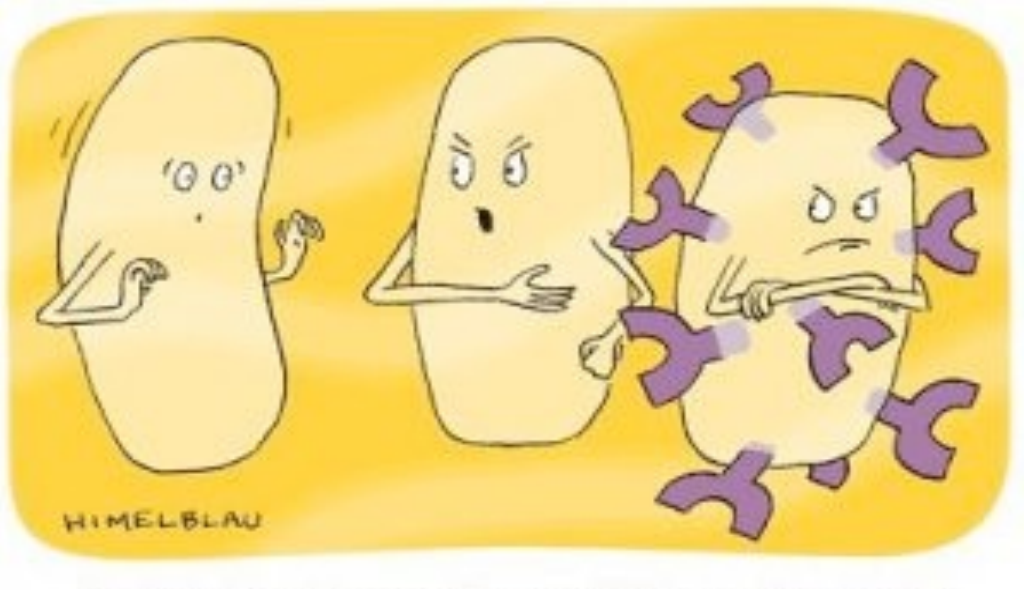
M2D2: Perform protein purification protocol

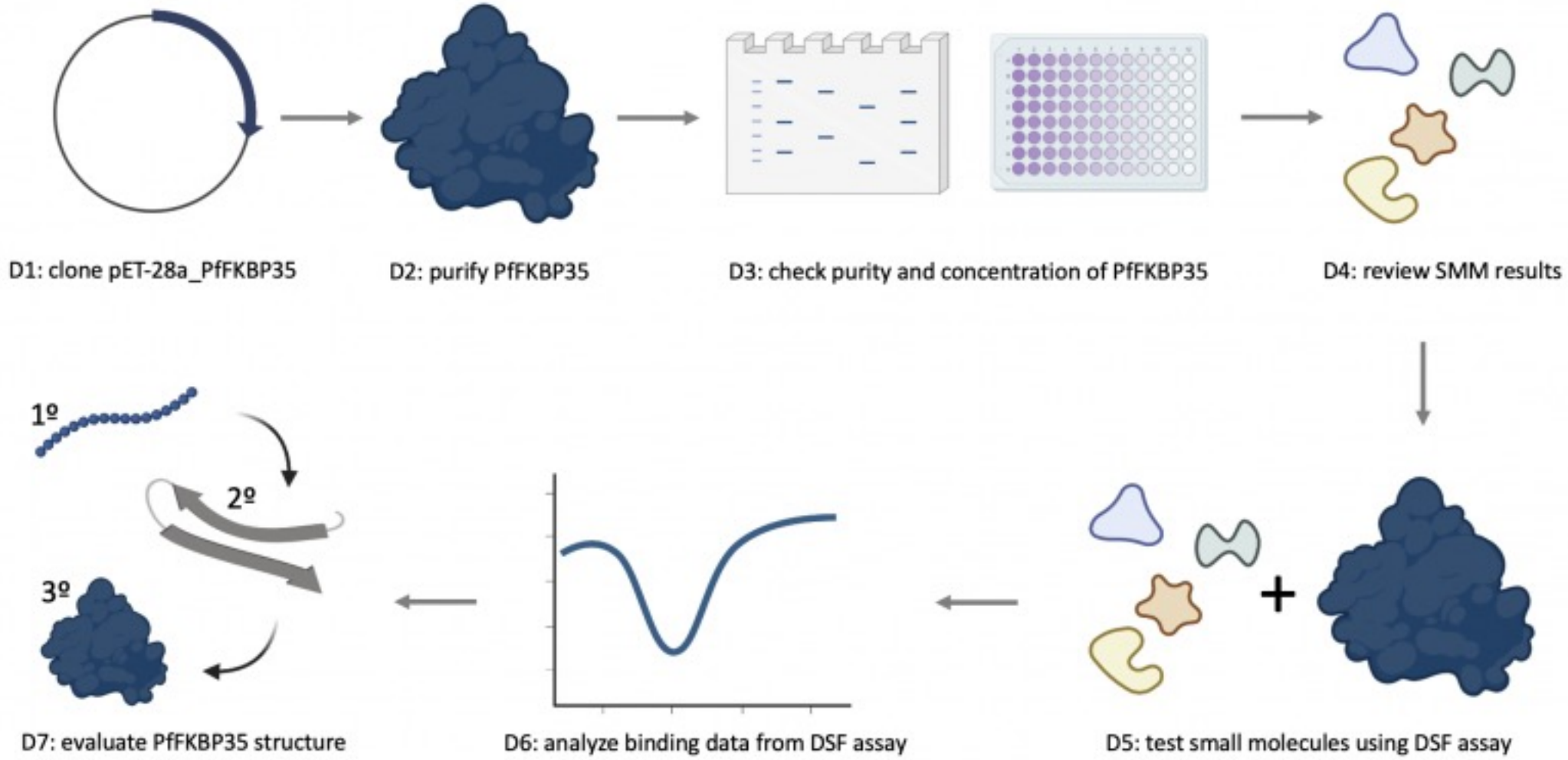
1. Prelab discussion
2. Protein purification
3. Assess RE digest



