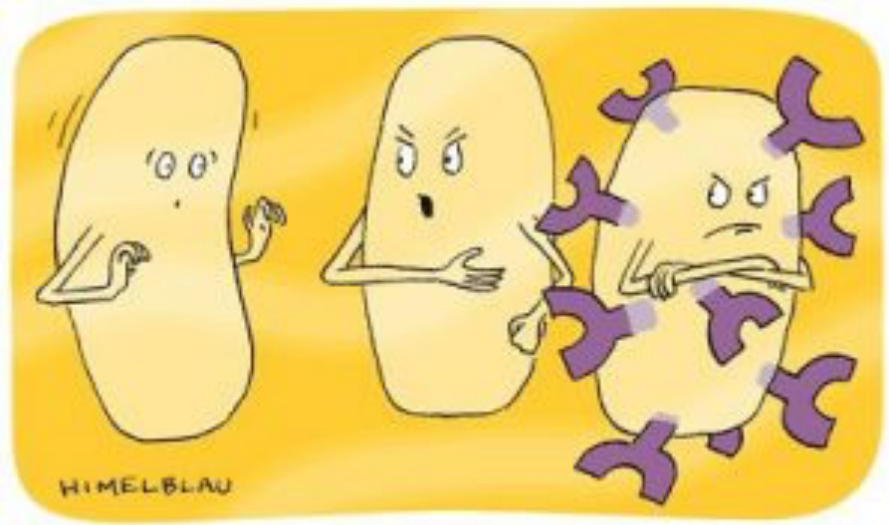
M2D1: Perform protein purification protocol

- Prelab discussion
- Purification demo
- Wiki protocol for purifying TDP43_RRM12 protein



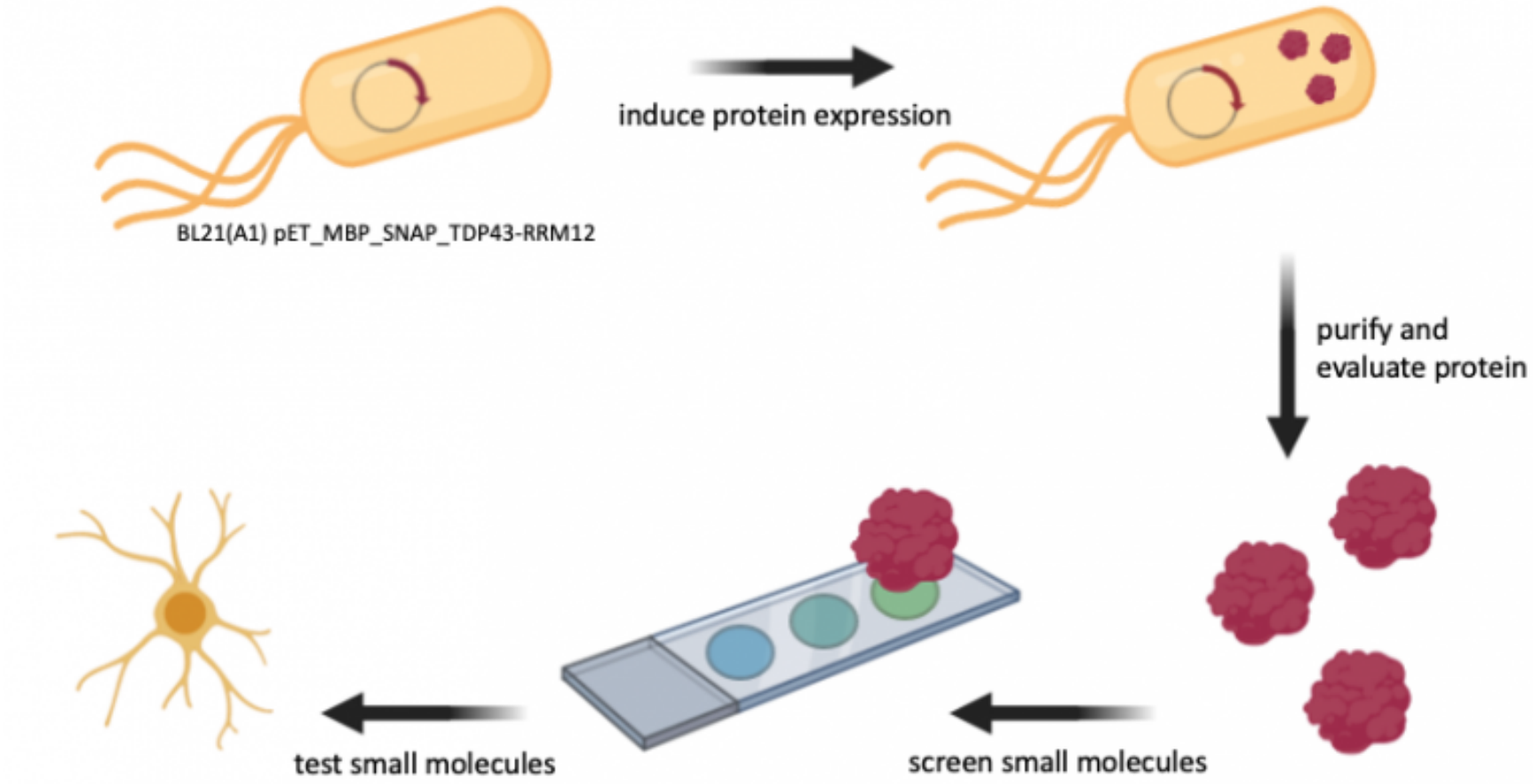
"Don't pick it up," I say, and he says, "It's just a *plasmid*, what harm could it do?" Well just look at him now....God knows *what* protein he's expressing!

