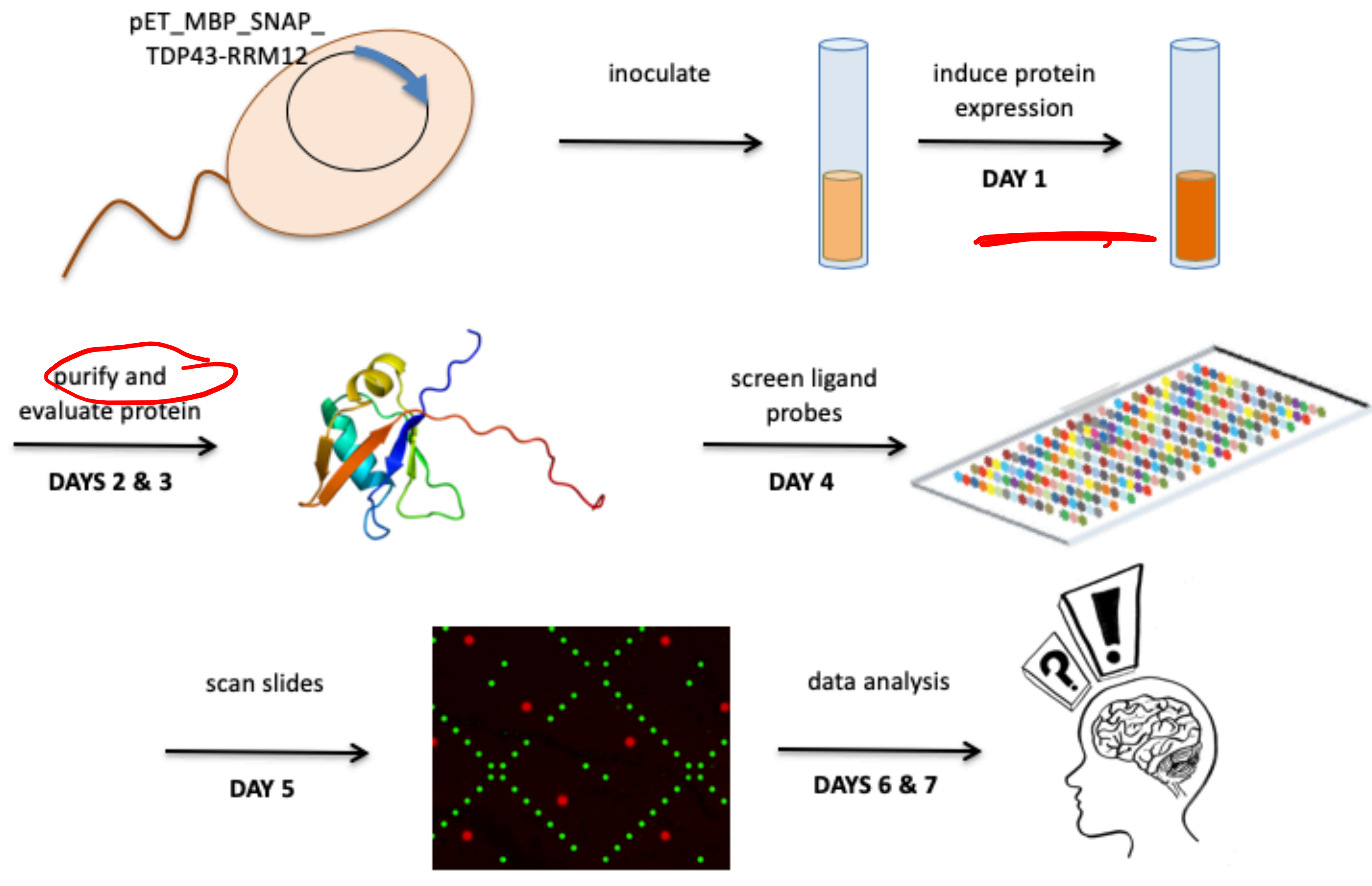
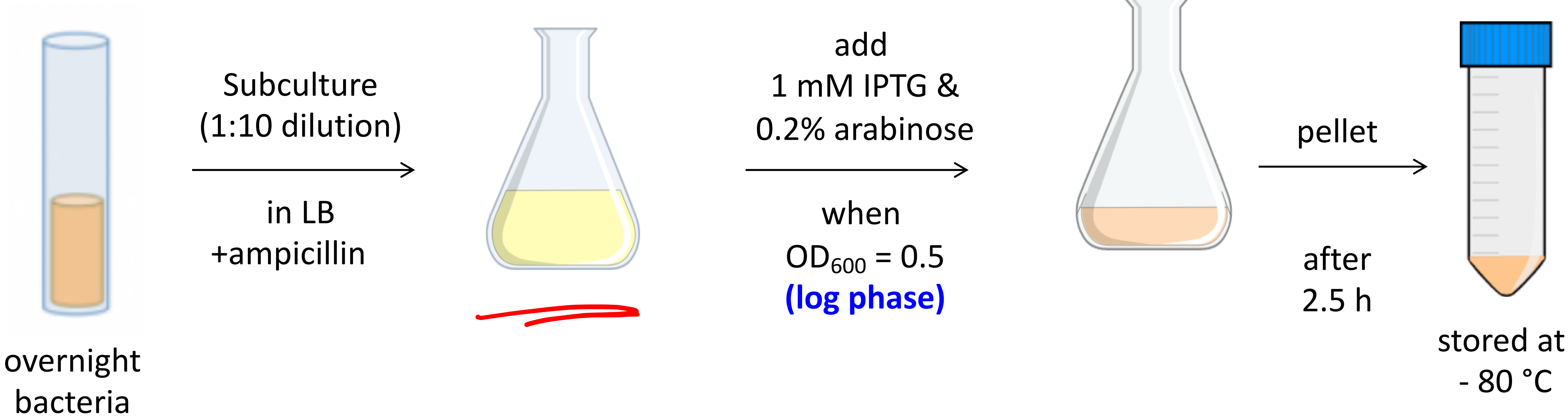
M1D2: Purify TDP43 protein

