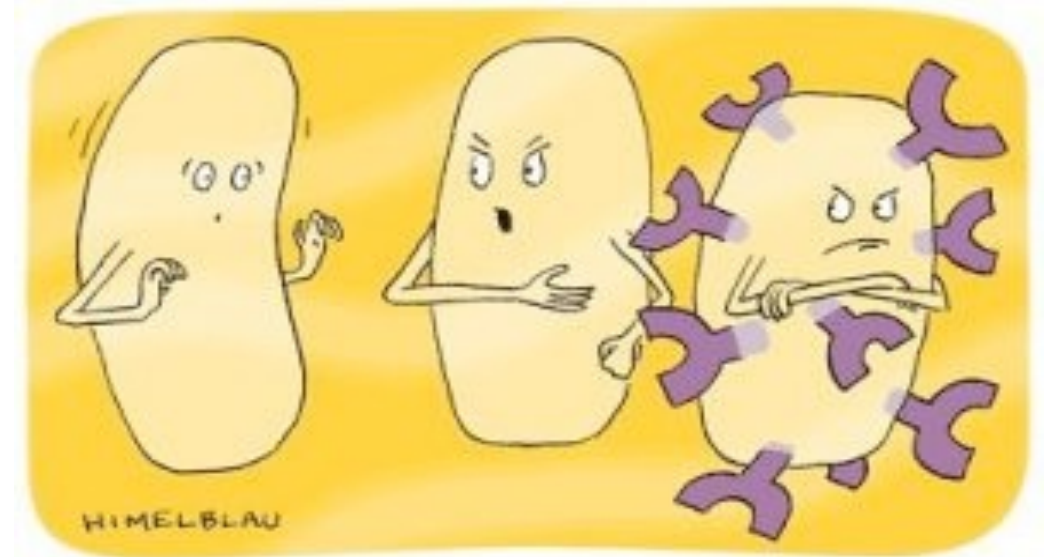


# M1D3: Induce and purify TDP43 protein

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!

