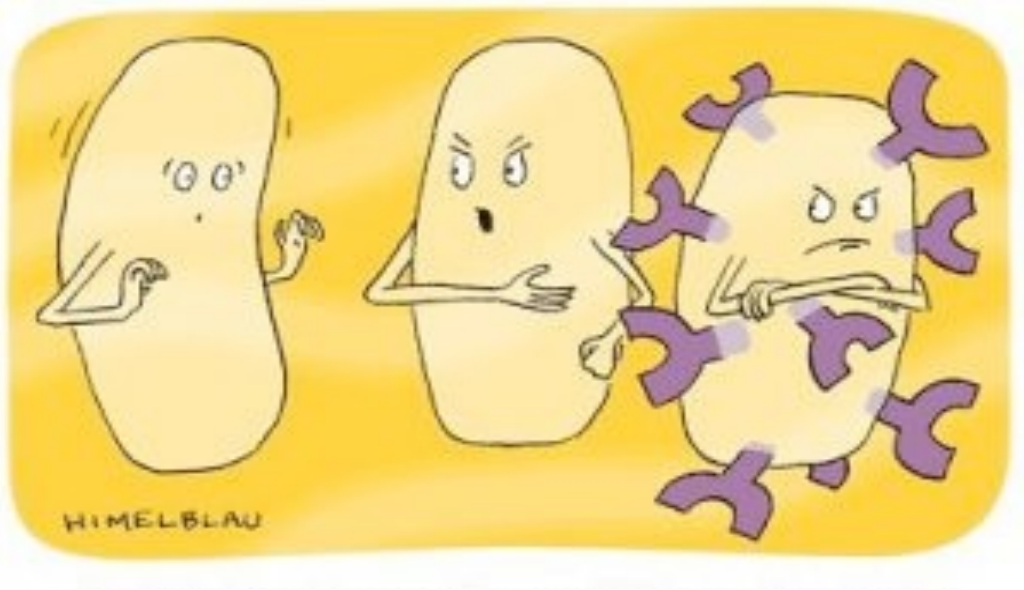
M2D2: Perform protein purification protocol

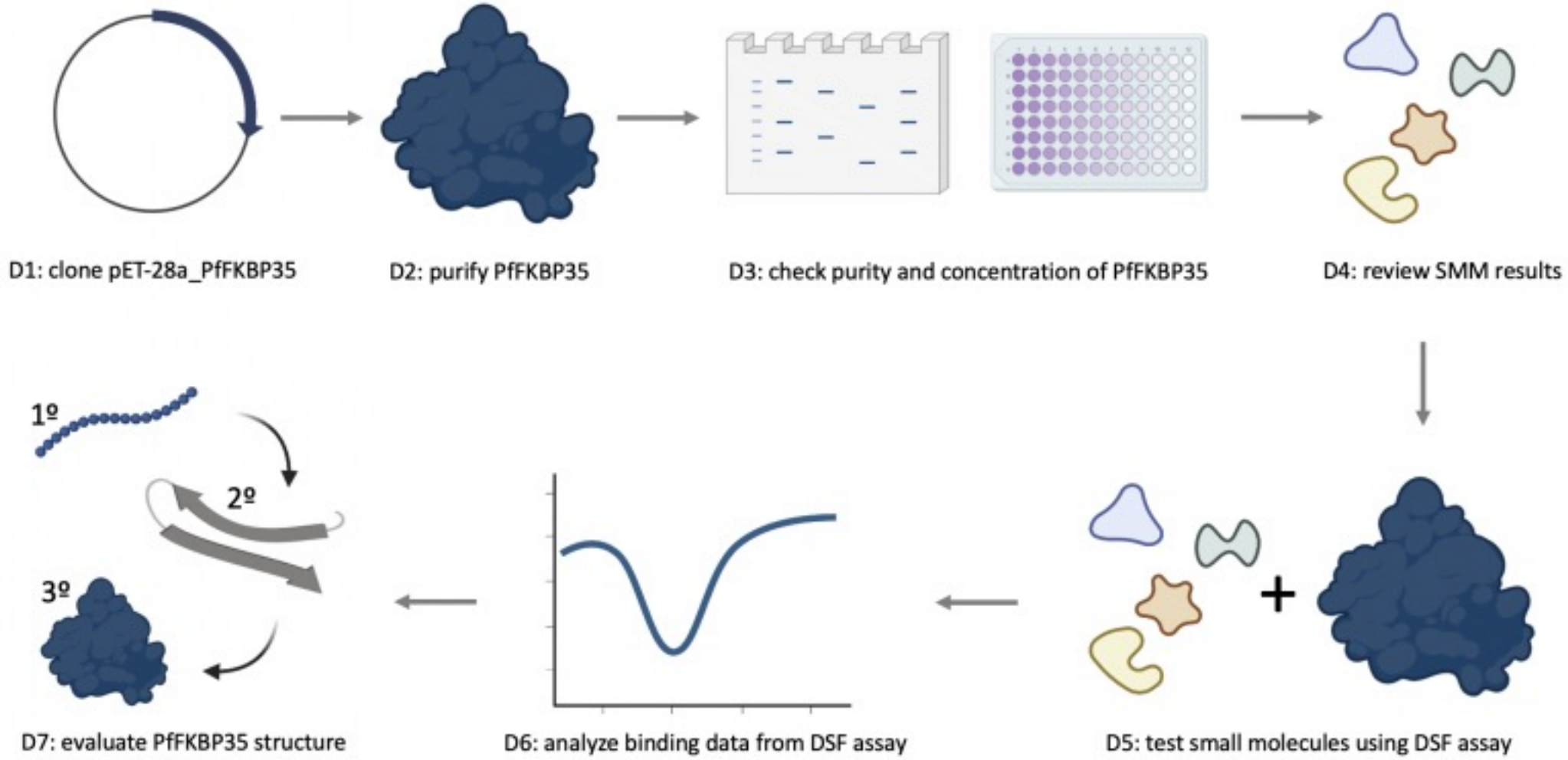
1. Prelab discussion
2. Protein purification
3. Assess RE digest



