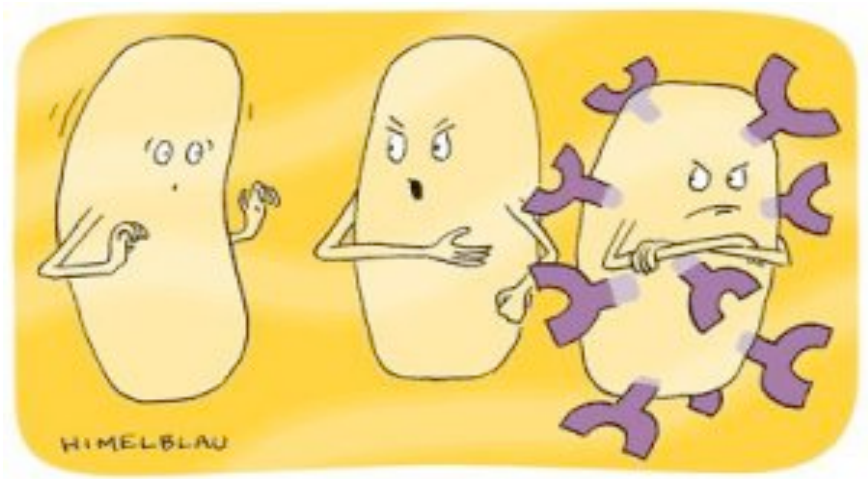
M1D2:
Purify TDP43 protein

1. Prelab discussion
2. Gel electrophorese confirmation digest
3. Purify TPD43 protein

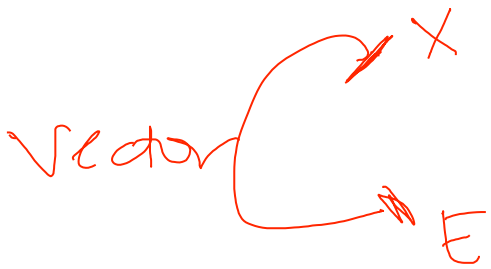


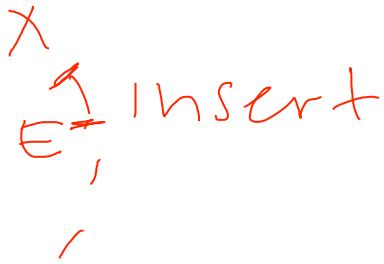
"Don't pick it up," I say, and he says, "It's just a *plasmid*, what harm could it do?" Well just look at him now…who knows *what* protein he's expressing!

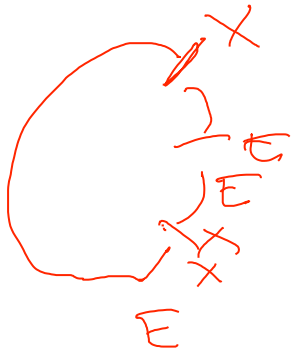
Ideally, 3:1 **molar** ratio of insert:backbone

Why perform confirmation digests?

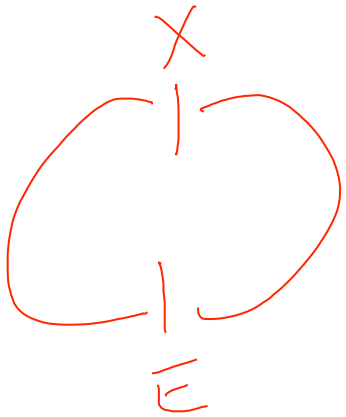
- Too much insert:





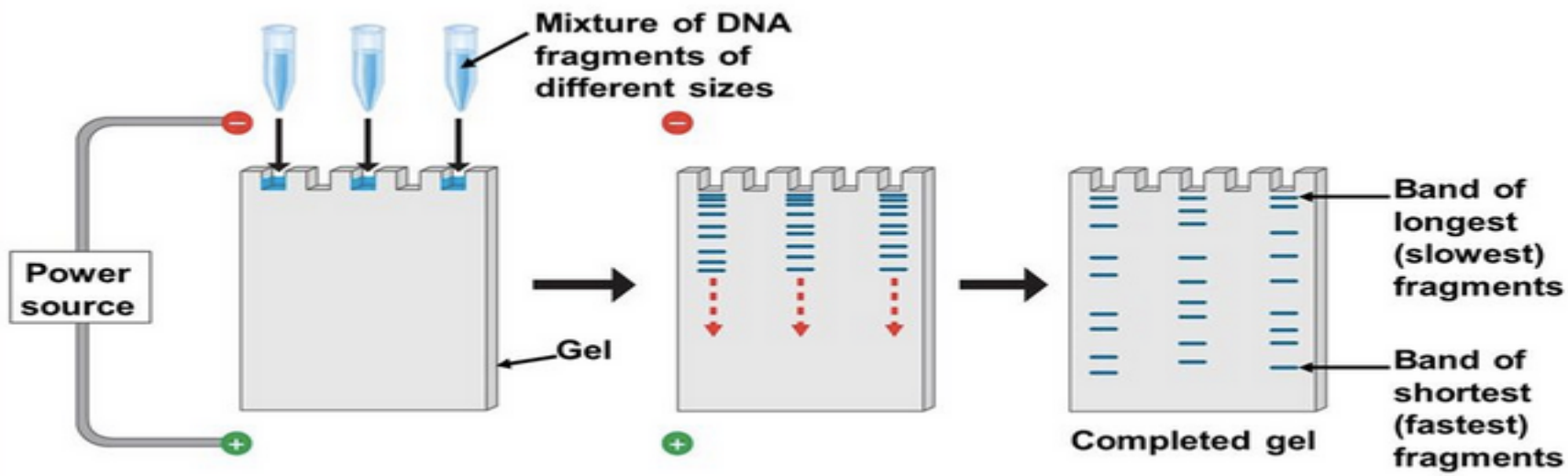


- Too much vector:

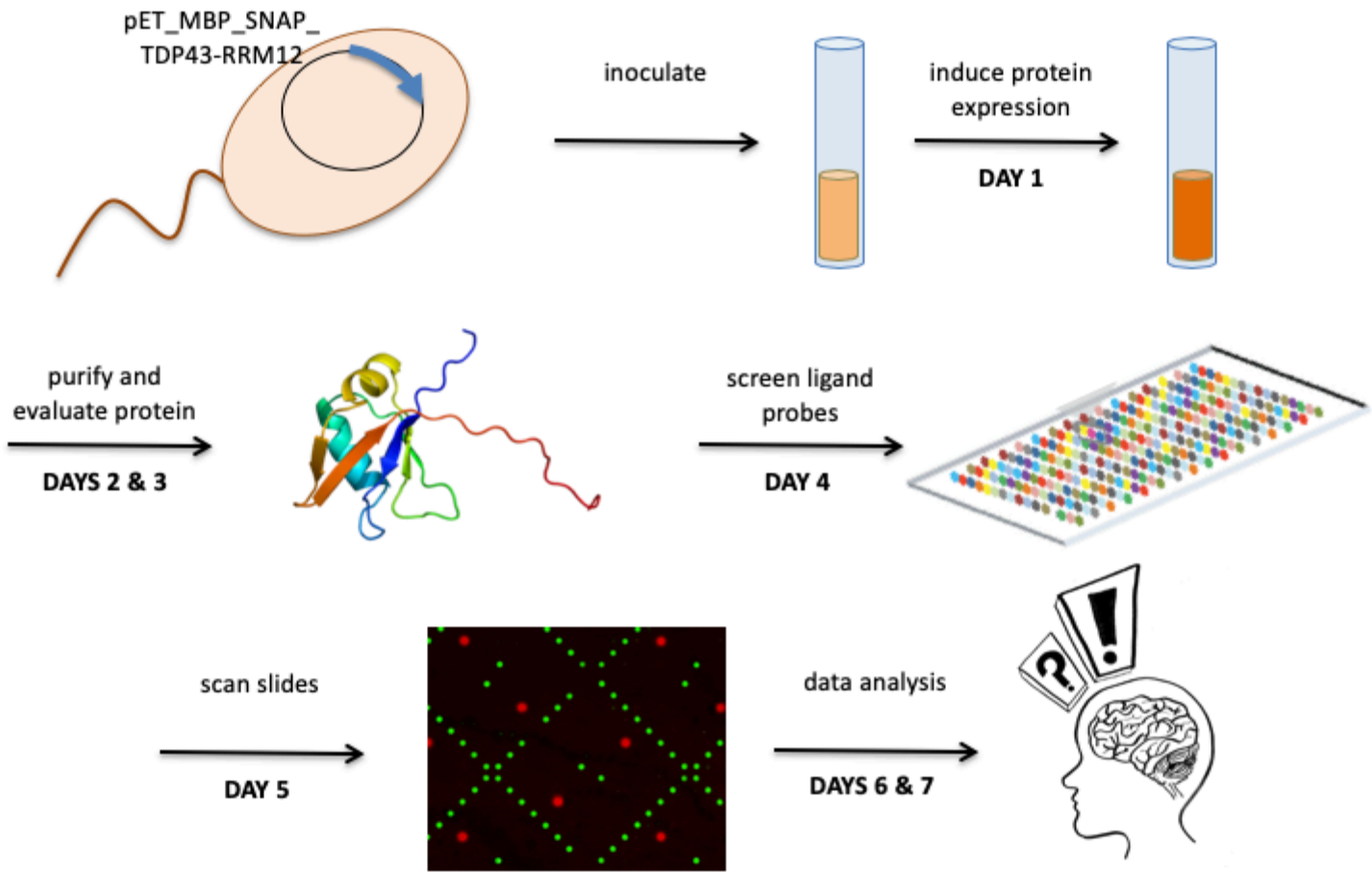


How will we visualize digest results?

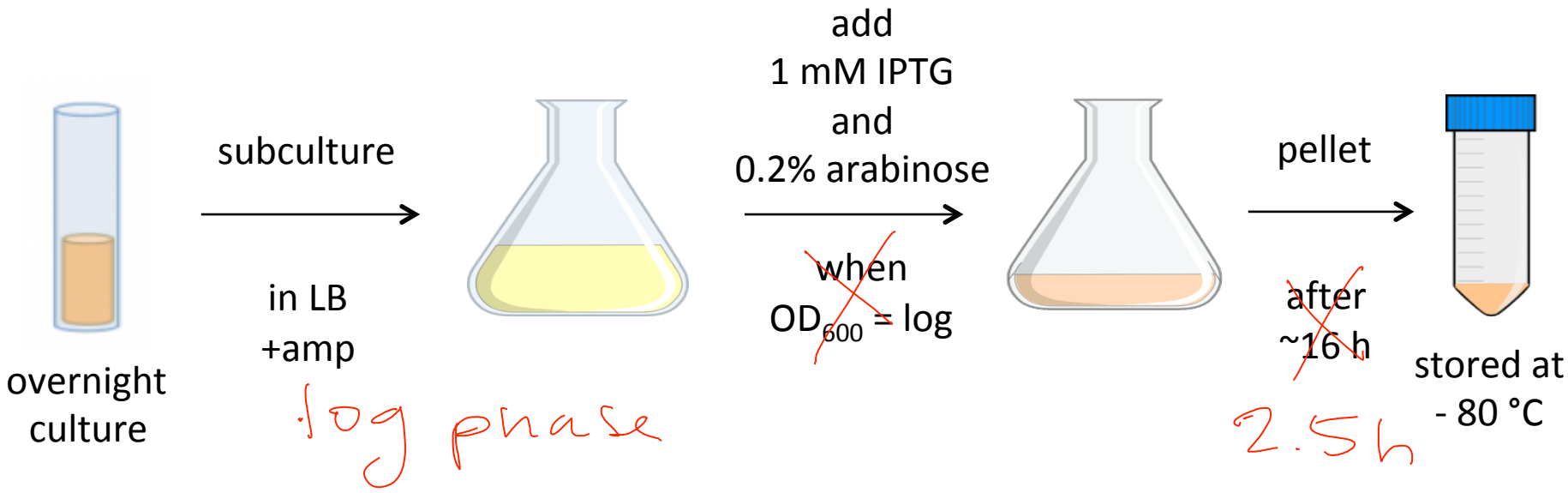
DNA fragments resolved using 1% agarose gel