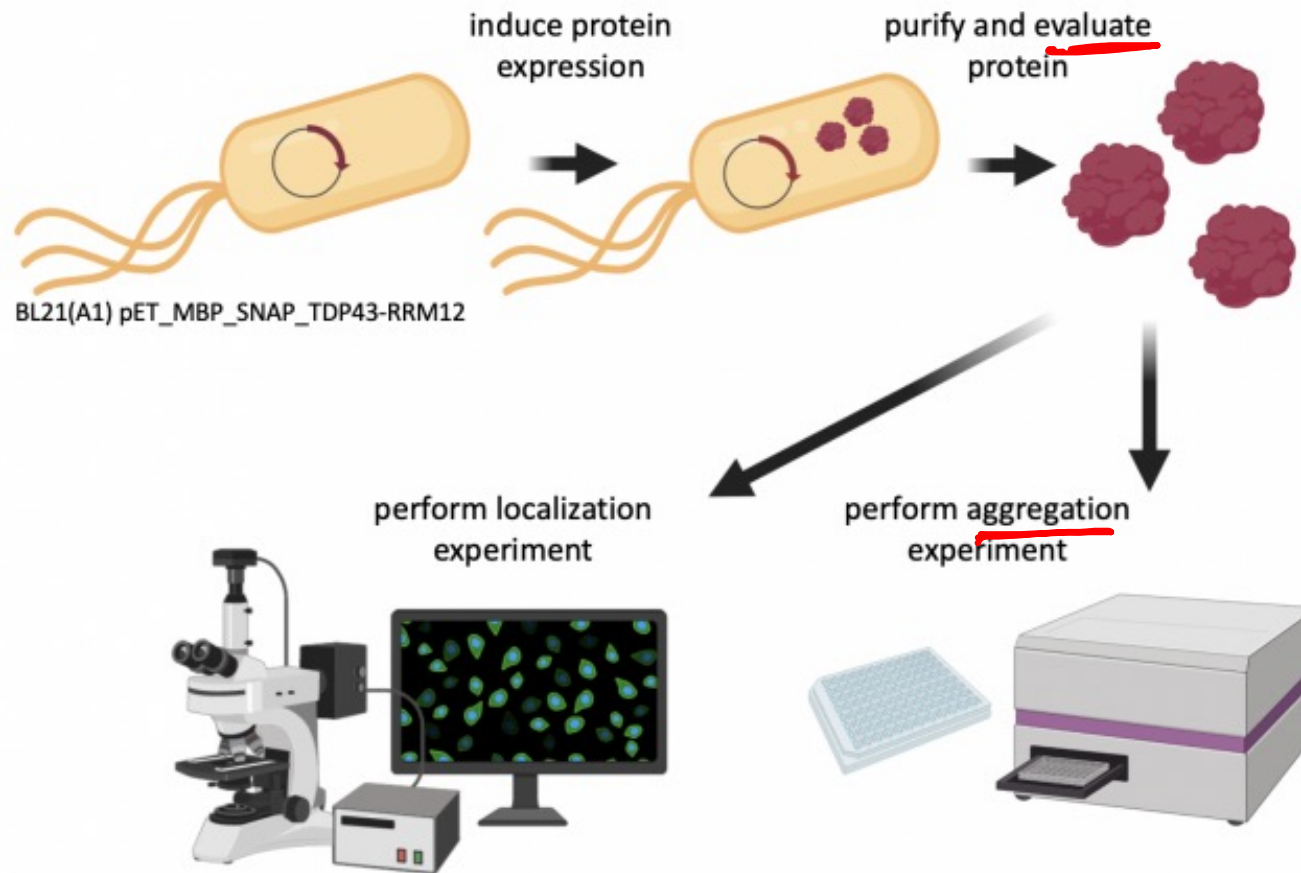
# Get started on protein purification

Post 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

*Transformation  
Bacteria*