1. Prelab discussion (2 parts)
2. Gel electrophoresis of pET_MBP_SNAP_TDP43-RRM12
3. Purification of TDP43-RRM12 from *E. coli* BL21(A1)

Mod1 Experimental Overview

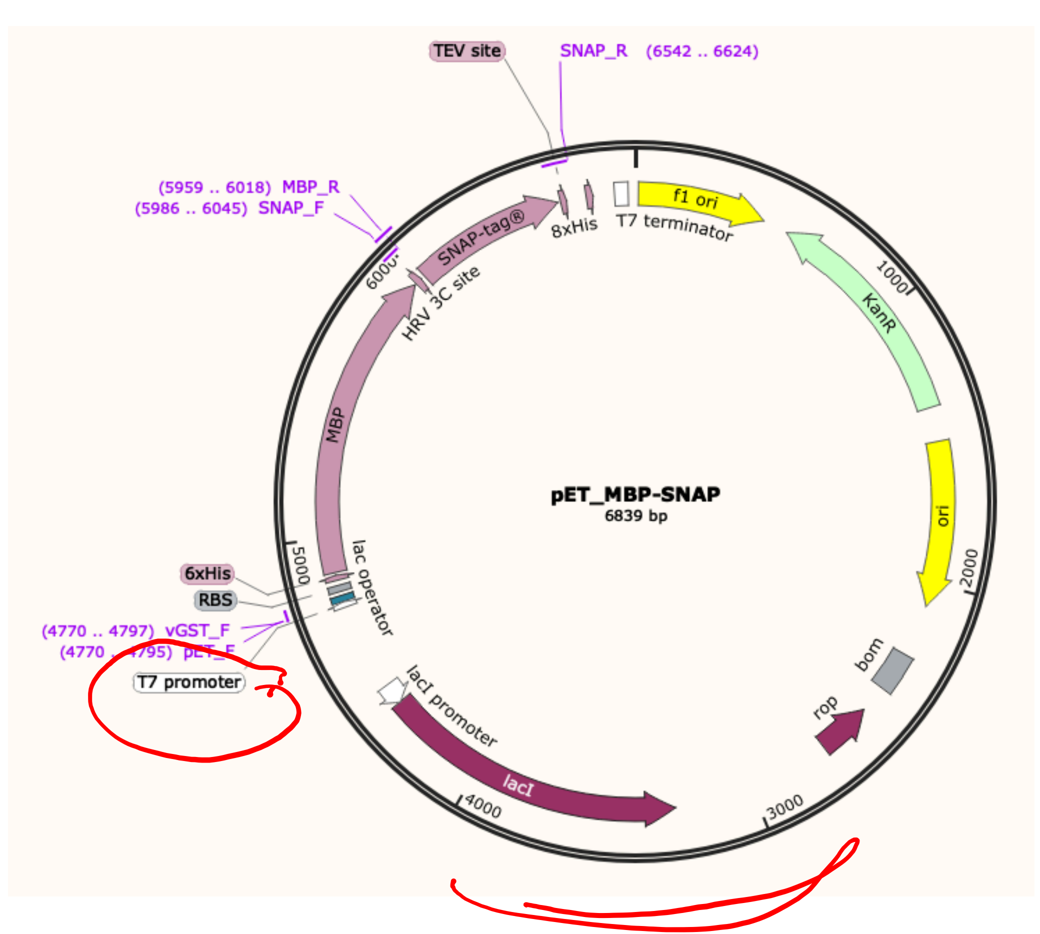


How did we induce protein expression?