Overview of Mod1 experiments

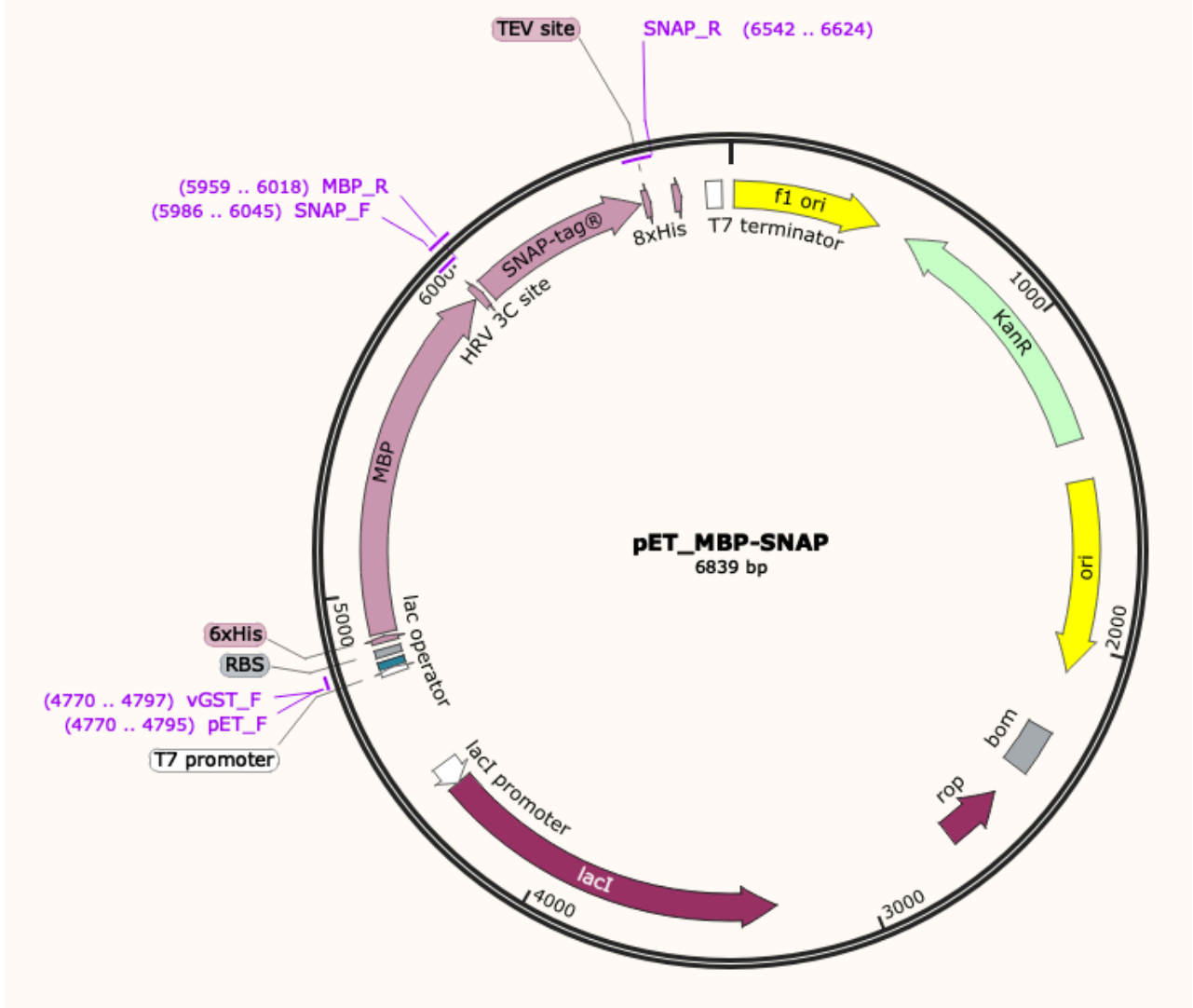


How did we induce protein expression?



In addition to your induced sample, you will also examine and un-induced sample for TDP43 expression

