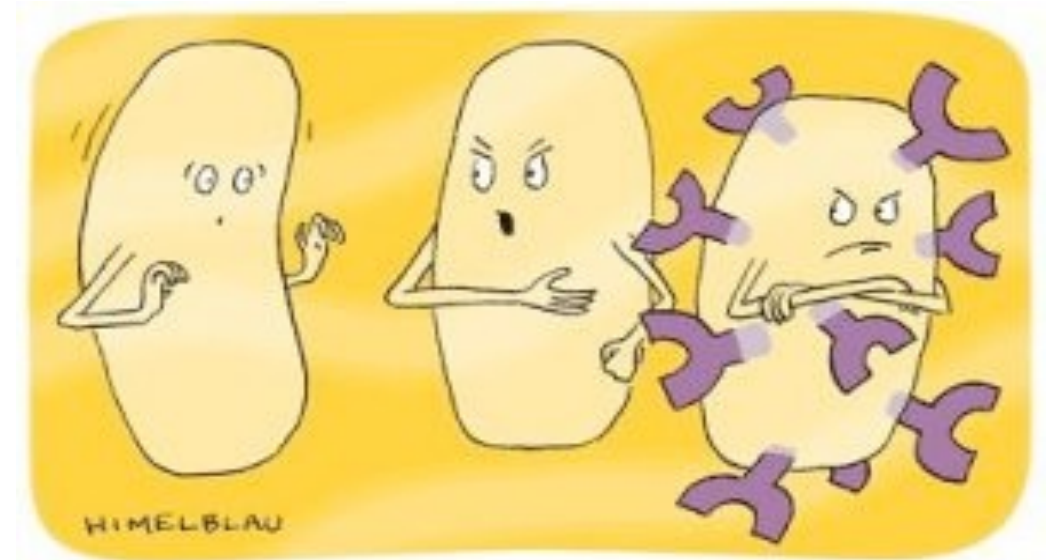# M1D3: Induce and purify TDP43 protein

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

Part 2

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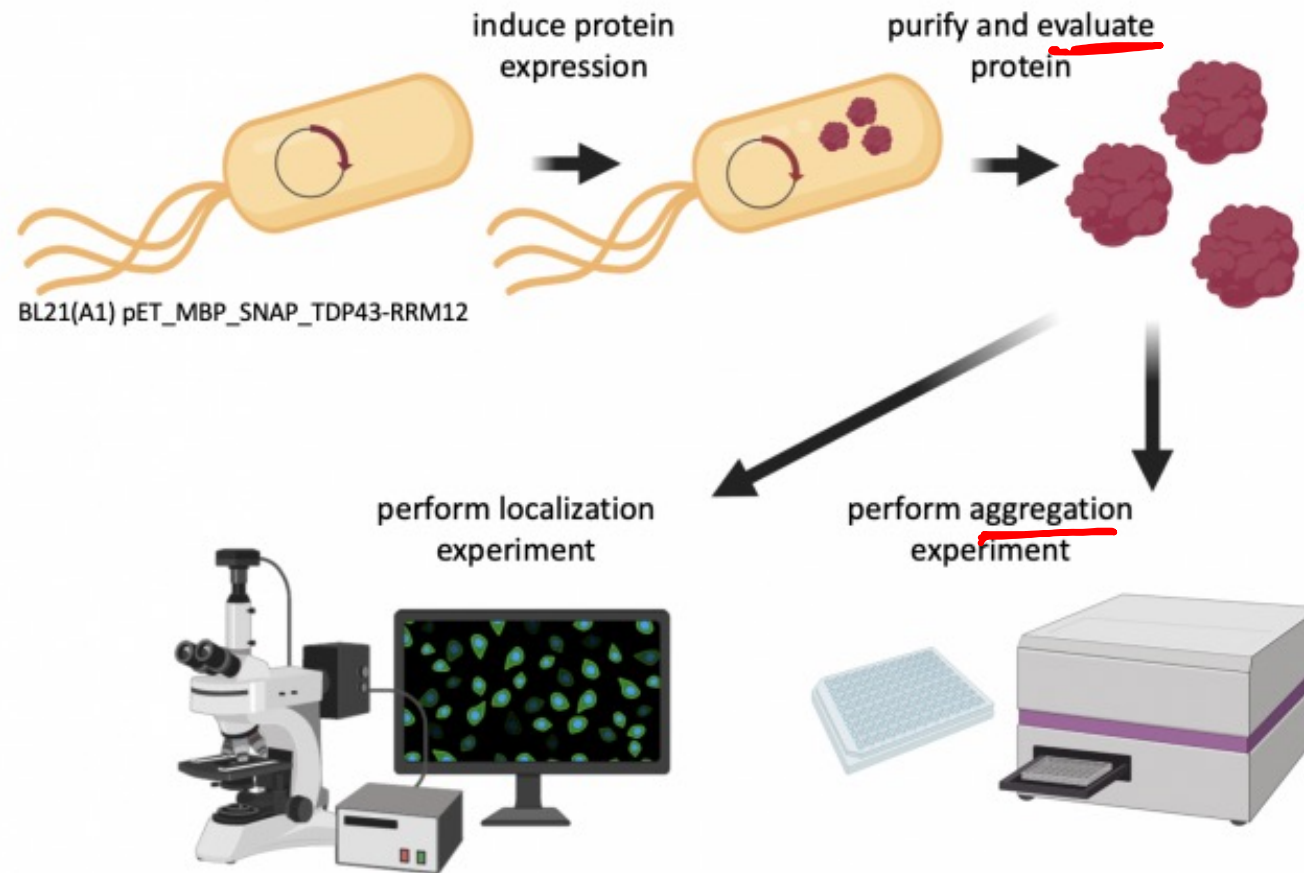