Important Dates for Mod 2!

- Research article (15%)
  - completed individually and submitted via Stellar
  - due 5/2
- Journal club presentation (15%)
  - completed individually and presented via Zoom
  - due 4/6 & 4/8
- Laboratory quizzes
  - scheduled for M2D4 and M2D7
- Notebook (10% collectively)
  - one entry will be graded by Jeff 24 hr after M2D7
- Blog (part of 5% Participation)
  - due 5/3

Overview of Mod2 Experiments

Research goal: Identify and characterize small molecule binders to a protein drug target

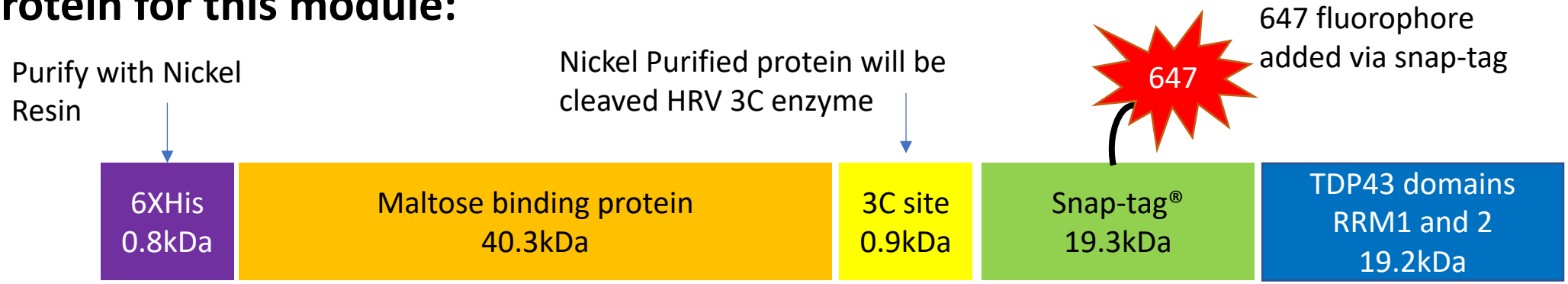


What is TDP-43 RRM12?

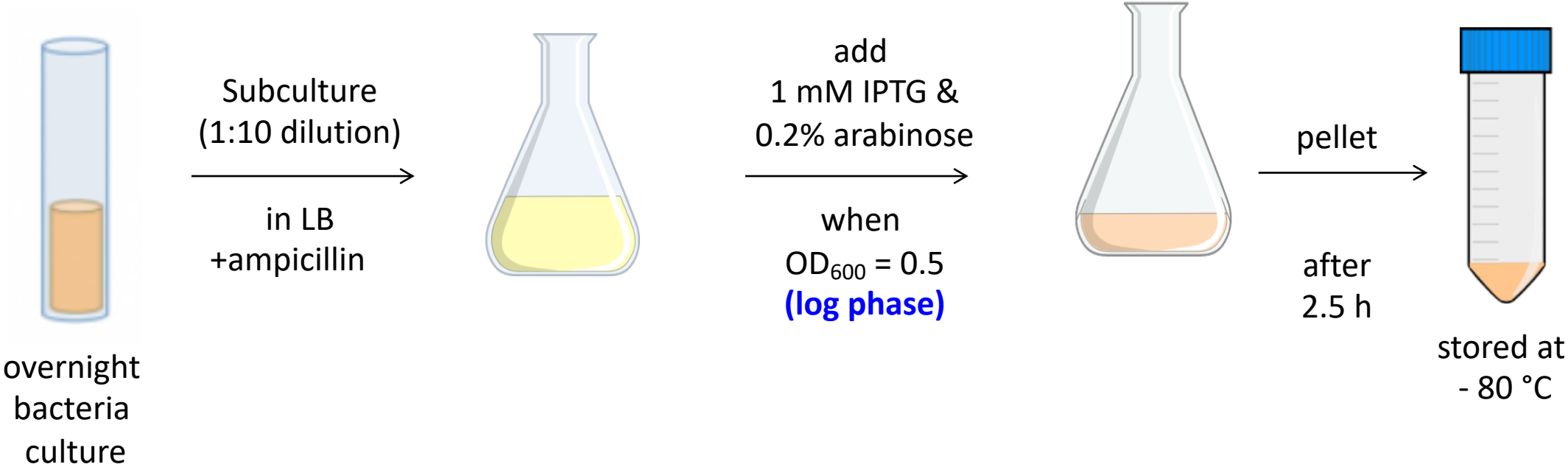
- TDP-43 is a DNA- and RNA-binding protein
  - Mainly localized to the nucleus
  - Can form aggregates and be aberrantly modified
  - Linked to amyotrophic lateral sclerosis (ALS) and frontotemporal dementia FTD)

- 4 main domains
  - N-terminal & C-terminal domains
  - 2 RNA recognition motifs (RRM1 and RRM2)