"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!

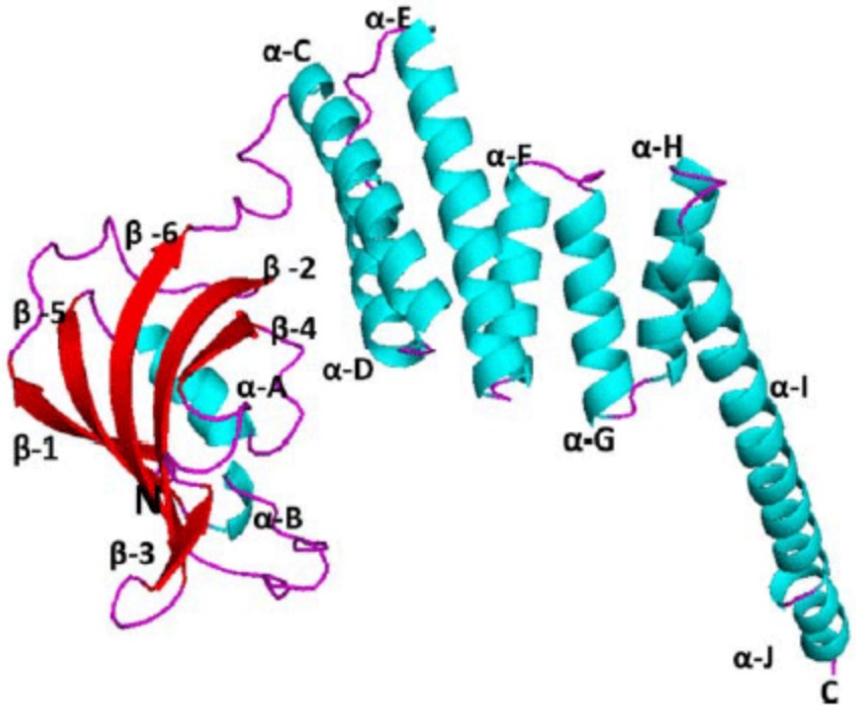
Overview of M2: drug discovery

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

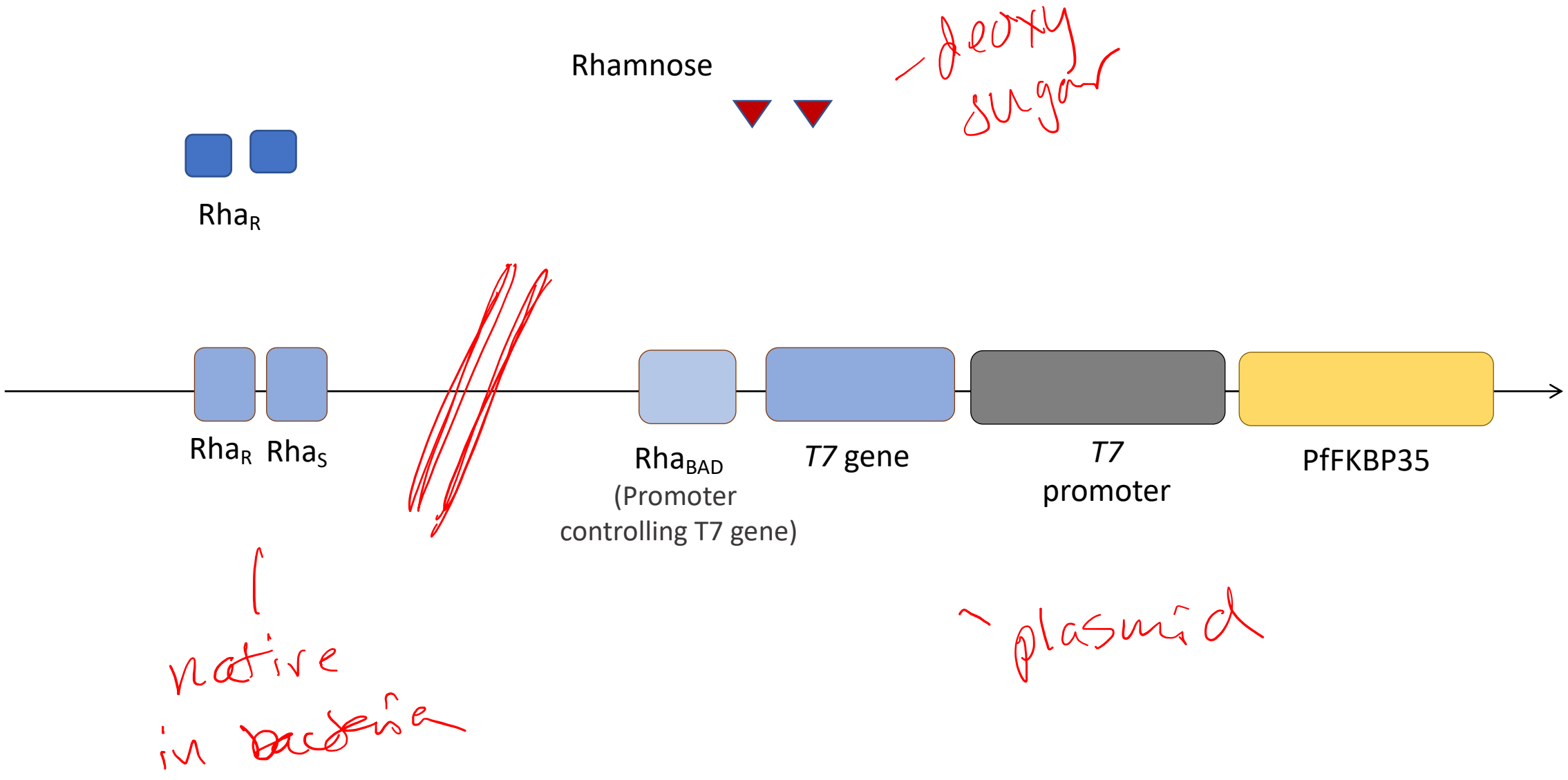


PfFKBP35

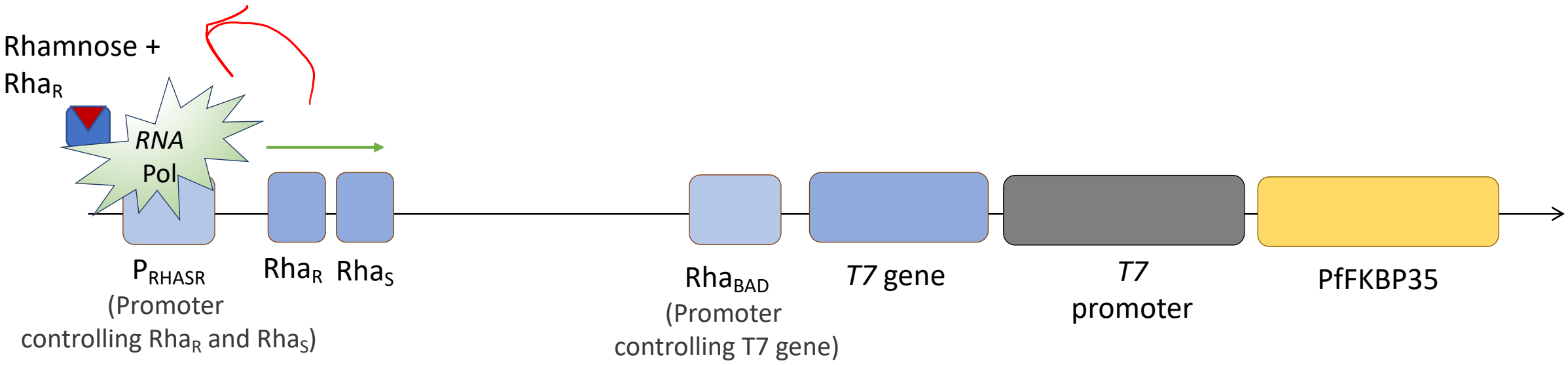
- Pf = Plasmodium falciparum
- Essential to parasite survival
- Known drug target for:
  - Rapamycin
  - FK506
- Problem: Has a human ortholog in FKBP12
  - FKBP12 -/- is embryonic lethal
- How can we target the parasite protein and not the human?



