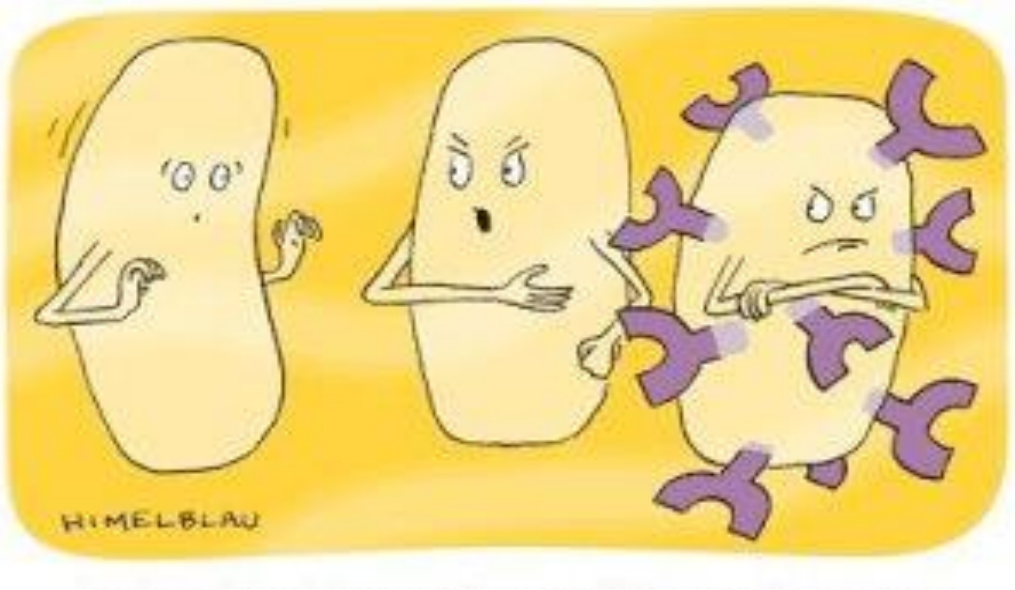
M2D2: Perform protein purification protocol

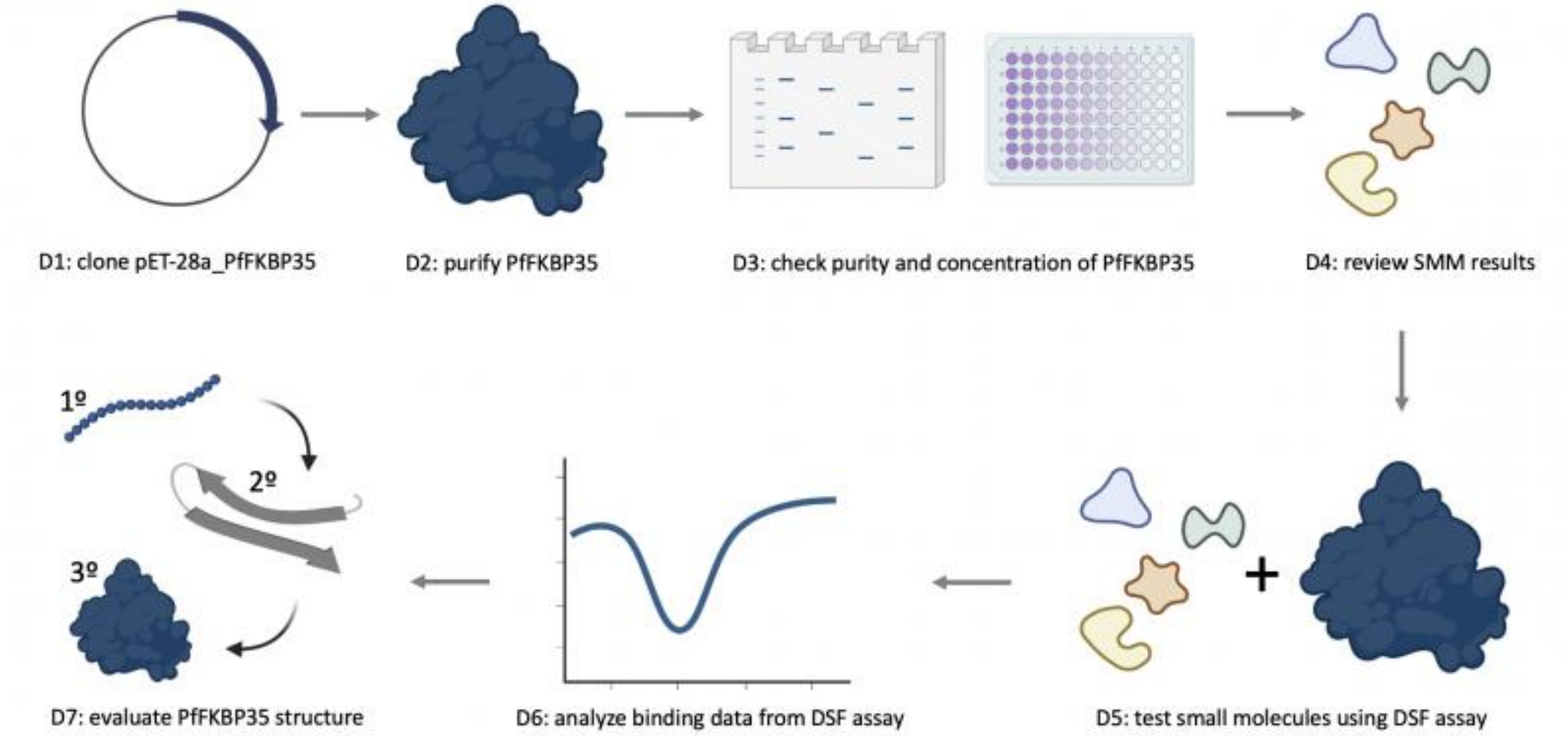
1. Prelab discussion
2. Protein purification
3. Assess RE digest



"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!

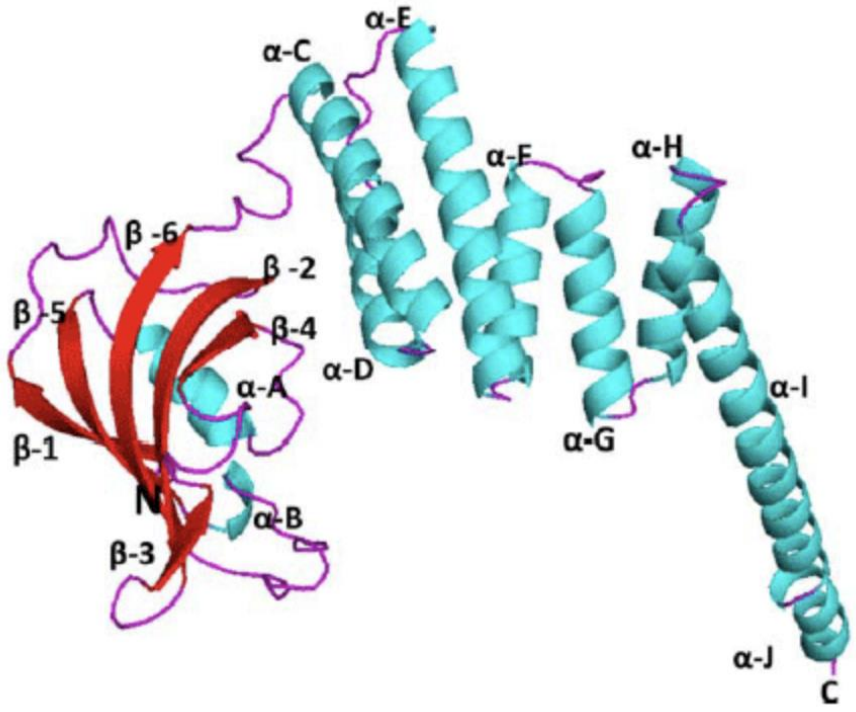
Overview of M2: drug discovery

Research goal: Test small molecules for binding to the *Plasmodium falciparum* FKBP35 protein using a functional assay.



PfFKBP35

- Pf = Plasmodium falciparum
- Essential to parasite survival
- Known drug target for:
  - Rapamycin
  - FK506
- Problem: Has a human ortholog in FKBP12
- How can we target the parasite protein and not the human?