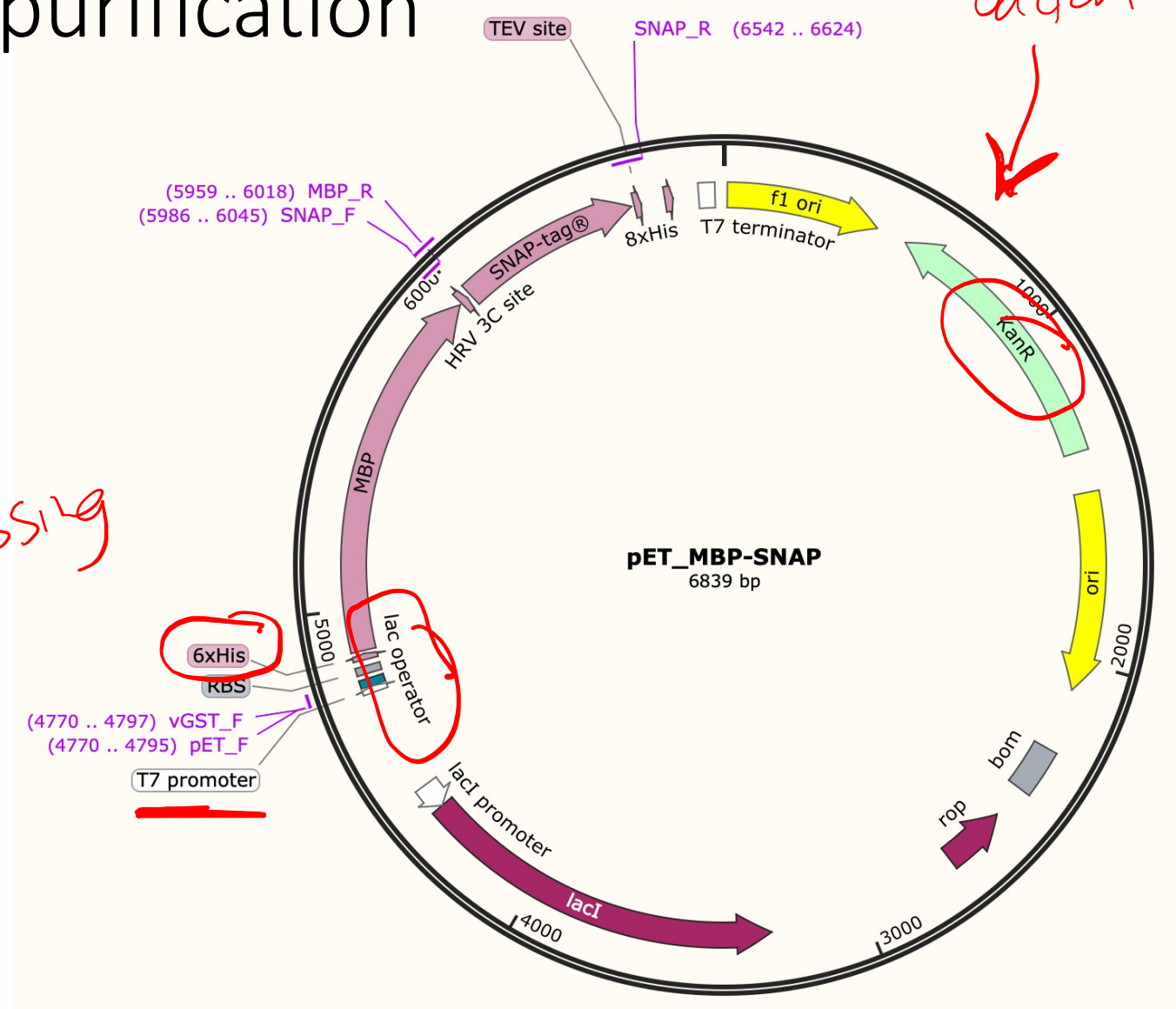
## Induction

- T7 promoter
- Lac operator
- Kanamycin

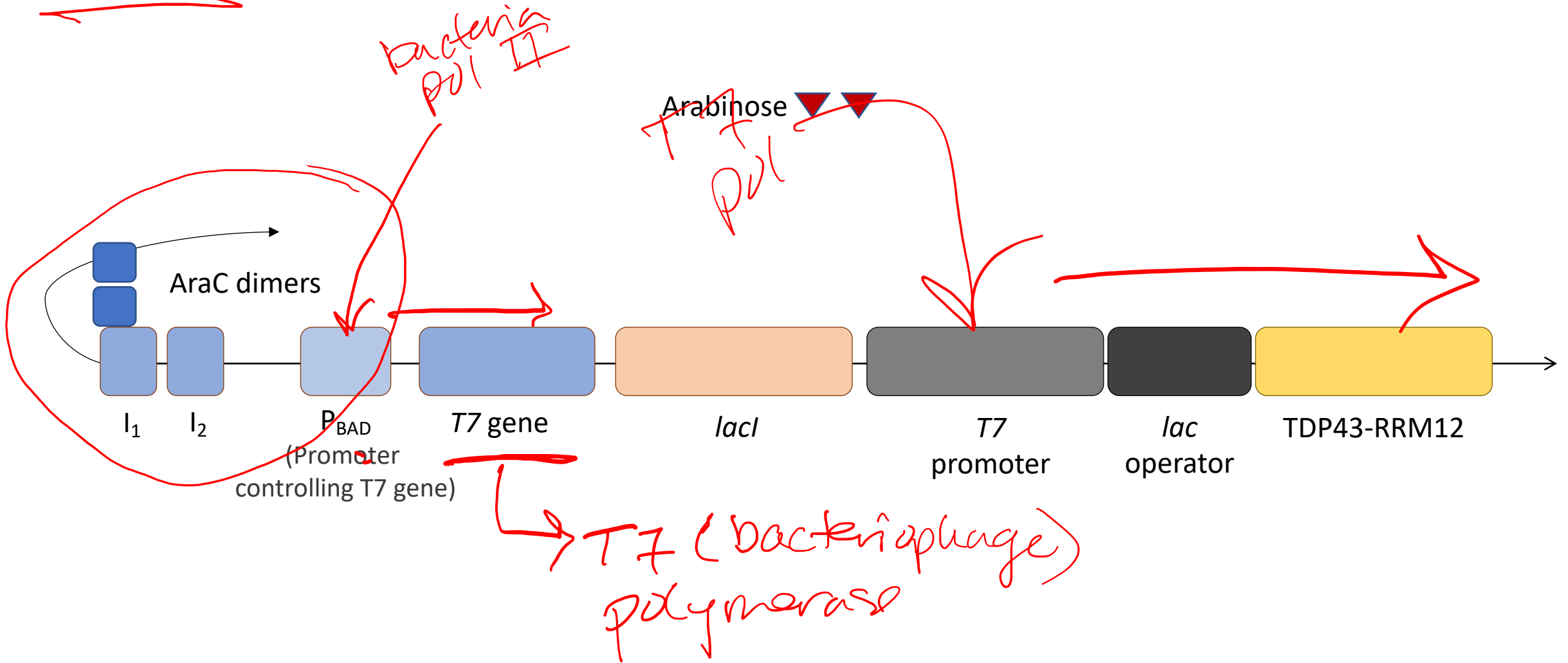
*- selection  
- select for  
bacteria expressing  
plasmid*