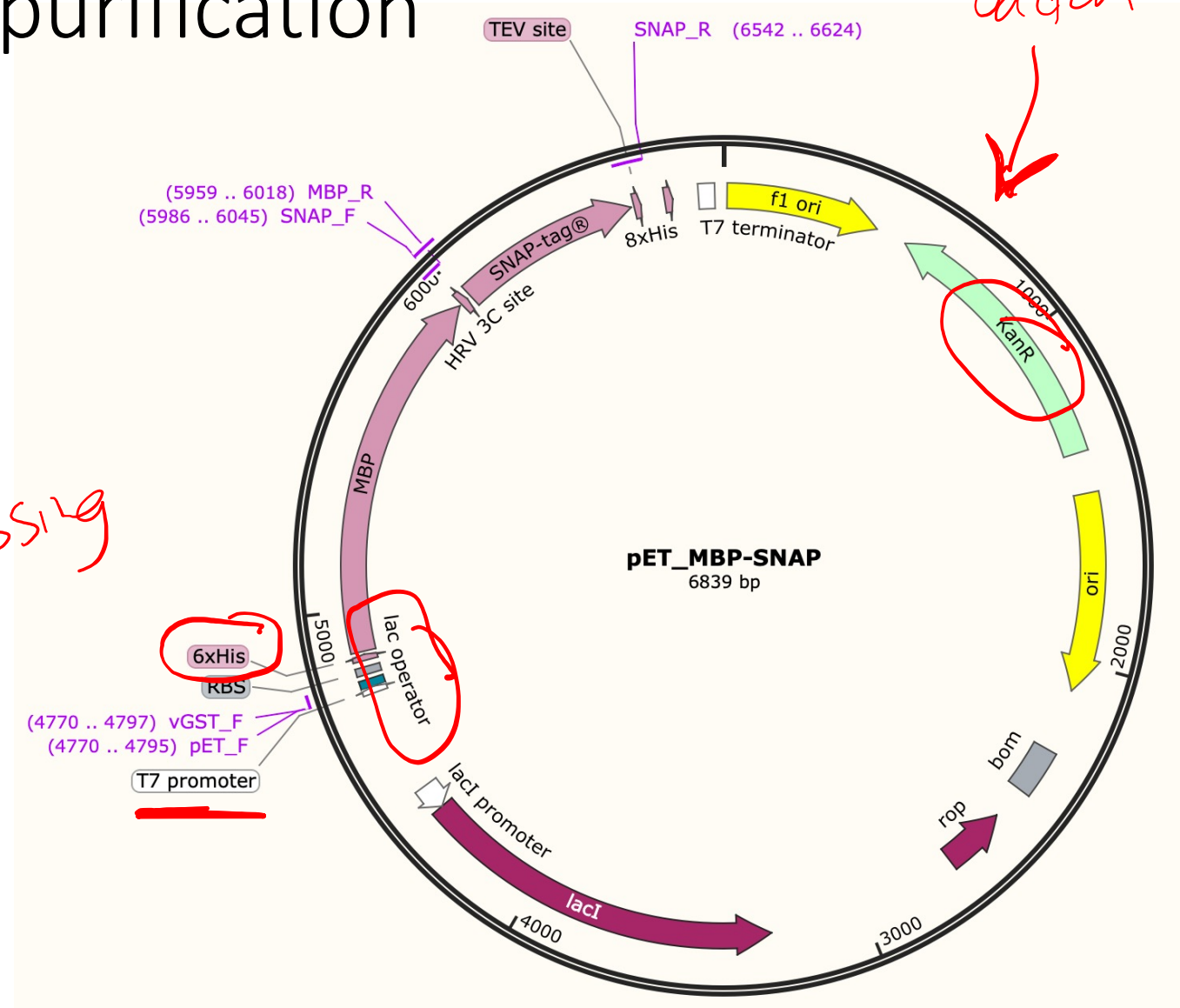# Use genetic features of the plasmid to control protein expression and purification
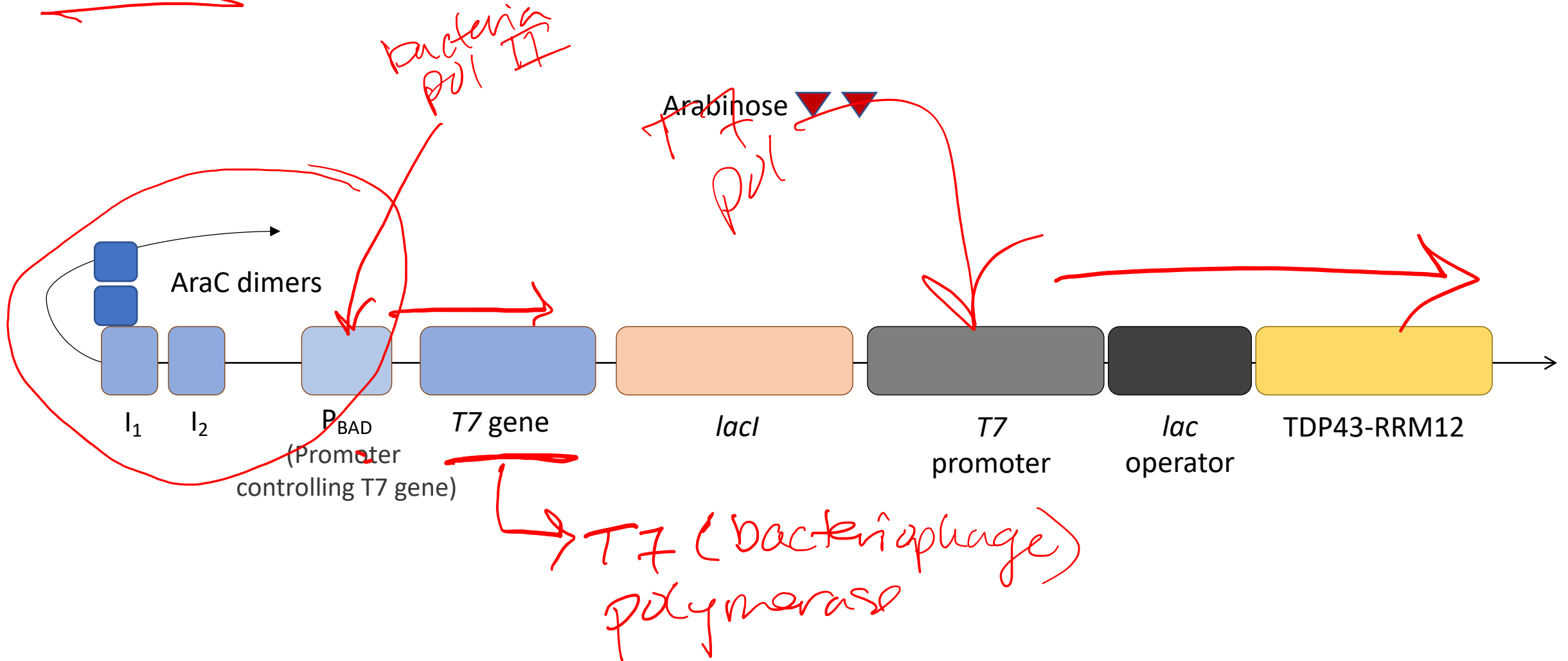
Induction