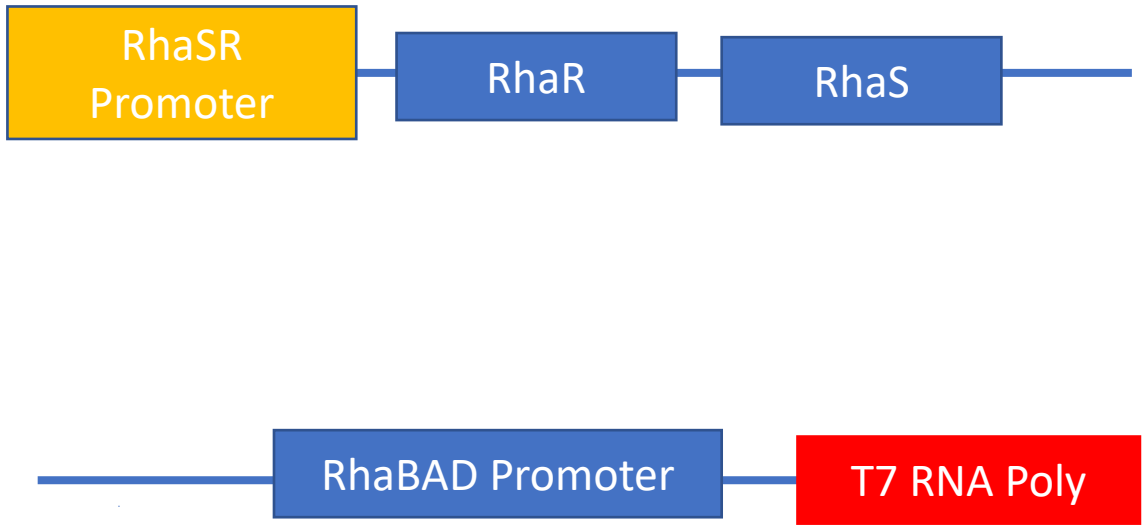
Very Brief Outline of Induction

1) Addition of rhamnose causes Genomic Rhamnose Operon to make T7 RNA Polymerase

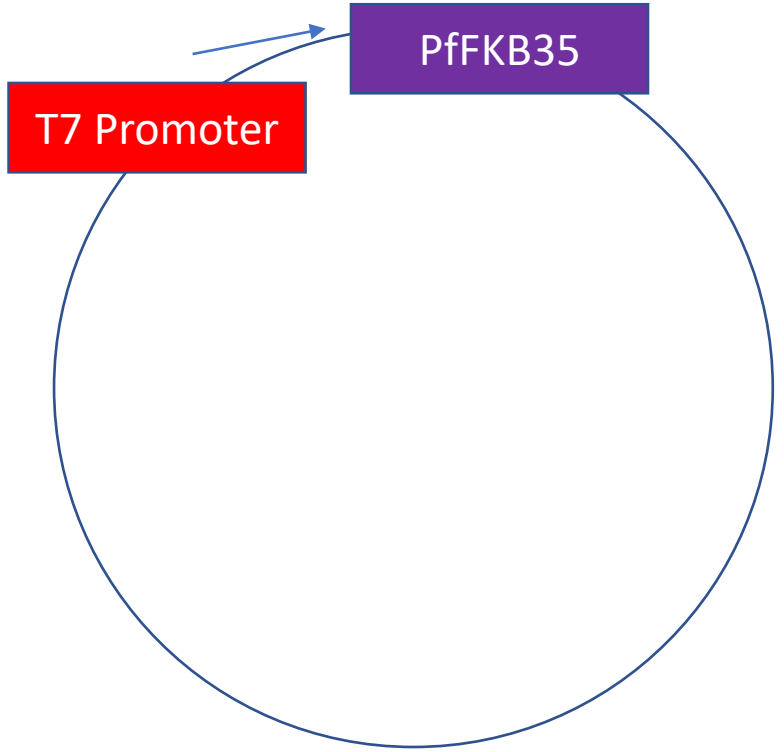
2) T7 RNA Polymerase binds T7 promoter and makes our protein

