify TDP43 protein

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** (come and lyse cells for 15 minutes on nutator at front bench
  - During lysis, Tyler 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

15 min Lyse & watch Tyler assemble column

Split lysate evenly & spin 30 minutes

Assemble Column

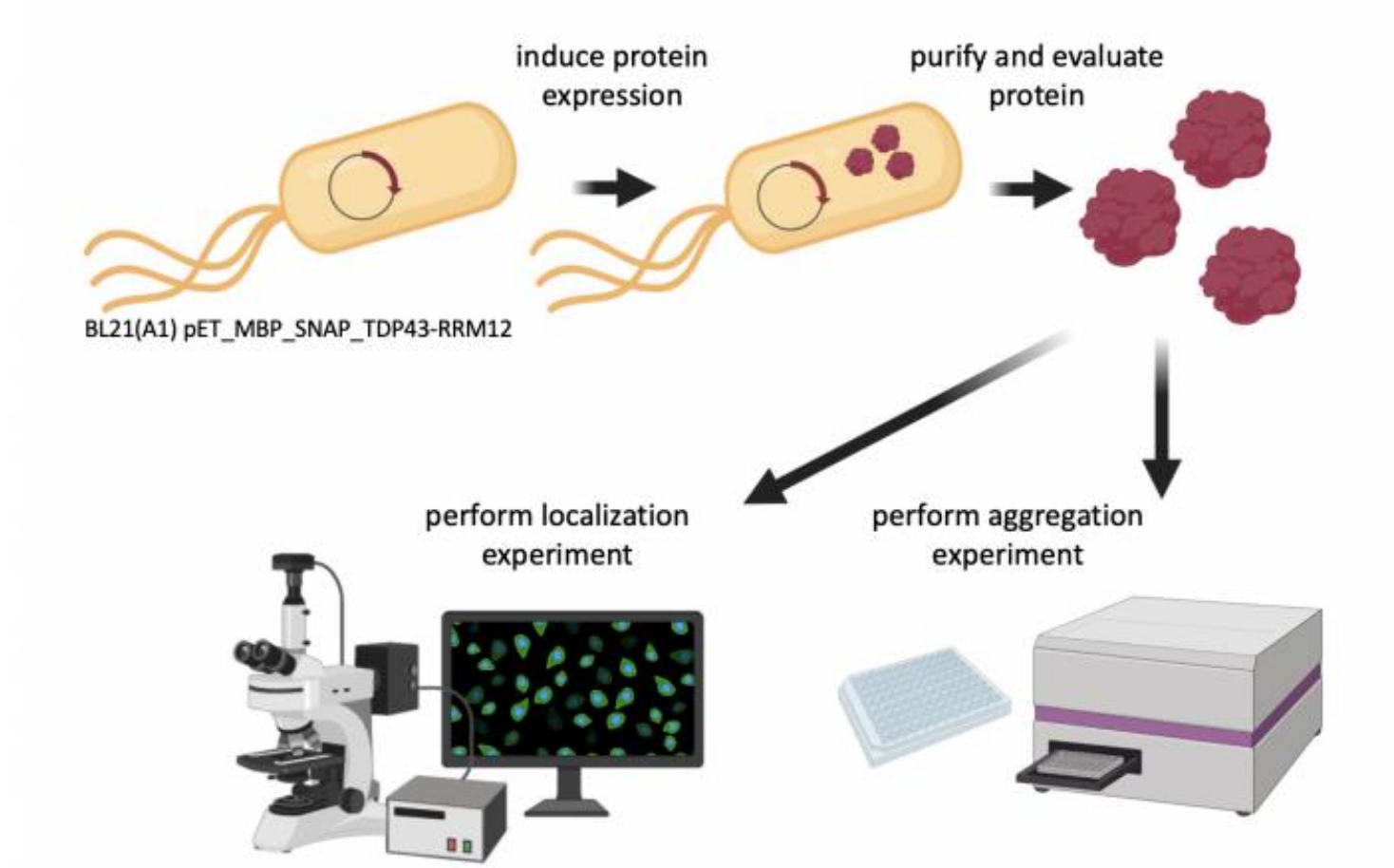
Save 30 ul of lysate