- T7 promoter
- Lac operator
- Kanamycin

— Selection
— select for bacteria expressing plasmid

Purification

- His-tag

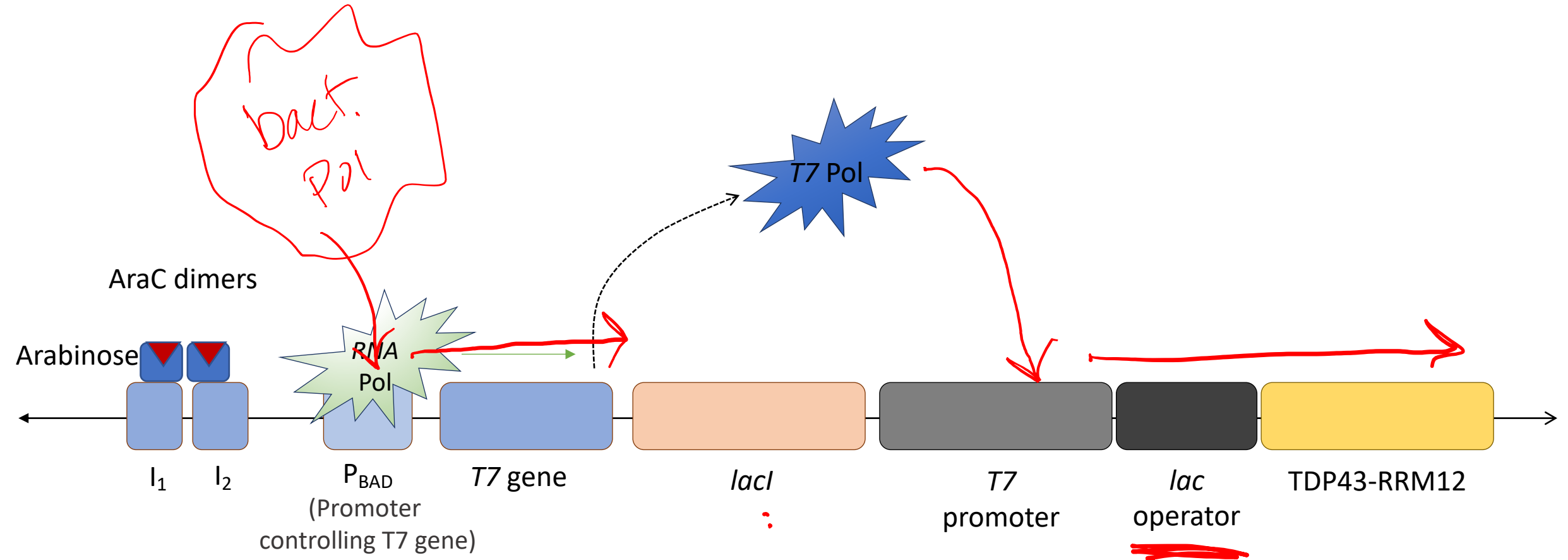pET_MBP-SNAP
6839 bp

Transform bacteria

Bacterial induction: How it begins…

AraC dimers

I₁ I₂ P_BAD
(Promoter controlling T7 gene)