Bacterial induction in ***our*** construct

1) Genomic Rhamnose operon

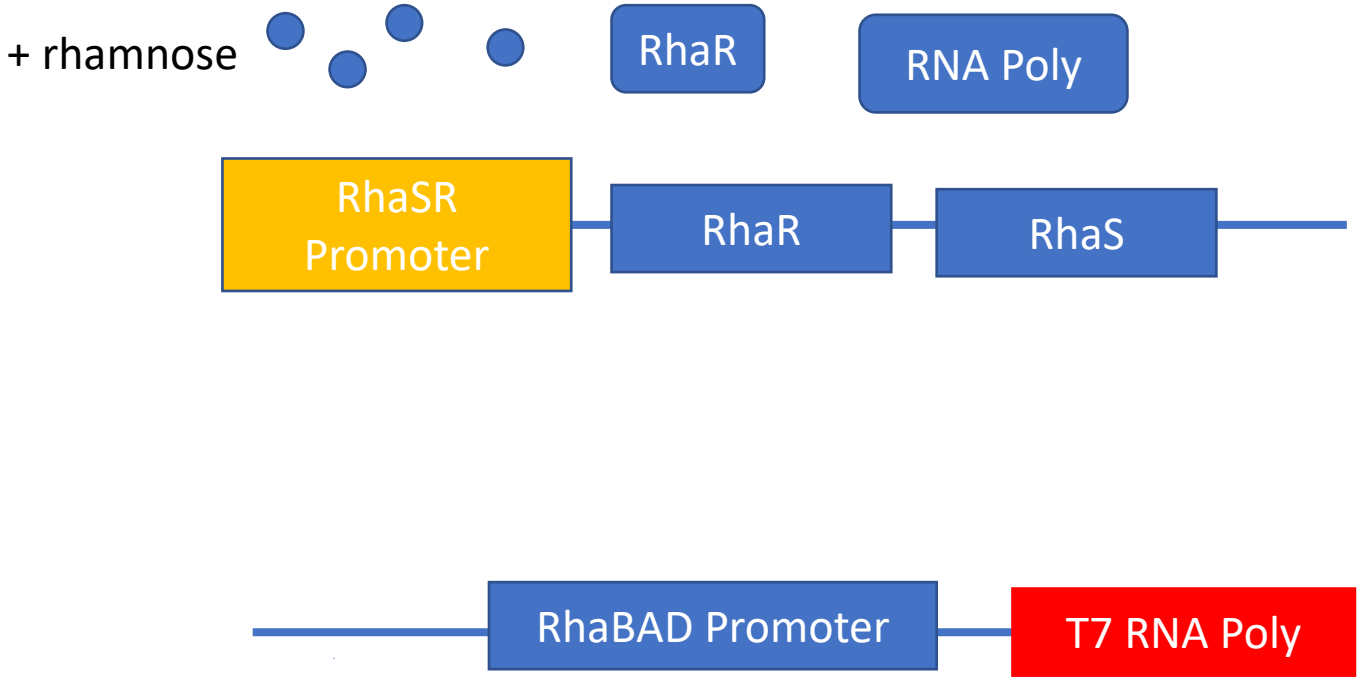


2) Pet28a-PfFKB35



Bacterial induction in ***our*** construct

1) Genomic Rhamnose operon



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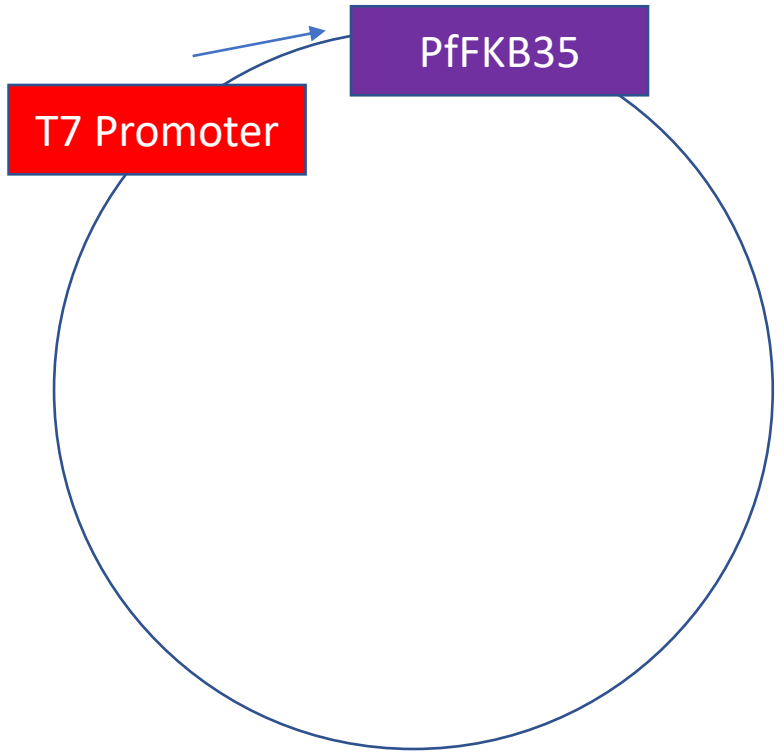
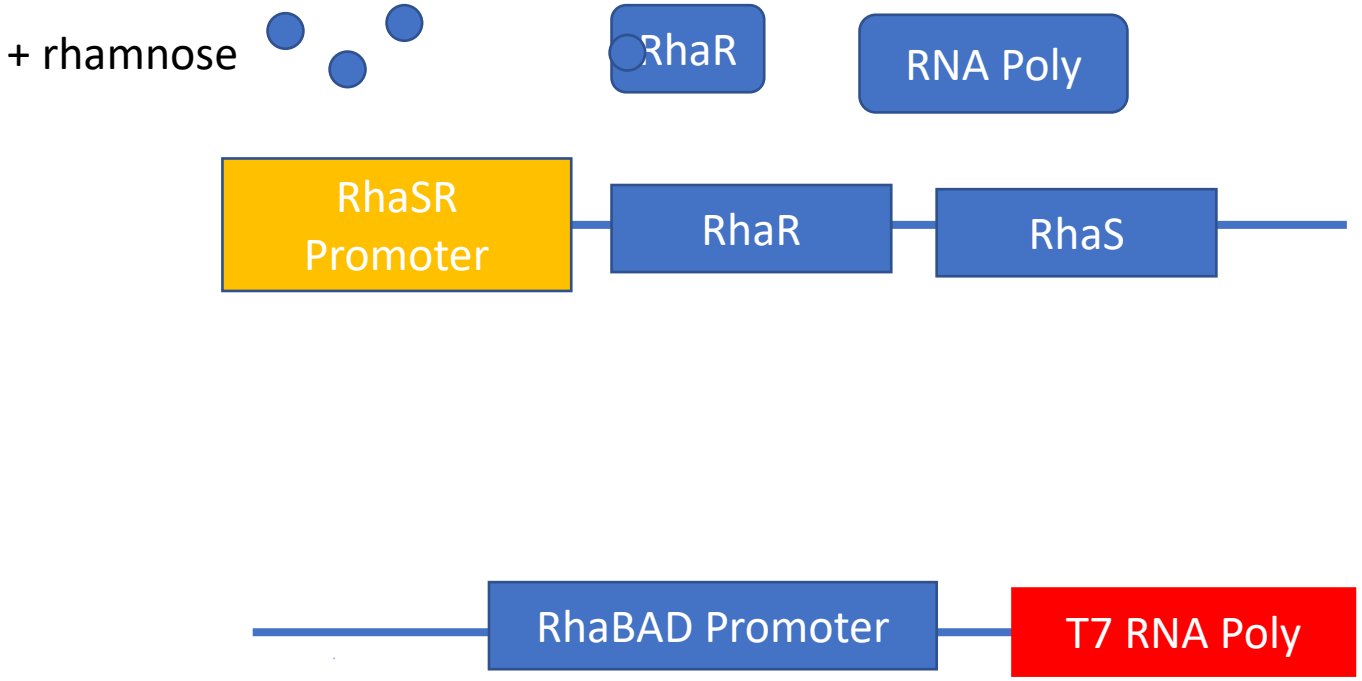


Bacterial induction in ***our*** construct

1) Genomic Rhamnose operon



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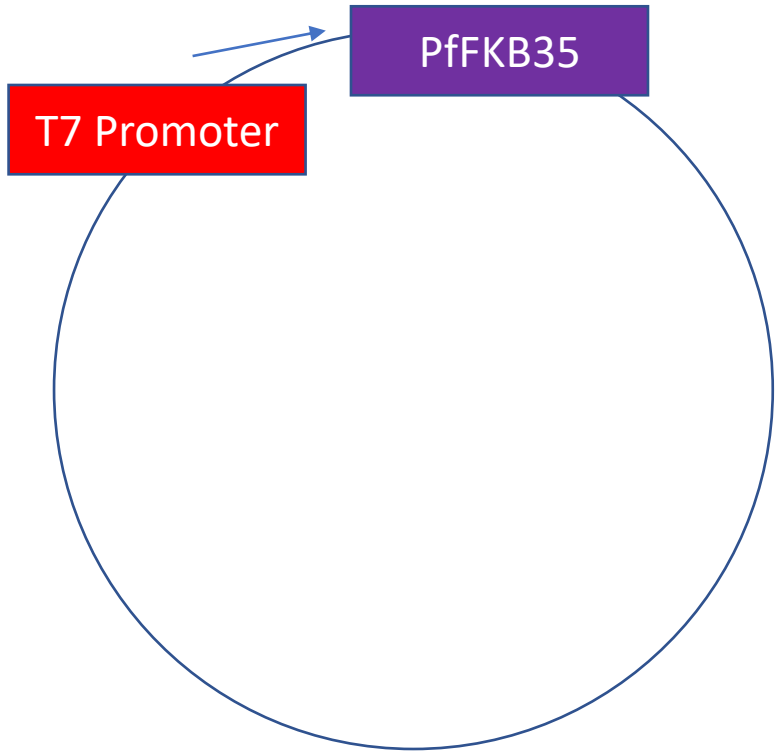

Bacterial induction in ***our*** construct

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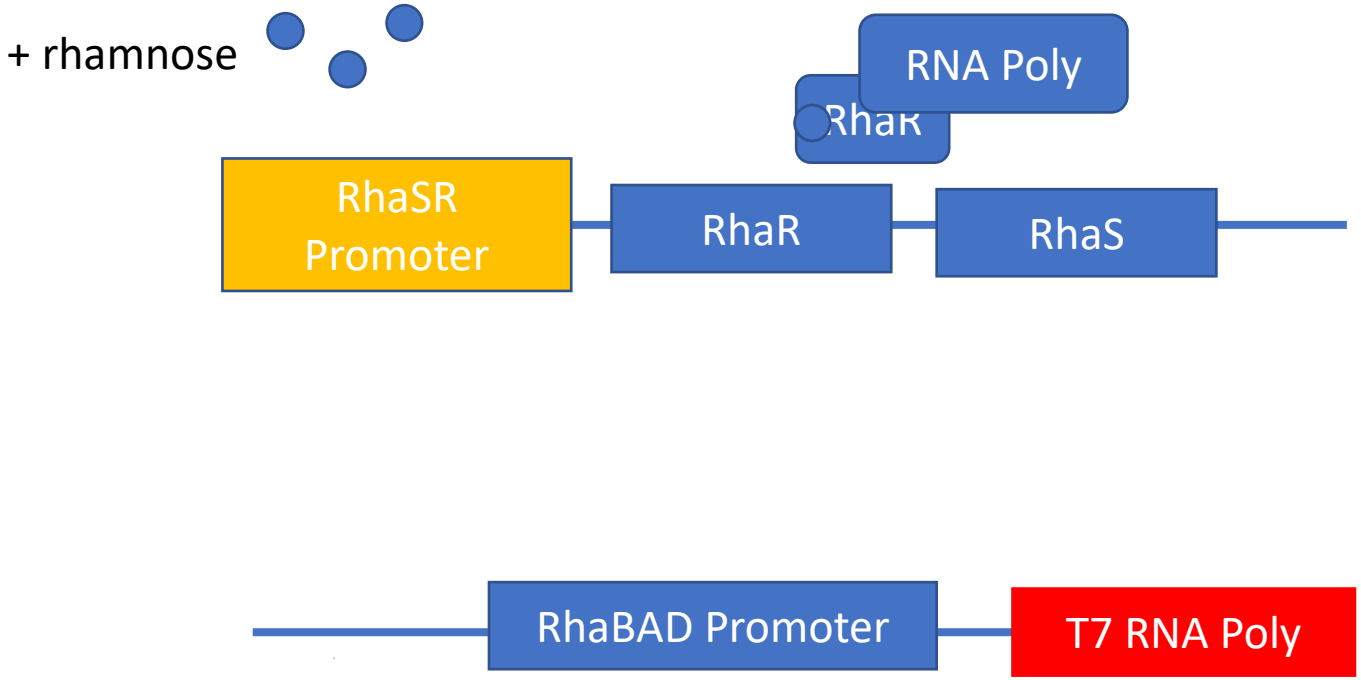