Incubate lysate in resin for 2 hours nutating @ 4C

Prelab II, Lecture, BREAK

Overview of Mod1 experiments

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



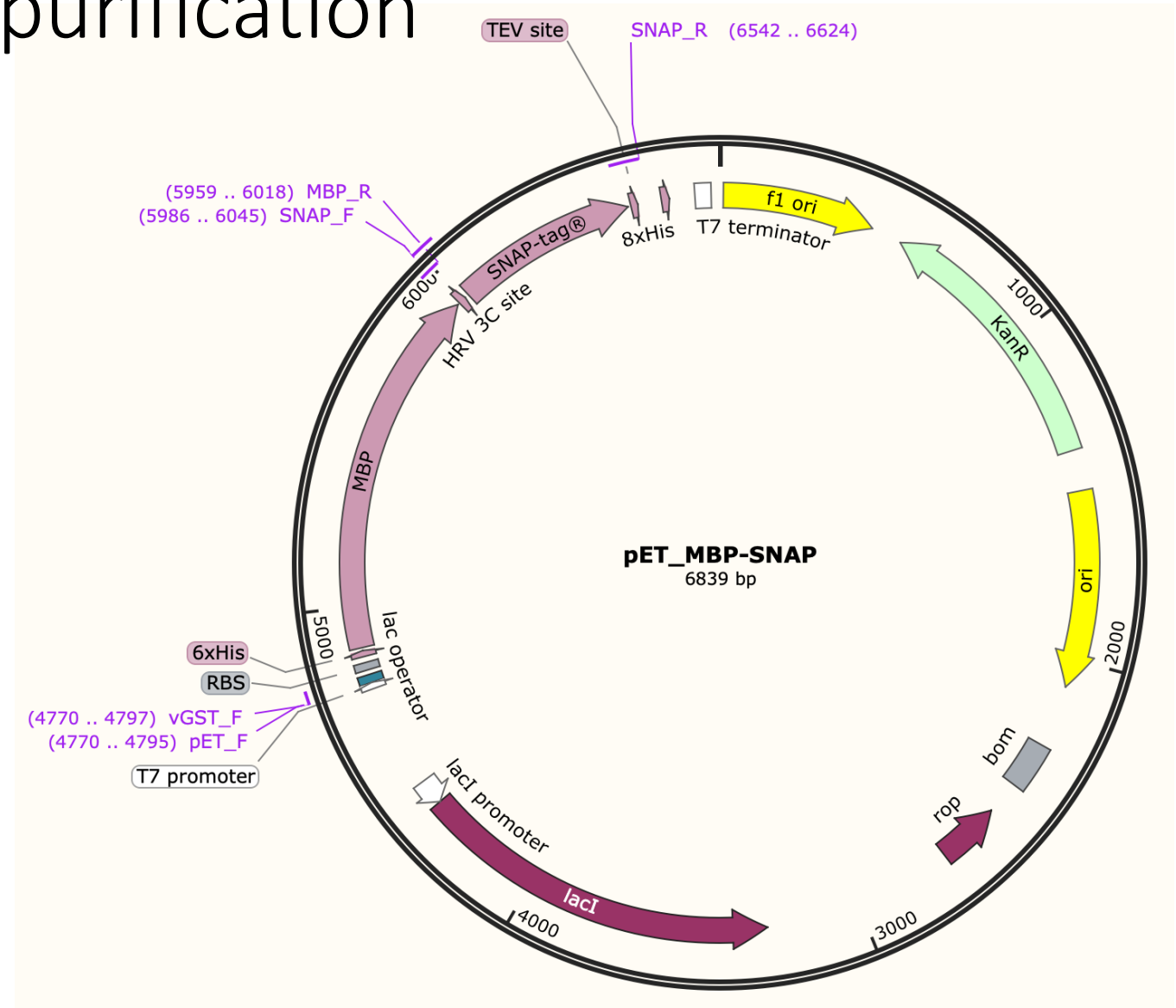
Use genetic features of the plasmid to control protein expression and purification

Induction

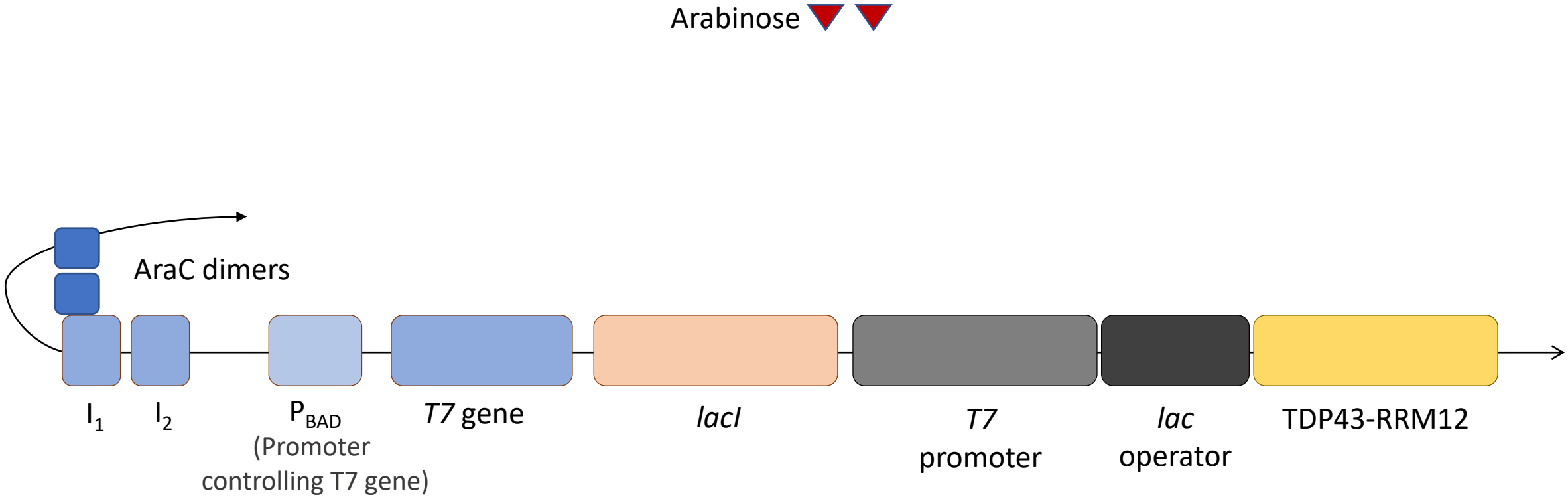
- T7 promoter
- Lac operator
- Kanamycin

