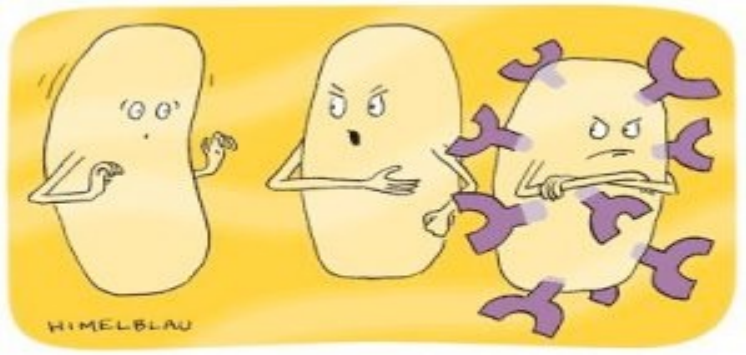
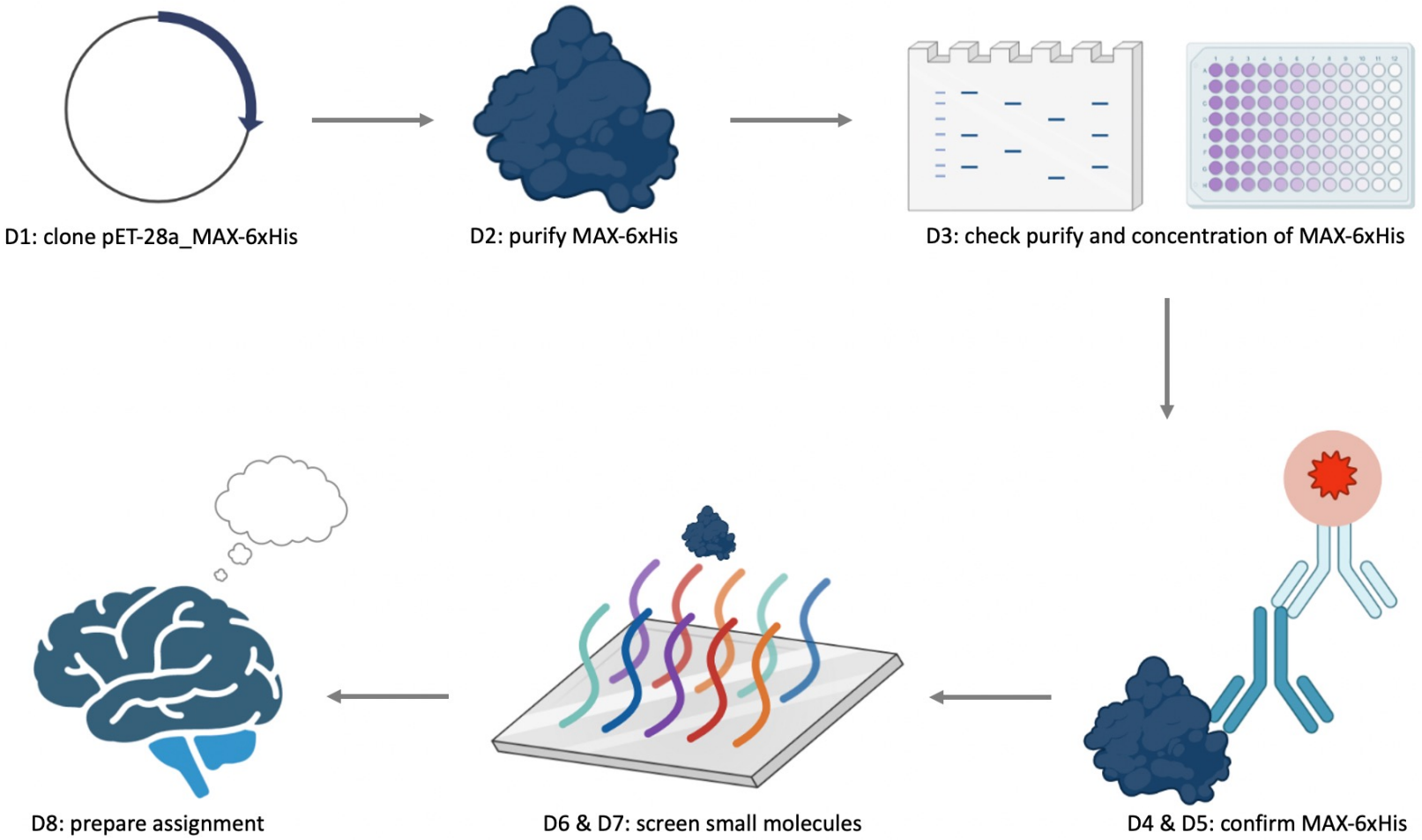
M1D2:
Perform protein purification protocol