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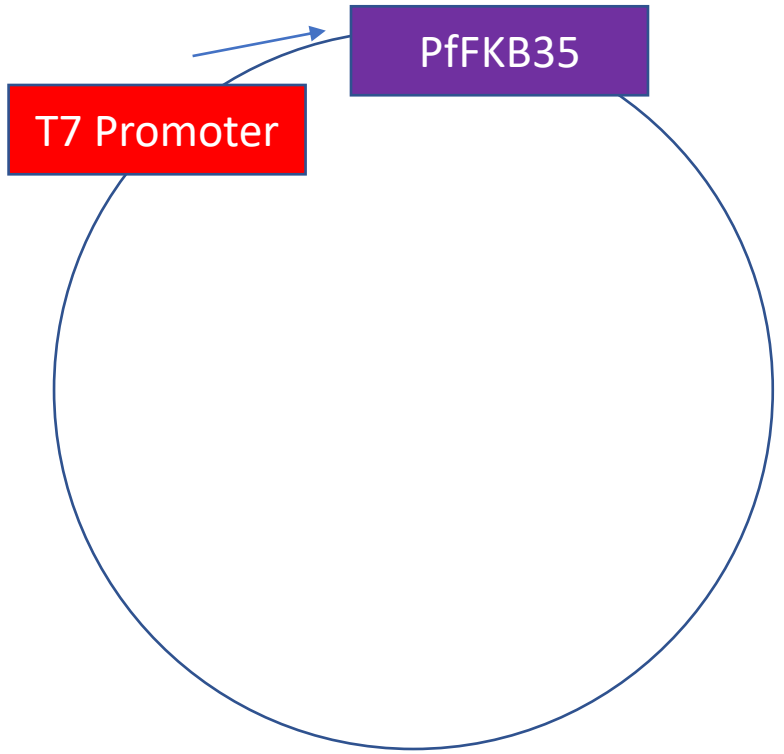

Bacterial induction in ***our*** construct

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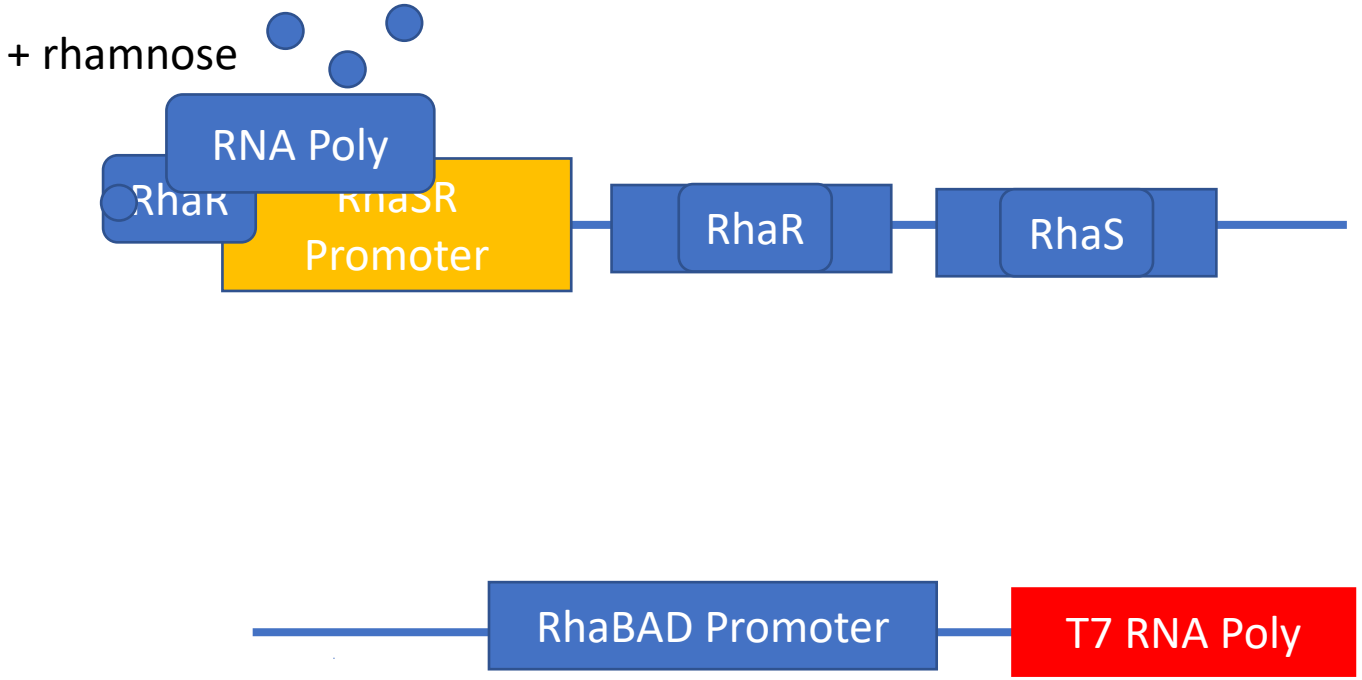

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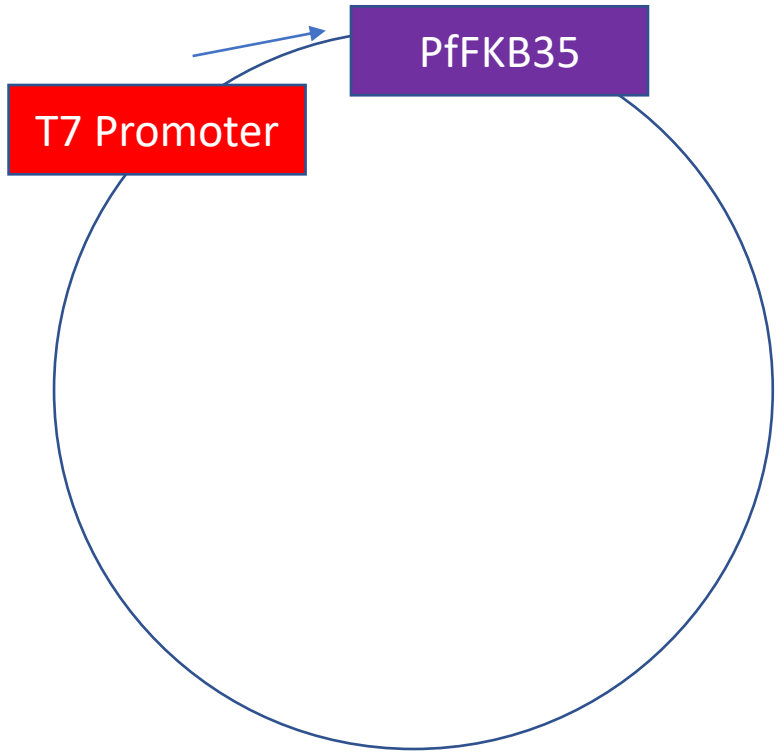