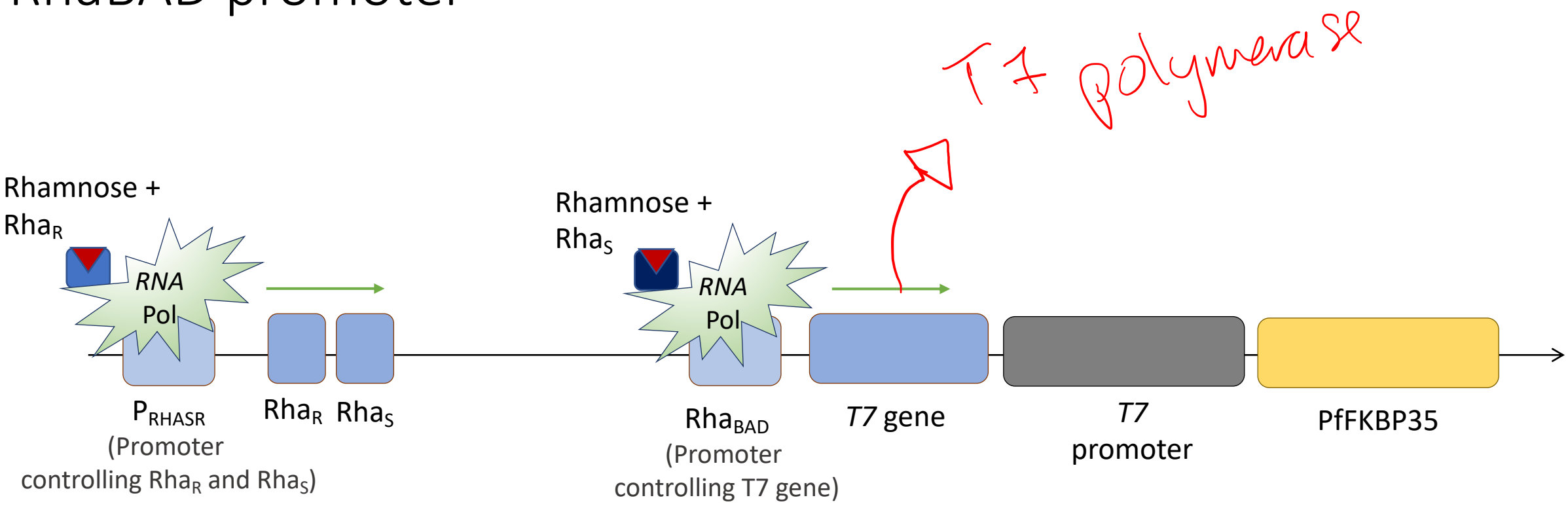
Bacterial induction: How it begins…



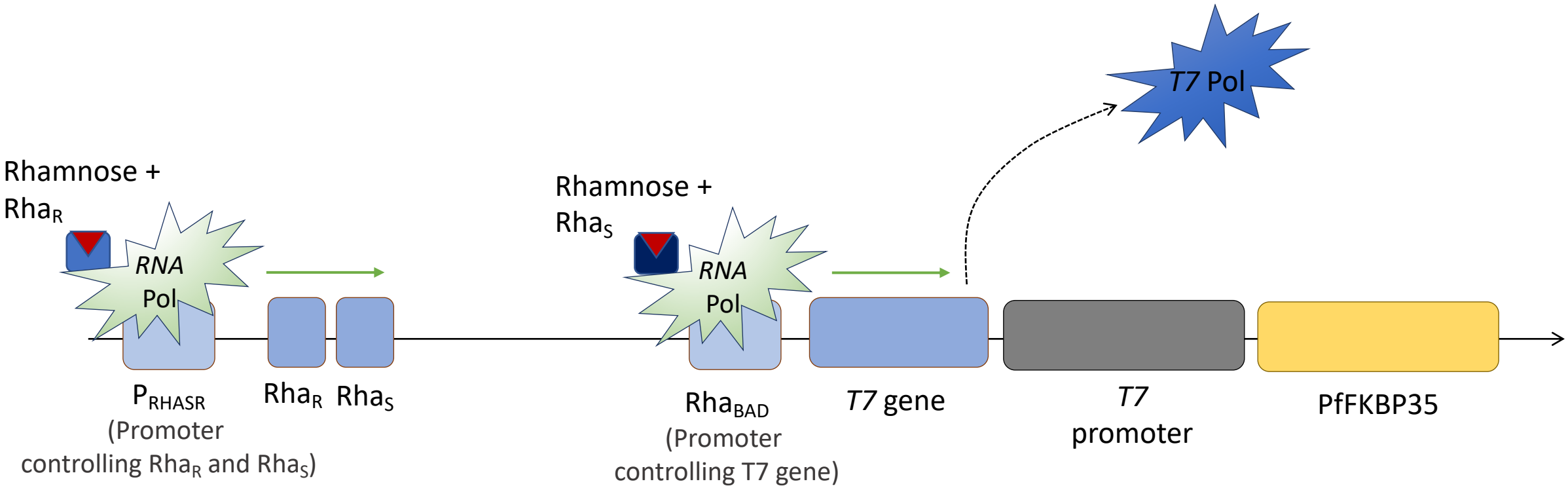
Bacterial induction: RhaR activates production of RhaR and RhaS