1. Prelab discussion
2. Purify MAX-6xHis protein
3. Electrophorese confirmation digest



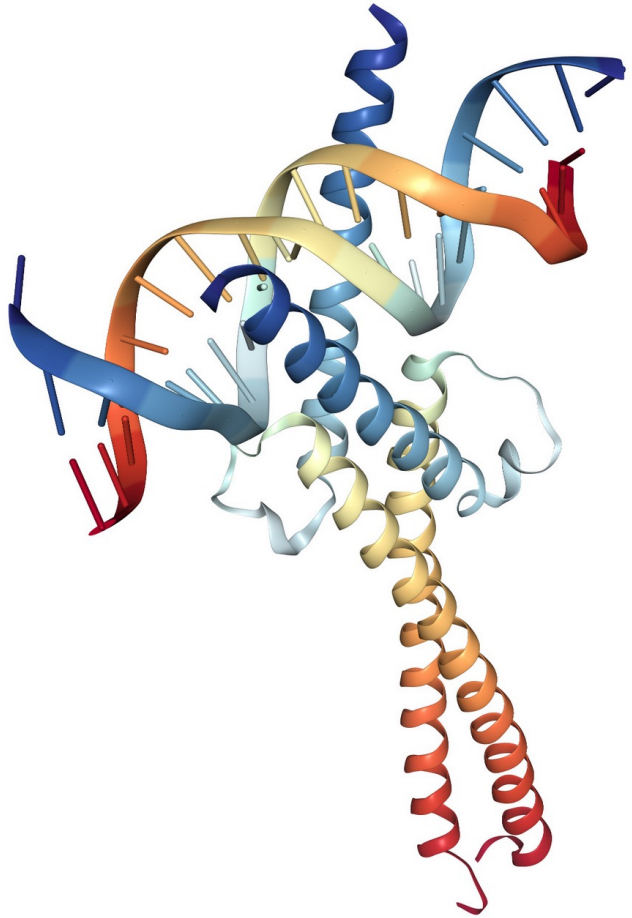
"Don't pick it up," I say, and he says, "It's just a *plasmid*, what harm could it do?" Well just look at him now…who knows *what* protein he's expressing!

Overview of Mod 1 experiments:

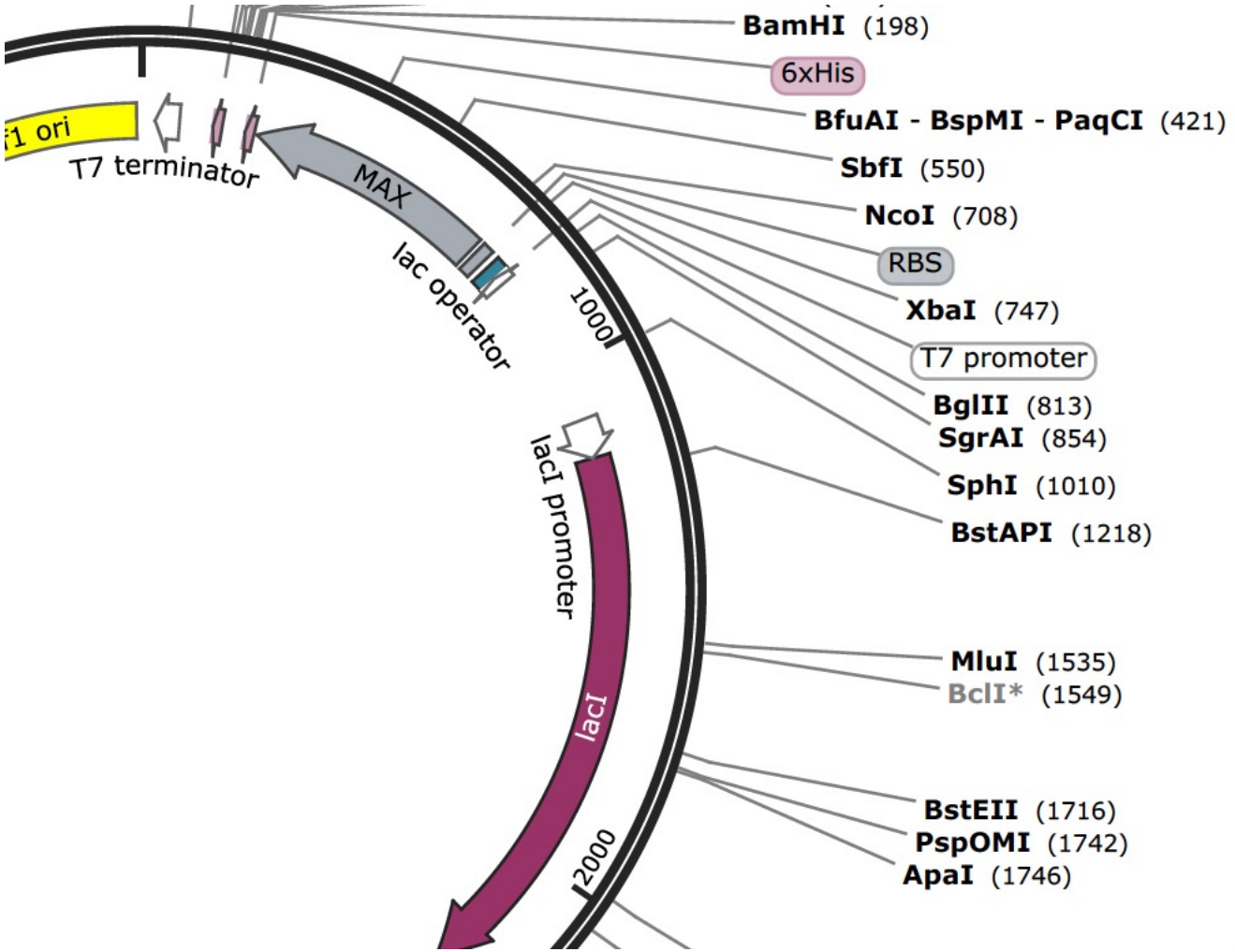


What is our protein of interest?