In addition to your induced sample, you will also examine and un-induced sample for TDP43_RRM12 expression

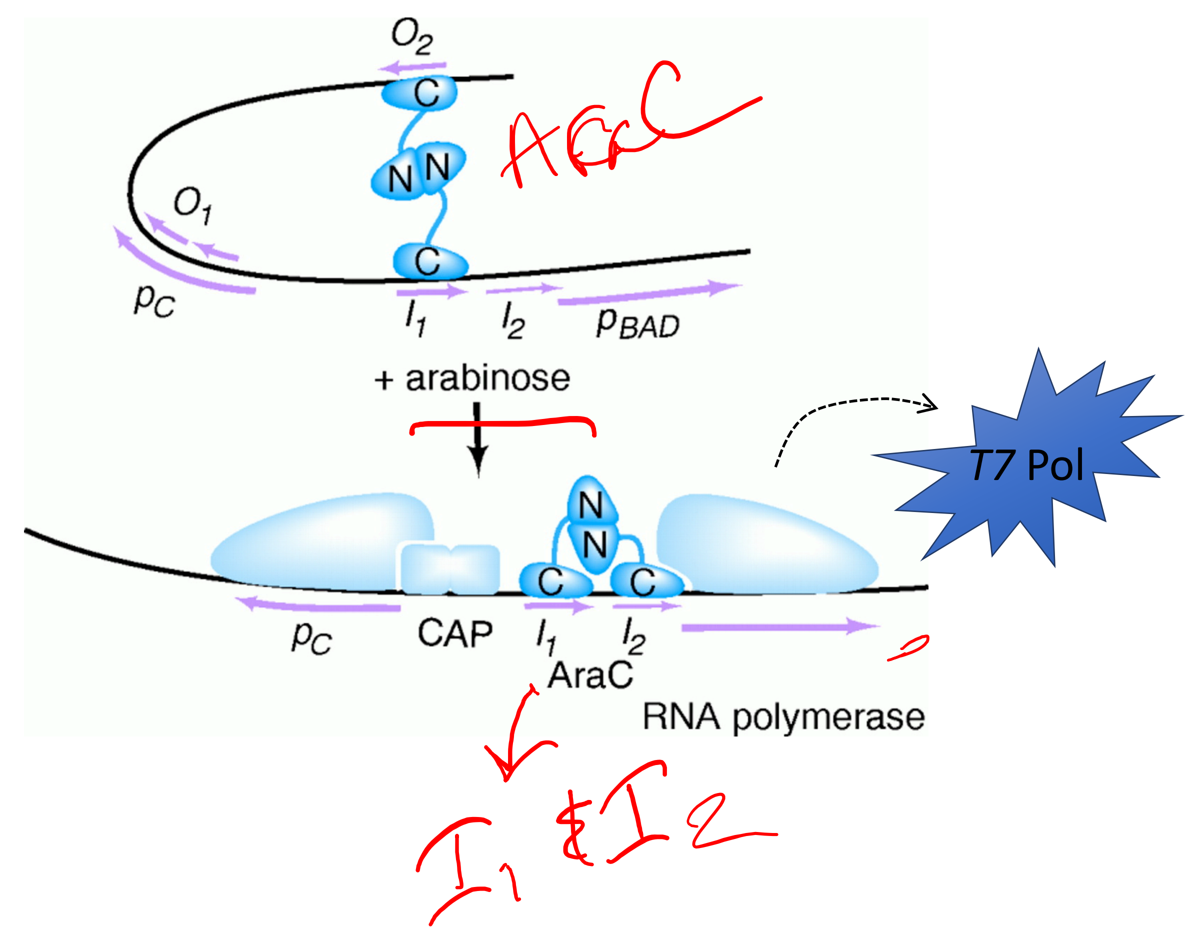
How do we induce protein expression with this vector?