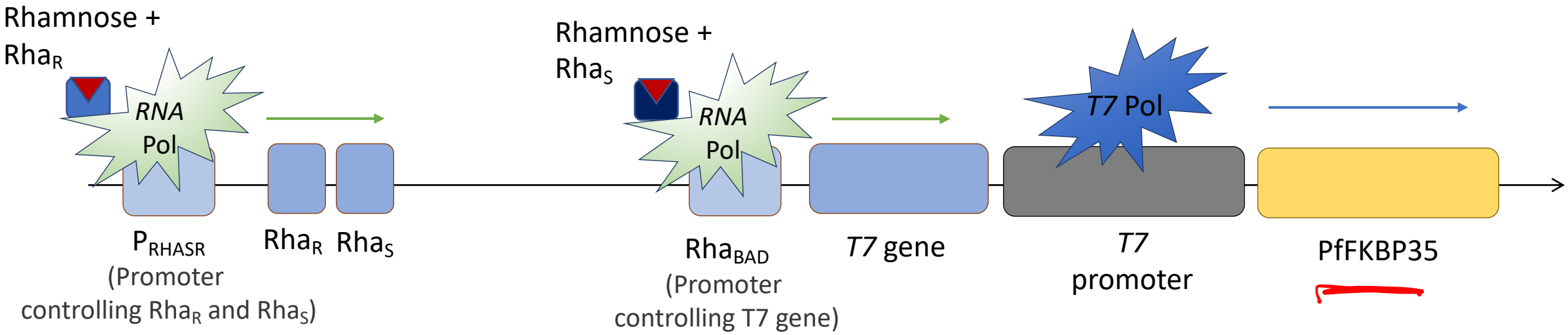
Bacterial induction: RhaS promotes RNA Pol binding to RhaBAD promoter



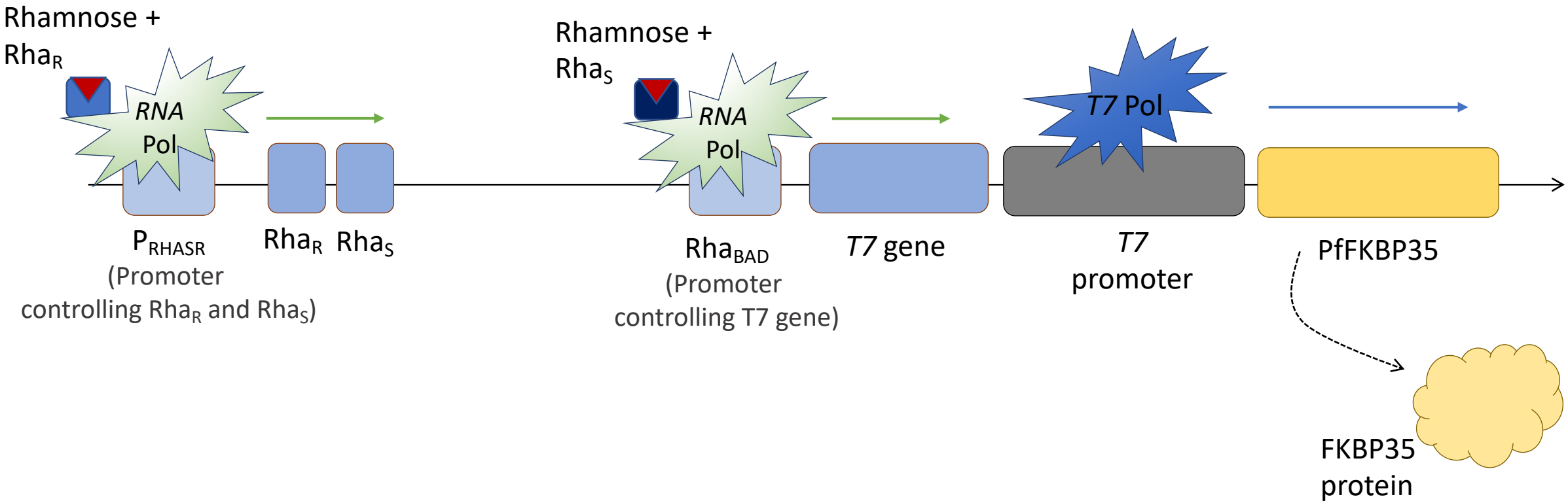
Bacterial induction: RhaBAD promoter controls T7 Pol production