- MAX functions as a transcription factor
  - Forms homodimers and heterodimers
  - Dimerizes with Myc, which is an oncogenic transcription factor
  - Homodimers and heterodimers compete for binding at promoters to provide regulatory system of target genees

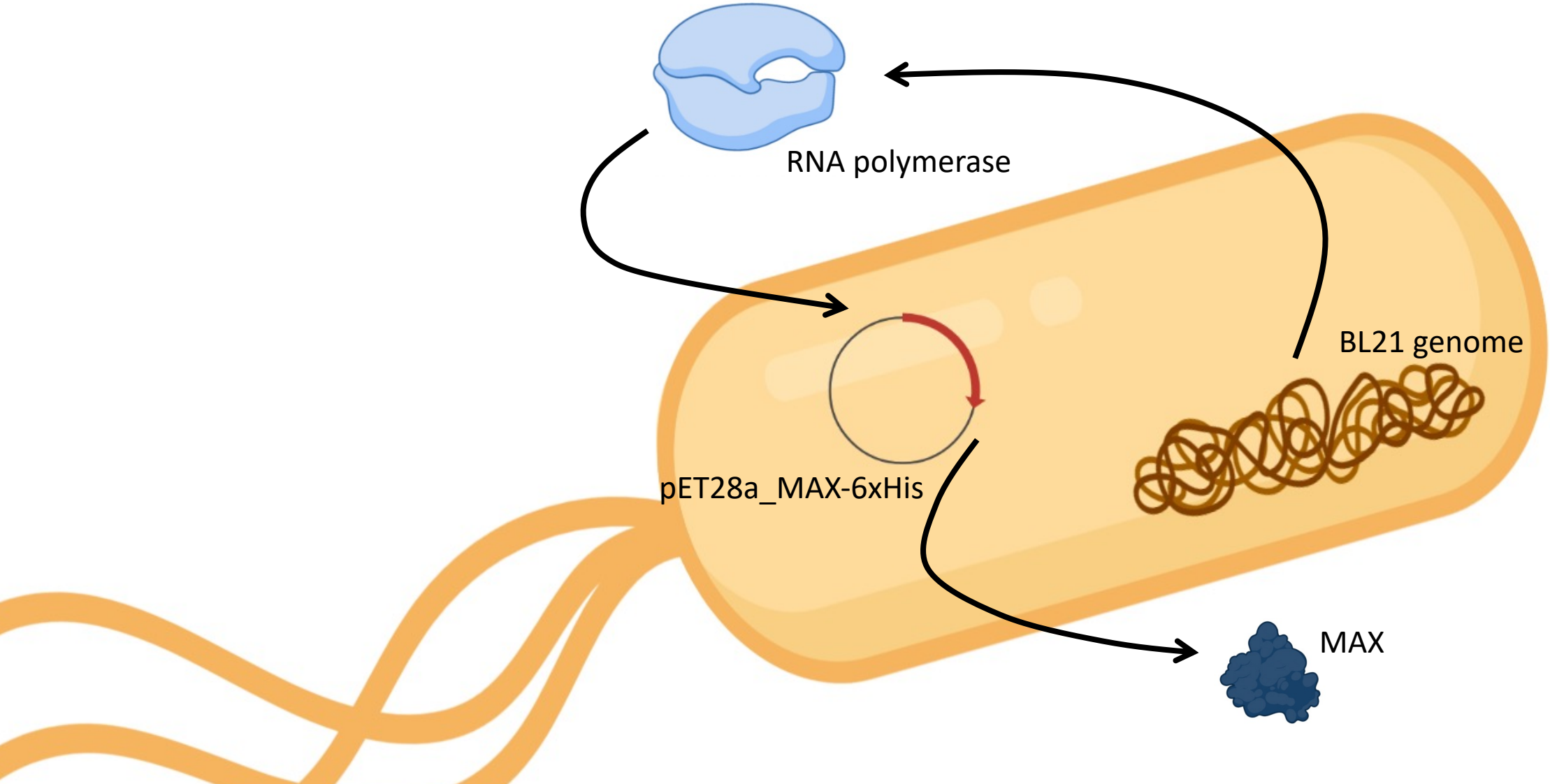


Closer look at pET28a_MAX-6xHis

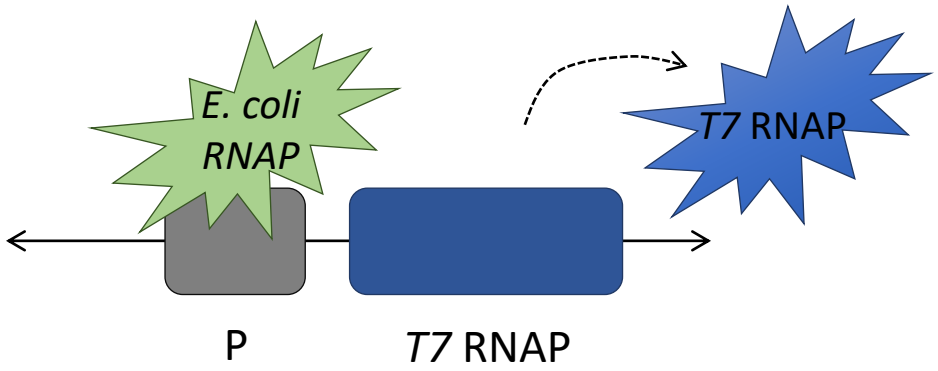