## Purification

- His-tag

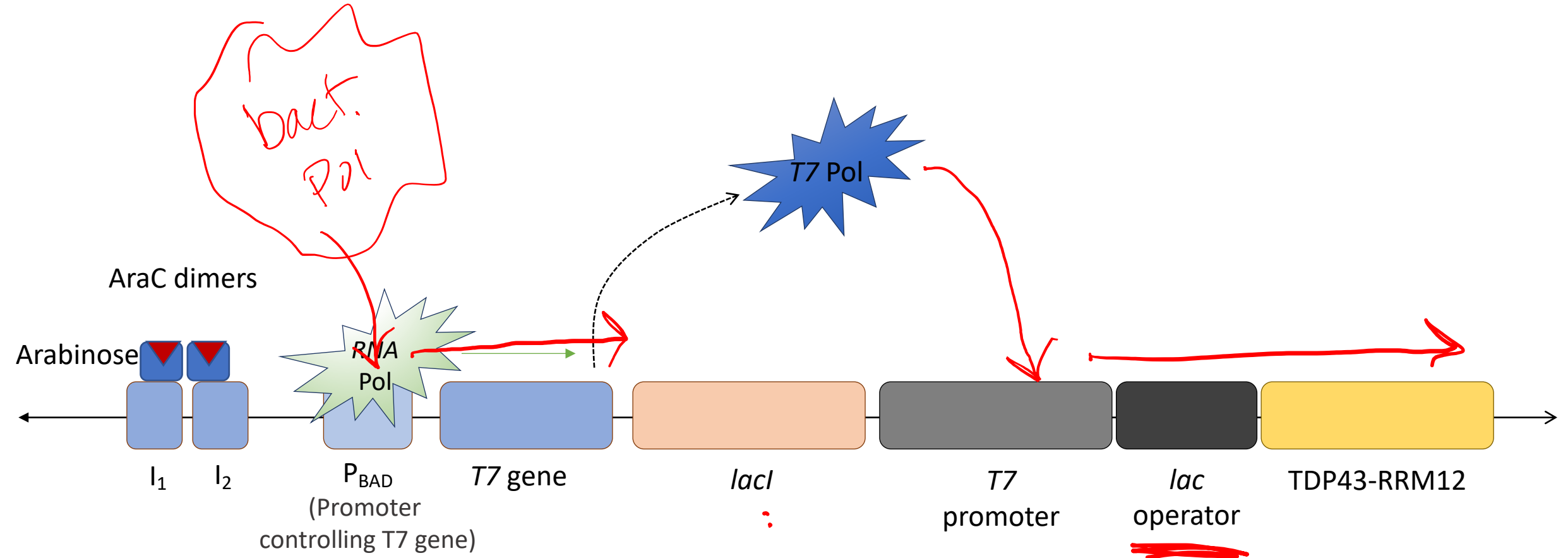


# Bacterial induction: How it begins...



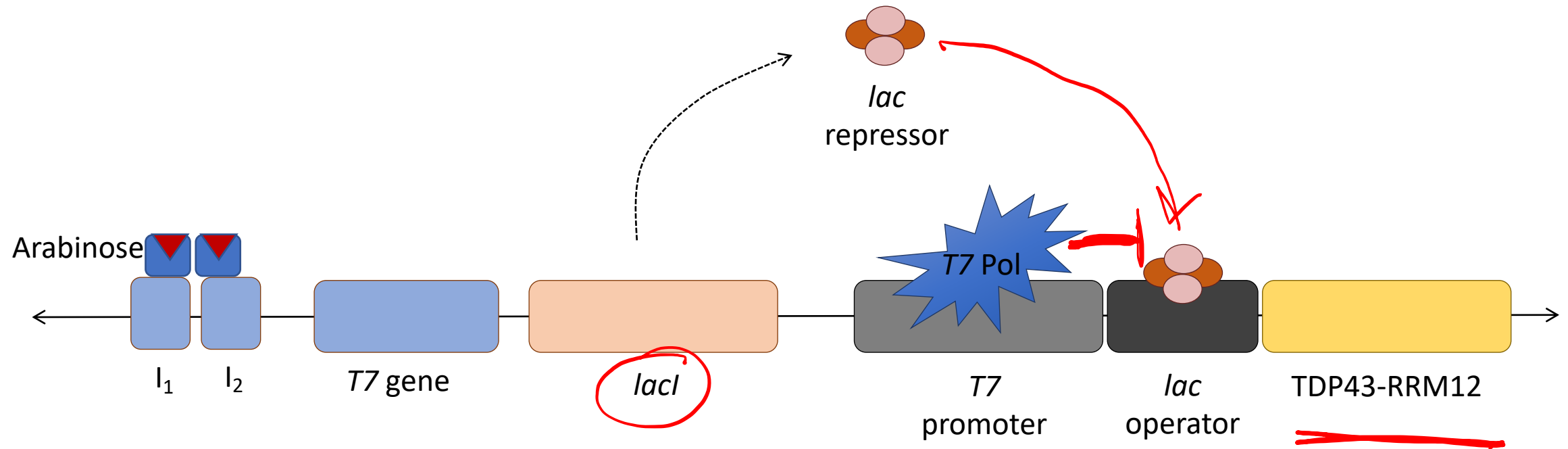
pET\_MBP\_SNAP\_TDP43-RRM12

# Bacterial induction: Arabinose controls T7 expression