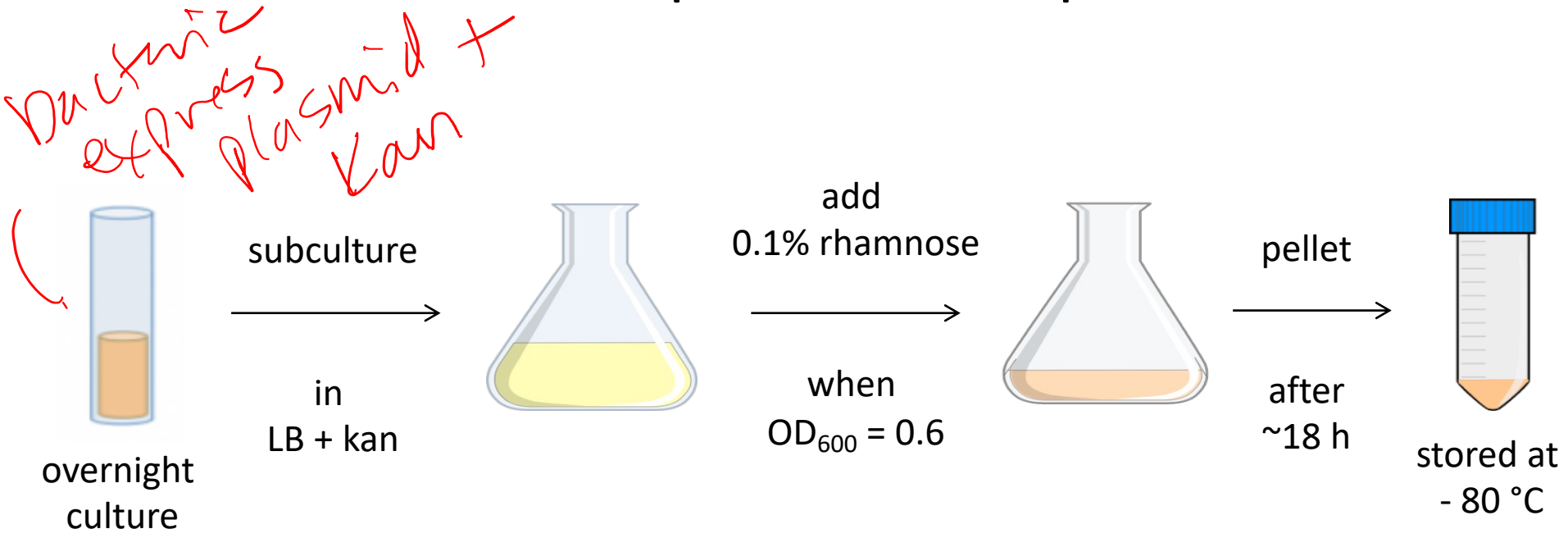
Bacterial induction: T7 Pol binds to the T7 promoter



Bacterial induction: T7 promoter controls FKBP35 production



How do we induce protein expression?



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


bacteria #
0.6 OD
induce
collect
lag
log
exponential
stationary
death
time

How will you purify PfFKBP35?

First, need to lyse cells to release proteins:

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

detergents/ buffers

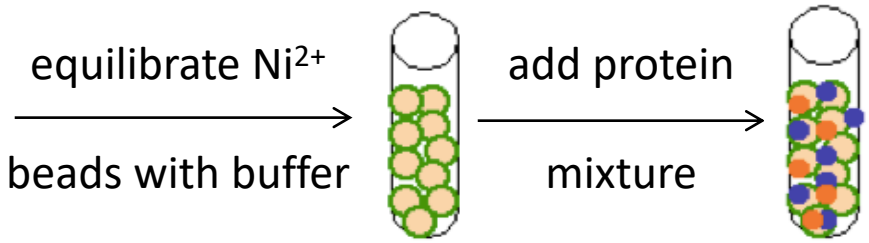
digest DNA

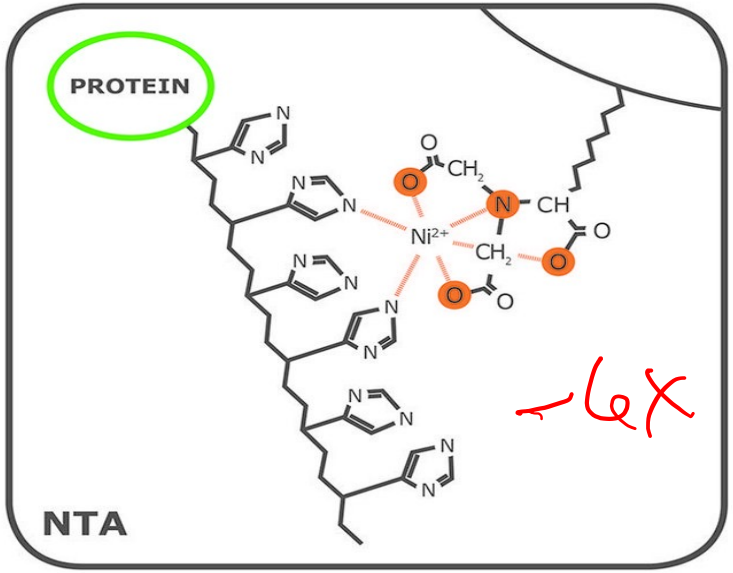
Break cell wall of bacteria

Preserve our POI throughout purification

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

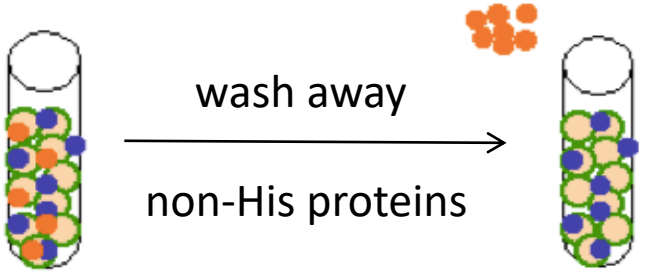
affinity purification

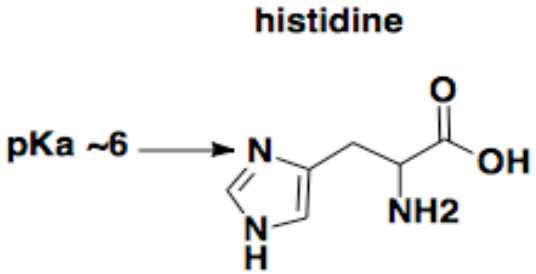


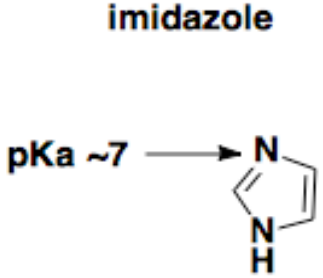


- Ni^{2+} chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand
- His tag chelates to Ni^{2+} causing protein to 'stick' to resin / column