- 6xHis
- *lac* operator
- *lac*I
- T7 promoter
- T7 terminator
- RBS

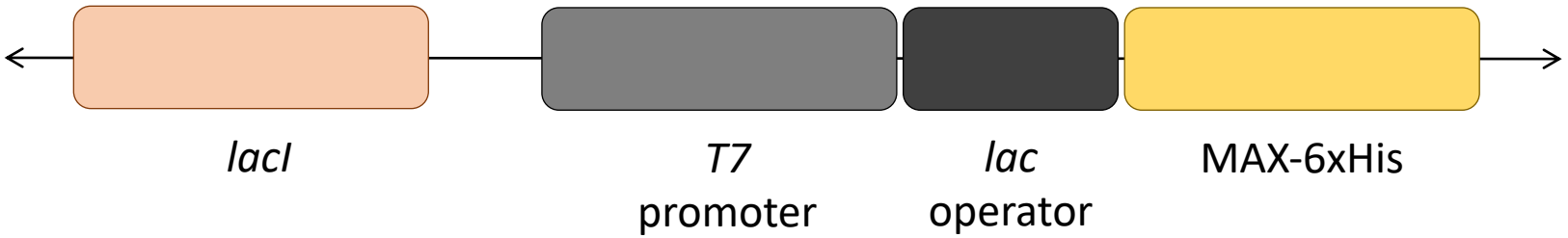
Overview of protein expression system



T7 RNA polymerase transcribes MAX-6xHis

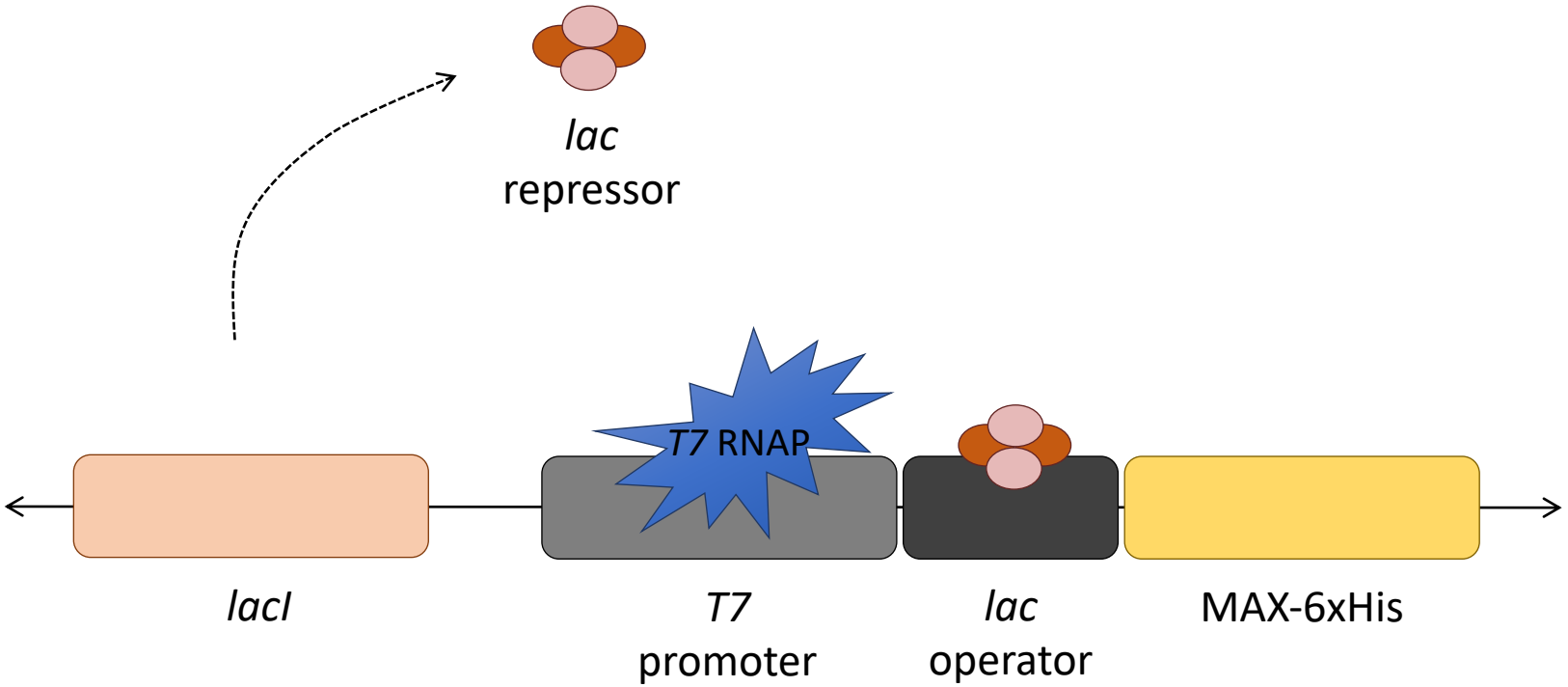


E. coli BL21



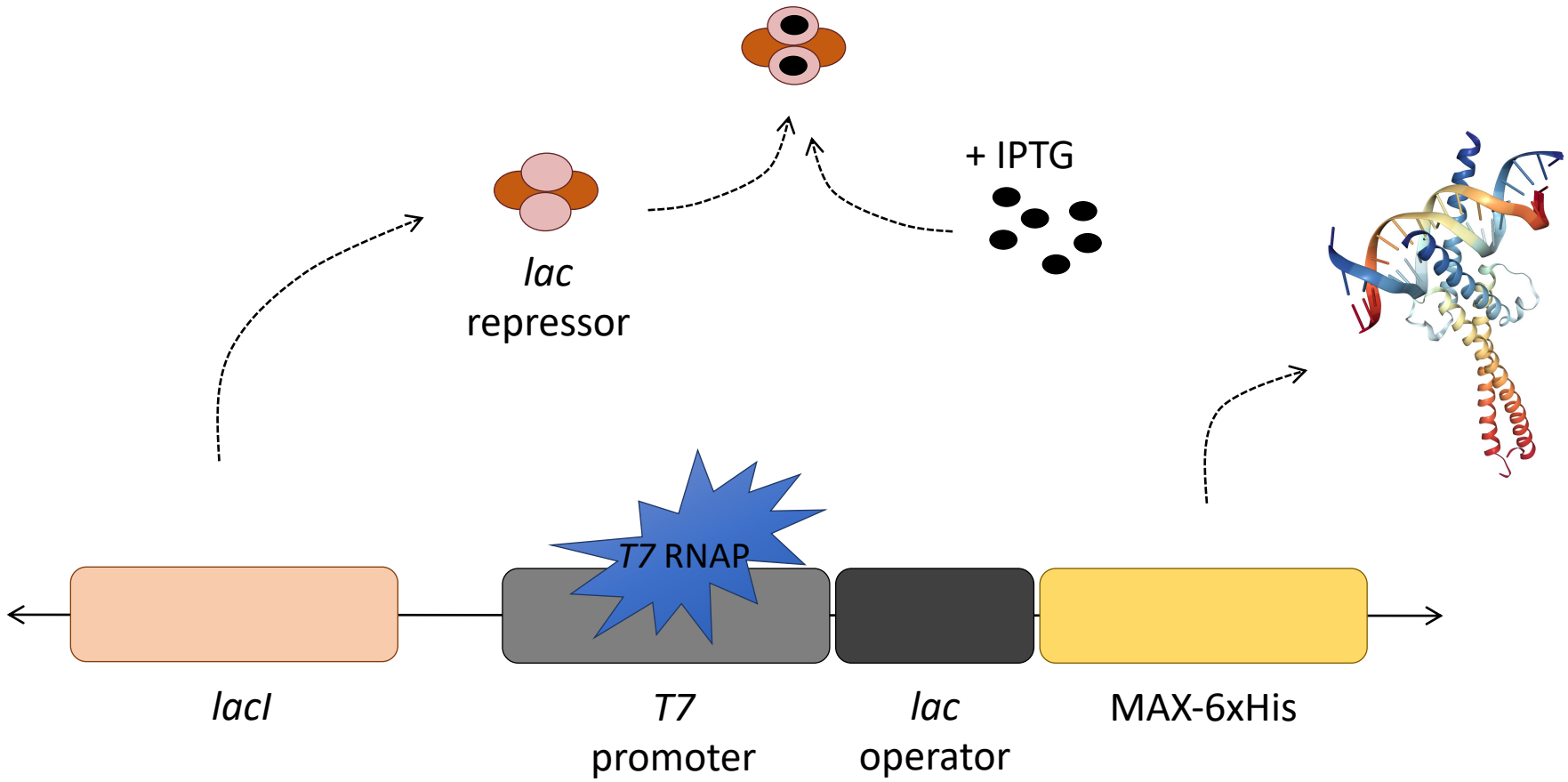
pET28a_MAX-6xHis

LacI repressor blocks transcription at *lac* operator