"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!

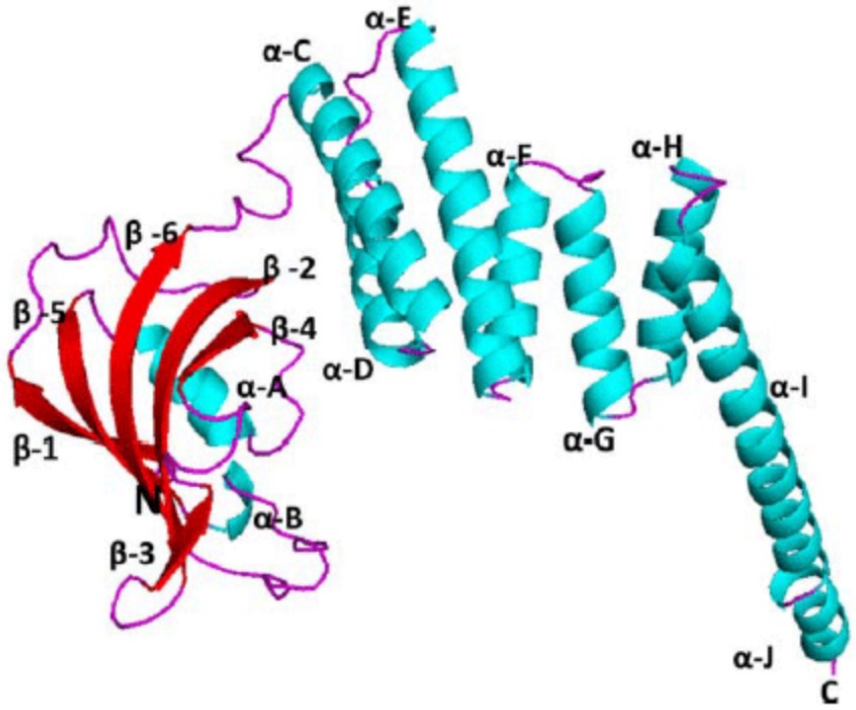
Overview of M2: drug discovery

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

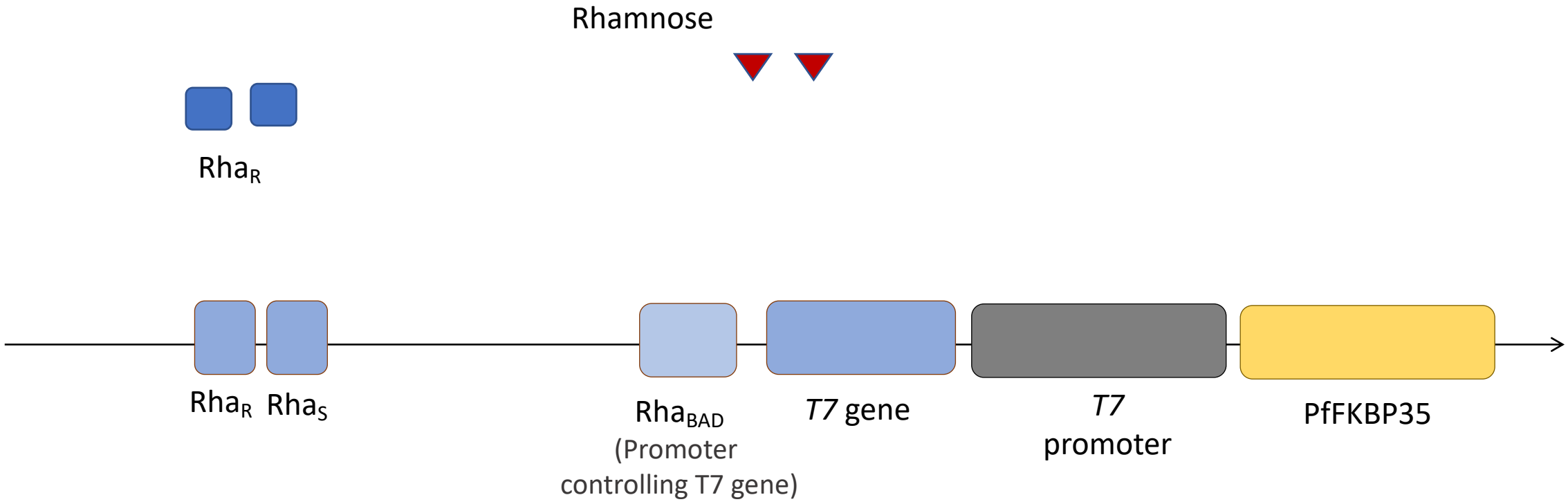


PfFKBP35

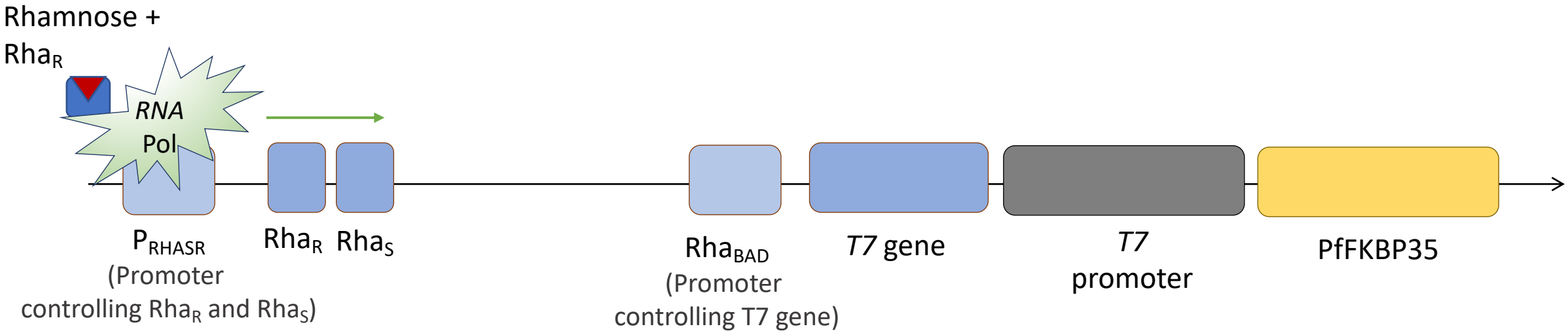
- Pf = Plasmodium falciparum
- Essential to parasite survival
- Known drug target for:
  - Rapamycin
  - FK506
- Problem: Has a human ortholog in FKBP12
  - FKBP12 -/- is embryonic lethal
- How can we target the parasite protein and not the human?