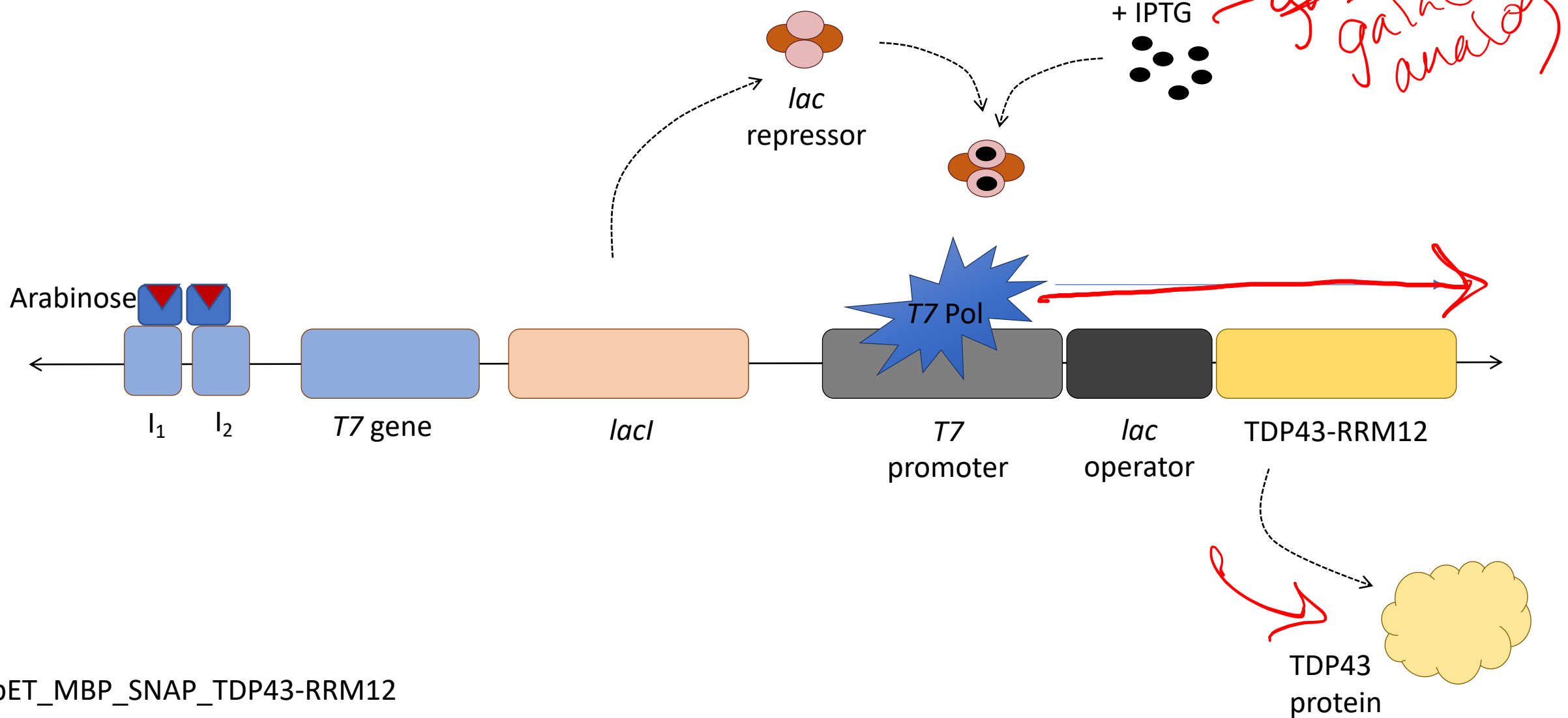
pET\_MBP\_SNAP\_TDP43-RRM12

# Bacterial induction: Lac repressor



pET\_MBP\_SNAP\_TDP43-RRM12

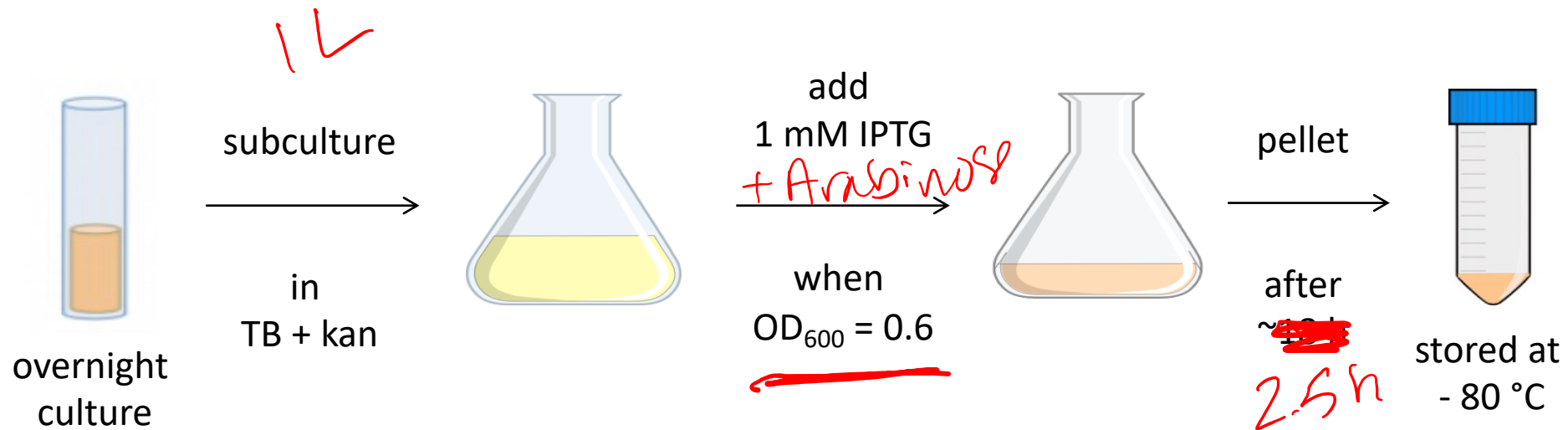
# Bacterial induction: IPTG removes lac repression