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



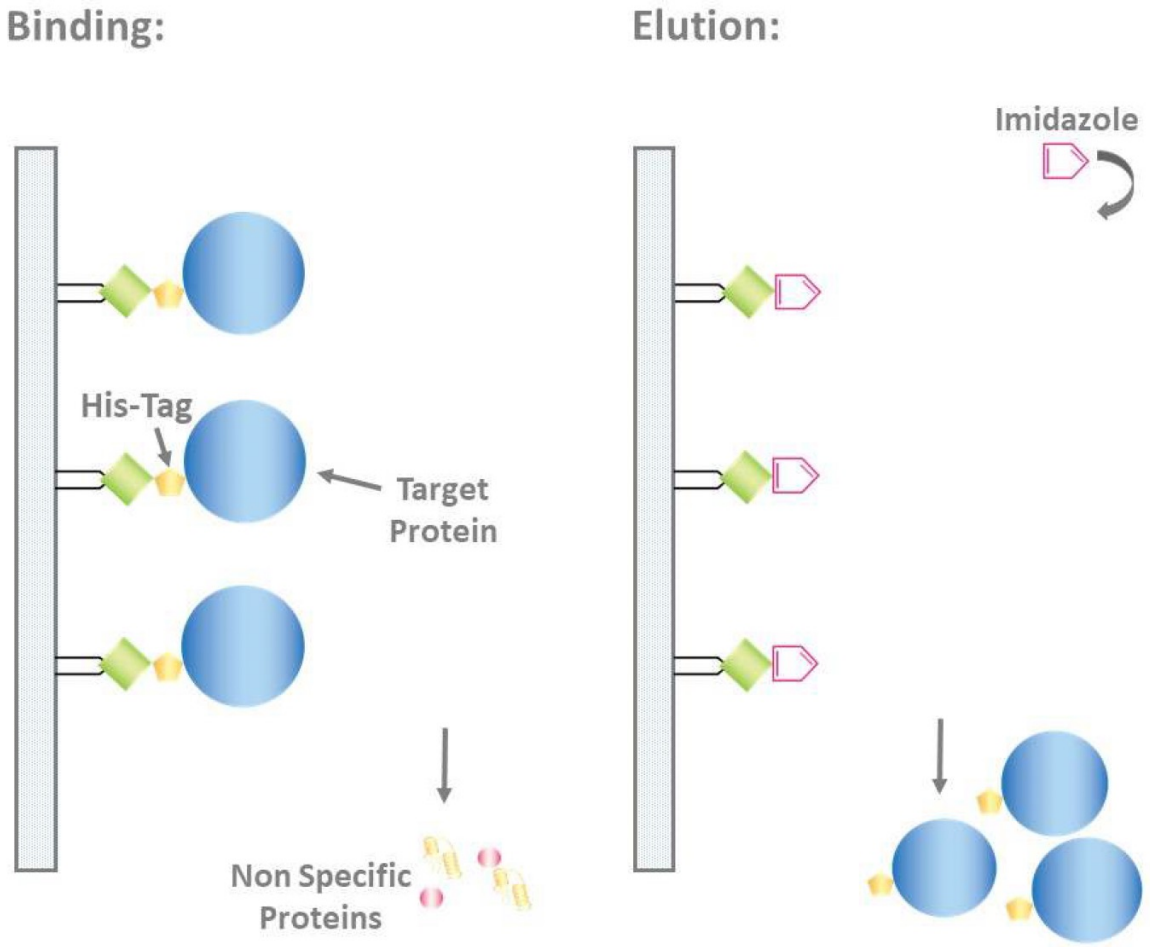




- Low concentration of imidazole included in wash buffer
- Imidazole competes for binding to Ni^{2+} resin
  - Low affinity binders / non-specific binders are outcompeted and released from the resin

– replace His

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

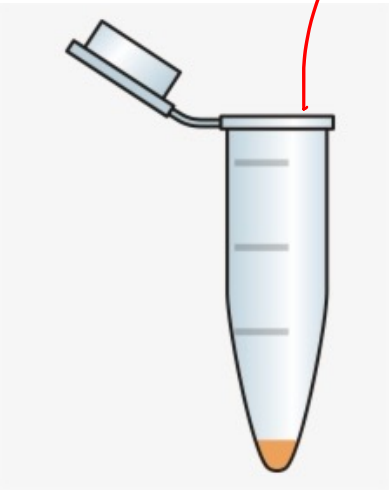
GoldBio

Purification process (and where you will save samples)