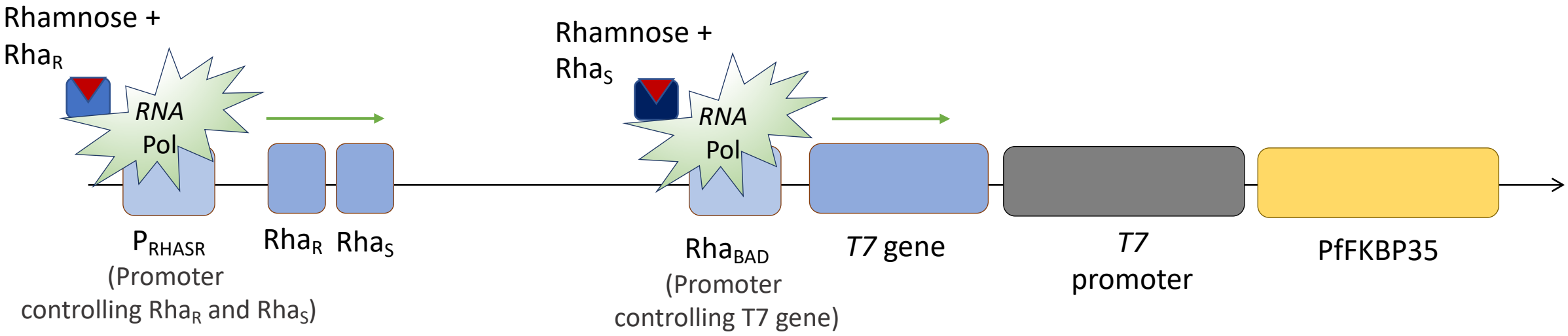
Bacterial induction: How it begins…



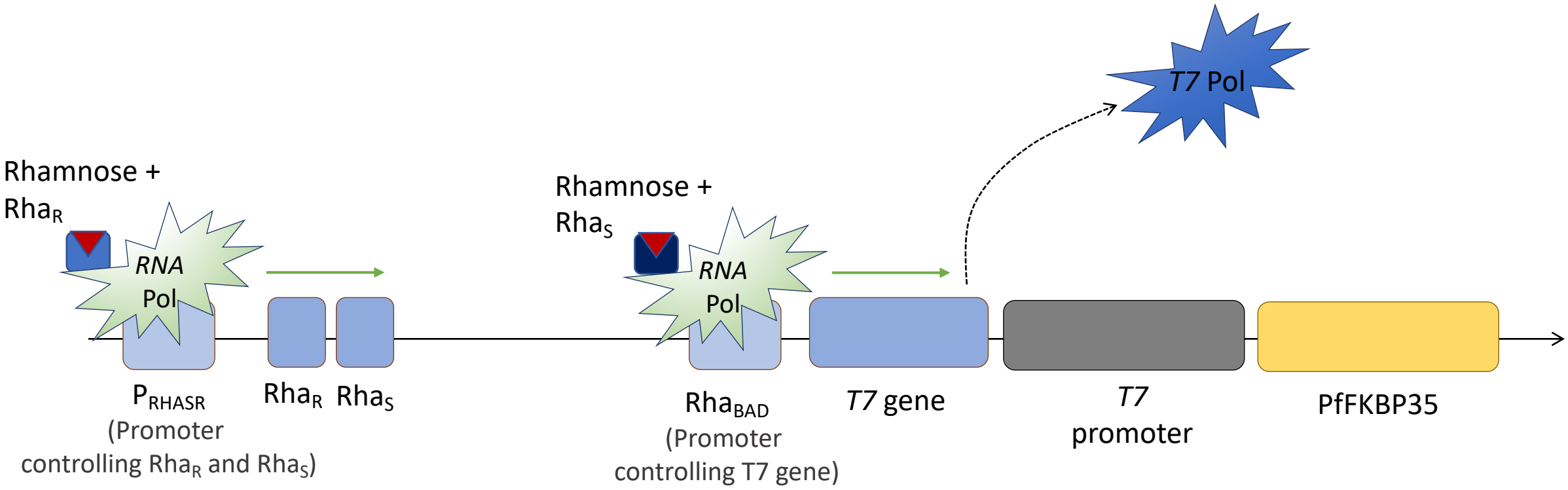
Bacterial induction: RhaR activates production of RhaR and RhaS



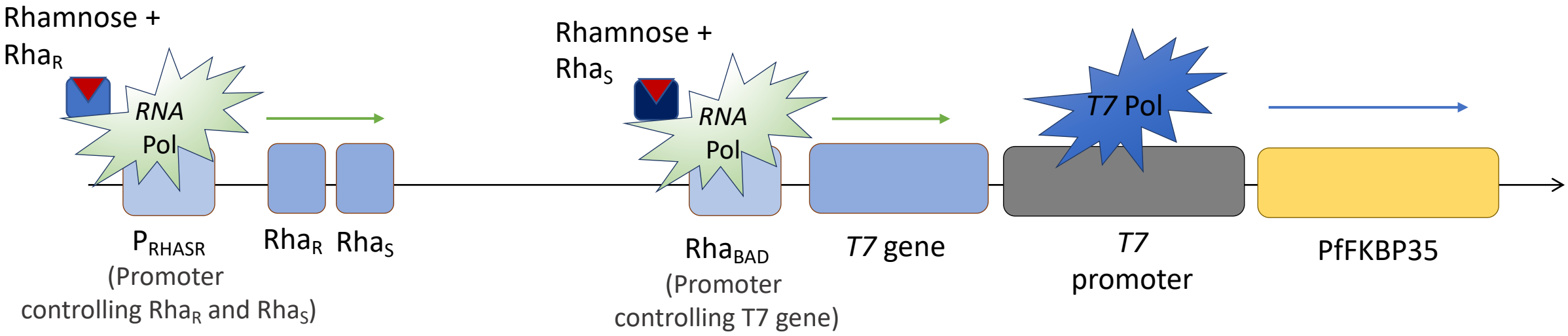
Bacterial induction: RhaS promotes RNA Pol binding to RhaBAD promoter