T7 gene

lacI

T7 promoter

lac operator

TDP43-RRM12

Arabinose
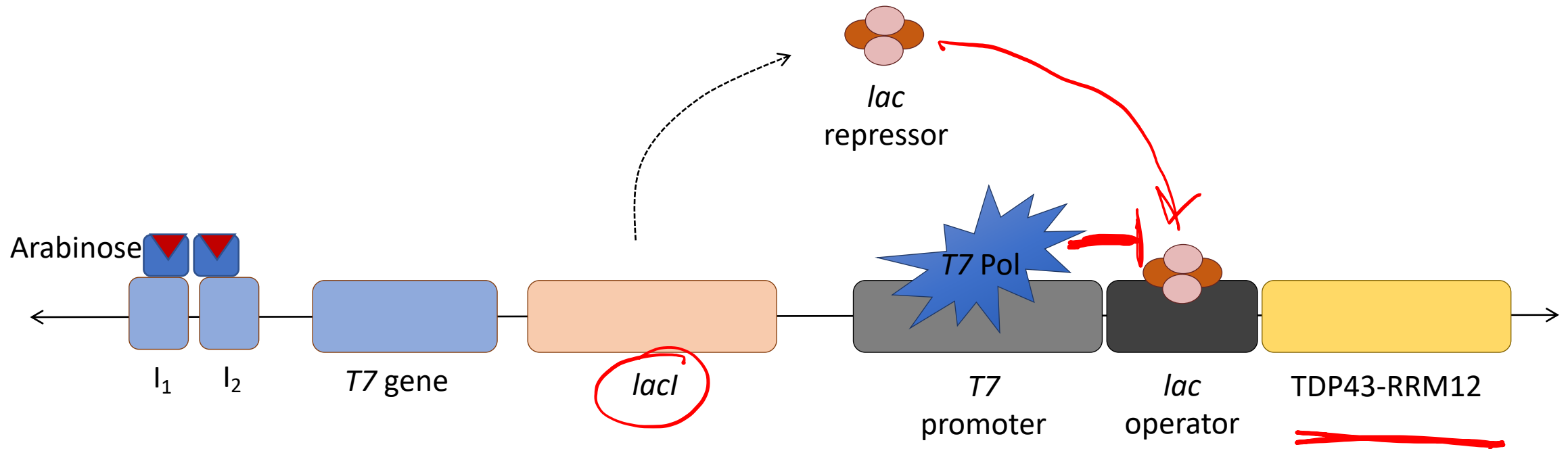
Bacteria Pol II

T7 Pol

T7 (bacteriophage) Polymerase

pET_MBP_SNAP_TDP43-RRM12
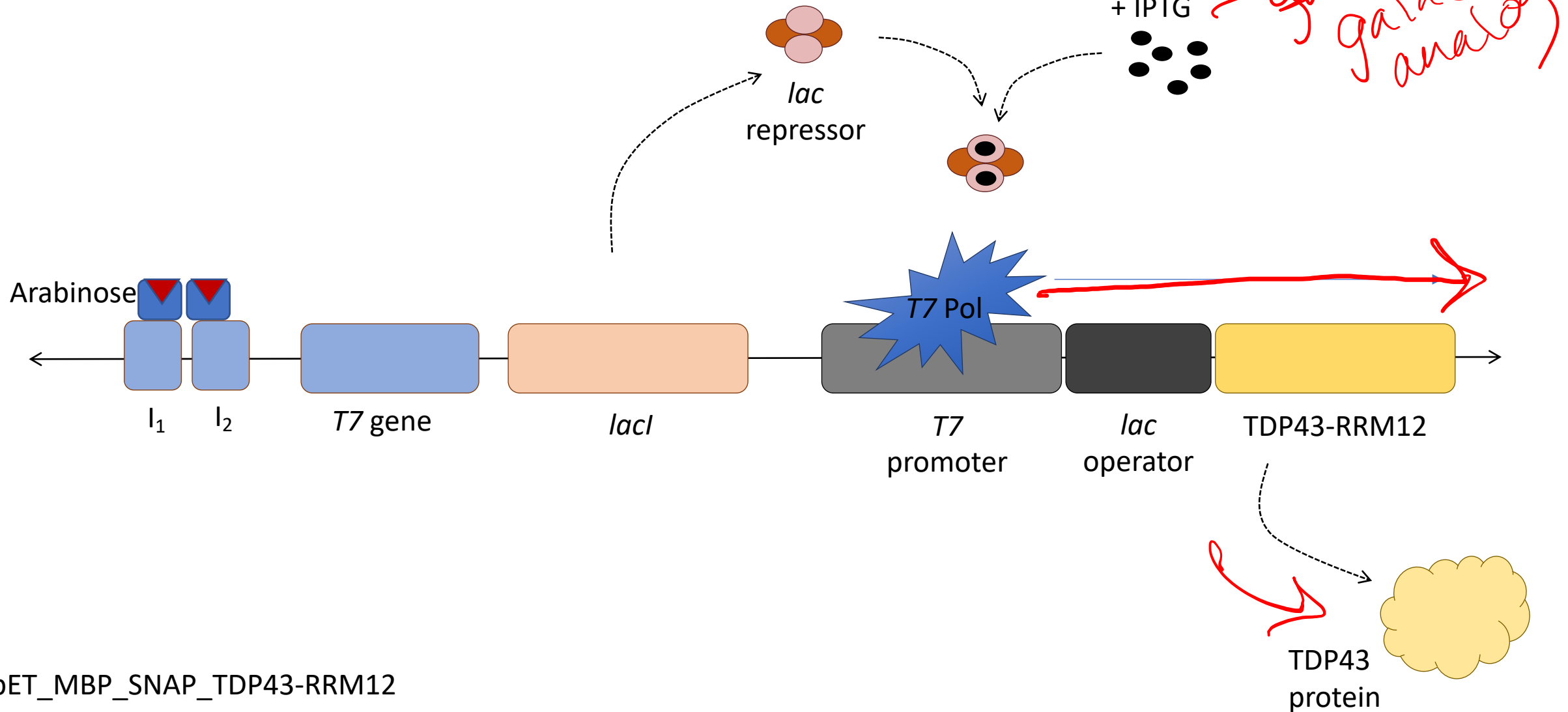
Bacterial induction: Arabinose controls T7 expression