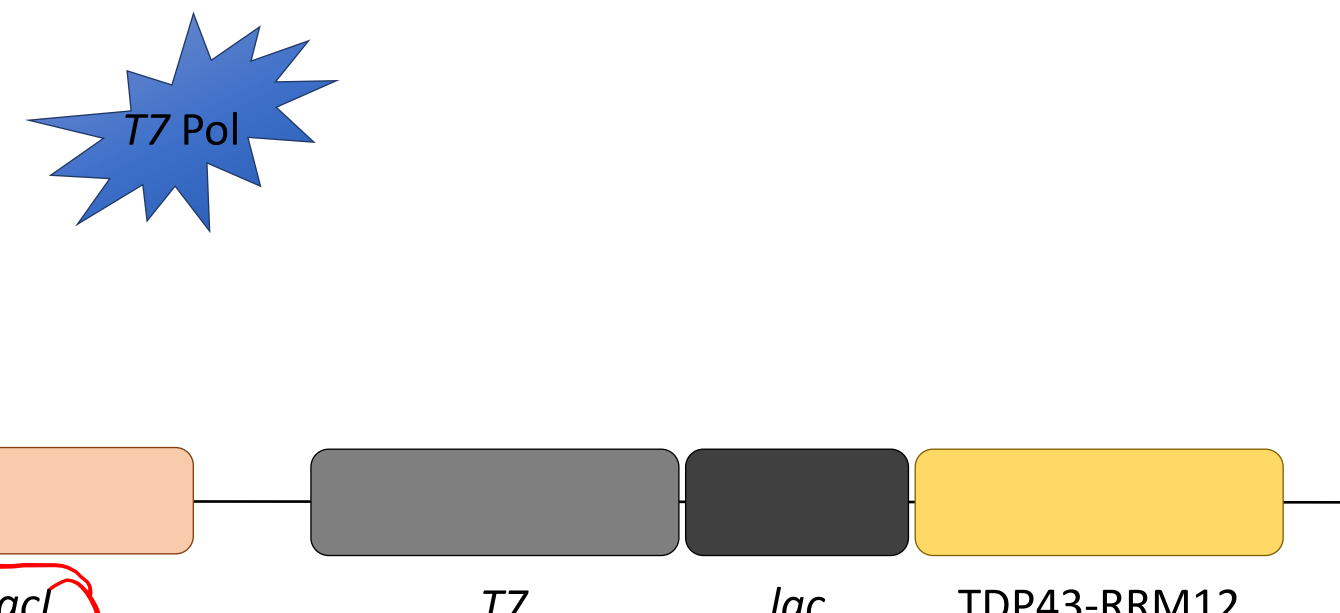
- Dual induction regulated by features encoded on the expression vector
  - T7 promoter → arabinose
  - *lac* operator → vector
  - IPTG