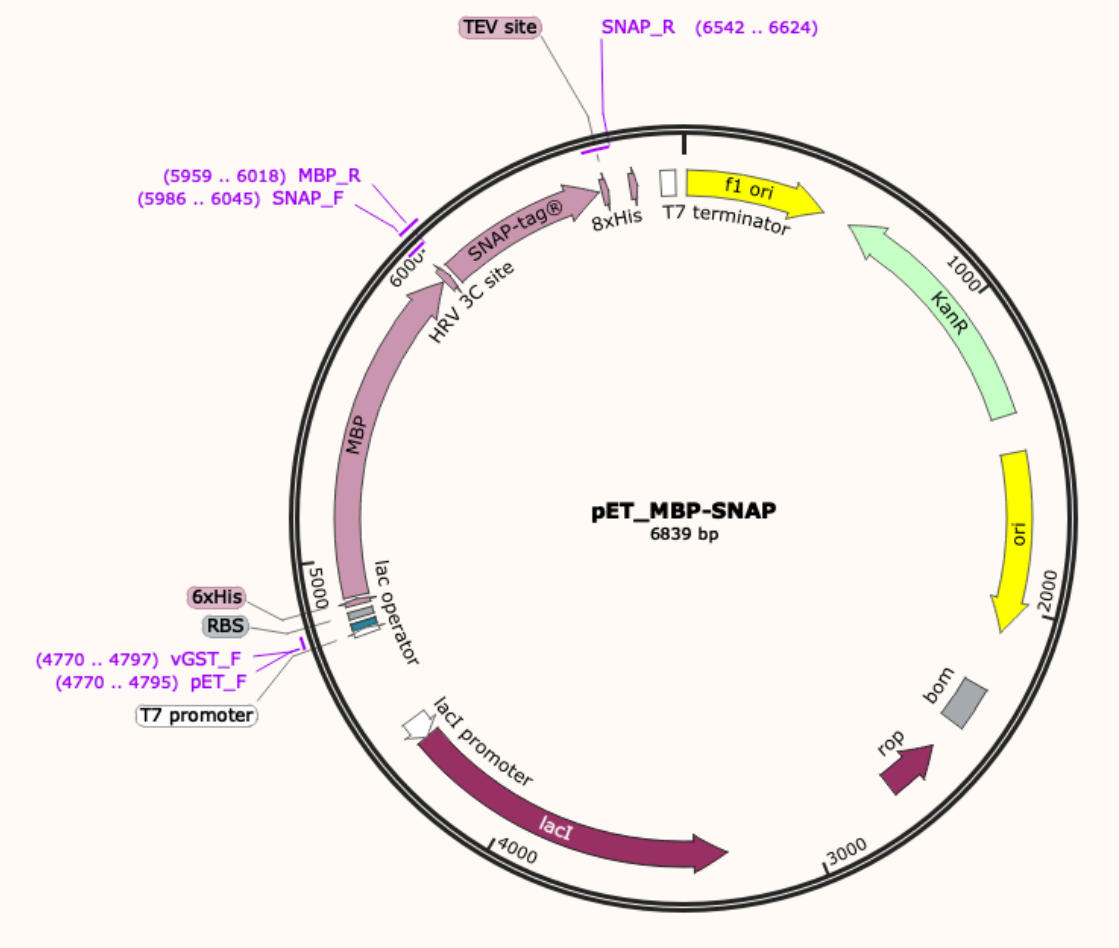
Our protein for this module:



How did we induce protein expression?

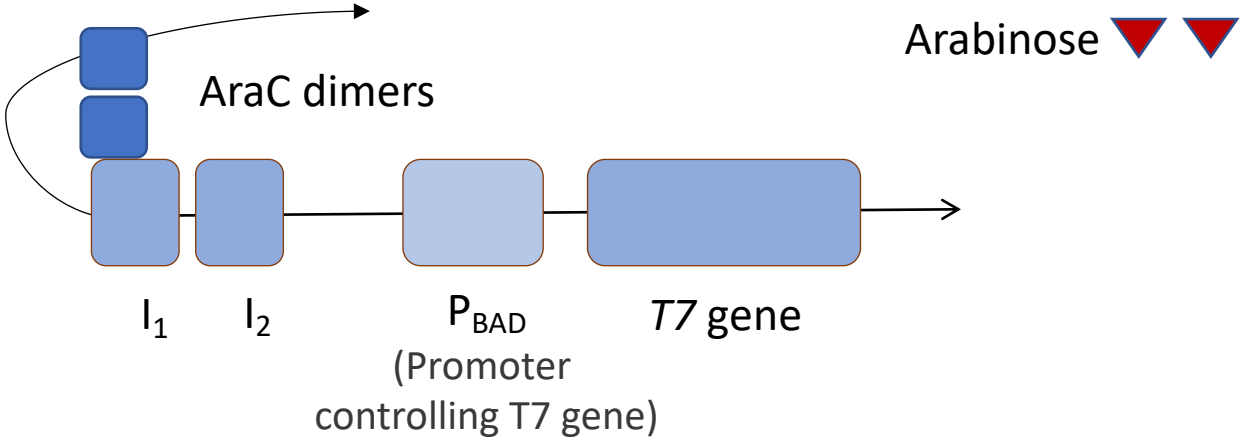


How do we induce protein expression with our plasmid?