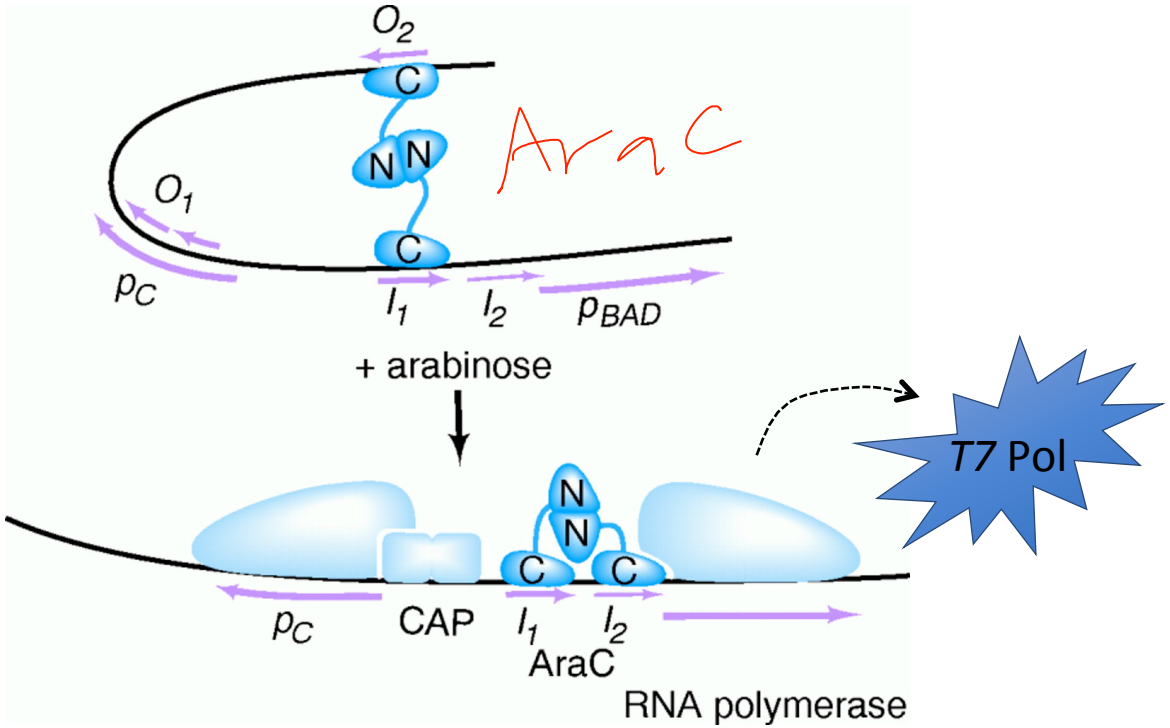
How is protein expression induced?



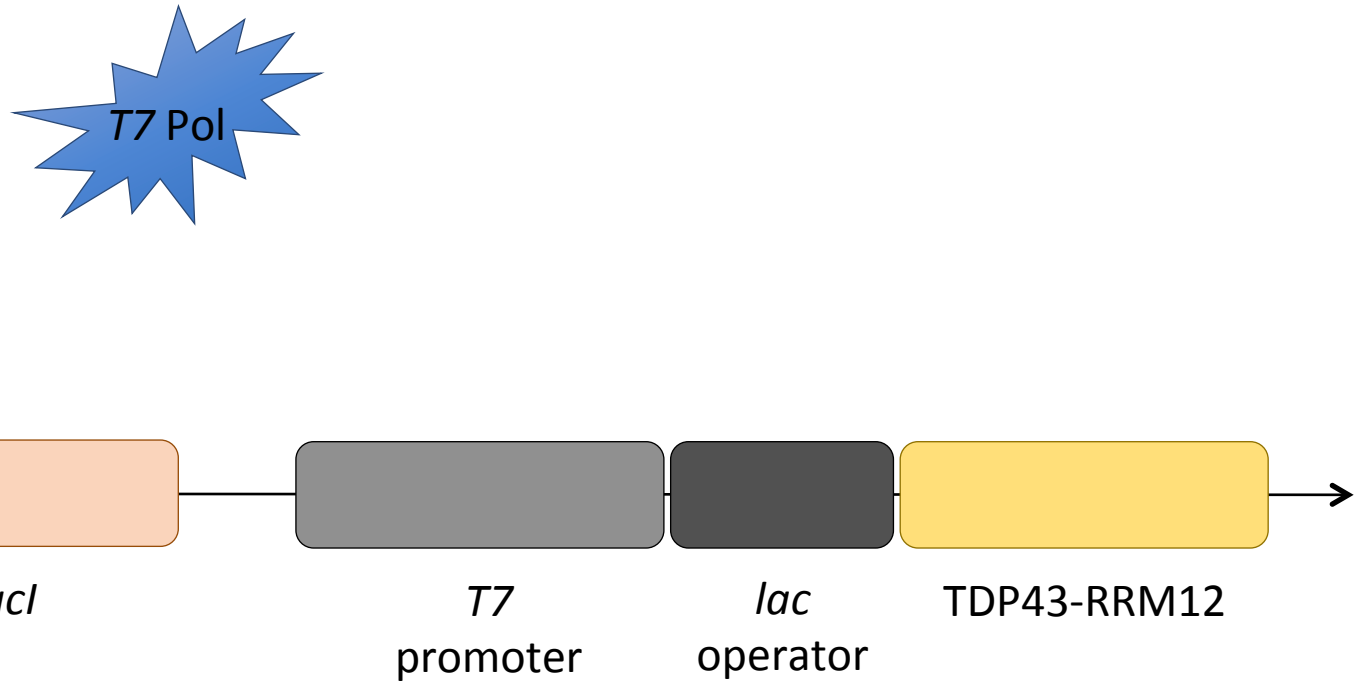
- Dual induction regulated by features encoded on the expression vector
  - T7 promoter plasmid
  - *lac* operator plasmid

BL21-A1 cells used to express TDP43-RRM12

- T7 RNA polymerase expressed from BL21-A1 genome
- Expression regulated by P_{BAD} via arabinose induction