pET\_MBP\_SNAP\_TDP43-RRM12

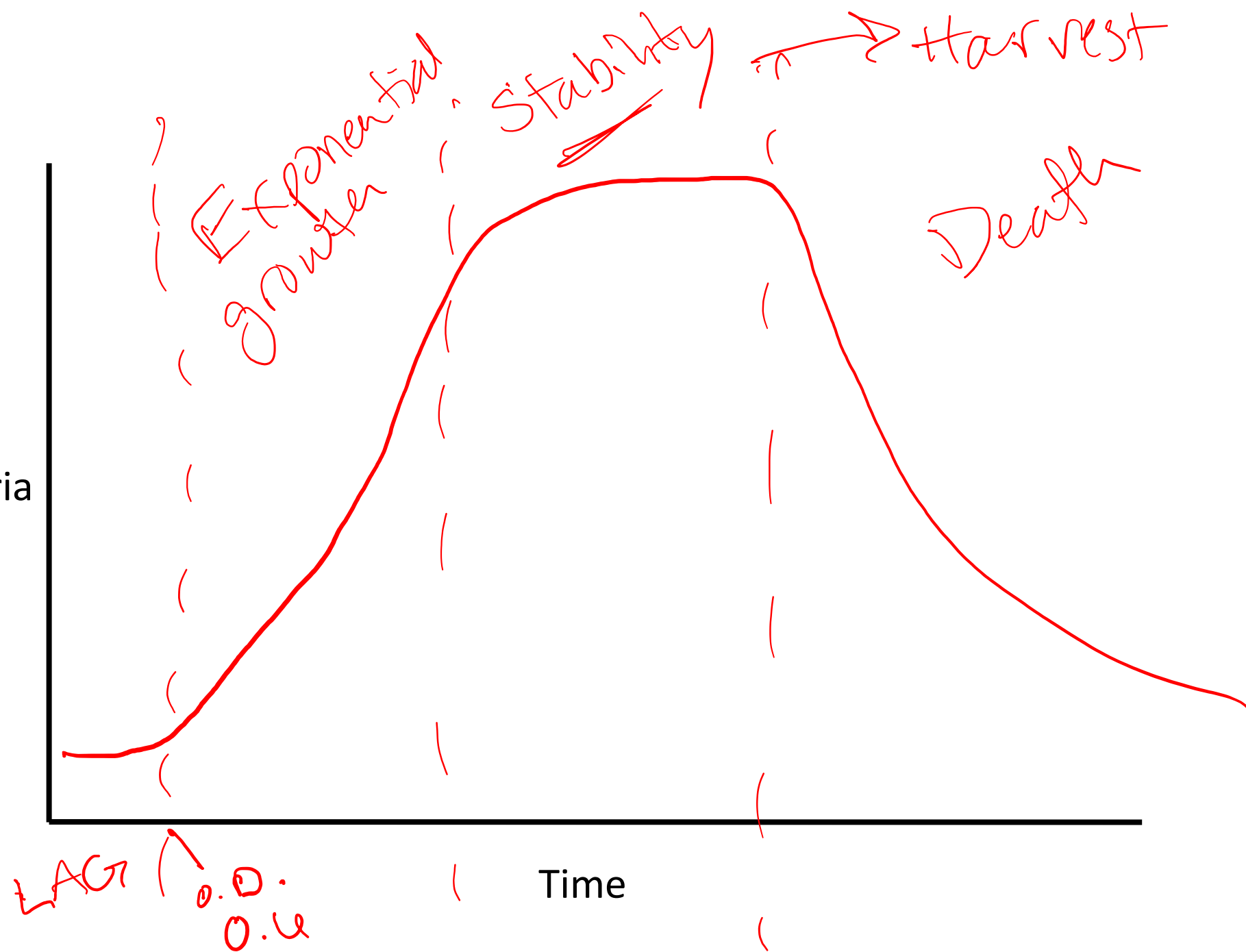


# How do we induce protein expression?



Why do we induce protein expression at OD<sub>600</sub> = 0.6?

# of bacteria



Time

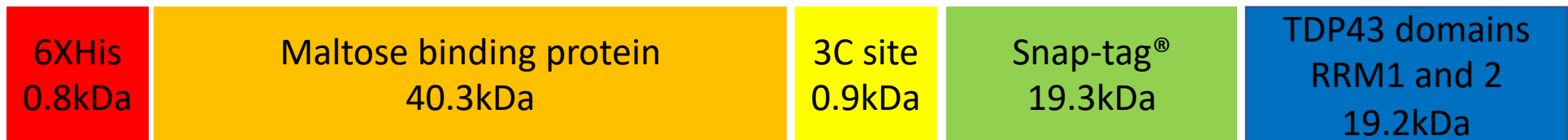
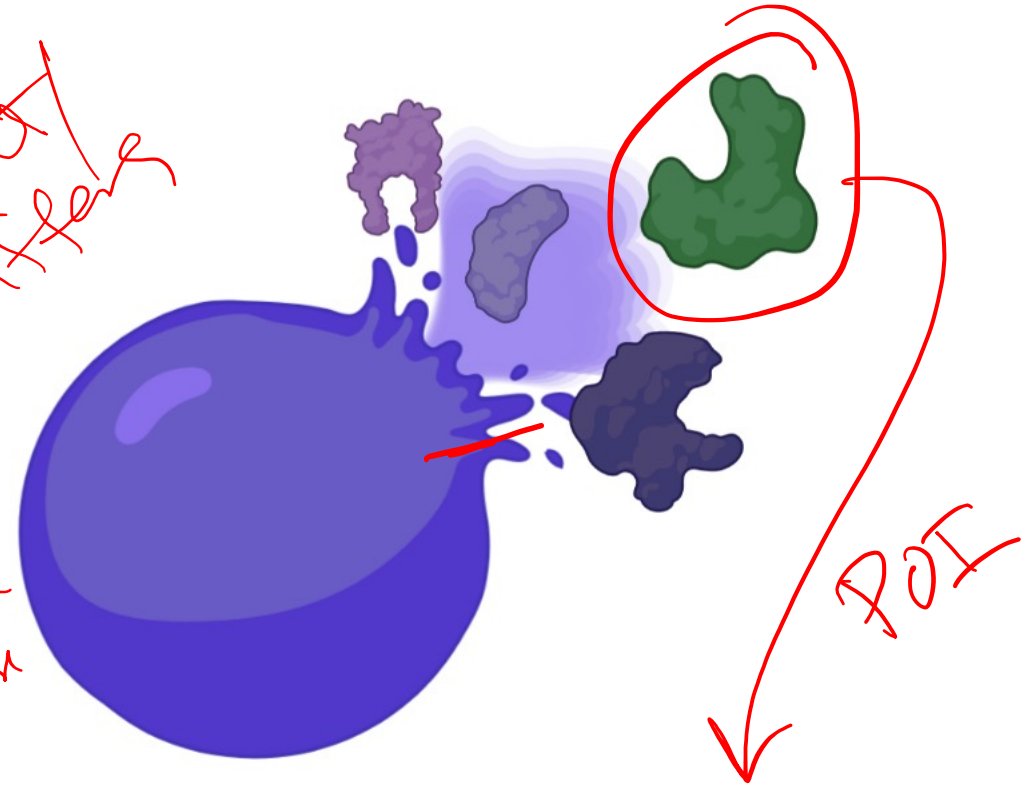
# How will you purify TDP43\_RRM12?