BL21-A1 cells used to express TDP43-RRM12

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

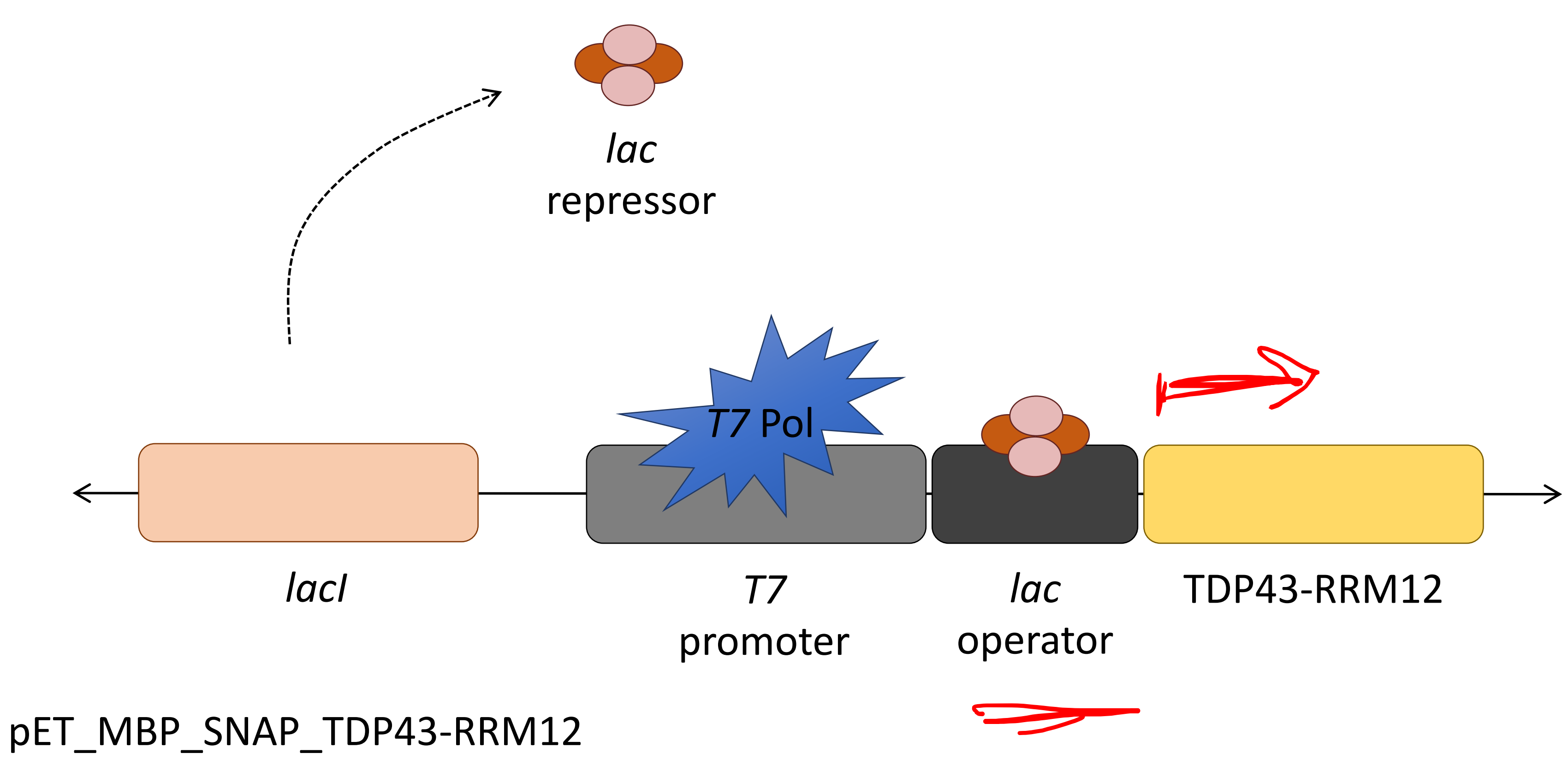


Lac system used to regulate TDP43 expression

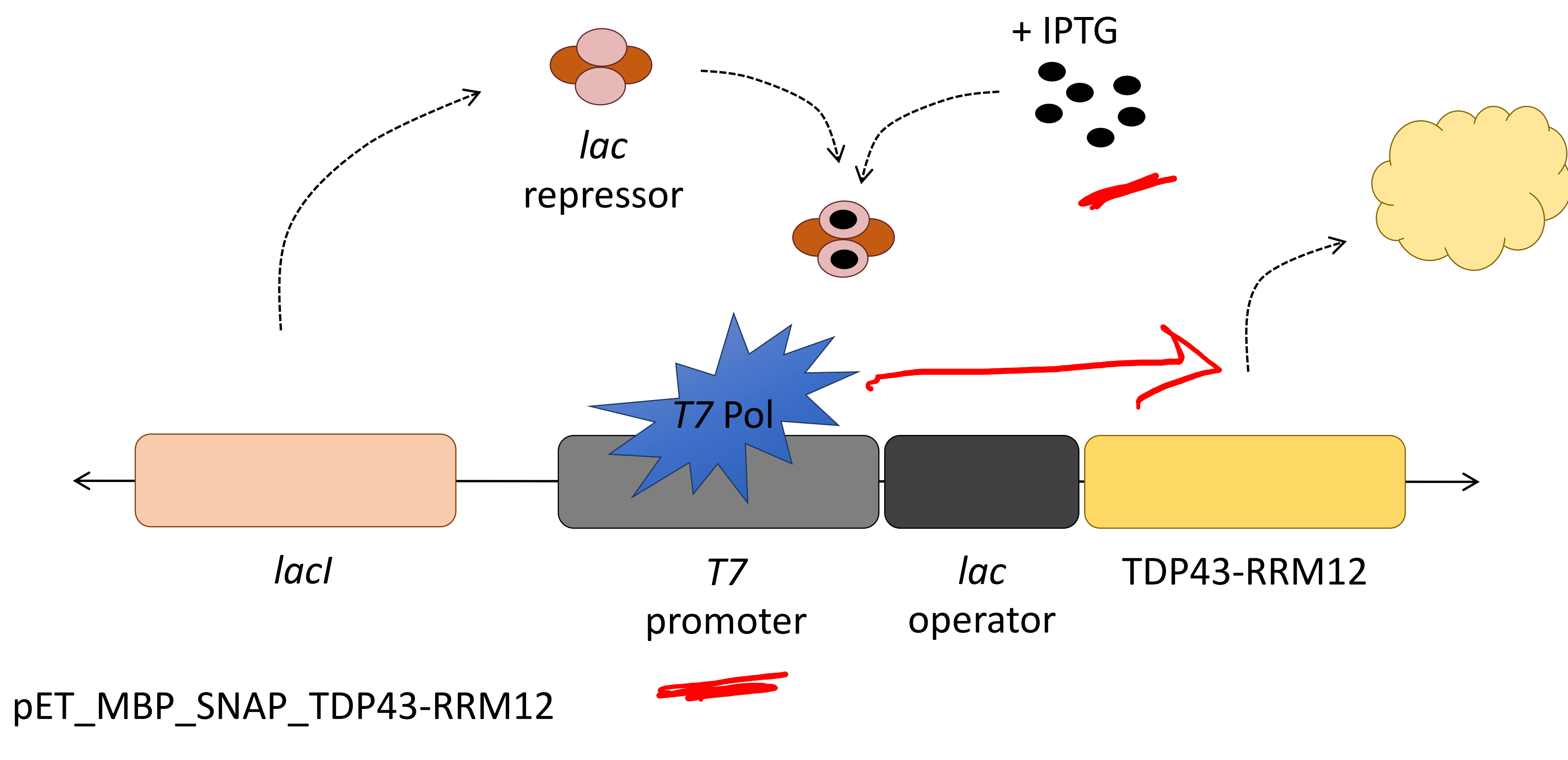


pET_MBP_SNAP_TDP43-RRM12

LacI repressor blocks transcription