Purification

- His-tag

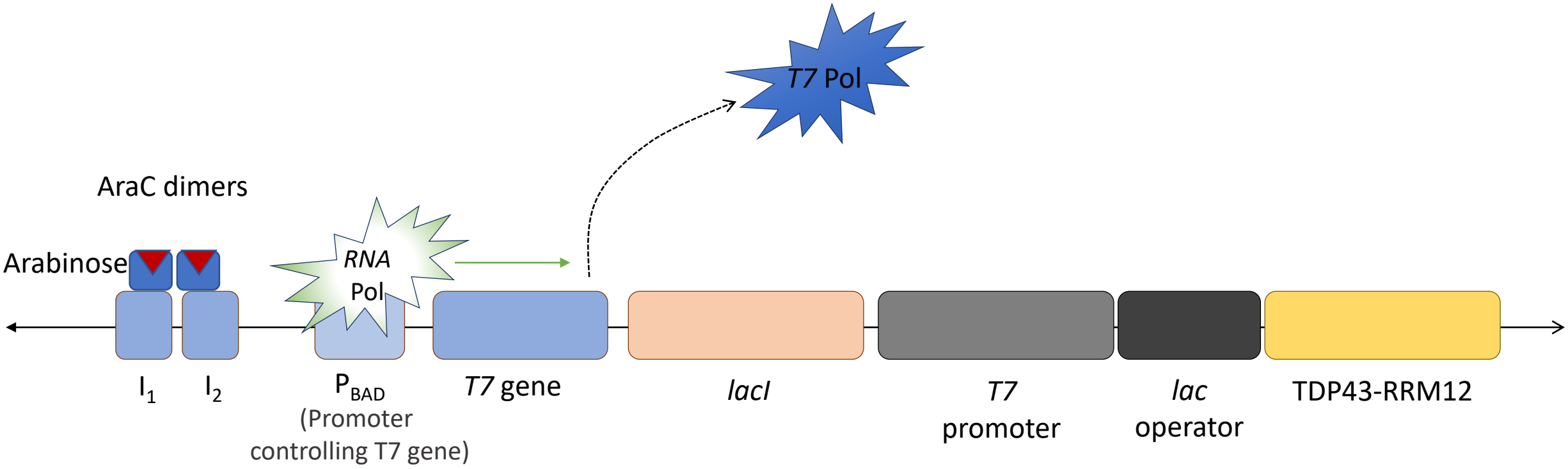


Bacterial induction: How it begins…



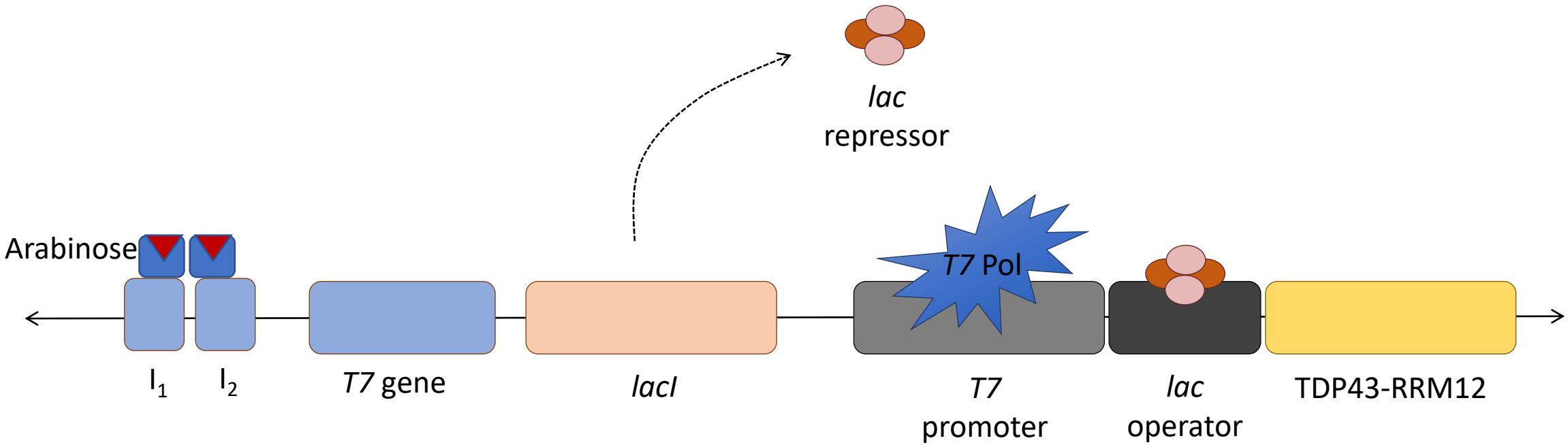
pET_MBP_SNAP_TDP43-RRM12

Bacterial induction: Arabinose controls T7 expression