pET_MBP_SNAP_TDP43-RRM12

Bacterial induction: Lac repressor

pET_MBP_SNAP_TDP43-RRM12
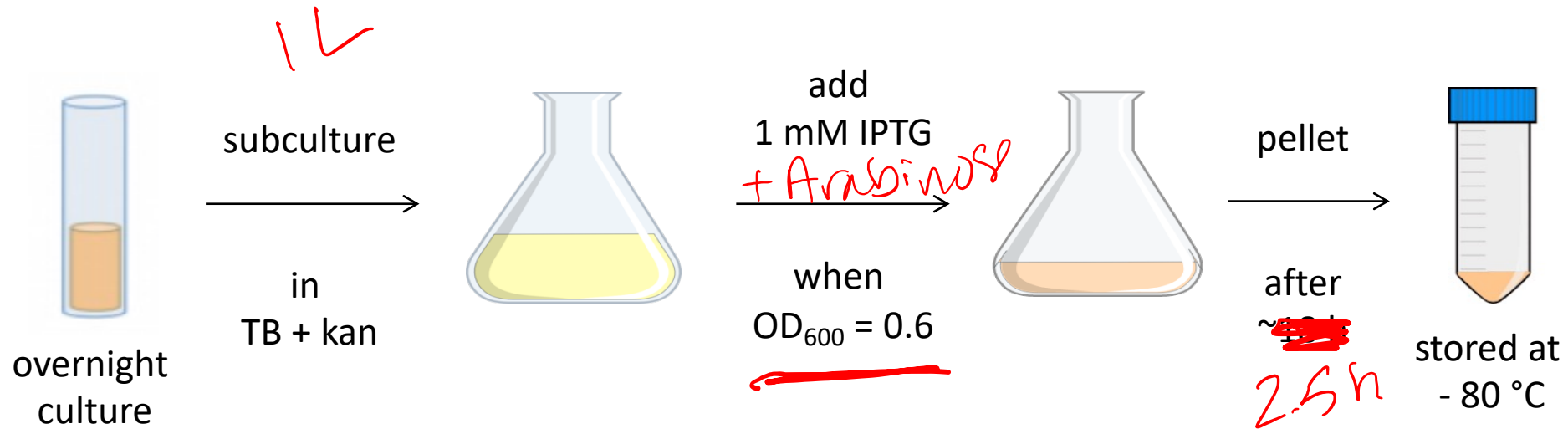
Bacterial induction: IPTG removes lac repression

galactose analog

+ IPTG

lac
repressor

T7 Pol

Arabinose

I$_1$ I$_2$ *T7* gene   *lacI*   *T7*
promoter   *lac*
operator   TDP43-RRM12

pET_MBP_SNAP_TDP43-RRM12

TDP43
protein

How do we induce protein expression?

overnight culture

1L

subculture

in TB + kan

add 1 mM IPTG

+Arabinose

when OD_{600} = 0.6

pellet

after 2.5h

stored at - 80 °C

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

# of bacteria

Time

Exponential growth

Stability

→ Harvest

Death

LAG

O.D.
0.4
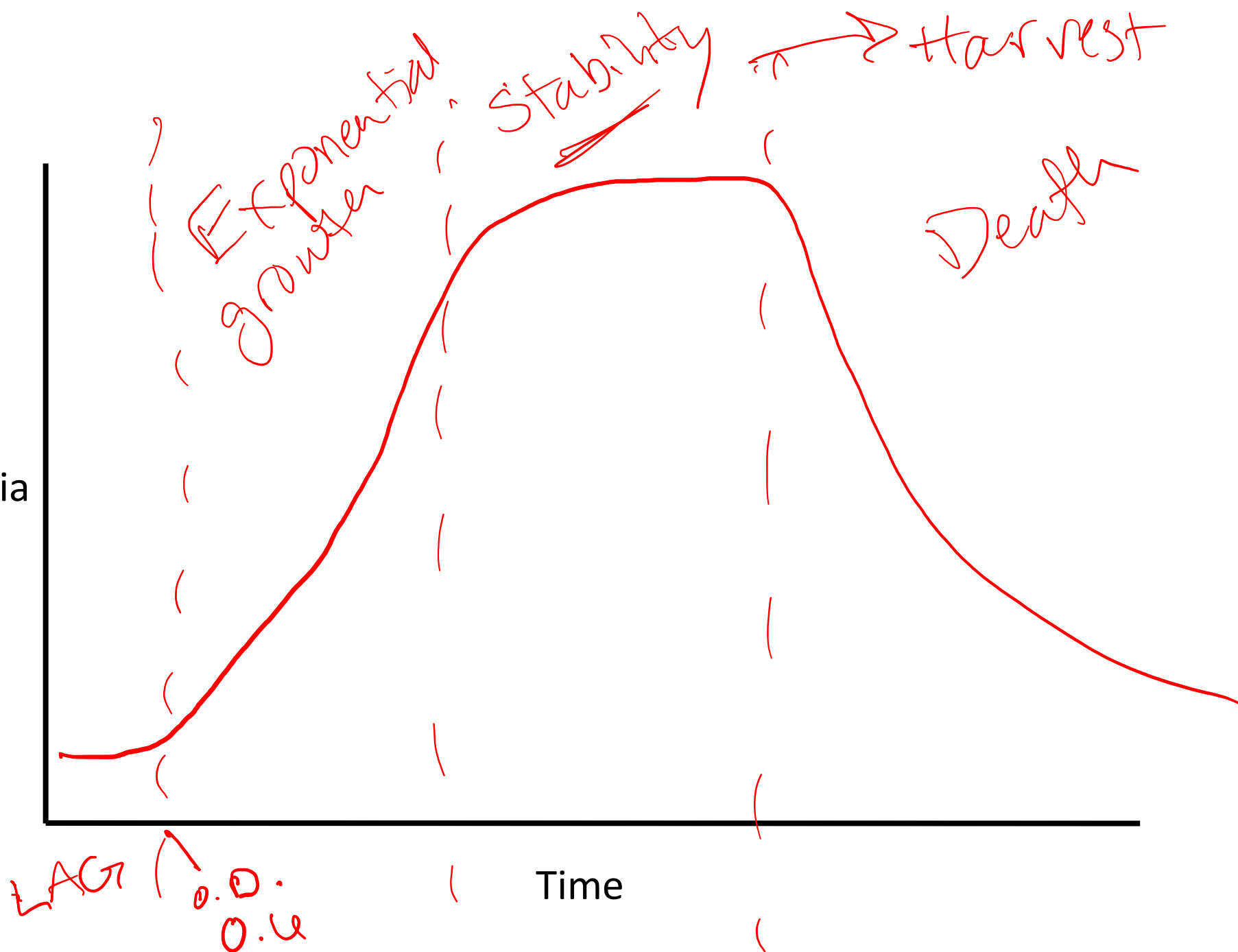
How will you purify TDP43_RRM12?