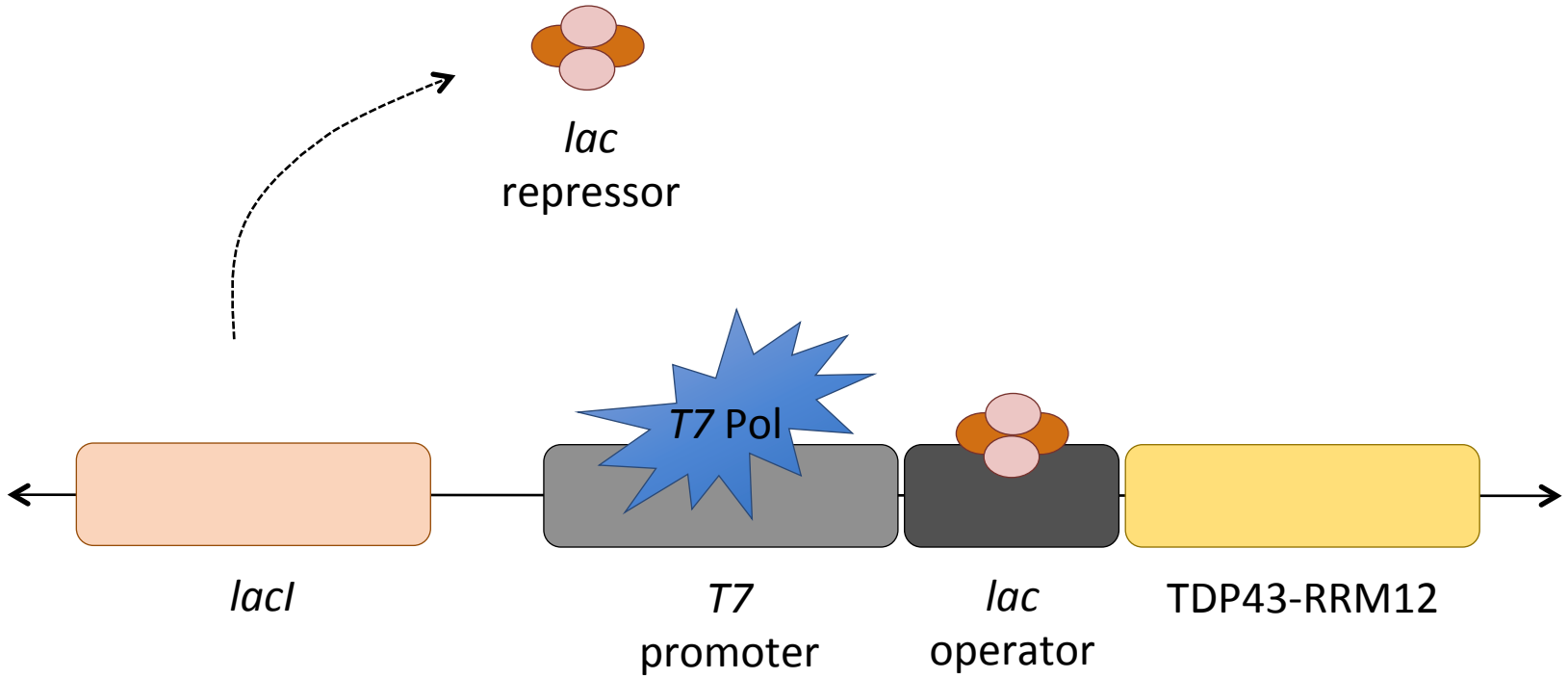


Lac system used to regulate TDP43 expression



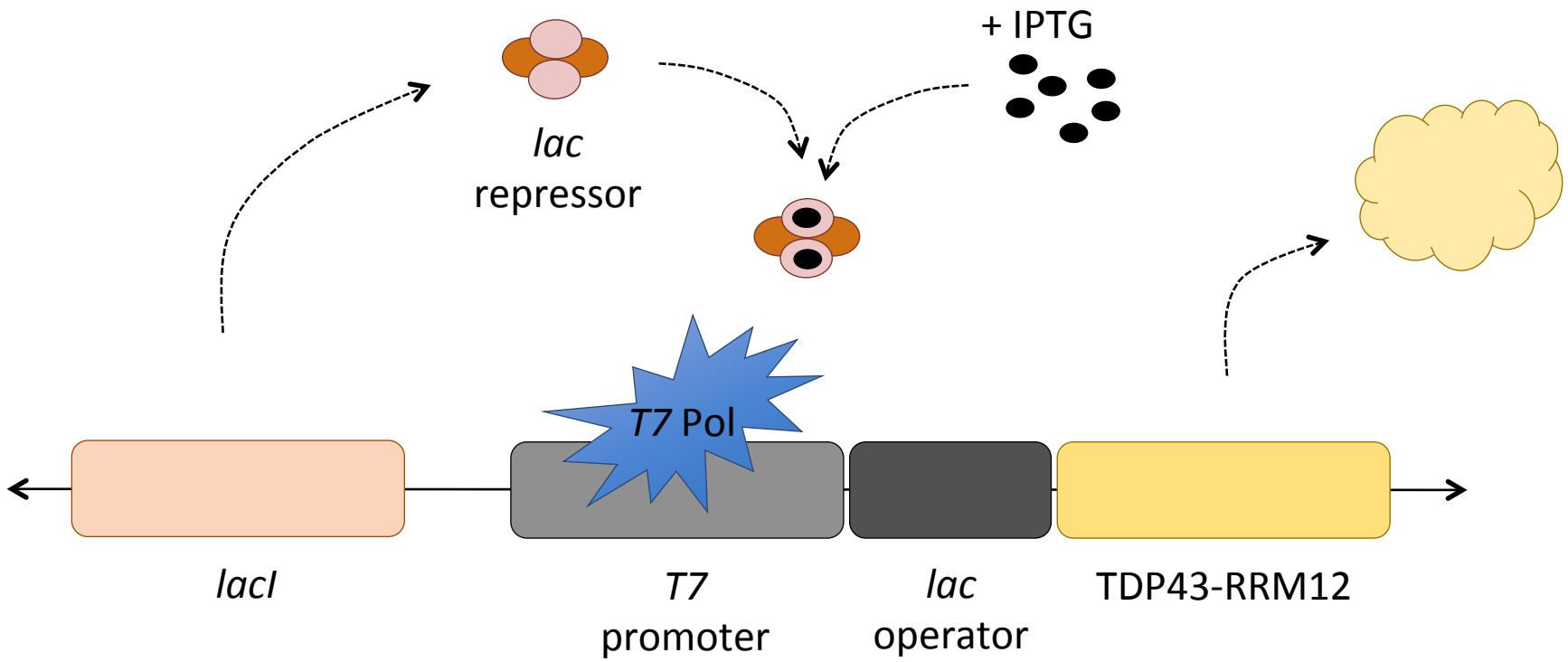
pET_MBP_SNAP_TDP43-RRM12

LacI repressor blocks transcription



pET_MBP_SNAP_TDP43-RRM12

IPTG ‘induces’ protein expression



pET_MBP_SNAP_TDP43-RRM12

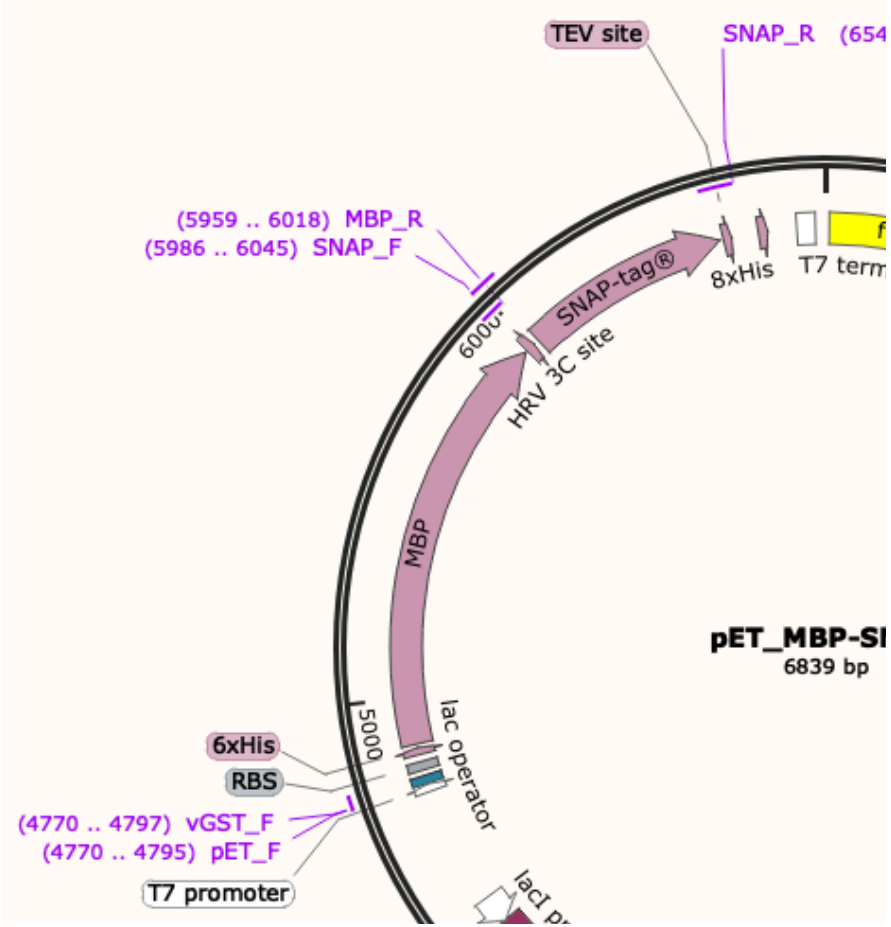
Quick review of induction system…

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