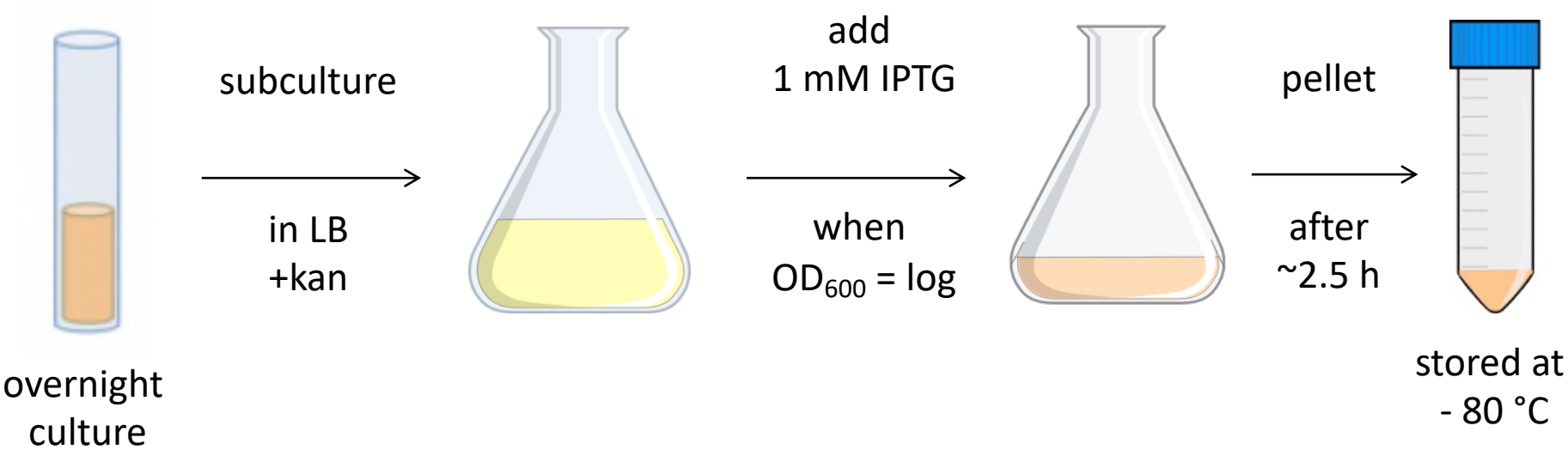
pET28a_MAX-6xHis

IPTG ‘induces’ MAX-6xHis expression



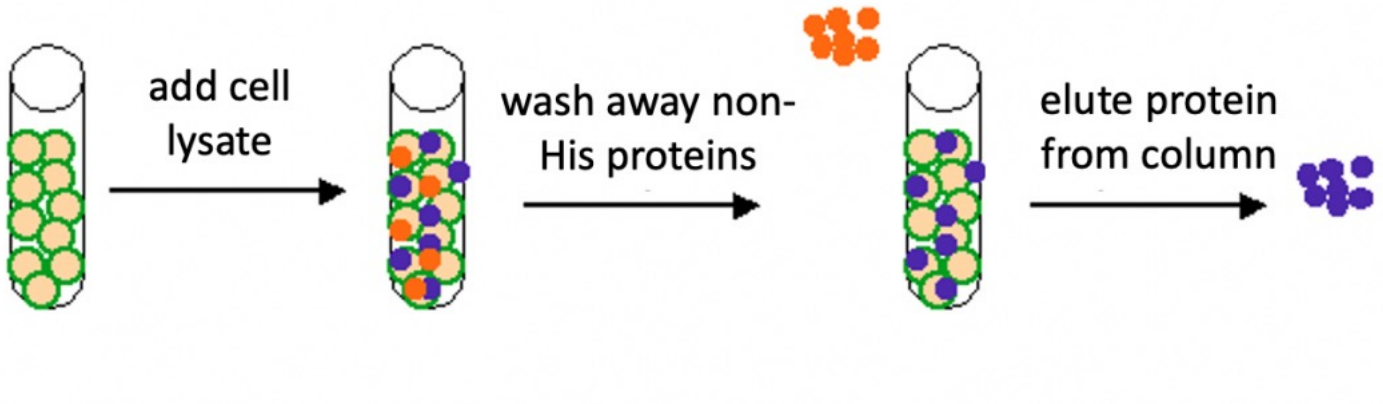
pET28a_MAX-6xHis

How did we induce protein expression?

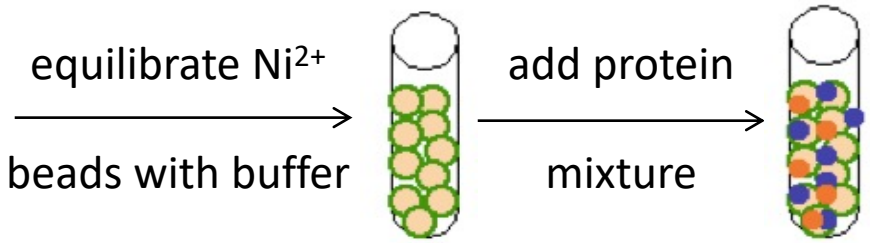


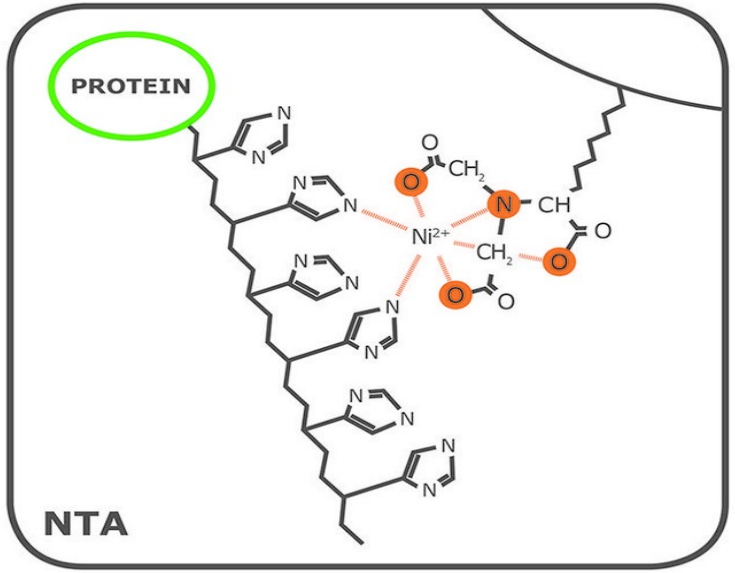
How will you purify MAX-6xHis?

- First, need to lyse cells to release proteins