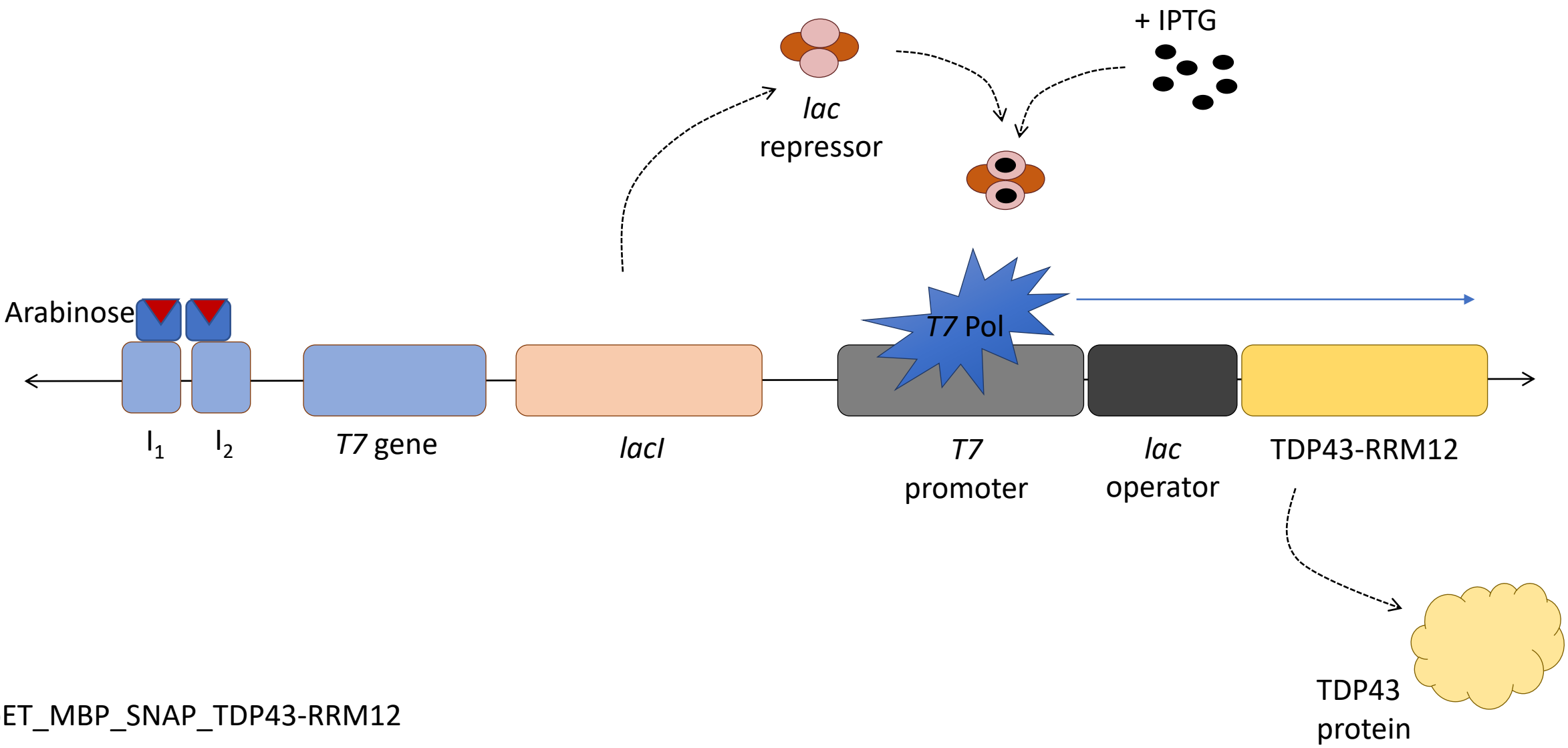
pET_MBP_SNAP_TDP43-RRM12

Bacterial induction: Lac repressor



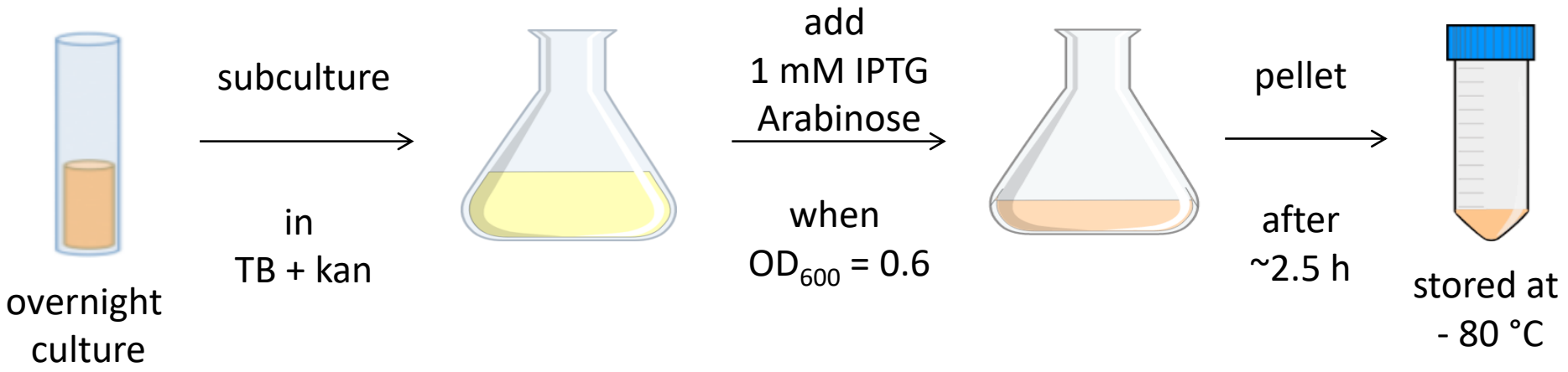
pET_MBP_SNAP_TDP43-RRM12

Bacterial induction: IPTG removes lac repression