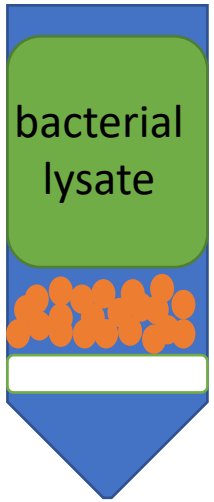
Pour supernatent

resin/agarose beads

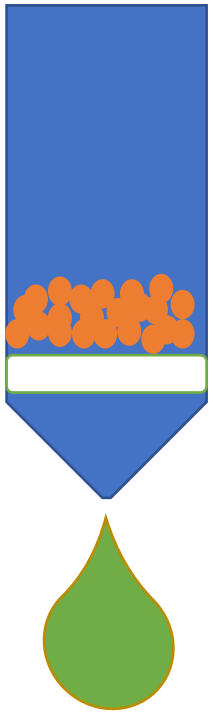
Pellet



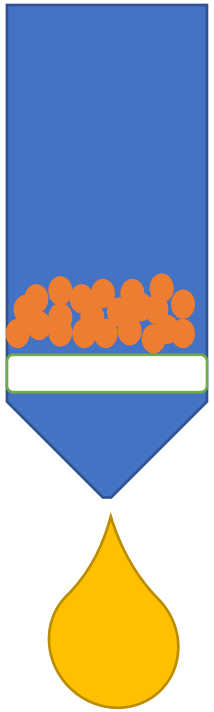
Lysate



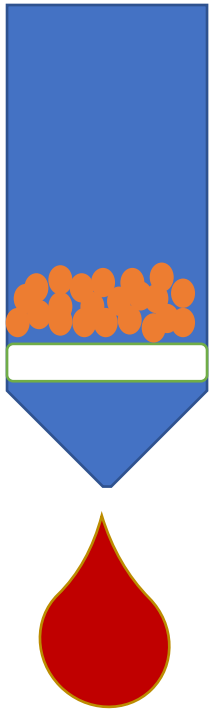
Flowthrough



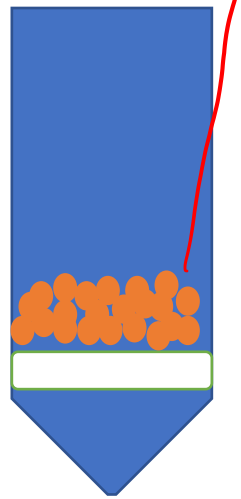
Wash



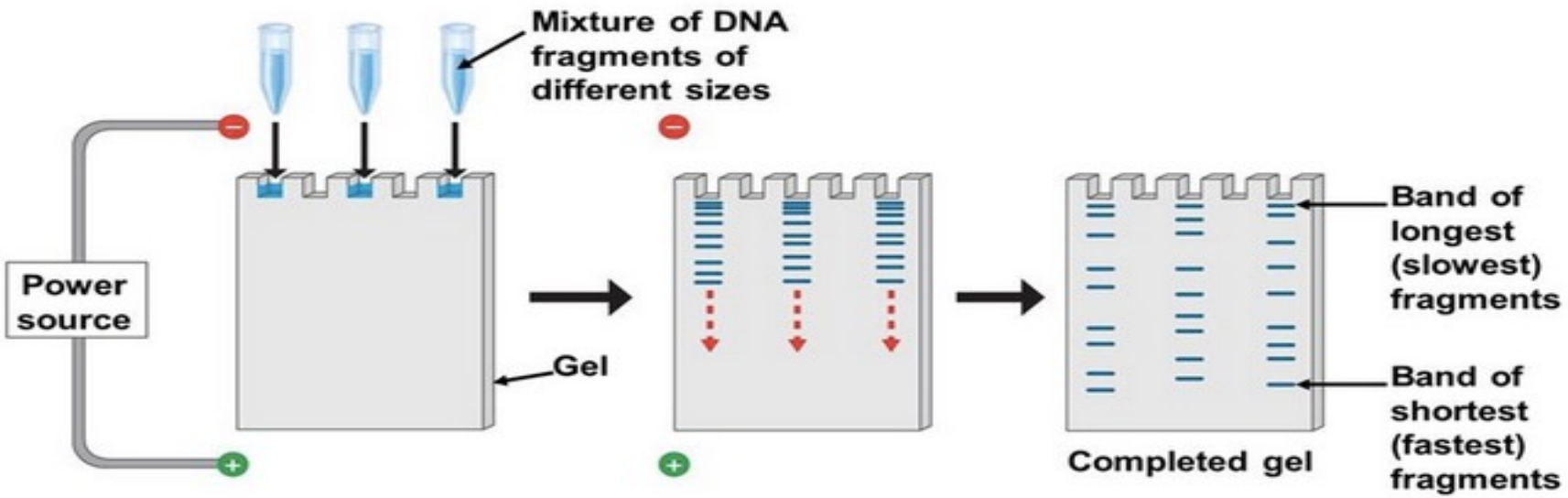
Elution



Slurry



DNA electrophoresis review



1% agarose gel

How do you visualize the migration through the gel?

tracking dye – bromophenol blue

How do you visualize DNA bands in the gel?

SYBR Safe

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