  - B-PER (Bacterial Protein Extraction Reagent):
  - Lysonase:
  - Proteinase inhibitor:



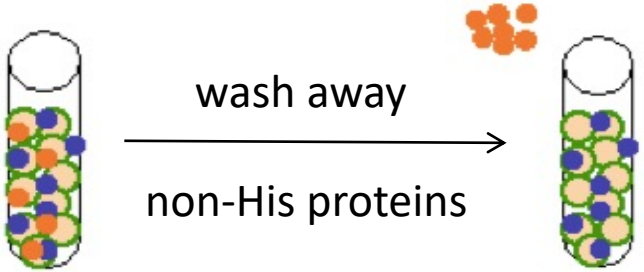
6xHis tag binds to Ni^{2+} resin / column

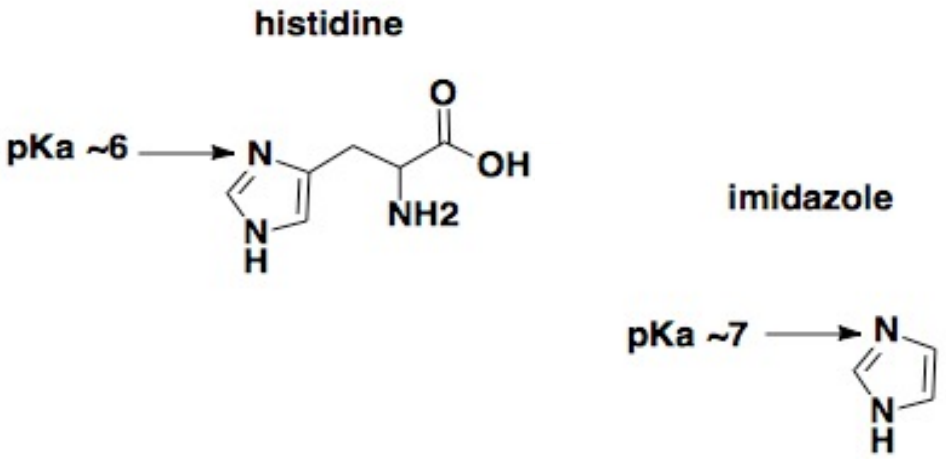




- Ni^{2+} chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand
- His tag chelates to Ni^{2+} causing protein to ‘stick’ to resin / column