IPTG 'induces' protein expression



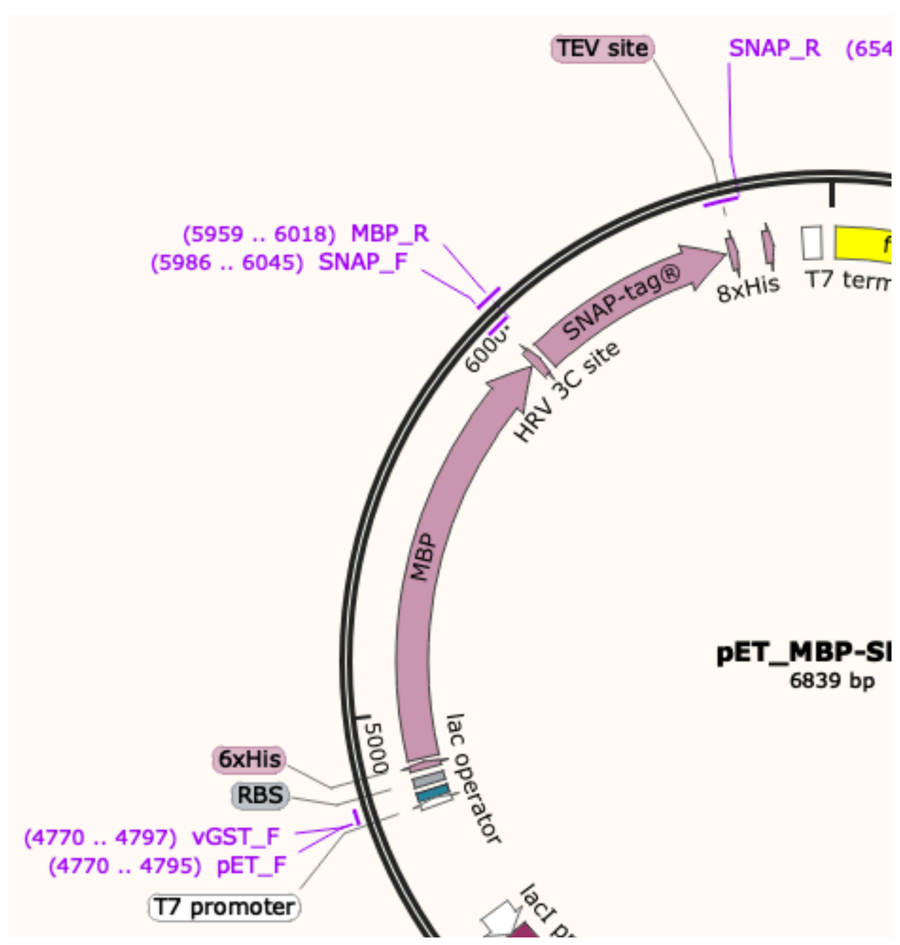
Quick review of induction system…

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

	- arabinose	+ arabinose
- IPTG	- T7 - TDP43	+ T7 - TDP43
+ IPTG	- -	

What are you *actually* expressing?