	- arabinose	+ arabinose
- IPTG	– T7 RNAP – TDP43	+ RNAP – TDP43
+ IPTG		

What are you *actually* expressing / purifying?

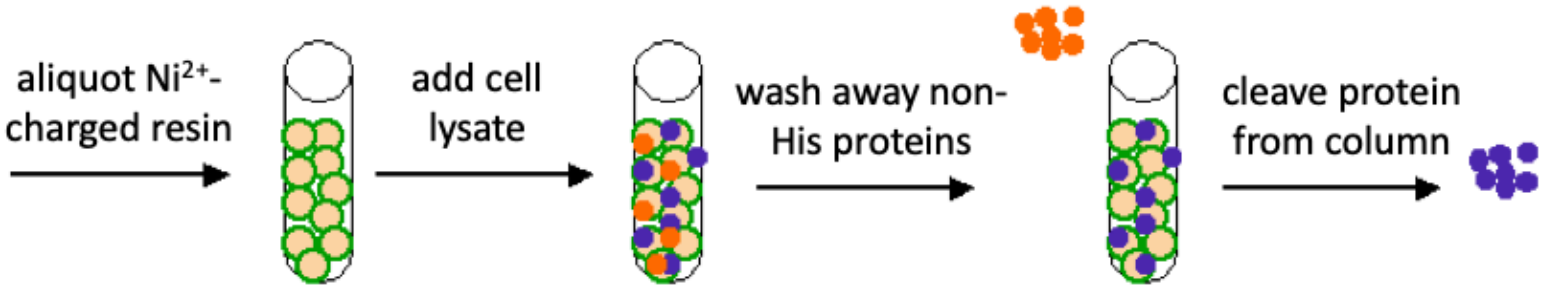
- Draw the TDP43-RRM12 protein product:
  - What additional features were added to TDP43-RRM12 during cloning?
  - What additional features are added to TDP43-RRM12 from the expression vector?



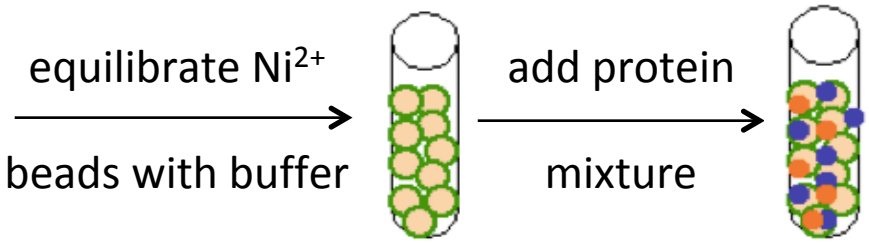
How will you purify TDP43-RRM12?