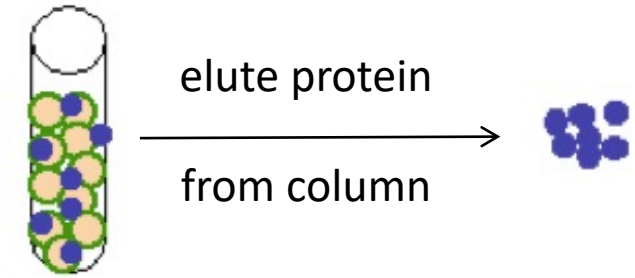
Non-specific binders washed from Ni^{2+} resin / column using imidazole

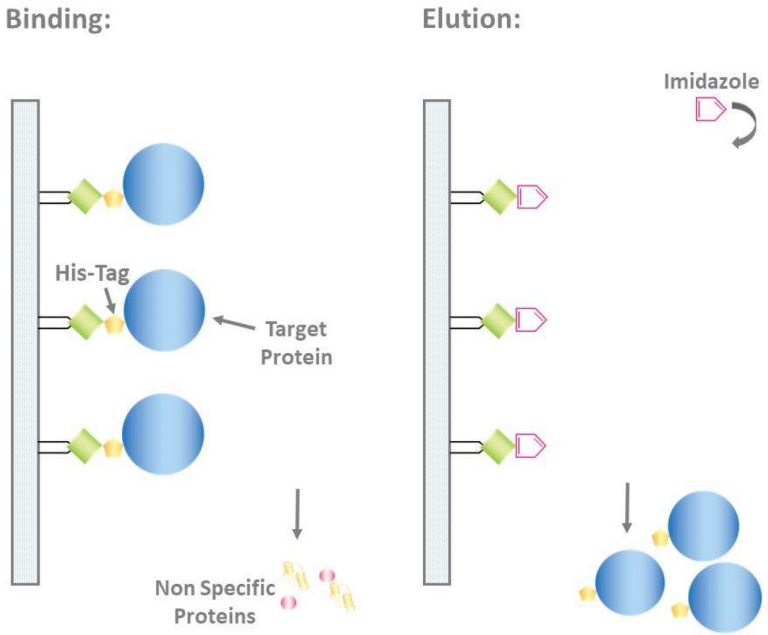




- Low concentration of imidazole included in wash buffer
- Imidazole competes for binding to Ni^{2+} resin
  - Low affinity binders / non-specific binders are outcompeted and released from the resin

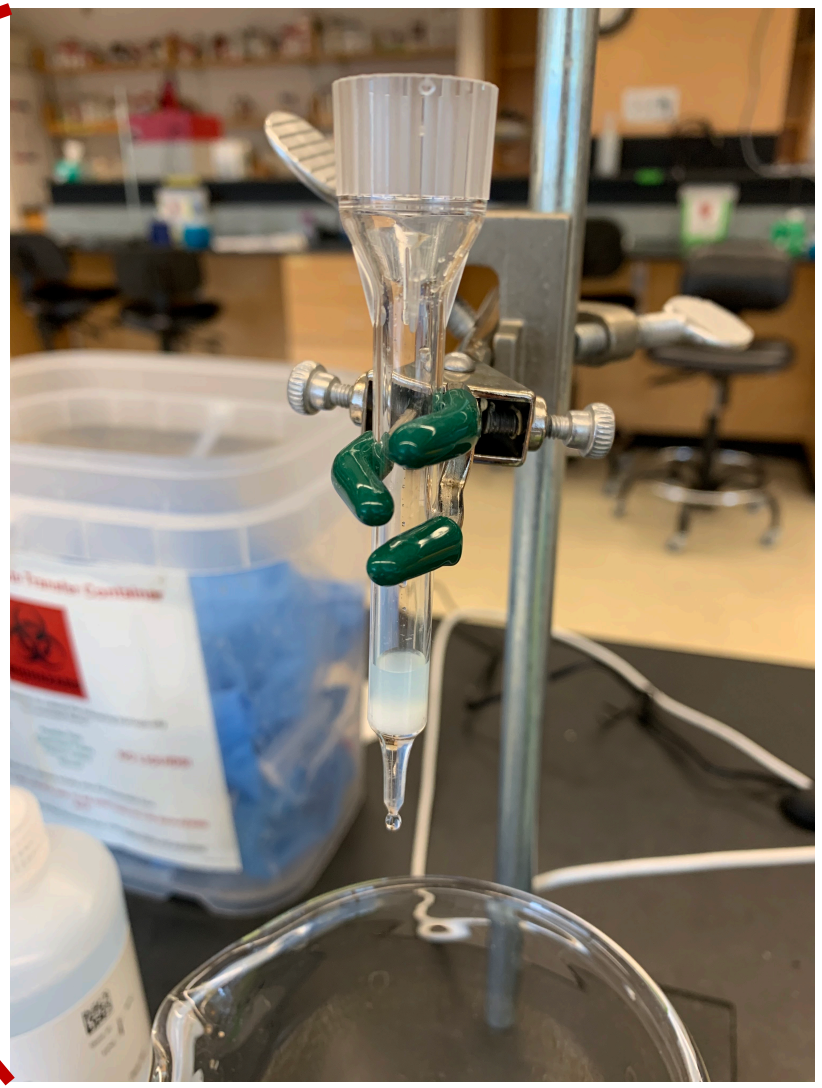
Imidazole used to elute protein from column