Bacterial induction in ***our*** construct

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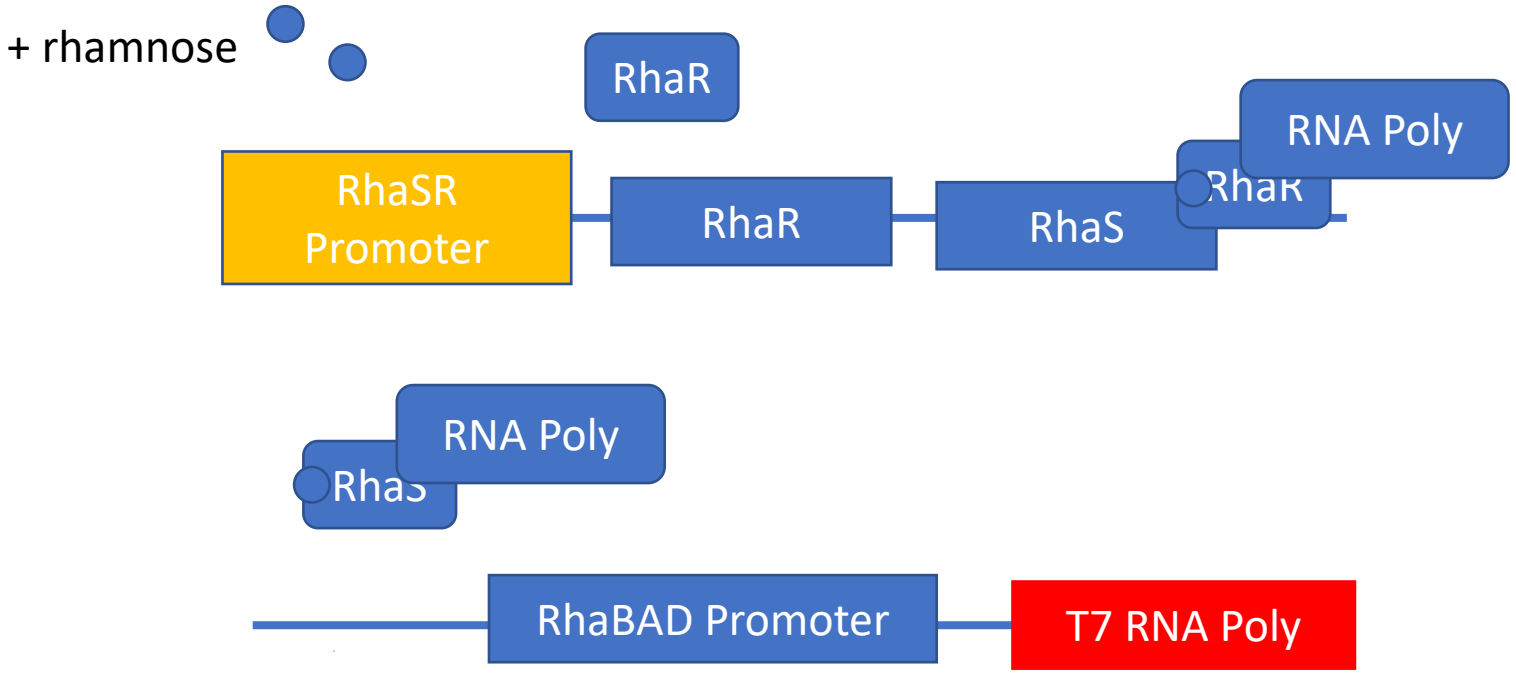

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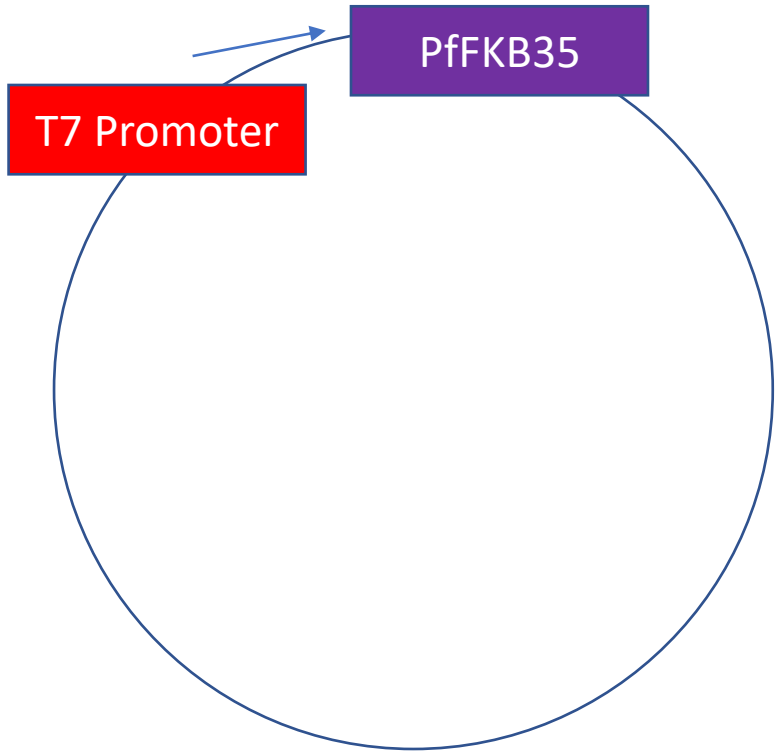
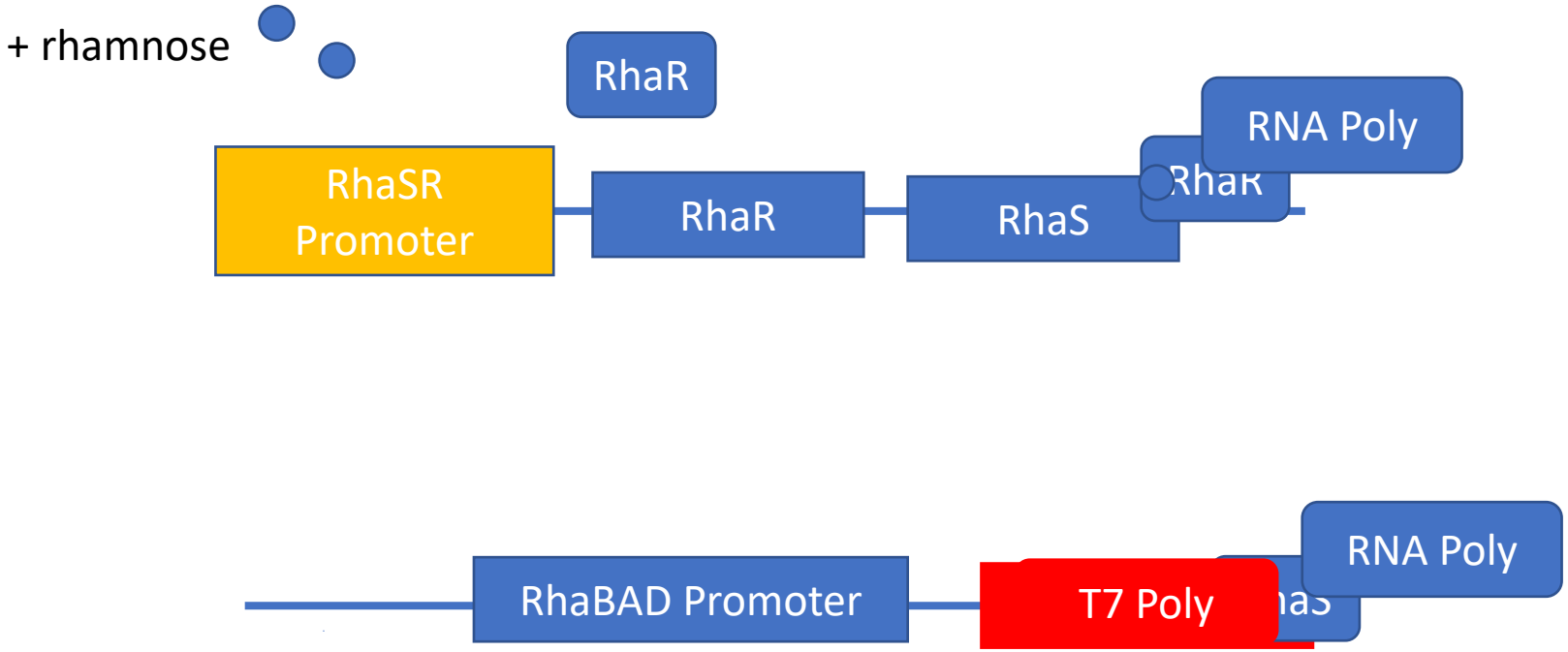