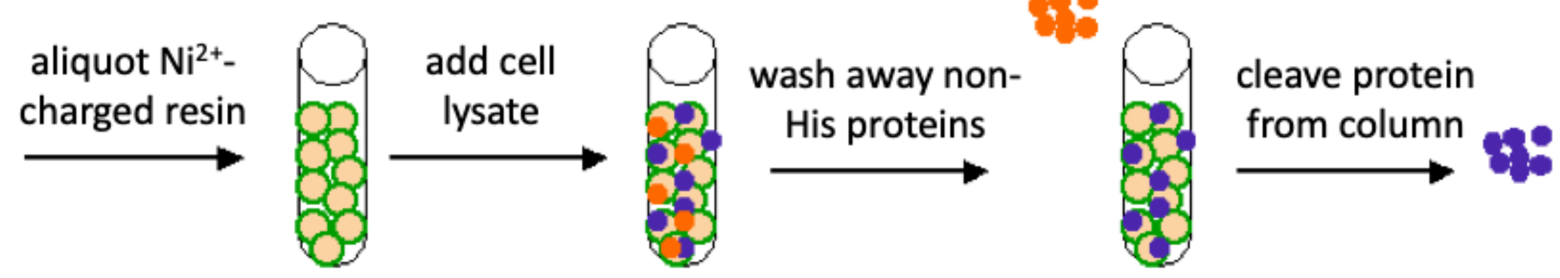
- Draw the TDP43-RRM12 protein product:
    - What additional features were added to TDP43-RRM12 during cloning?
    - What additional features are added to TDP43-RRM12 from the expression vector?



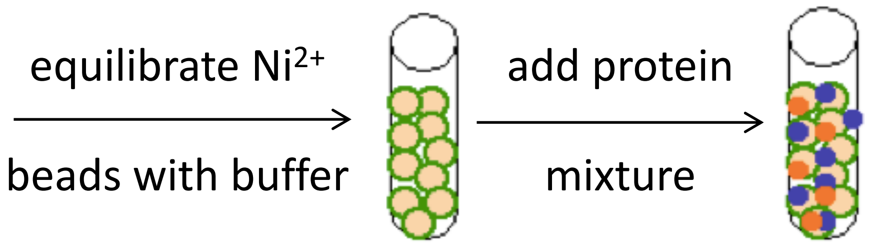
How will you purify TDP43-RRM12?

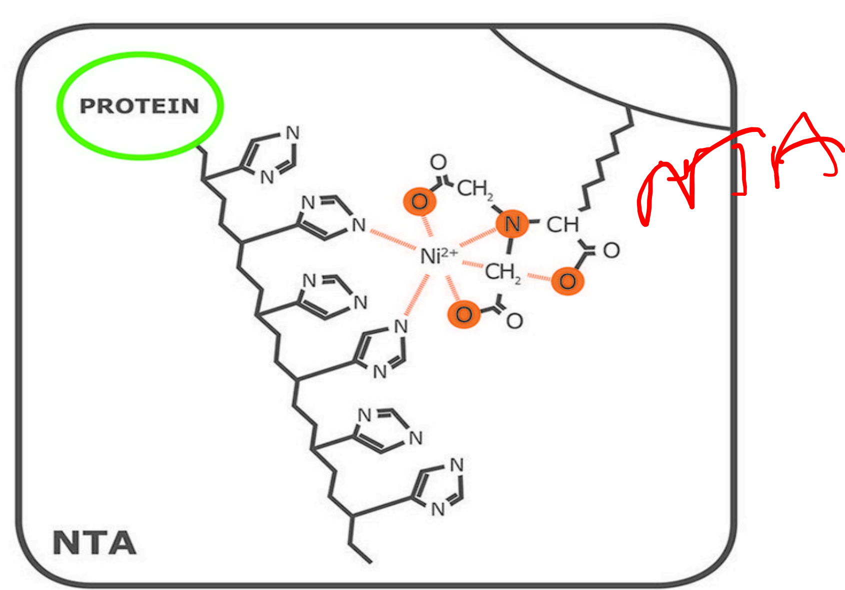
- First, need to lyse cells to release proteins
  - Lysonase/benzonase: chemical disruption of cell membrane and RNA

  - Sonication: physical disruption of cell membrane



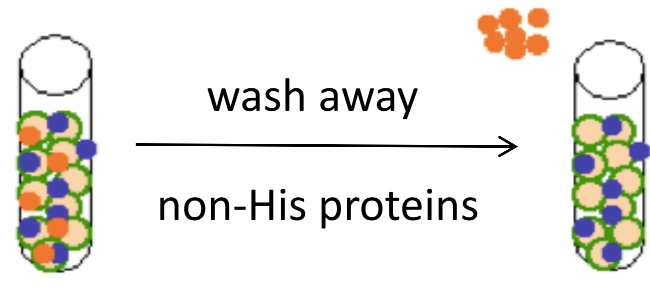
6xHis tag binds to Ni^{2+} resin / column