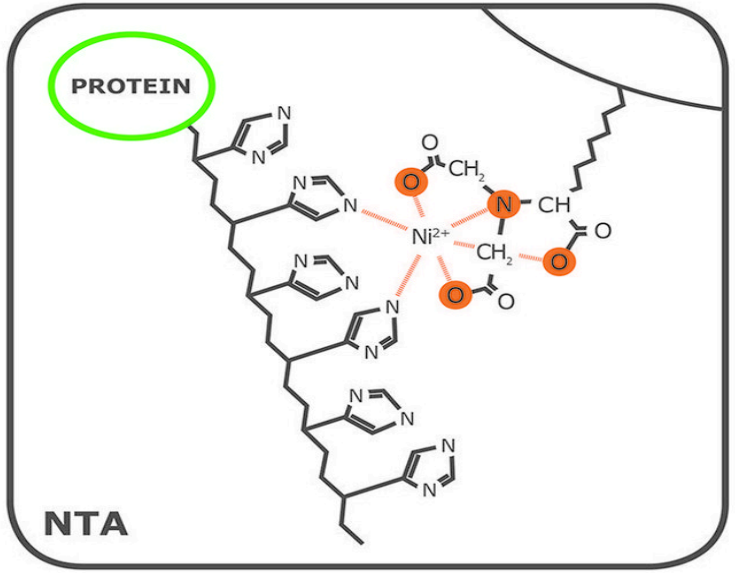
- First, need to lyse cells to release proteins
  - Lysonase: chemical disruption of cell membrane

  - Sonication: physical disruption of cell membrane



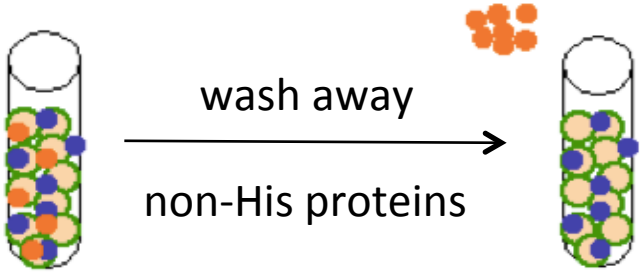
6xHis tag binds to Ni^{2+} resin / column

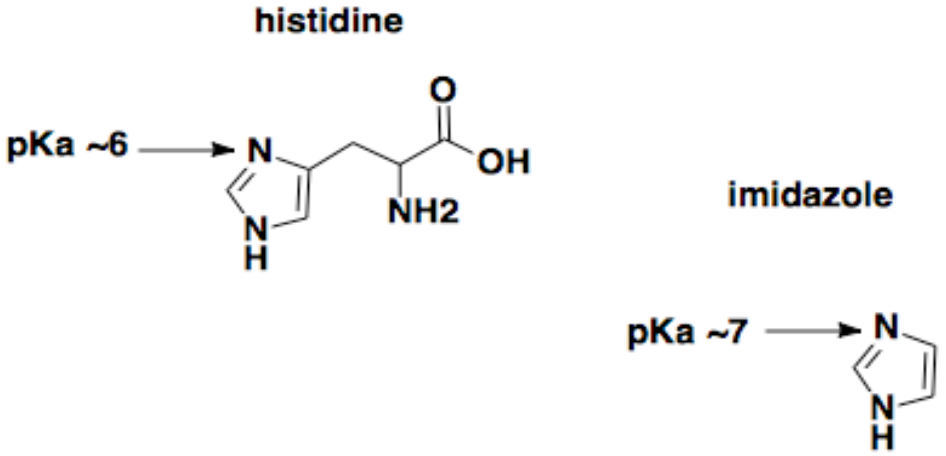




- Ni^{2+} chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand
- His tag chelates to Ni^{2+} causing protein to ‘stick’ to resin / column

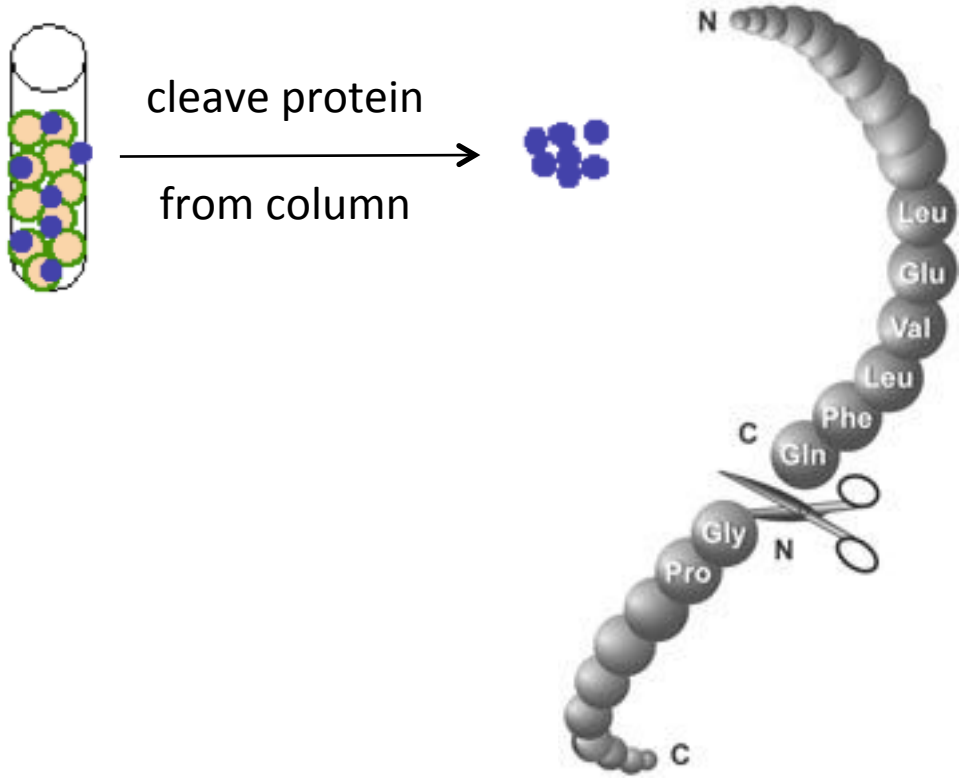
Non-specific binders washed from Ni^{2+} resin / column using imidazole