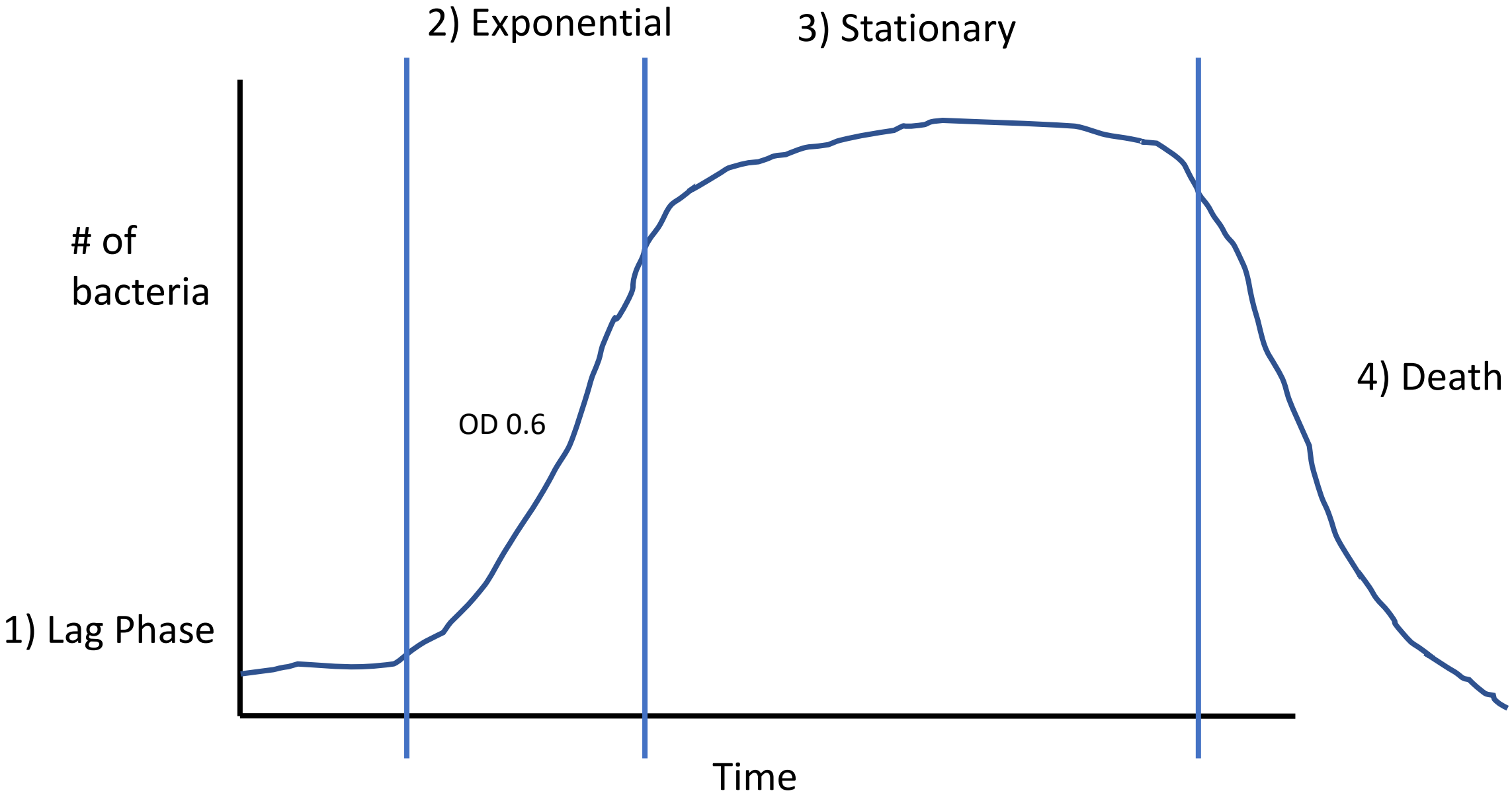


pET_MBP_SNAP_TDP43-RRM12

How do we induce protein expression?

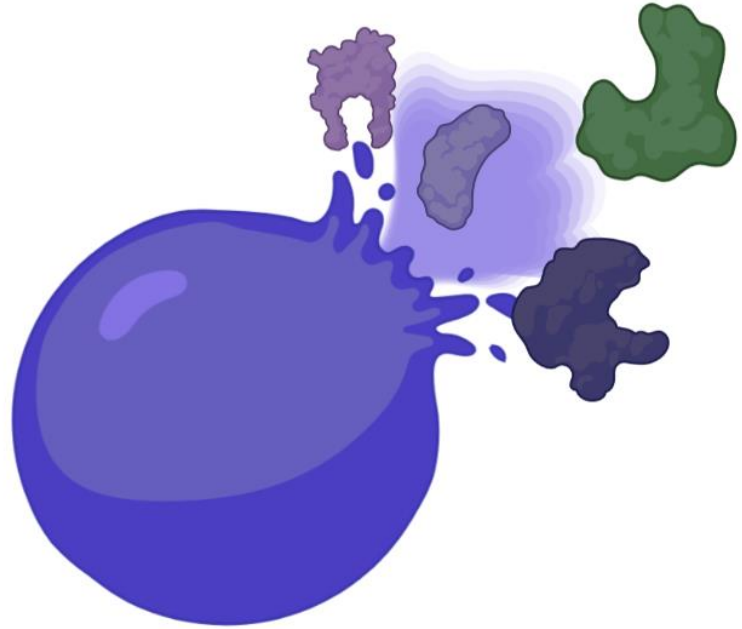


Why do we induce protein expression at OD_{600} = 0.6?