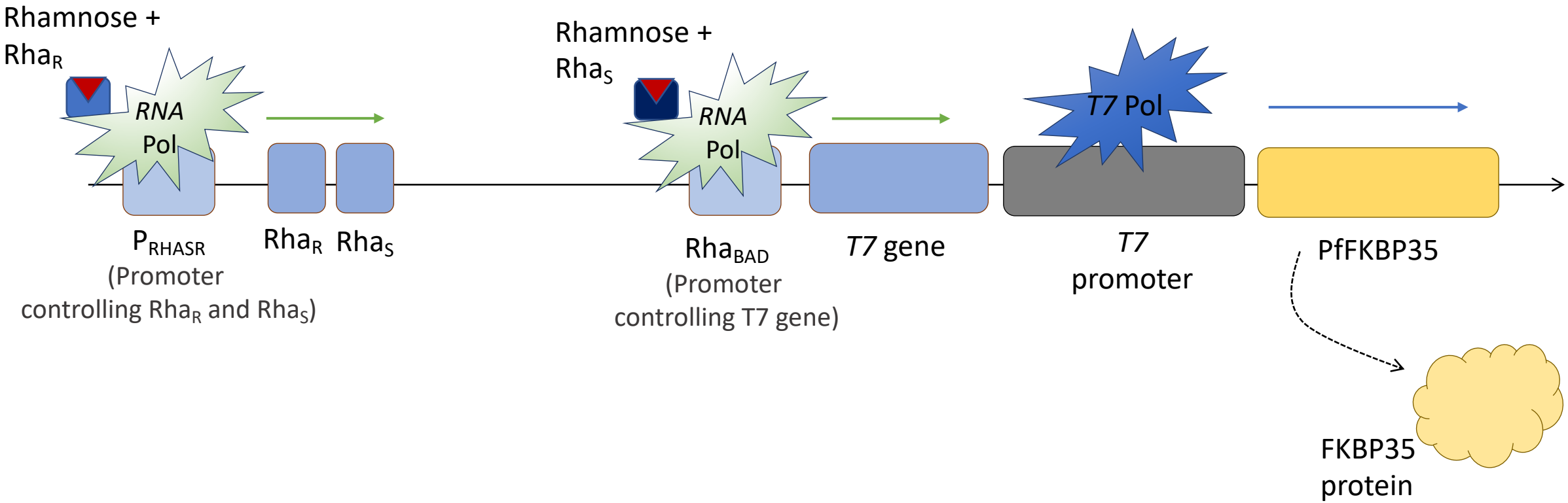
Bacterial induction: RhaBAD promoter controls T7 Pol production



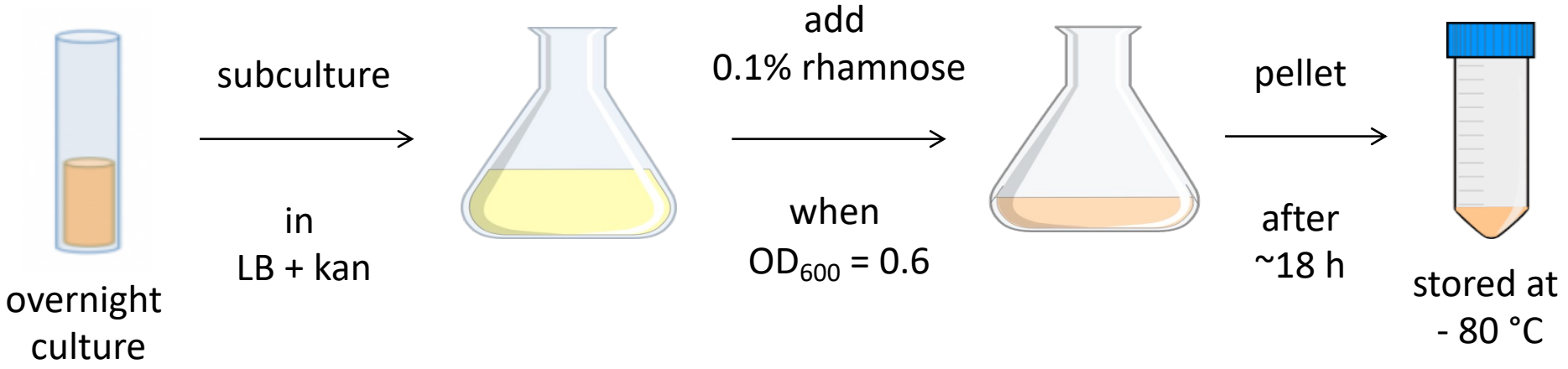
Bacterial induction: T7 Pol binds to the T7 promoter



Bacterial induction: T7 promoter controls FKBP35 production



How do we induce protein expression?