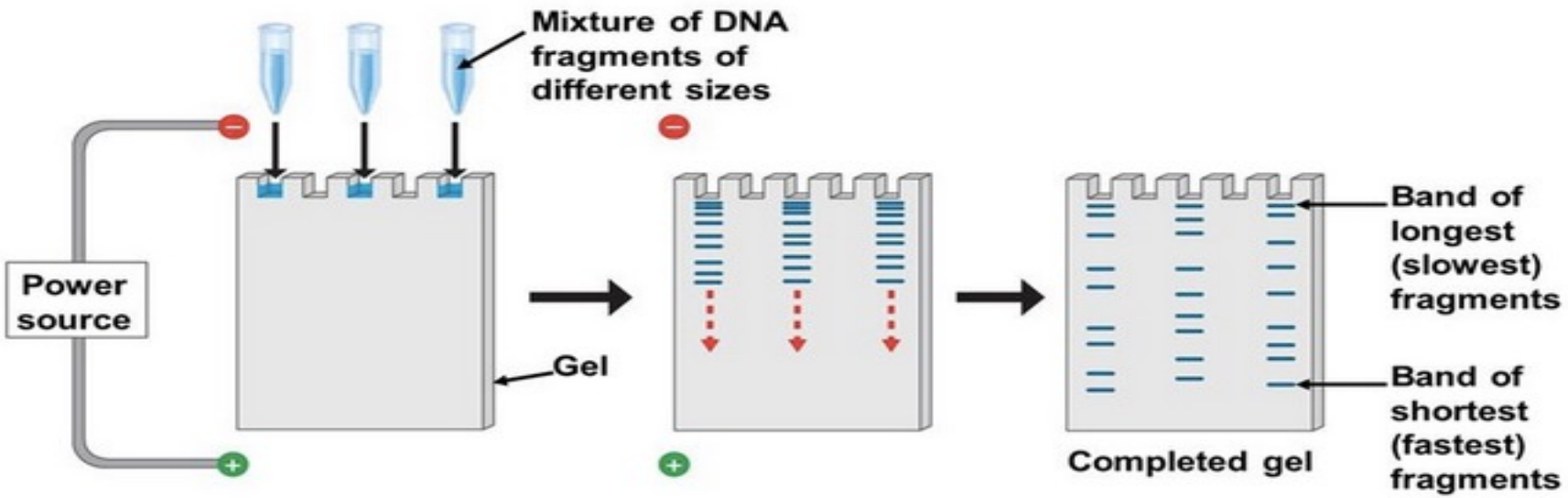


- Elution buffer contains higher concentration of imidazole compared to wash buffer
- Increased concentration allows imidazole to out-compete 6xHis for binding to Ni^{2+} resin

Demonstration of protein purification



Wrap-up of confirmation digest



- How do you visualize migration through the gel?

- How do you visualize DNA bands in the gel?

For today…

- Start protein purification protocol
- Complete gel electrophoresis during lysis incubation
- Be sure to clearly label all tubes containing protein purification aliquots!

For M1D3…

- Draft a figure of your confirmation digest results for your Data summary
  - All figures should include a TITLE and CAPTION

Notes on figure making:

- Image should not be the entire page
  - Only needs to be large enough to be clear
- Title should be conclusive
  - Don’t include what you did, rather include what you found / discovered
- Caption should not include methods details
  - Define abbreviations, symbols, etc.

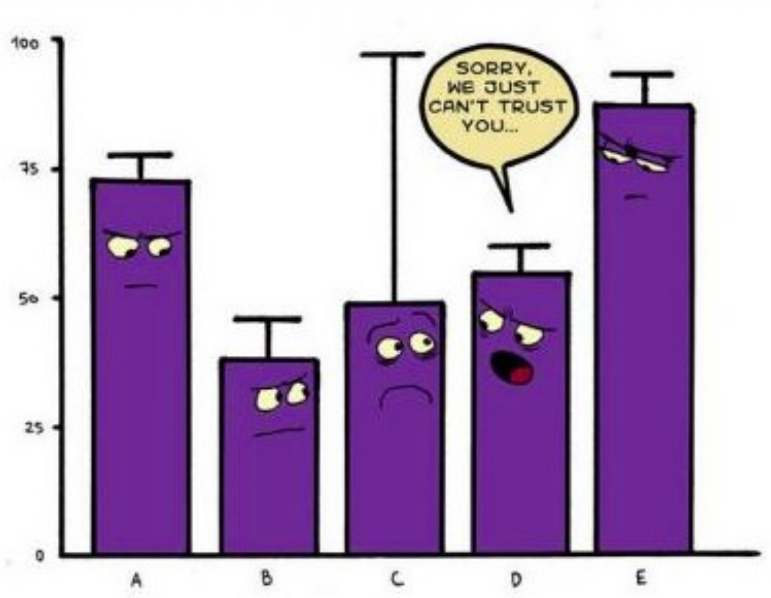


Figure X: Title is the take-home message of the experimental data.
Caption includes all of the details necessary to understand the data presented in the figure…not methods!!