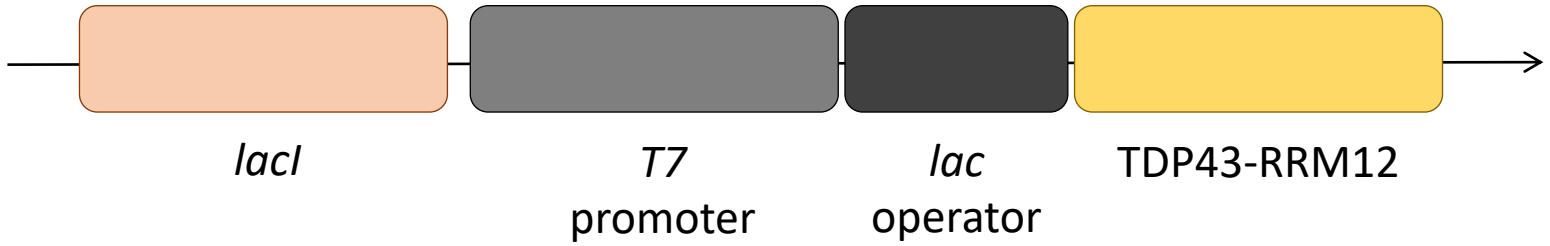


- Dual induction regulated by features encoded on the expression vector
  - T7 promoter
  - *lac* operator

Bacterial induction: How it begins…

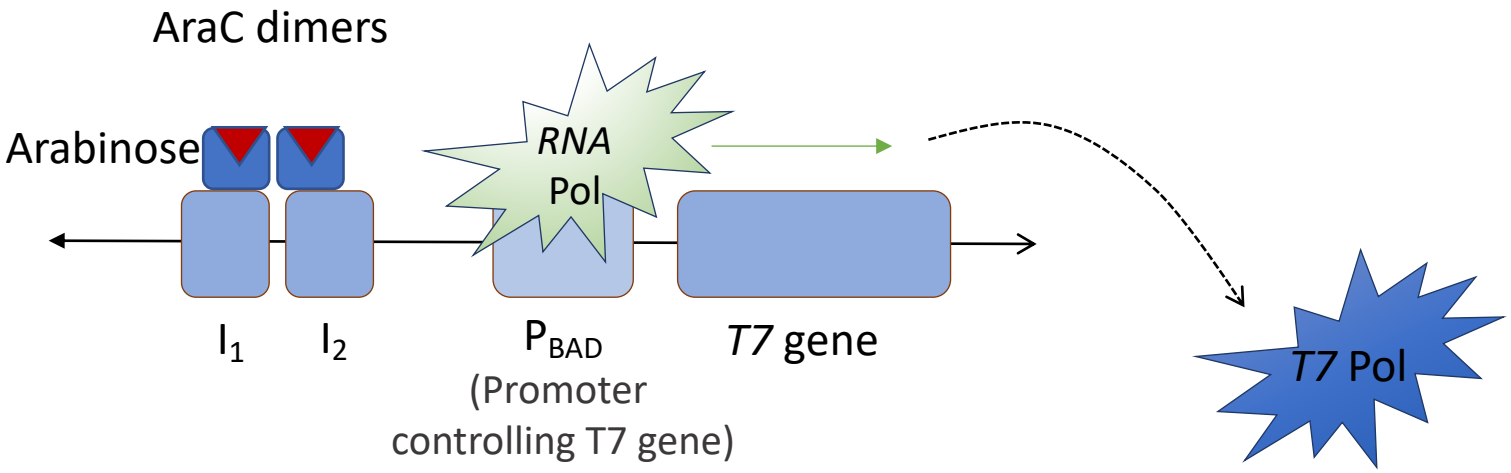


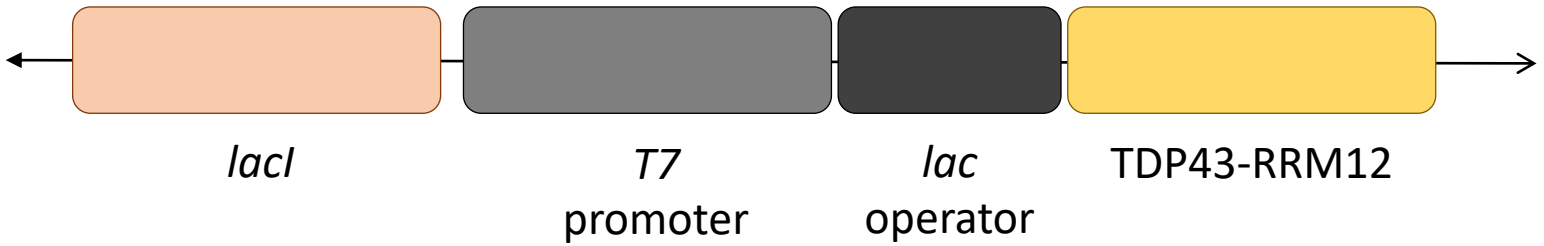
- We transformed BL21-A1 bacteria with our TDP43-RRM12 plasmid
- The BL21-A1 bacteria has been modified to express T7 RNA polymerase
- Expression of T7 polymerase is regulated by P_{BAD} via arabinose induction