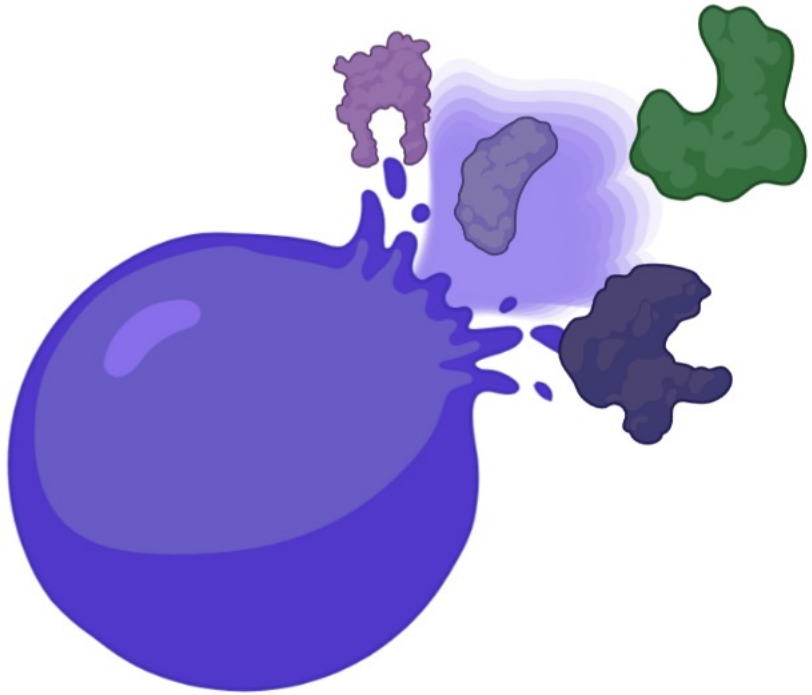


Why do we induce protein expression at OD_{600} = 0.6?

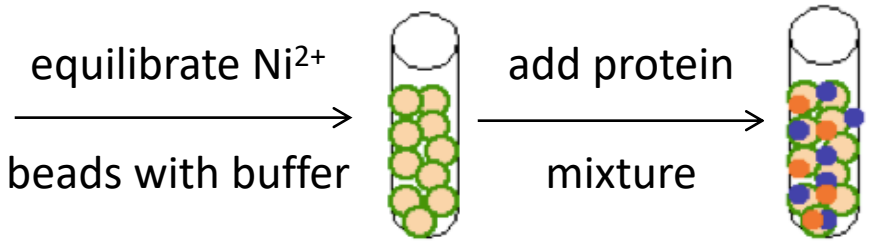
How will you purify PfFKBP35?

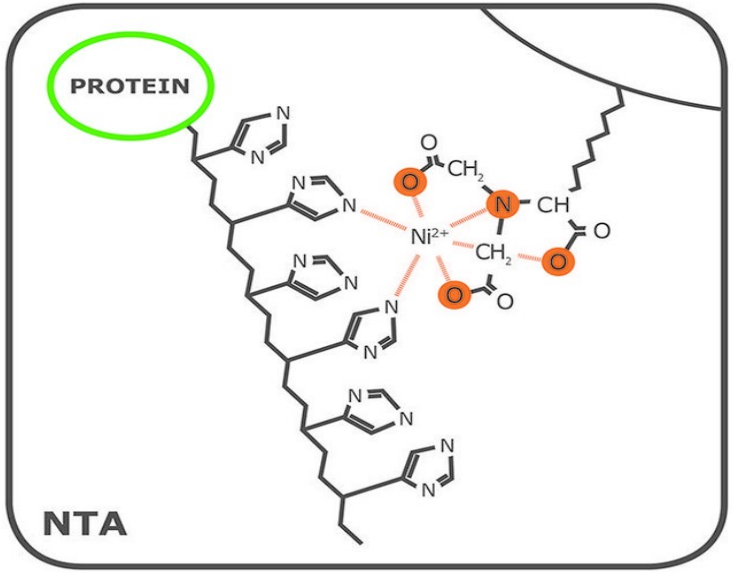
First, need to lyse cells to release proteins:

- B-PER bacterial extraction reagent
- Lysozyme + DNaseI
- Protease Inhibitor Cocktail



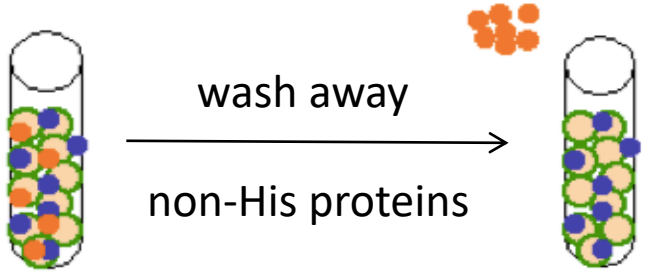
6xHis tag binds to Ni^{2+} resin / column to allow purification of protein of interest

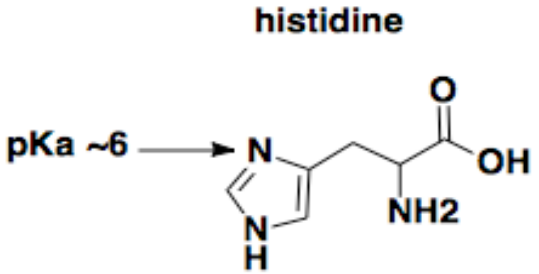


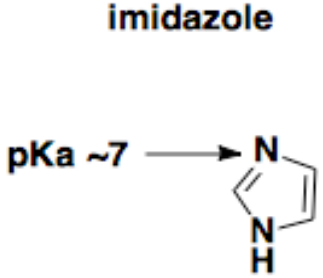


- Ni^{2+} chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand
- His tag chelates to Ni^{2+} causing protein to ‘stick’ to resin / column