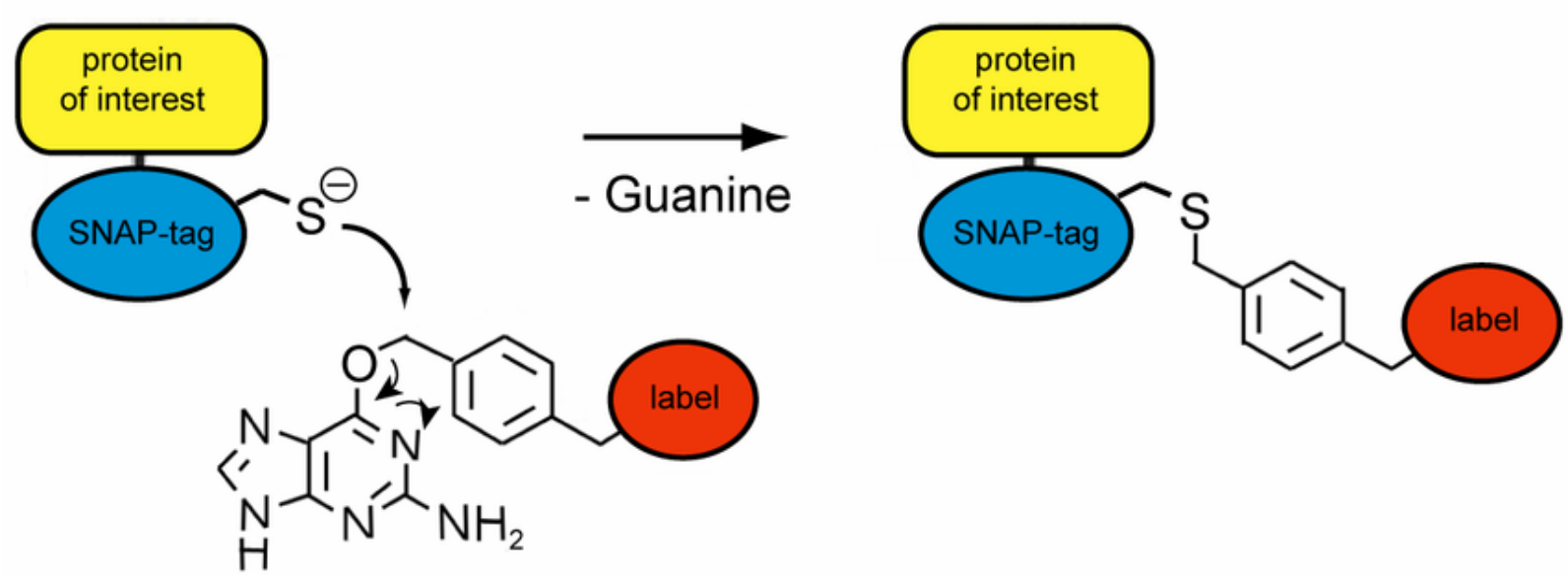
- Low concentration of imidazole included in wash buffer
- Imidazole competes for binding to Ni^{2+} resin
  - Low affinity binders / non-specific binders are outcompeted and released from the resin

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



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

What is the SNAP sequence?



For today…

- Will begin with Part #2 (protein purification)
  - Complete Part #1 (electrophoresis) during ~2 hr incubation at Part #4, Step #8
- At 4:30p begin Part #4, Step #9
- Wipe benchtops and empty waste buckets!

For M1D3…

- Draft a figure of your confirmation digest results for your Data summary
  - ALL figures must include a TITLE and a 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.

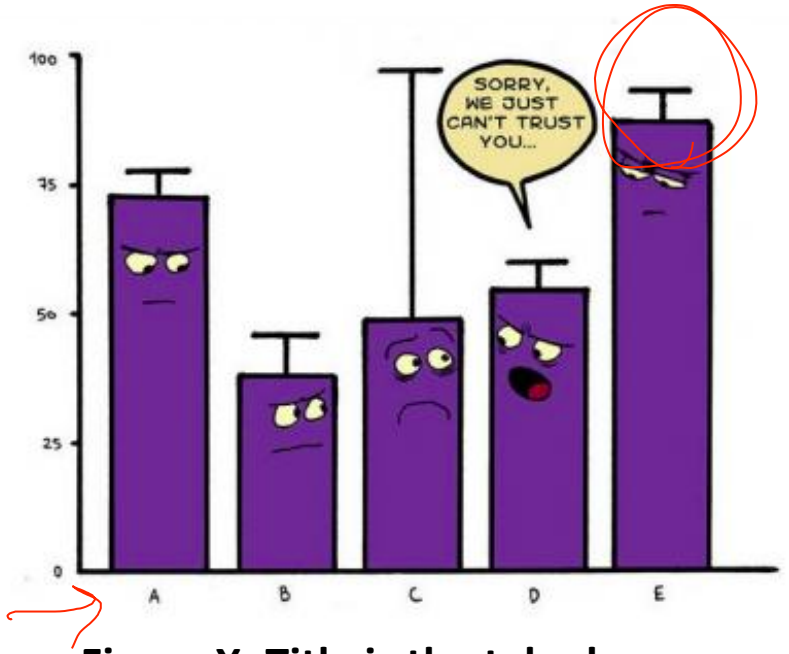


Figure X: Title is the take-home message of the experimental data.
Caption includes all of the details necessary to understand the data presented in the figure…not methods!!