Bacterial induction in ***our*** construct

1) Genomic Rhamnose operon



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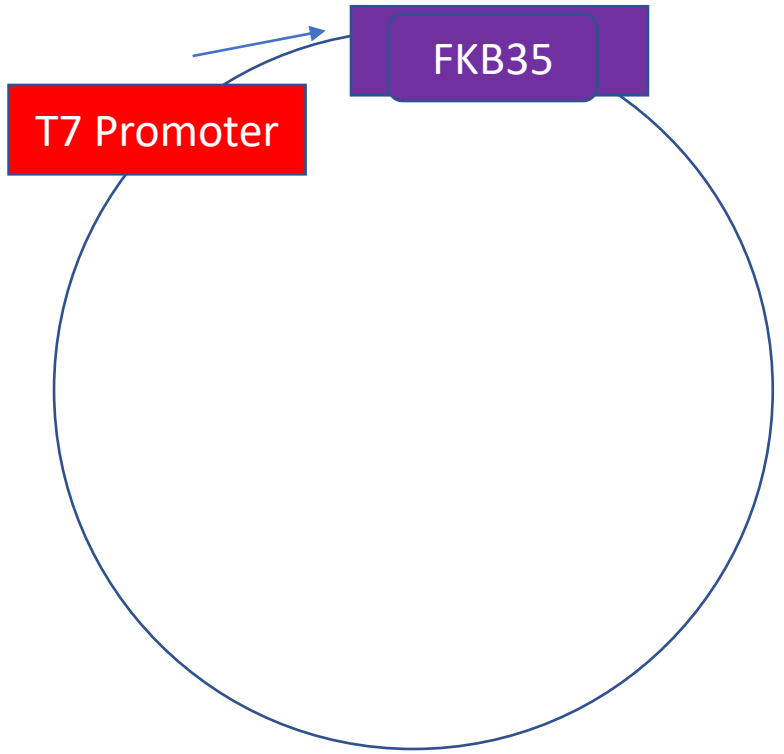
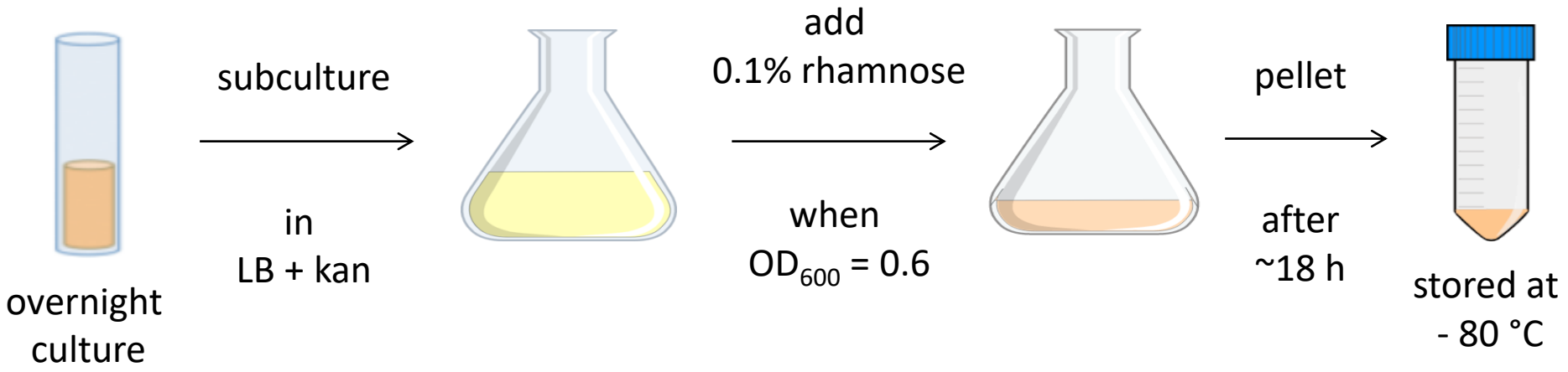


Less Brief Outline of Induction

1) Rhamnose binds RharR protein that recruits RNA poly to the RharSR promoter **in the genome**
2) RharR & RharS get made
3) Rhamnose binds RharS that recruits RNA poly to the RhaBAD promoter
4) RhaBad promoter makes T7 RNA Poly
5) T7 RNA Polymerase binds T7 promoter **on our plasmid** and makes our protein

How do we induce protein expression?

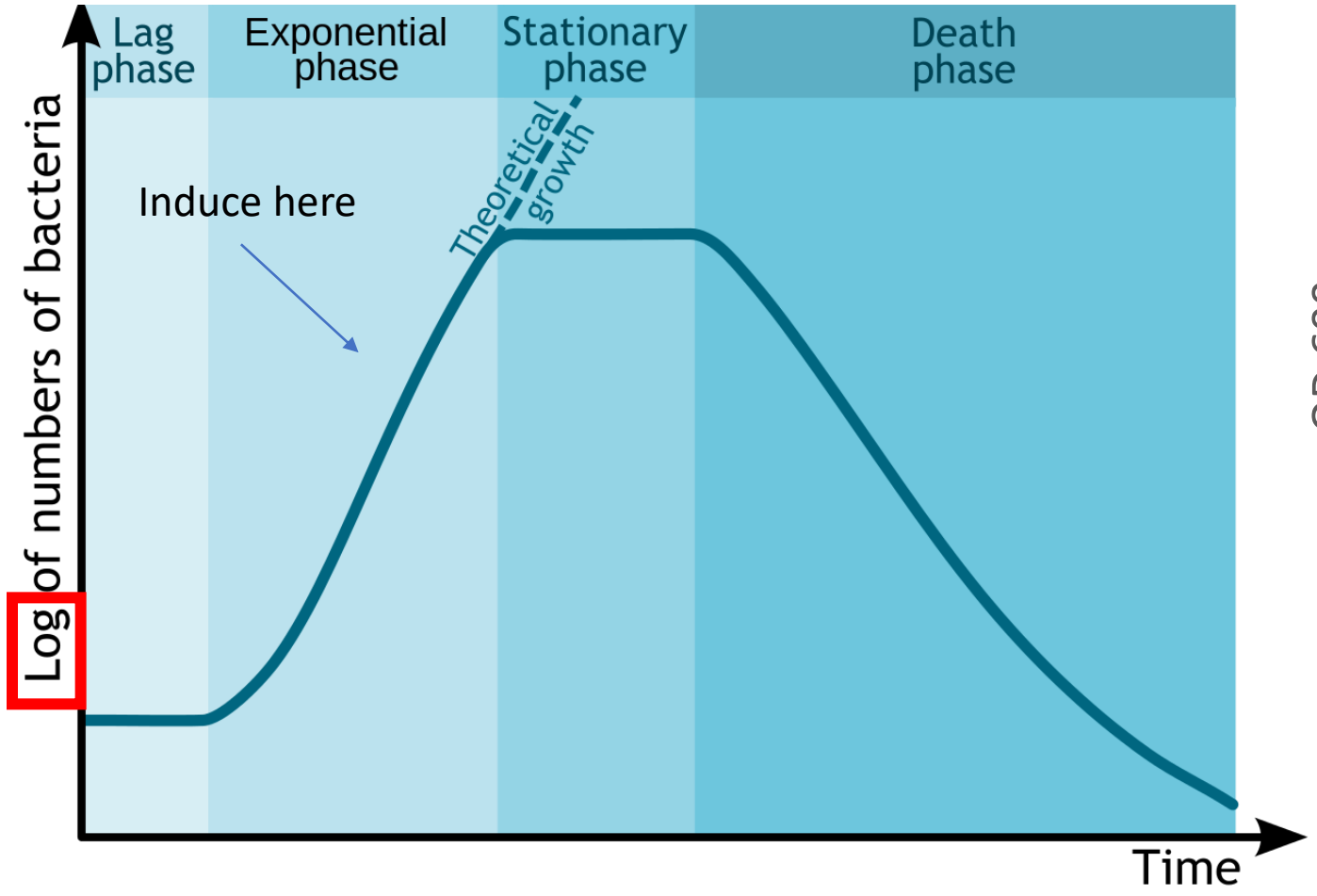


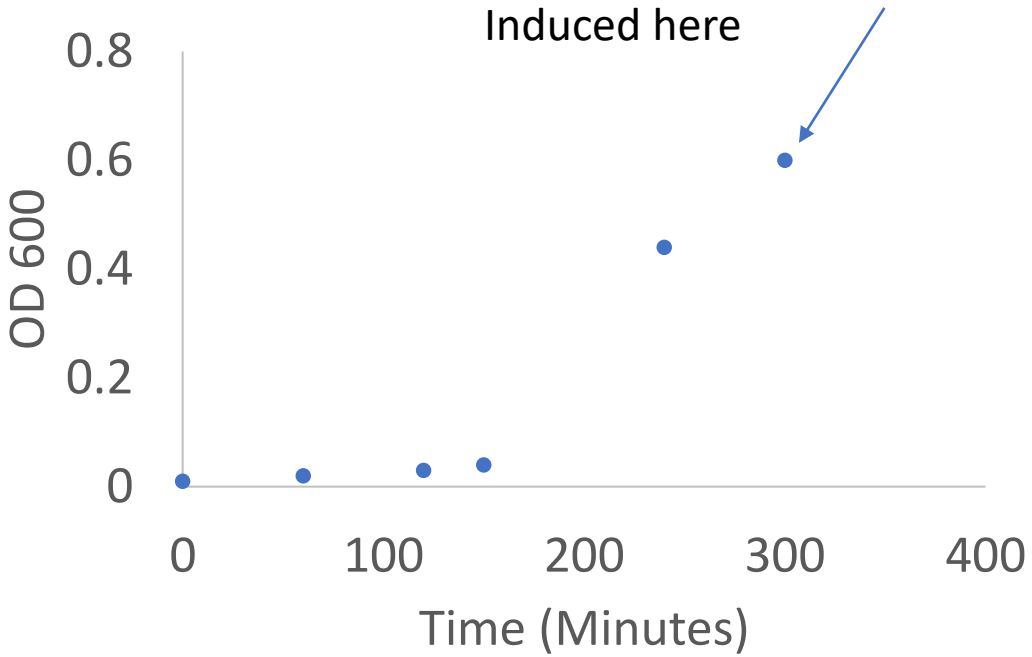
Why do we add kanamycin to our culture?