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

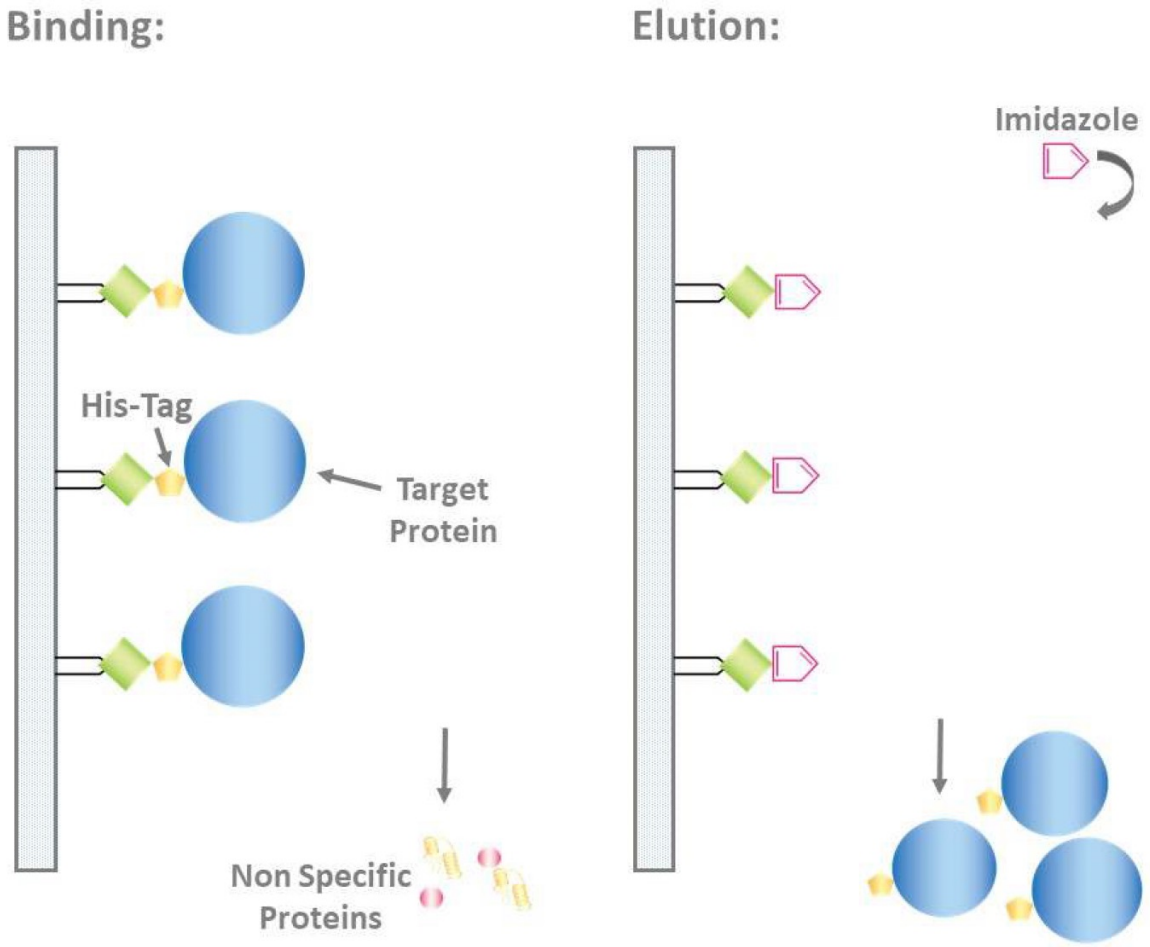






- Low concentration of imidazole included in wash buffer
- Imidazole competes for binding to Ni^{2+} resin
  - Low affinity binders / non-specific binders are outcompeted and released from the resin

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

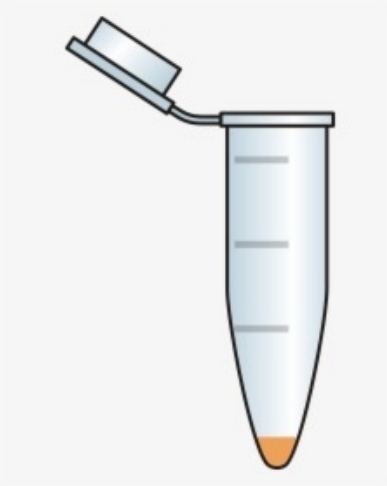


- Similar concept to wash
  - Wash uses 50mM imidazole
  - Elution used 250mM imidazole
- Instead of competing away non-specific binding, we can now out-compete the His Tag