2) Exponential
3) Stationary
# of bacteria
4) Death
OD 0.6
1) Lag Phase
Time

How will you purify TDP43_RRM12?

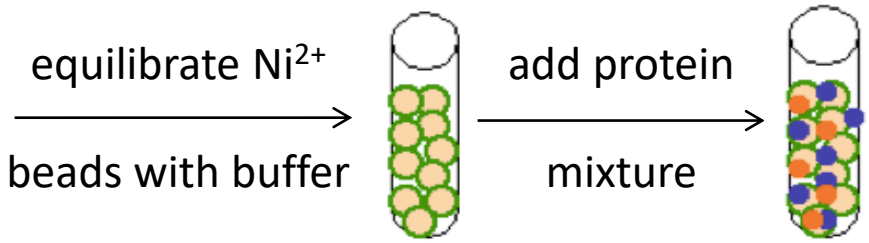
First, need to lyse cells to release proteins:

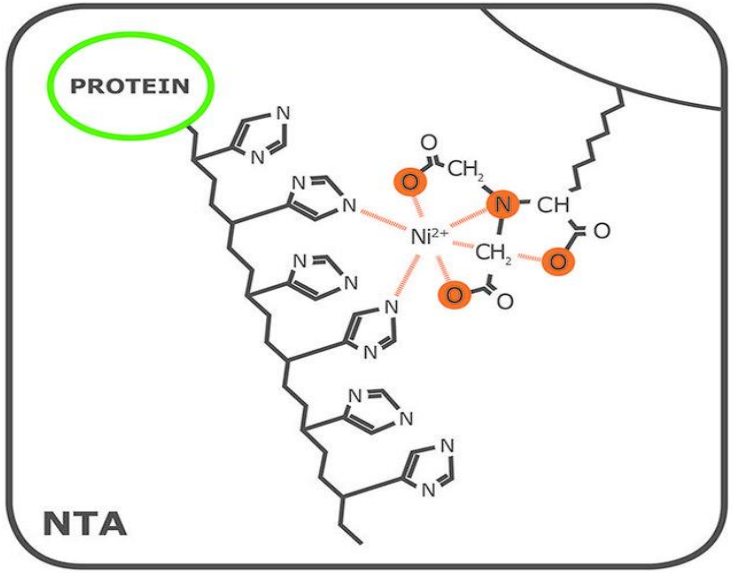


- B-PER bacterial extraction reagent
  - Detergents, Buffers
- Lysonase & Benzonase
  - Cell wall & DNAse/RNAse
- Protease Inhibitor Cocktail Prevents degradation

6XHis 0.8kDa	Maltose binding protein 40.3kDa	3C site 0.9kDa	Snap-tag® 19.3kDa	TDP43 domains RRM1 and 2 19.2kDa
Affinity tag	MBP solubliziaiton tag	Cleavage	Alexafluor 647	**P**rotein **O**f **I**nterest

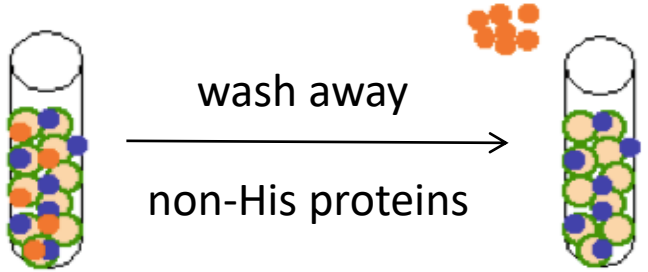
6xHis tag binds to Ni^{2+} resin / column to allow purification of protein of interest



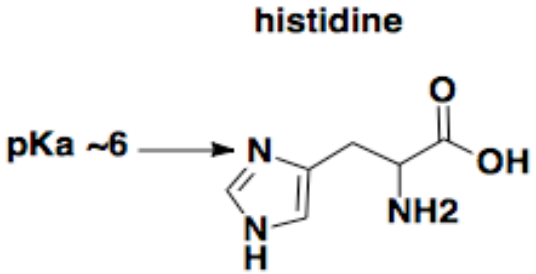


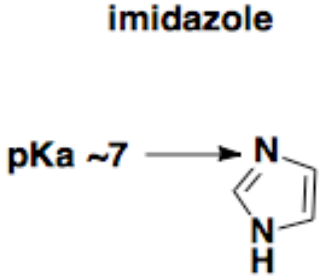
- Ni^{2+} chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand
- His tag chelates to Ni^{2+} causing protein to ‘stick’ to resin / column