First, need to lyse cells to release proteins:

- B-PER bacterial extraction reagent
- Lysozyme — *Lysozyme + Benzonase*  
*↳ break cell wall*
- Protease Inhibitor Cocktail  
*↳ keep protein from degrading*

*→ Detergent  
Buffers*

*DNase  
RNase*



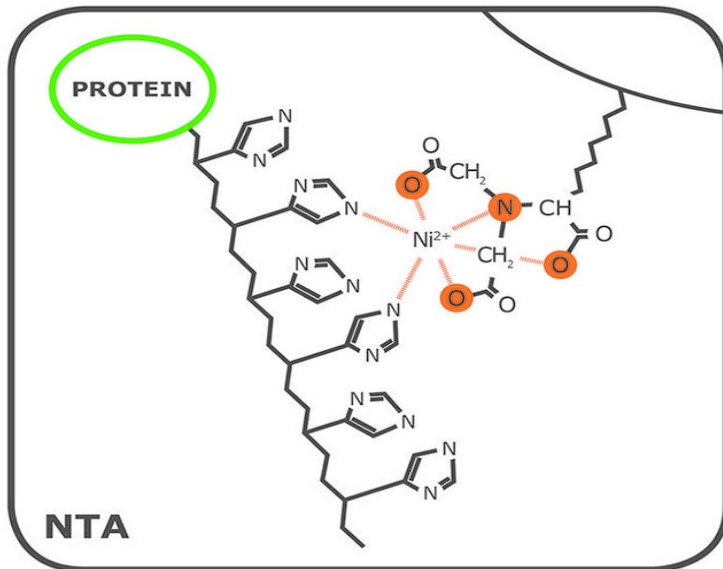
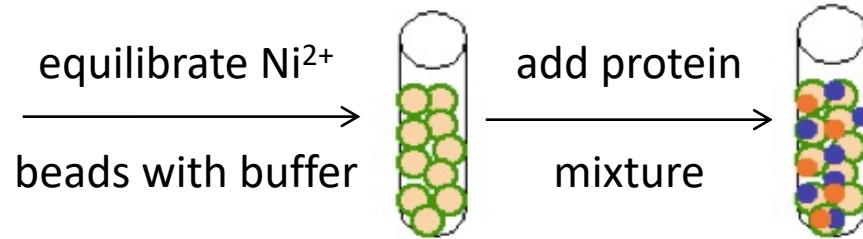
*MBP*

*HRV  
3C*

*Alexa 647*

Recombinant protein of TDP43\_RRM12

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

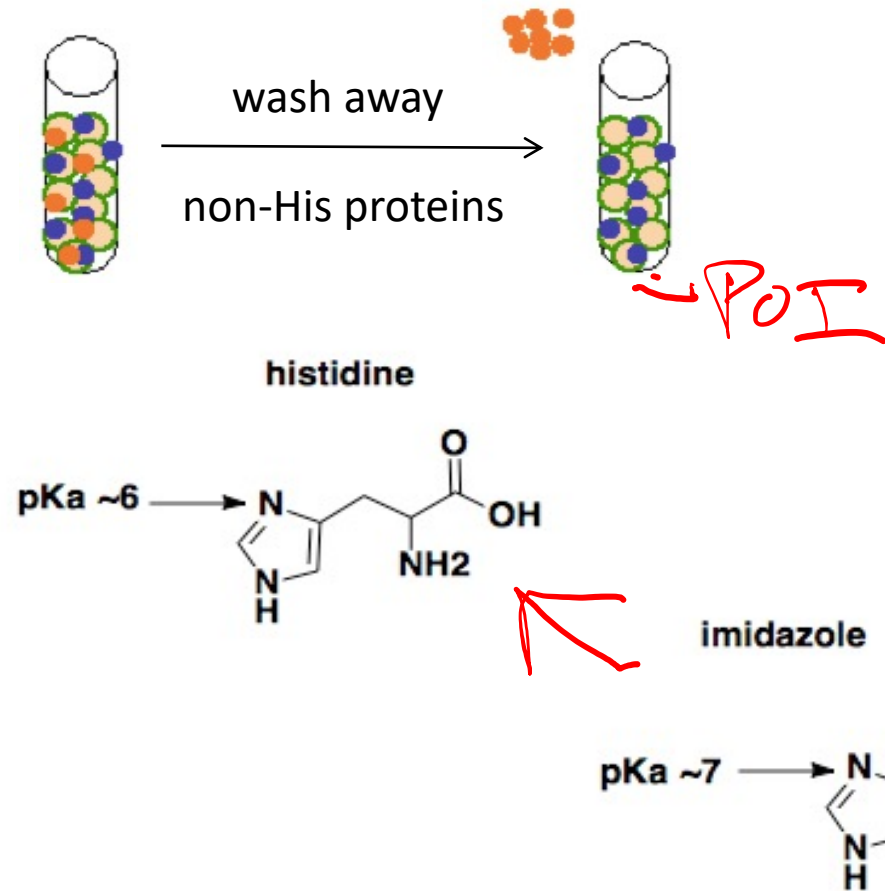


- $\text{Ni}^{2+}$  chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand

- His tag chelates to  $\text{Ni}^{2+}$  causing protein to 'stick' to resin / column

↳ 6x His tag

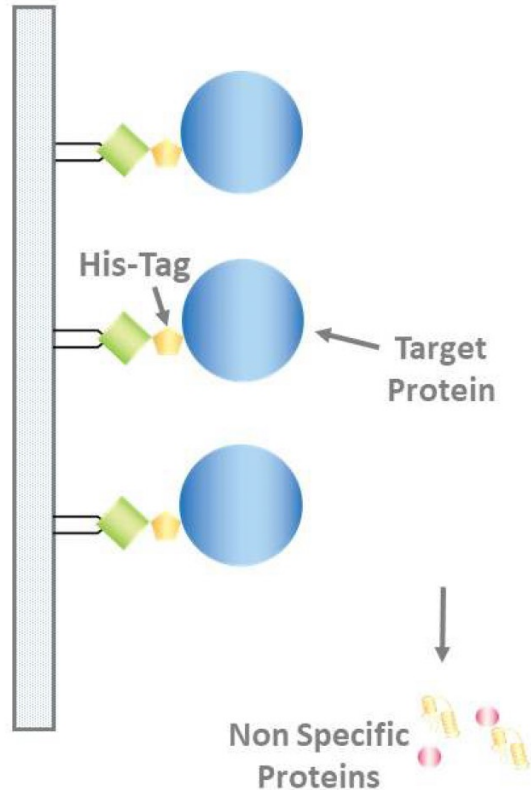
# Non-specific binders washed from Ni<sup>2+</sup> resin / column using a low concentration of imidazole