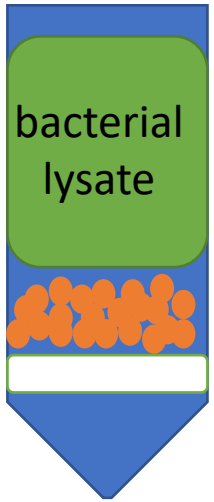
GoldBio

Purification process (and where you will save samples)

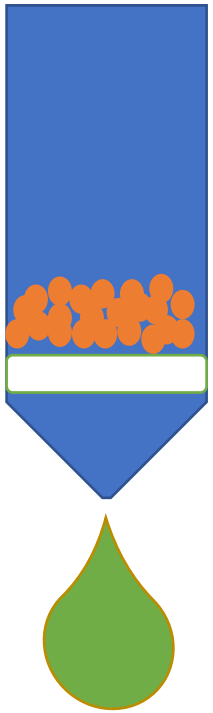
Pellet



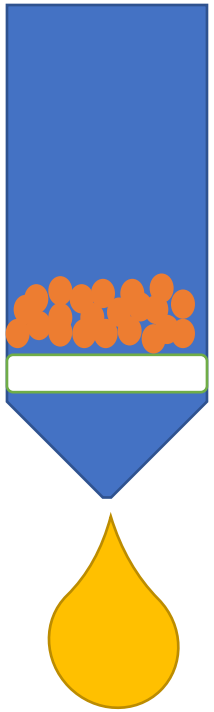
Lysate



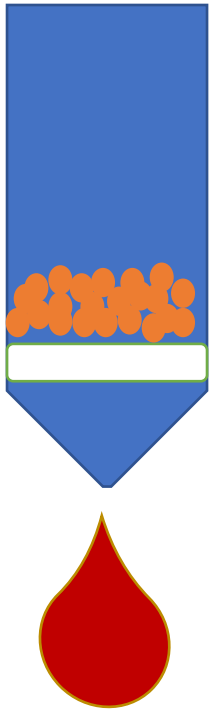
Flowthrough



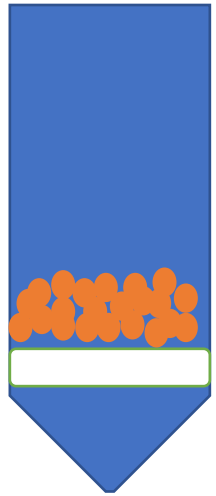
Wash



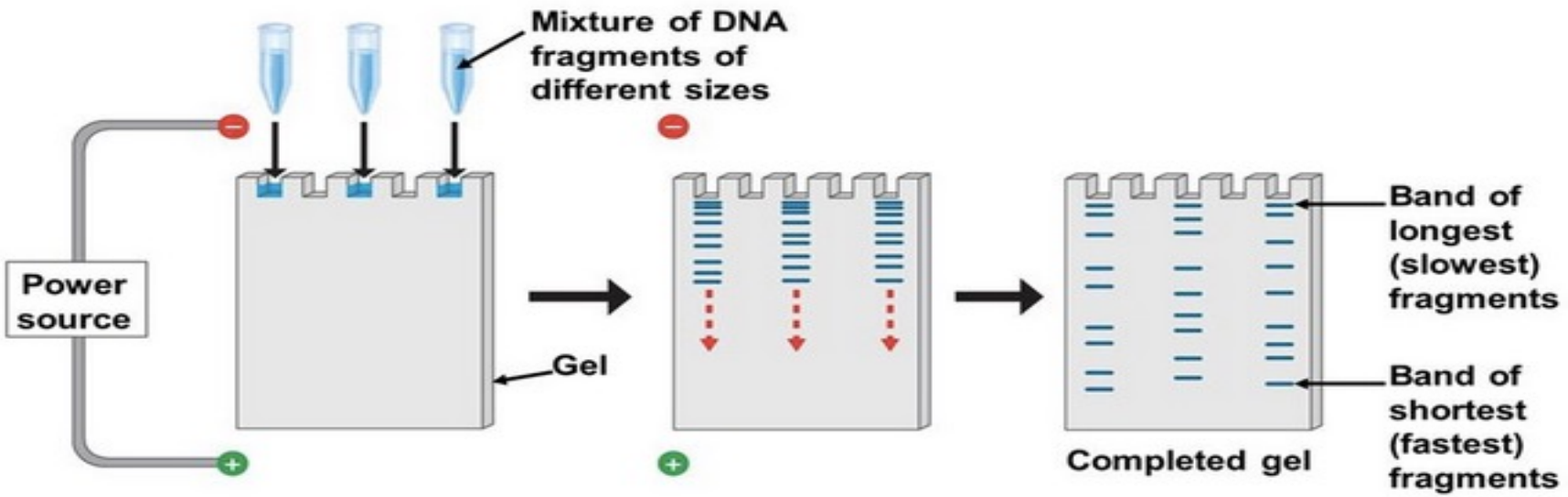
Elution



Slurry



DNA electrophoresis review



How do you visualize the migration through the gel?

How do you visualize DNA bands in the gel?

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