pET_MBP_SNAP_TDP43-RRM12

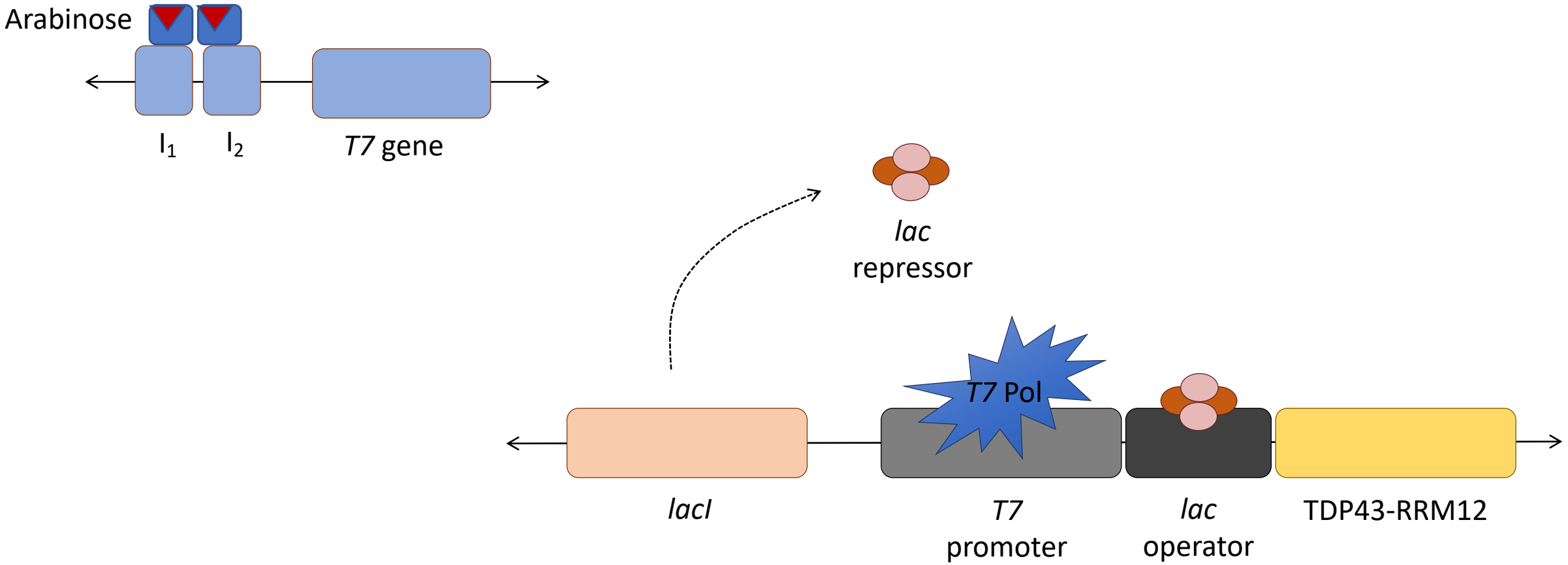
Bacterial induction: Arabinose controls T7 expression