- Low concentration of imidazole included in wash buffer
- Imidazole competes for binding to Ni<sup>2+</sup> 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<sup>2+</sup> resin / column

Binding:

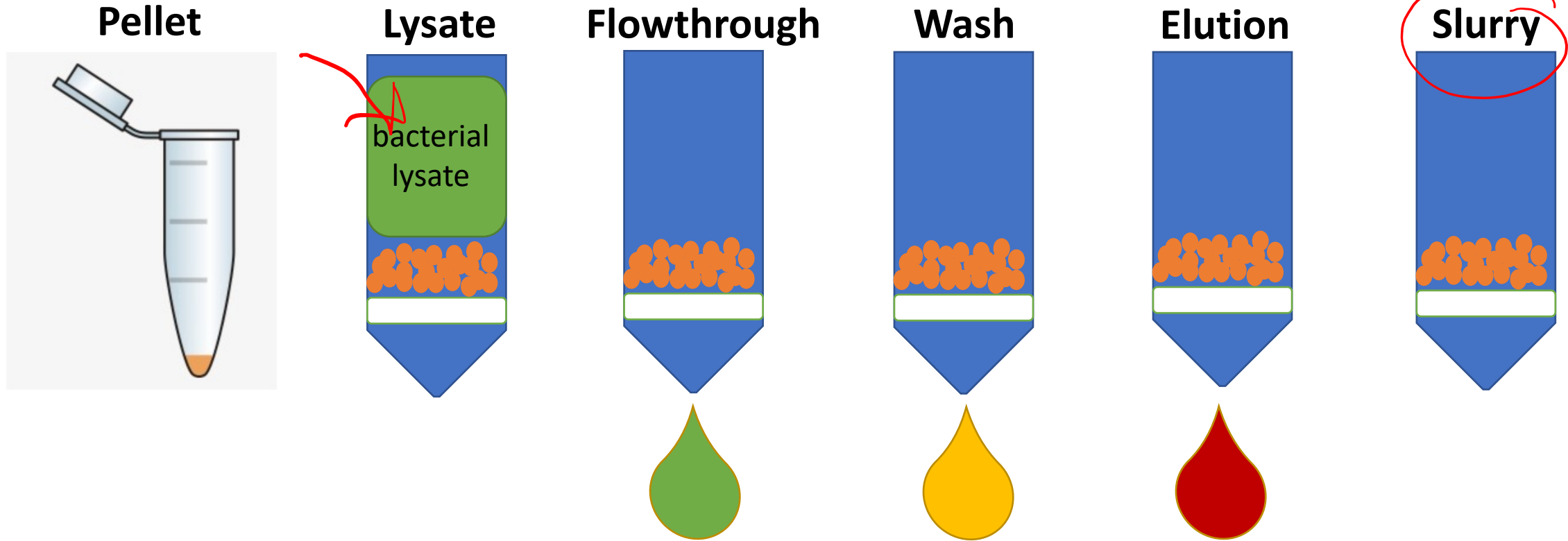


Elution:



- 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

# Purification process (and where you will save samples)



## 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

paragraph

## Include enough information to replicate the experiment

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

— bottom of wiki

## 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

~~MIQ3~~

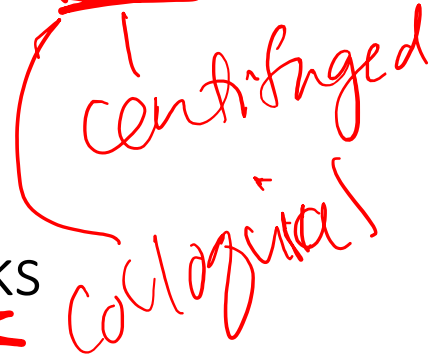
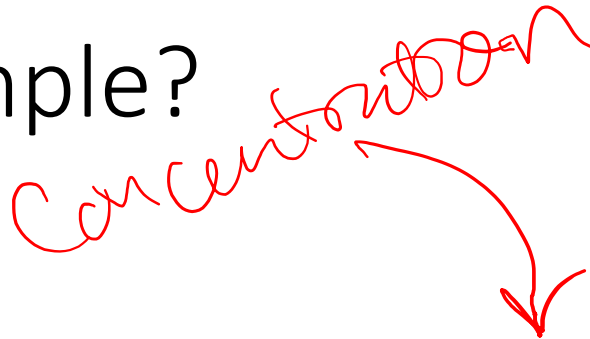
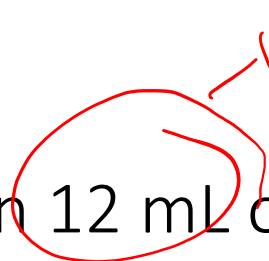
- Include parts of the protocol that the teaching faculty completed, but do not say “completed by teaching faculty.”

# How can you improve this example?

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

down the cells and counted them with a hemocytometer.

were incubated in 37 C incubator."



Flasks



UP 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?