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

Addition of Rhamnose to induce protein expression occurs during the Exponential/log phase of growth

(Or Log Phase)



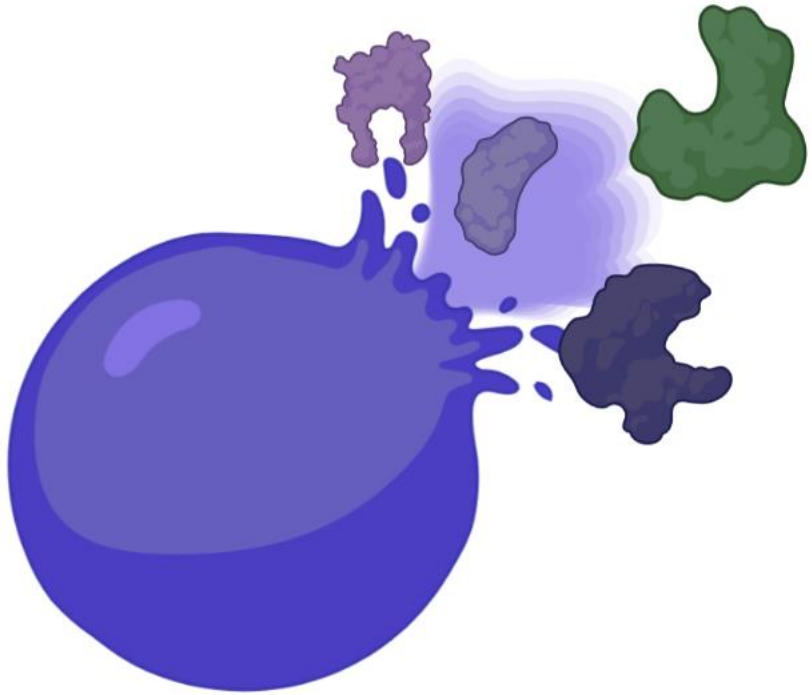


How will you purify PfFKBP35?

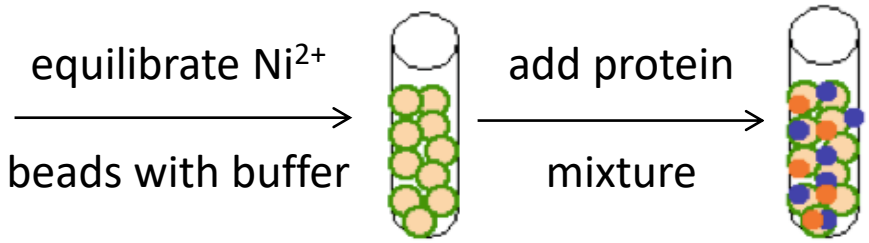
First, need to lyse cells to release proteins:

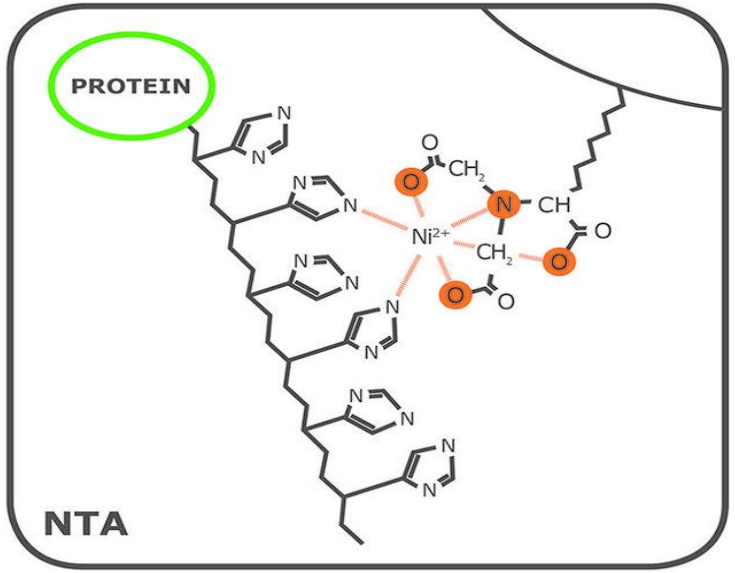
- B-PER bacterial extraction reagent
  - Detergents & Buffers
- Lysozyme + DNaseI
  - Breaks down cell walls, digests DNA
- Protease Inhibitor Cocktail
  - Why?

Preserves our protein



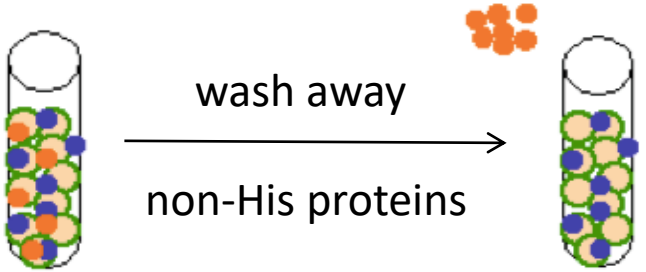
6xHis tag binds to Ni^{2+} resin / column to allow purification of protein of interest via affinity purification

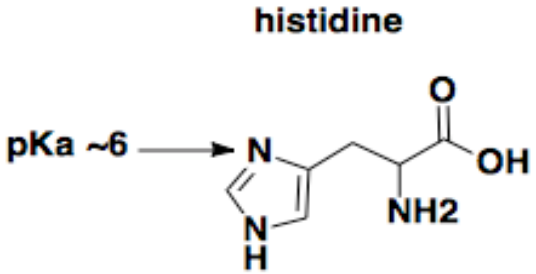


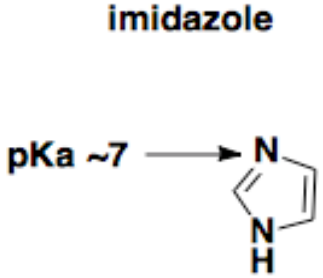


- Ni^{2+} chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand
- His tag chelates to Ni^{2+} causing protein to ‘stick’ to resin / column