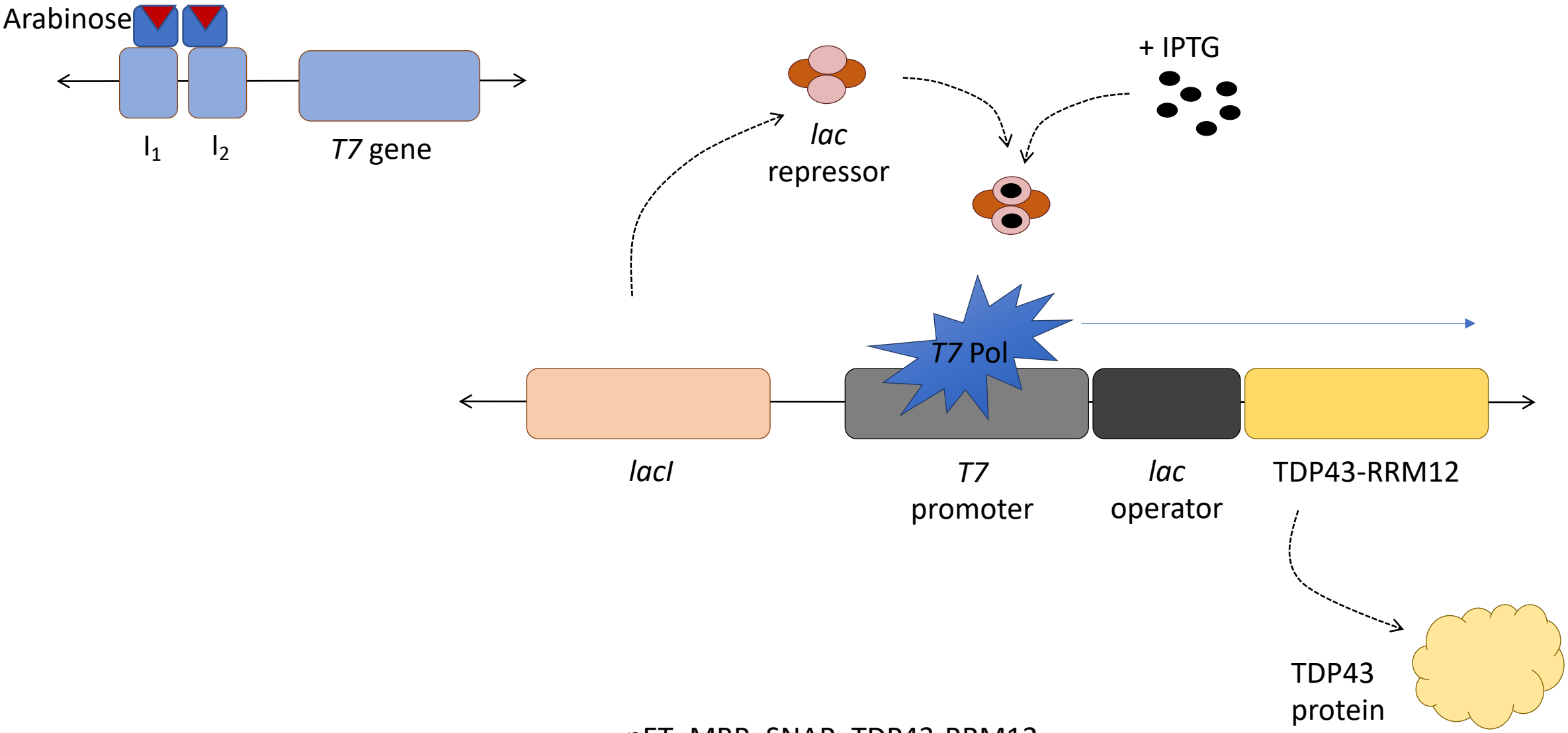
pET_MBP_SNAP_TDP43-RRM12

Bacterial induction: Lac repressor



pET_MBP_SNAP_TDP43-RRM12

Bacterial induction: IPTG removes lac repression



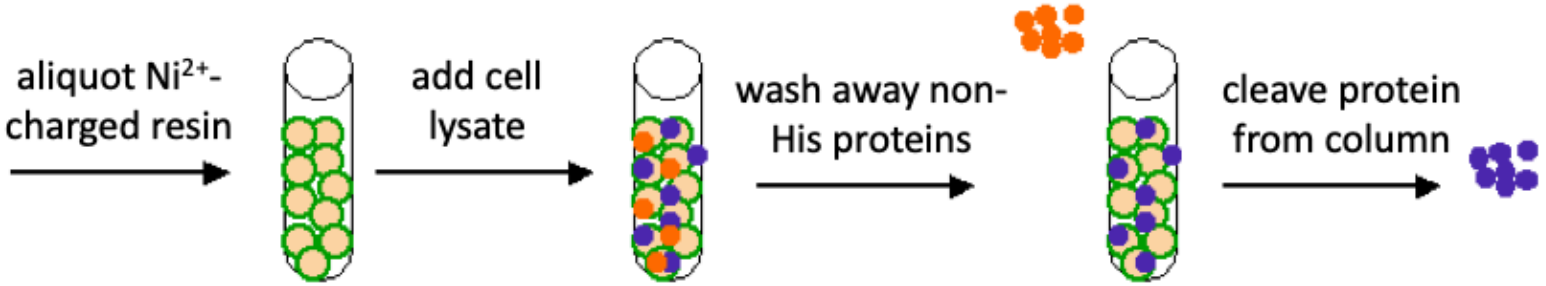
pET_MBP_SNAP_TDP43-RRM12

Quick review of induction system…

- When is T7 RNAP transcribed?
- When is TDP43-RRM12 transcribed?

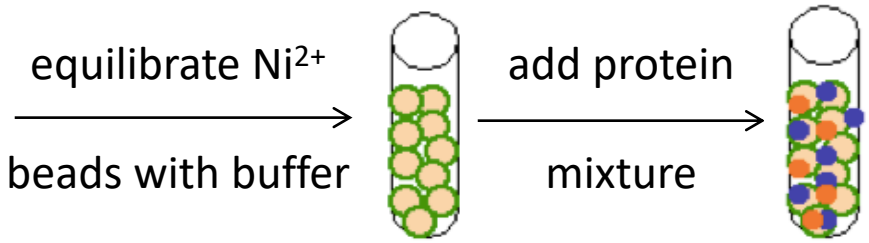
	- arabinose	+ arabinose
- IPTG		
+ IPTG		

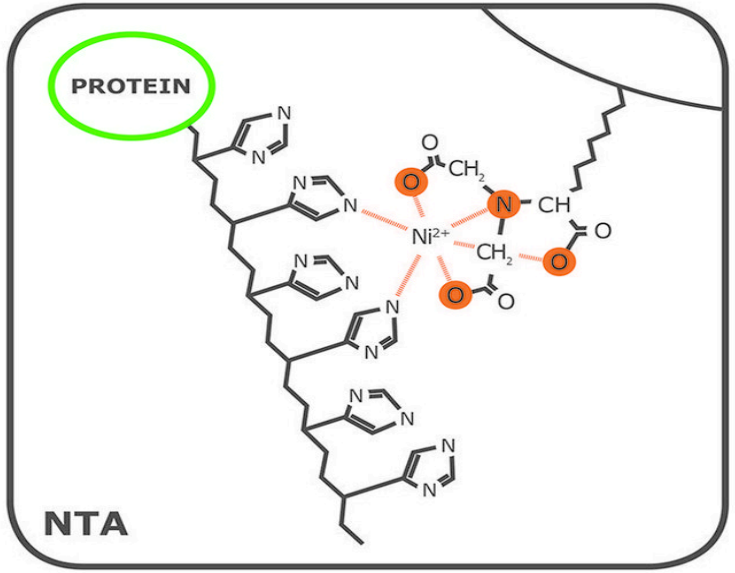
How do we purify TDP43-RRM12?