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



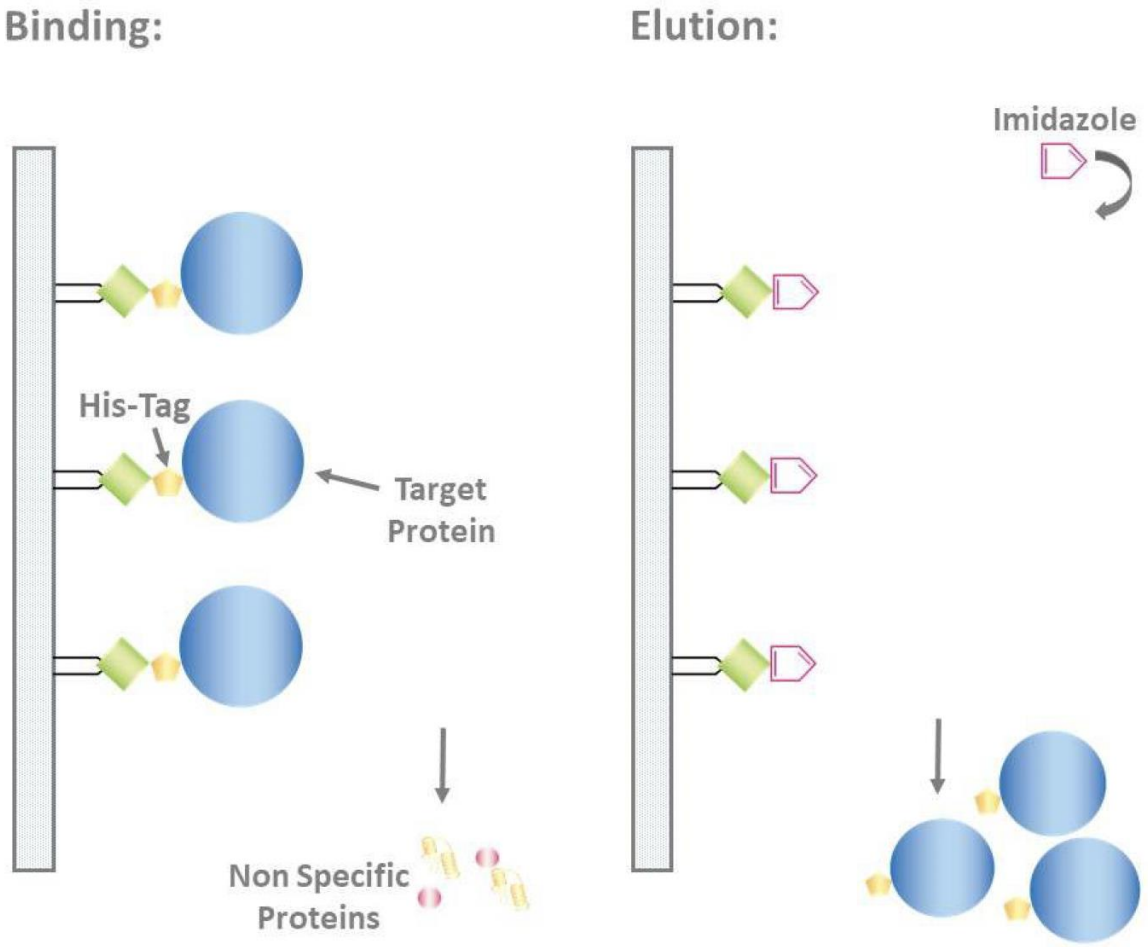
POIs remain after imidazole wash





- 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

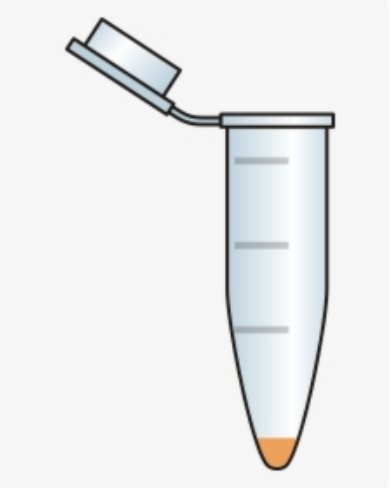


- Similar concept to wash
  - Wash uses 10mM imidazole
  - Elution used 250mM imidazole
- Instead of competing away non-specific binding, we can now out-compete the His Tag

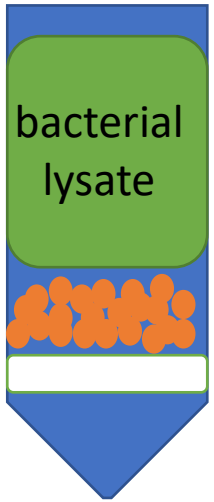
GoldBio

Purification process (and where you will save samples)

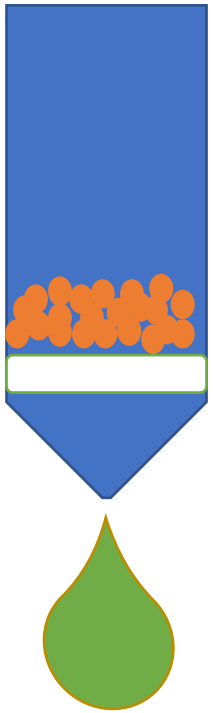
Pellet



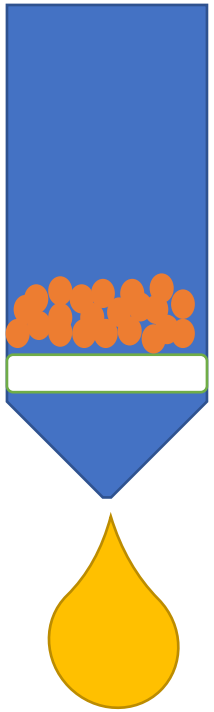
Lysate



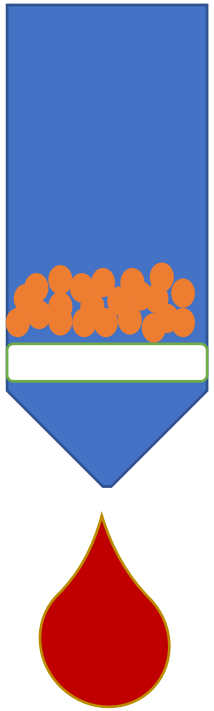
Flowthrough



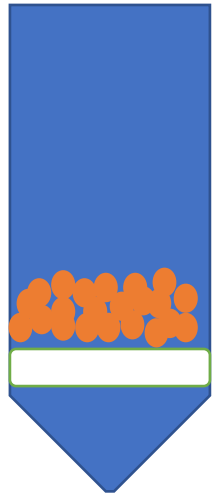
Wash



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."