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

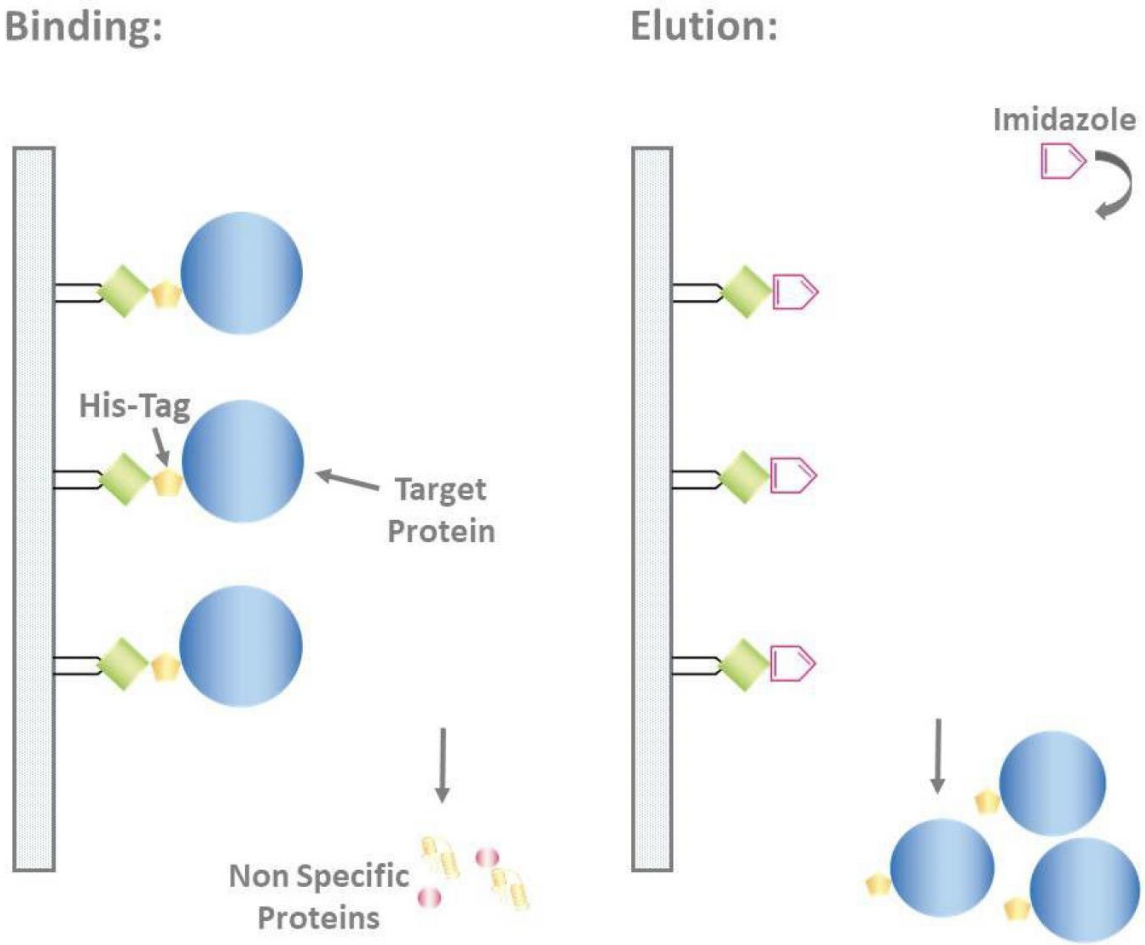






- Low concentration of imidazole included in wash buffer
- Imidazole competes for binding to Ni^{2+} resin
  - Low affinity binders / non-specific binders are outcompeted and released from the resin

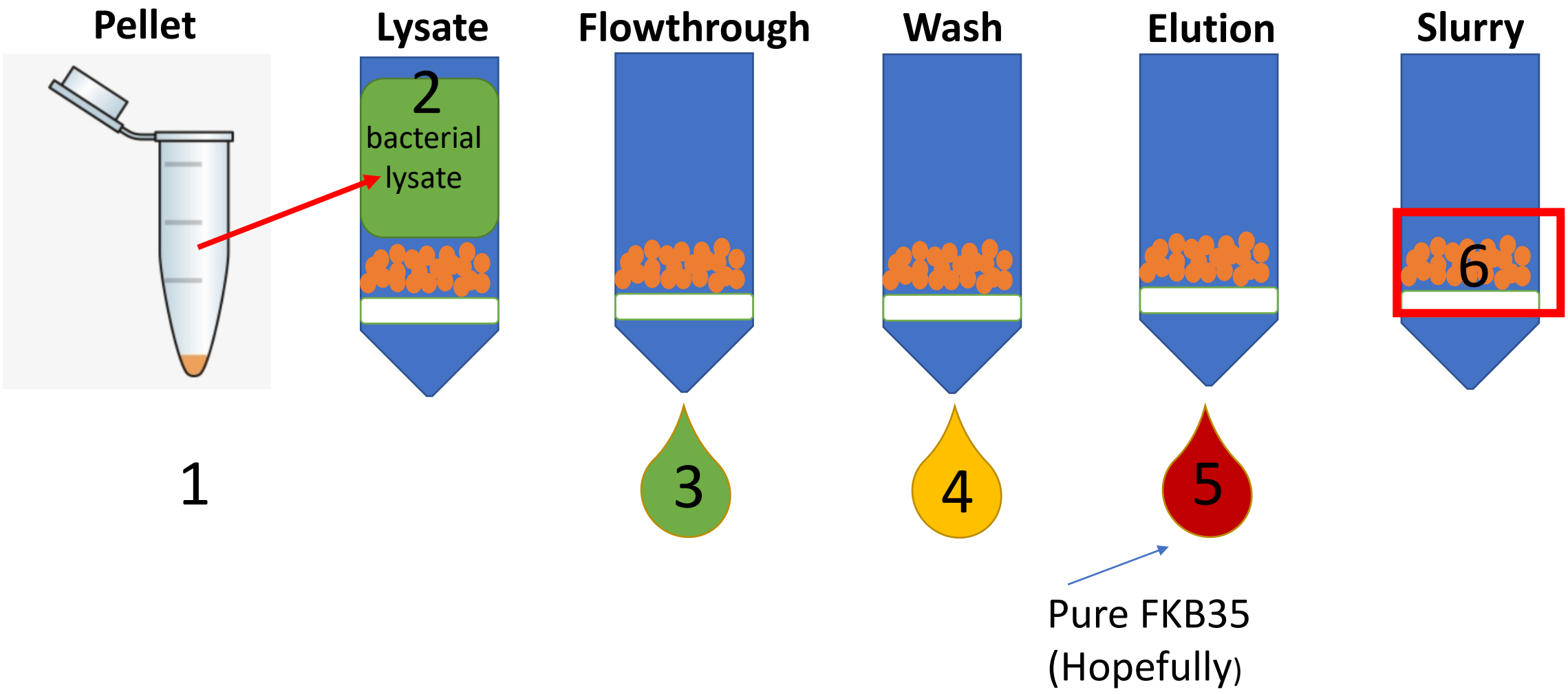
High concentration of imidazole is used to elute the protein from the Ni^{2+} resin / column



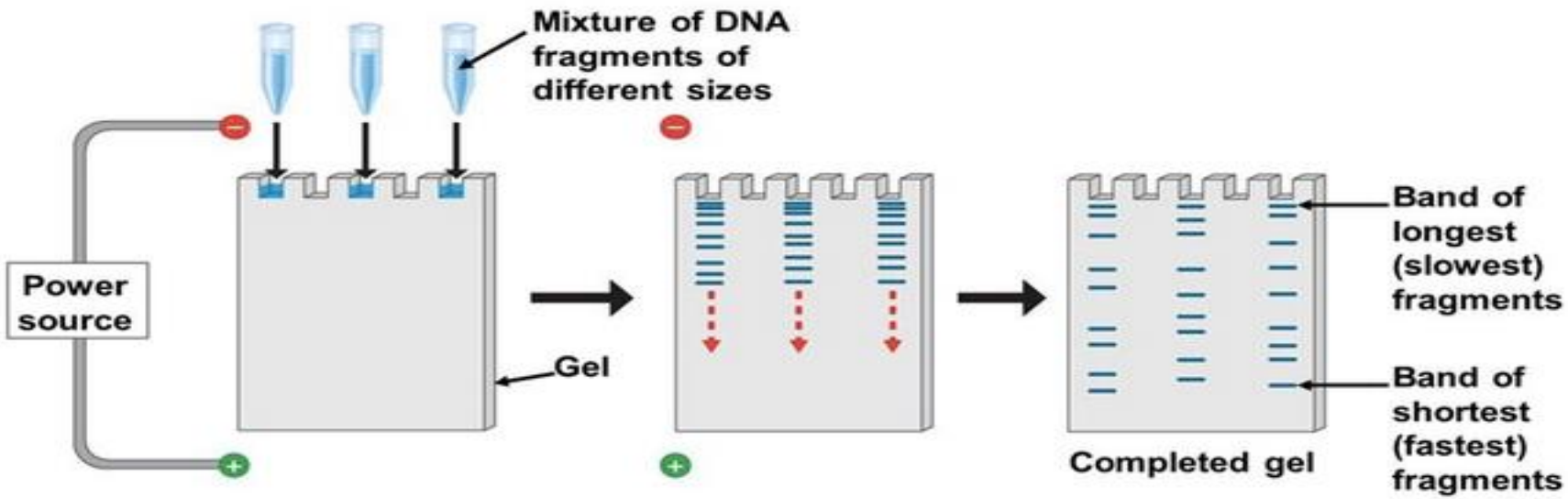
- Similar concept to wash
  - Wash uses 50mM imidazole
  - Elution used 250mM imidazole
- Instead of competing away non-specific binding, we can now out-compete the His Tag

GoldBio

Purification process (and where you will save samples)



DNA electrophoresis review



How do you visualize the migration through the gel?

Tracking dye / Dye front – Bromophenol blue

How do you visualize DNA bands in the gel?

SYBR Safe DNA Stain

For today…

1. Purify your protein for validation assay
2. During a centrifugation step, electrophorese your RE digest

For M2D3…

1. Answer question prompts on the wiki homework to think about how you will create a story from figures in the paper