- First, need to lyse cells to release proteins
  - Lysonase= lysozyme/benzonase: chemical disruption of cell membrane and RNA

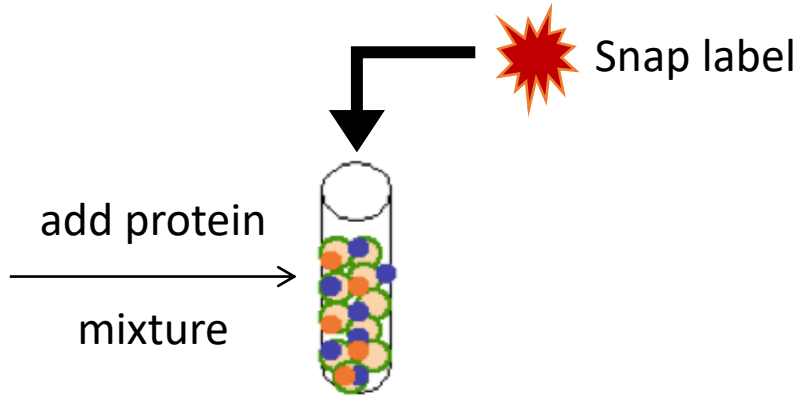
6xHis tag binds to Ni^{2+} resin / column





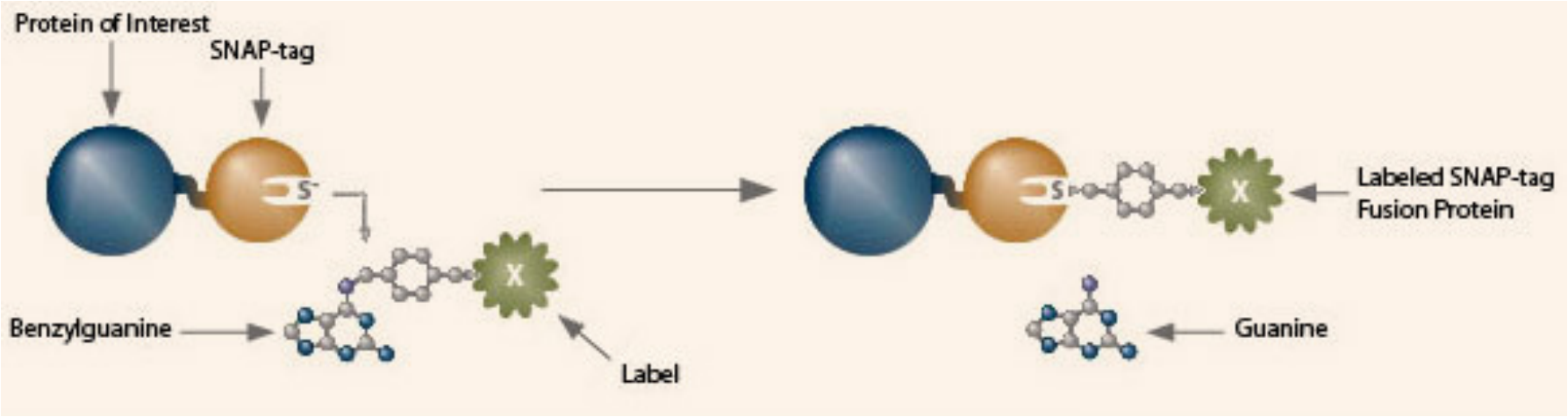
- Ni^{2+} immobilized on agarose resin via nitrilotriacetic acid (NTA) ligand chelation
- His tag chelates to Ni^{2+} causing protein to ‘stick’ to resin / column

How does SNAP labeling work?

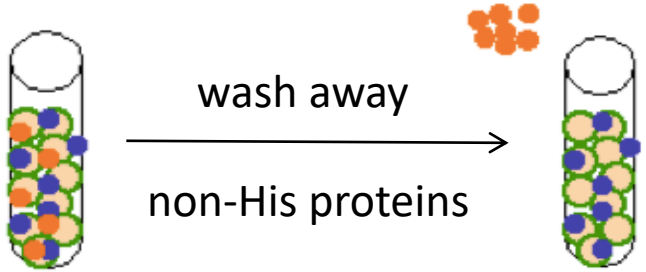


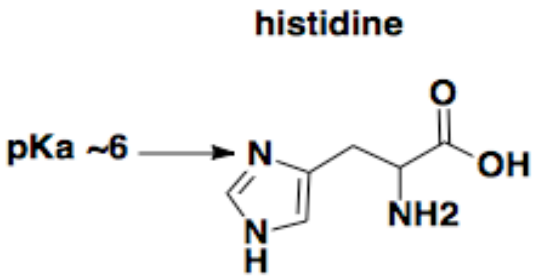
- Snap-tag based on DNA repair protein that repairs alkylated bases

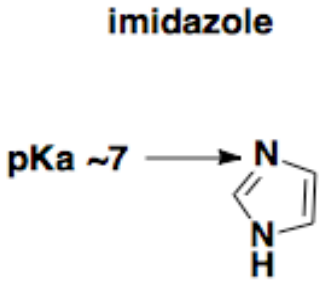
- Nucleophilic substitution reaction results in fluorophore binding to Snap-tag