First, need to lyse cells to release proteins:

(handwritten: → Detergent Buffers)

- B-PER bacterial extraction reagent

- Lysonase — *(handwritten: Lysozyme + Benzonase → break cell wall)*

- Protease Inhibitor Cocktail

(handwritten: → keep protein from degrading, DNAse RNase)

(handwritten: POI)

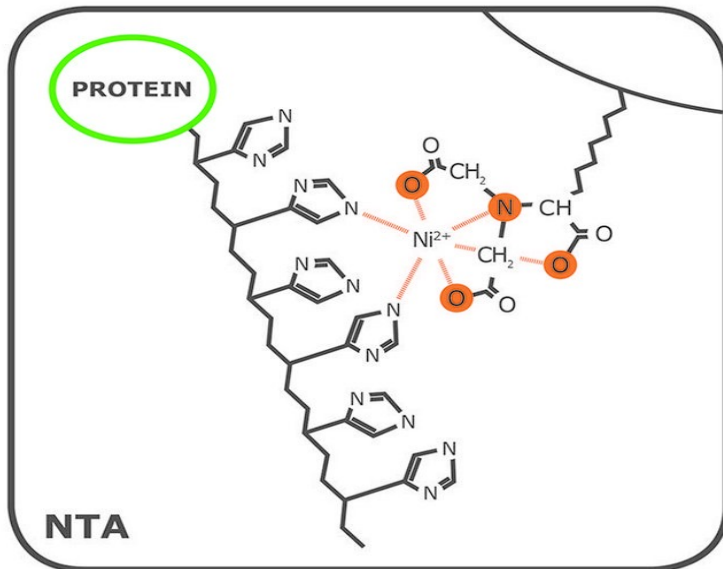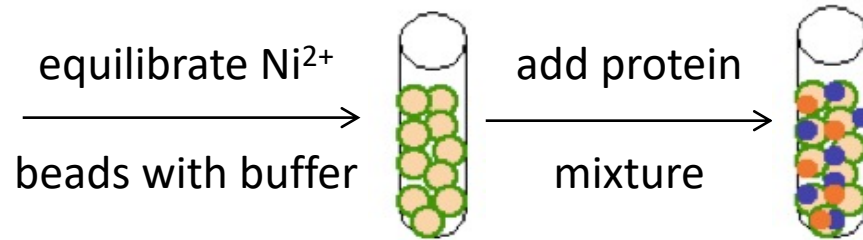| 6XHis 0.8kDa	Maltose binding protein 40.3kDa	3C site 0.9kDa	Snap-tag® 19.3kDa	TDP43 domains RRM1 and 2 19.2kDa

(handwritten: MBP) *(handwritten: HRV 3C)* *(handwritten: Alexa 647)*
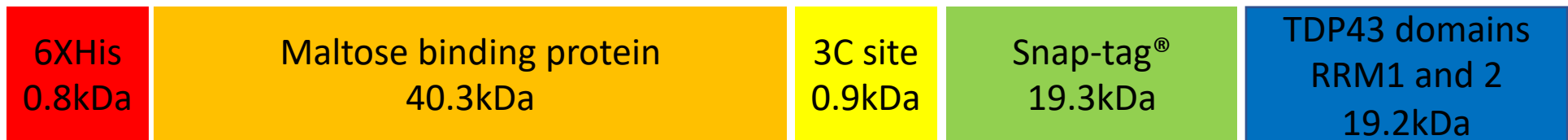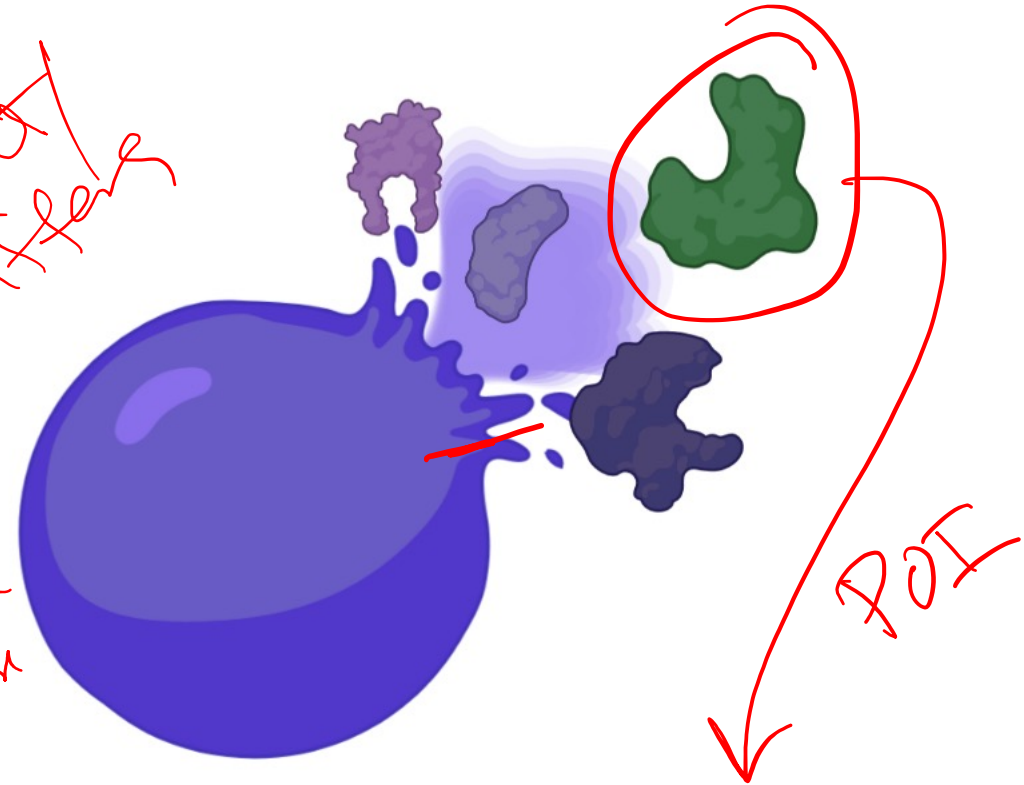
Recombinant protein of TDP43_RRM12