How can you improve this example?

What cells? From where were the cells attained?

How much? What else was added to the media?

"Cells were grown in 12 mL of RPMI supplemented with FBS. We spun

Volume here does not have context as based on the flask used. When might flask / plate size be helpful??

Define all abbreviations and include supplier / manufacturer.

Use passive voice and avoid jargon!

down the cells and counted them with a hemocytometer. Flasks

Be specific about the purpose of each of the steps used…cells were harvested using centrifugation (be sure to include speed and time) then counted using a hemocytometer. And what else was used? At what final concentration / percent?

Be specific about the subject of each action / step.

were incubated in 37 C incubator."

Specific location / equipment used is not important, just the temperature conditions. What other growth conditions were maintained?

What is the purpose of the Background & Motivation section?

What *specific* information should be included in the Background & Motivation section?

Notes on Background & Motivation section…

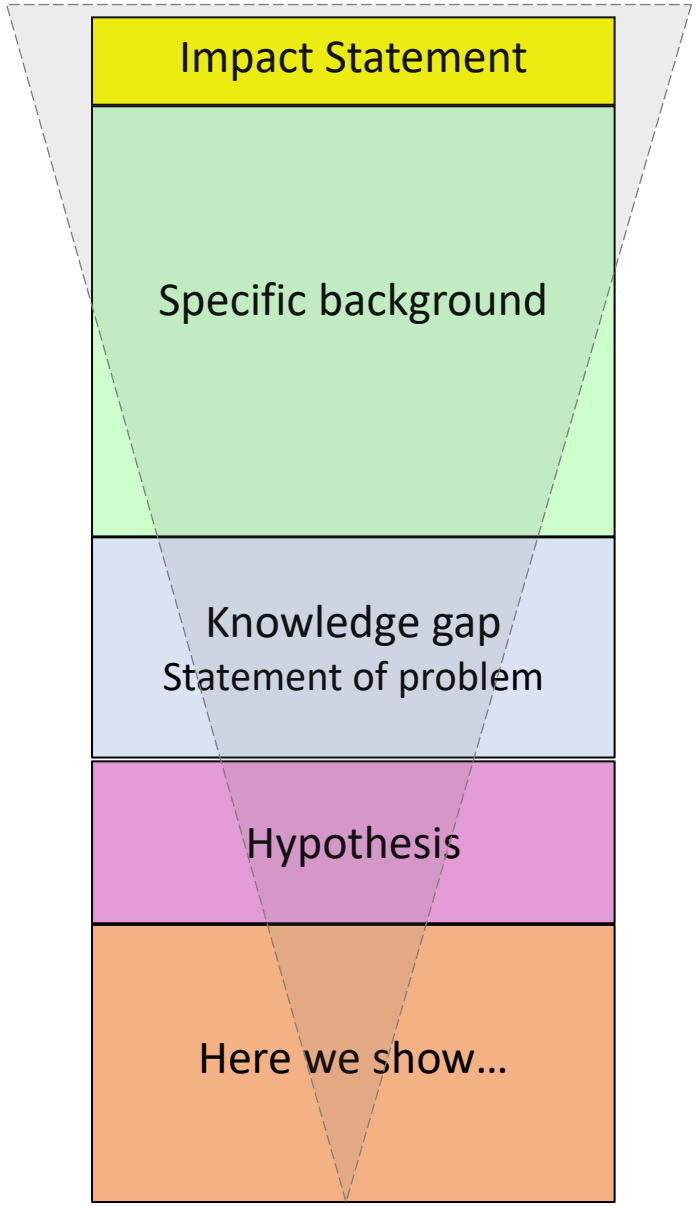
- Anchor your research in a general topic that is important to a broad audience
  - Focus on describing what is currently known in the field
  - Reference the relevant research in the field
- Connect your research to the general topic
  - Minimum essential information
  - Introduce specific technologies necessary for understanding your specific project
- Address how you will expand on what is currently known
  - Include evidence of incompleteness of current understanding
  - Motivate your investigation
  - Include a clear hypothesis / research goal
- Provide a preview of your findings and the implications
  - Tie back to the initial general topic
  - Avoid including extensive methods details

Notes on topic sentences…

- Topic sentence = first sentence of each paragraph

- Should 'funnel' from big picture topic to your specific research question / project
  - Provide only the background needed to understand research / problem / goal
  - Clearly state what is not currently known
  - Address how you will fill knowledge gap
  - Provide preview of your results

- Include references!!



How should you introduce your story?

1st paragraph: what is the big picture / problem?

2nd paragraph: what is currently known?

3rd (or 4th) paragraph: what is your research question?

4th (or 3rd) paragraph: how will you address your question?

5th paragraph: here we show…