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

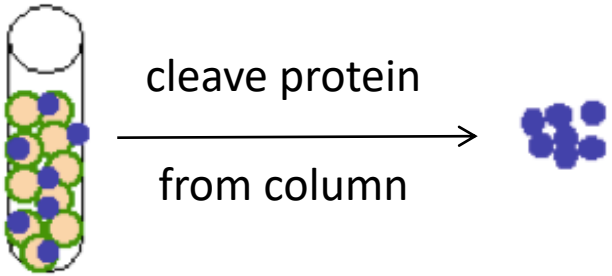




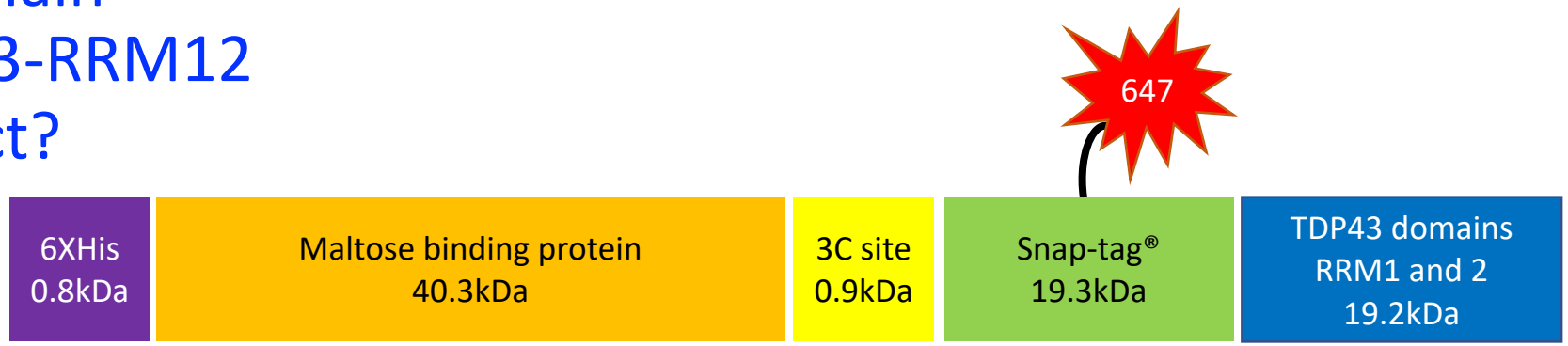


- Low concentration of imidazole included in wash buffer
- Imidazole competes for binding to Ni^{2+} resin
  - Low affinity binders / non-specific binders are outcompeted and released from the resin

HRV 3C cleavage reaction used to release protein from resin / column



What sequences remain associated with the TDP43-RRM12 purification product?



For today…

- Watch demo of protein purification
- Work through M2D1 wiki protocol

- Work on Data Summary!

For M2D2…

- Select an article for journal club!
  - Only one article can be selected per person in this section

- Make an experimental schematic of protein purification

Creating an experimental schematic

- An experimental schematic details key points of an experimental protocol
  - Even though more details are included, the goal is still to document key points only

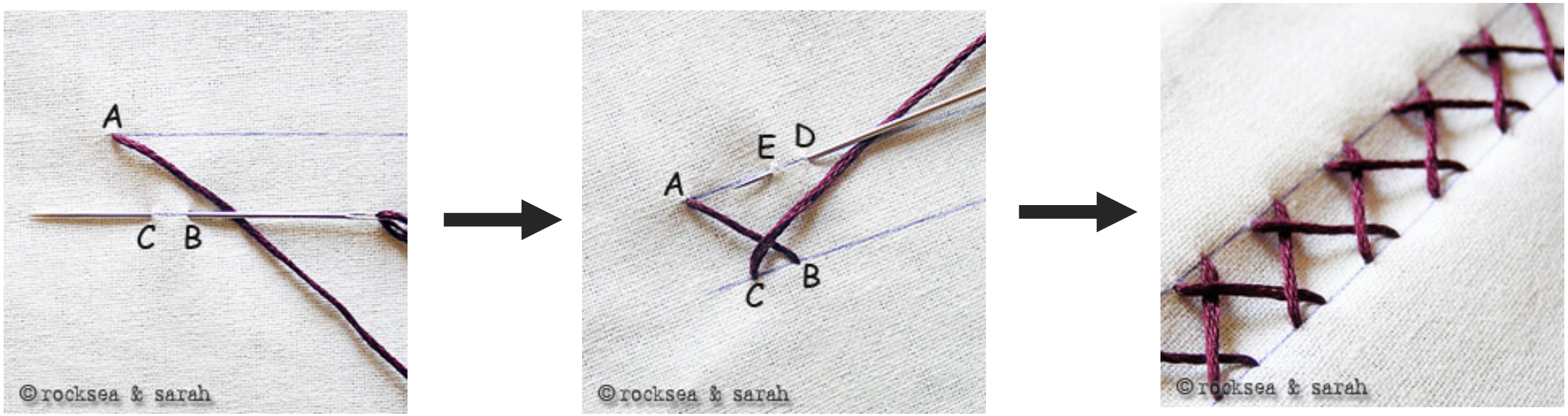


Figure X: Workflow to create the herringbone stitch using embroidery
The herringbone stitch is used to create a border in embroidery. To perform this stitch, the thread is pulled through the fabric, back to front, along the top border at point A. Reinsert the needle in the fabric along the bottom border to create a single short stitch between points B and C. Repeat this process along the top border at points D and E. Continue this pattern until the desired length has been obtained.

Demonstration of protein purification