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

equilibrate Ni^{2+} beads with buffer
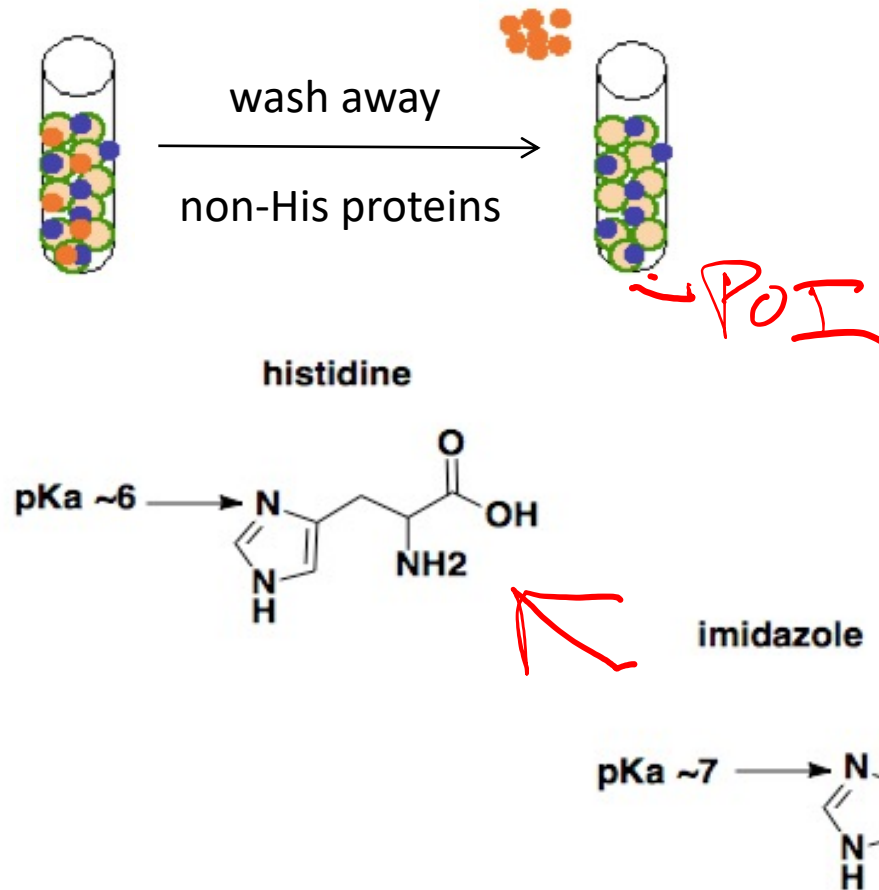
add protein mixture

PROTEIN

NTA

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

- His tag chelates to Ni^{2+} causing protein to 'stick' to resin / column
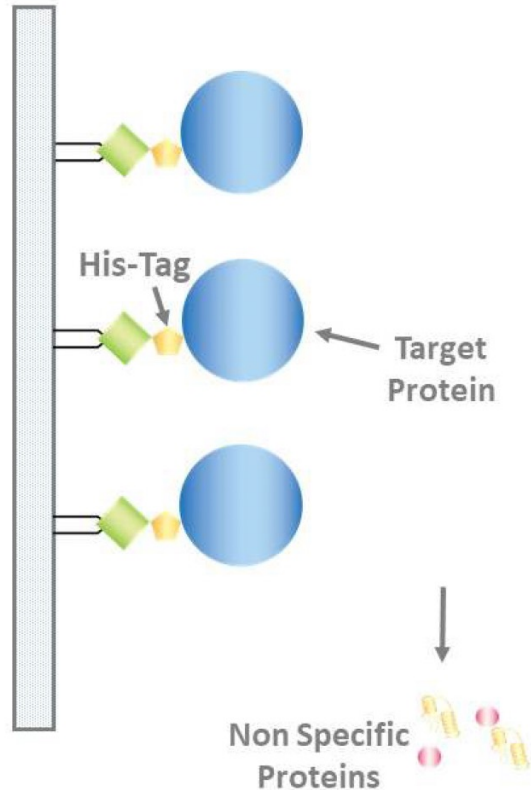
6x His tag

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

Binding:

Elution:

His-Tag

Target Protein

Imidazole

Non Specific Proteins
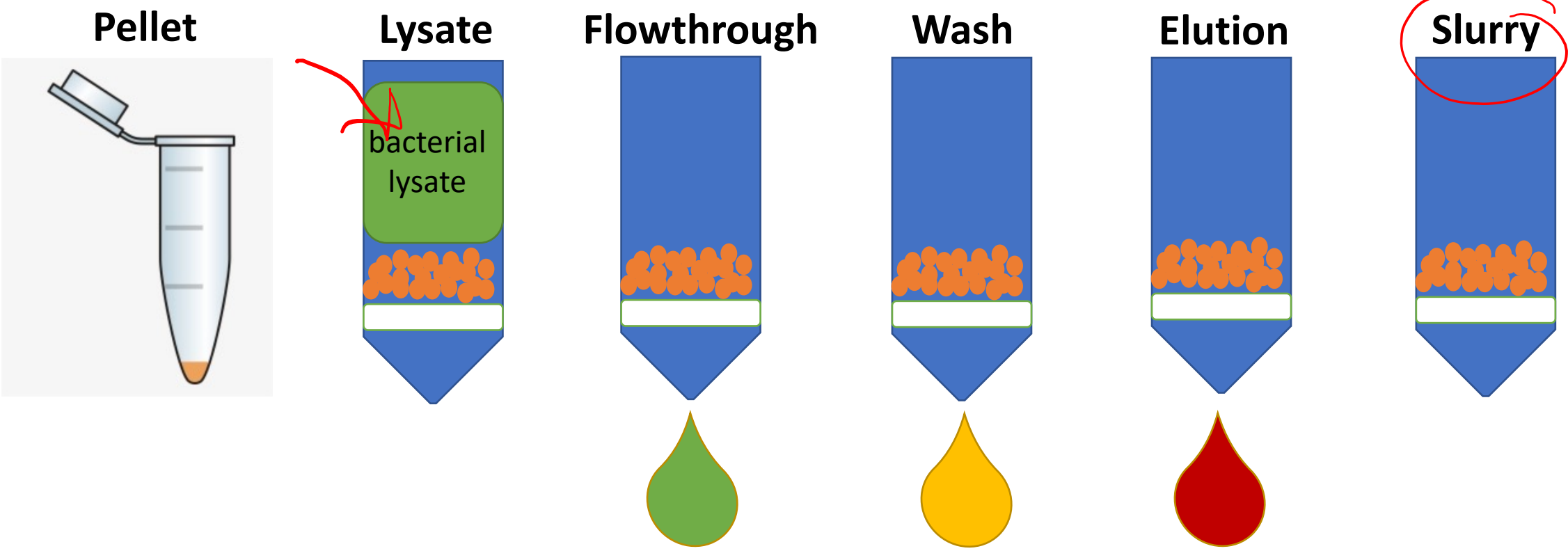
- Similar concept to wash
  - Wash uses 10mM imidazole
  - Elution used 250mM imidazole

- Instead of competing away non-specific binding, we can now out-compete the His Tag

GoldBio

Purification process (and where you will save samples)

resuspend in PBS

Pellet   **Lysate**   **Flowthrough**   **Wash**   **Elution**   **Slurry**

bacterial lysate

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 → *induction is part of protocol / methods*
  - 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) — *bottom of wiki paragraph*
- 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 → *this was done ...*

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."

M123 (crossed out)

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."

[Handwritten annotations in red:]

no volume

concentration

not past tense

centrifuged (colloquial)

not specific — cells

or How long? — culture conditions info

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!!
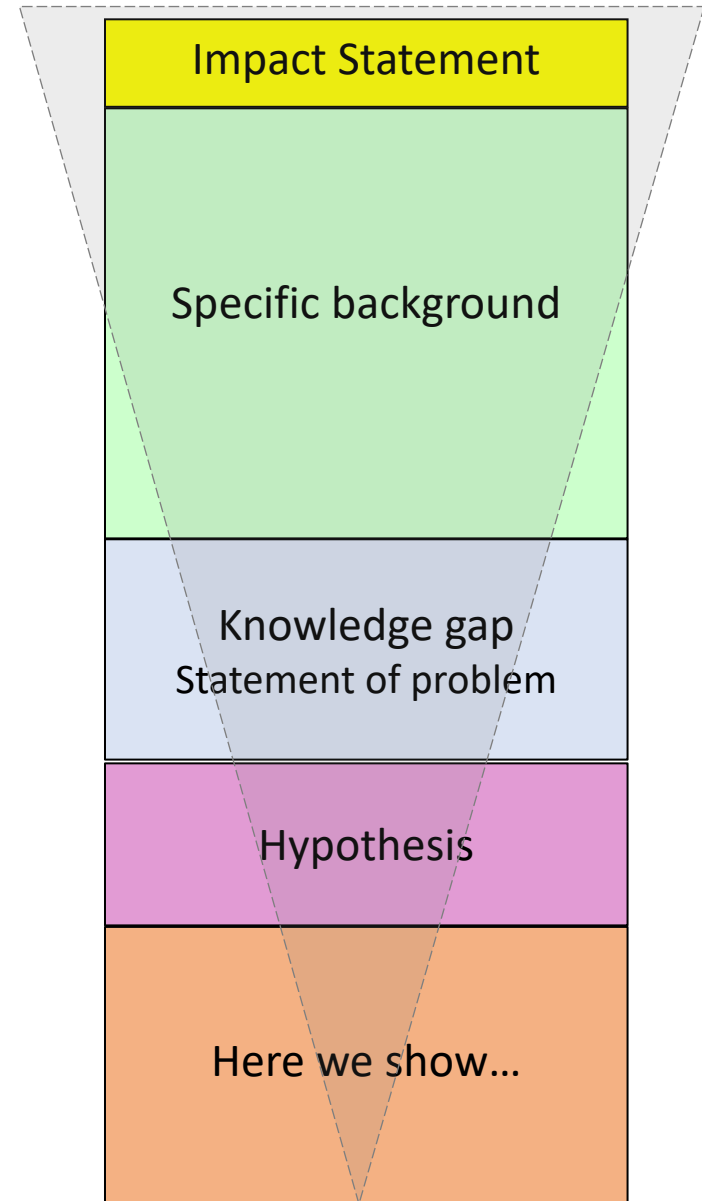
How should you introduce your story?

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...