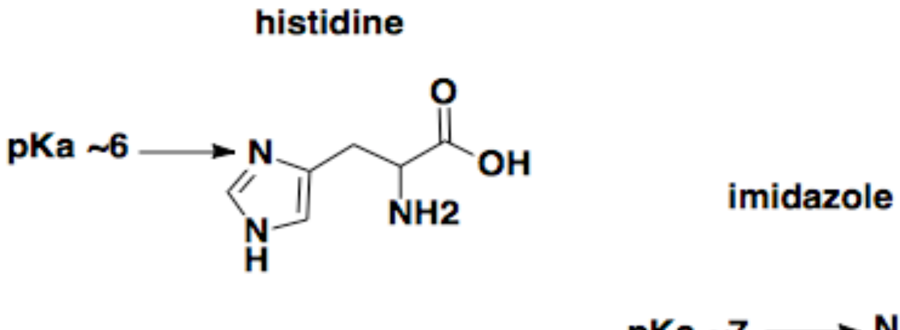




- Ni^{2+} chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand
- His tag chelates to Ni^{2+} causing protein to 'stick' to resin / column

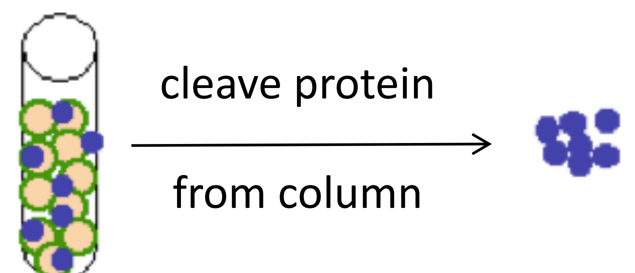
Non-specific binders washed from Ni^{2+} resin / column using imidazole





- 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

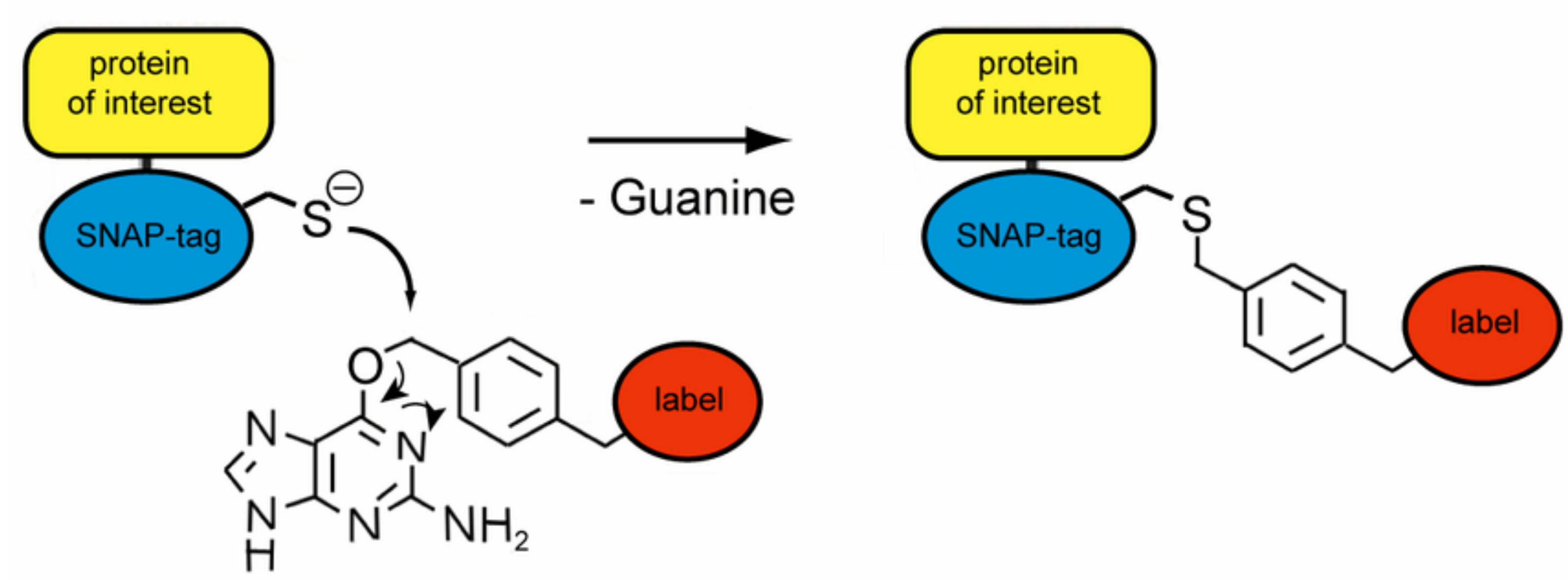


What sequences remain associated with the TDP43-RRM12 purification product?

HRV3C Protease

X - Leu - Glu - Val - Leu - Phe - Gln - Gly - Pro - X

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
- Upload the figure (one group member only) to Stellar
  - DOCUMENT title: Group Color_Digest Figure

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.

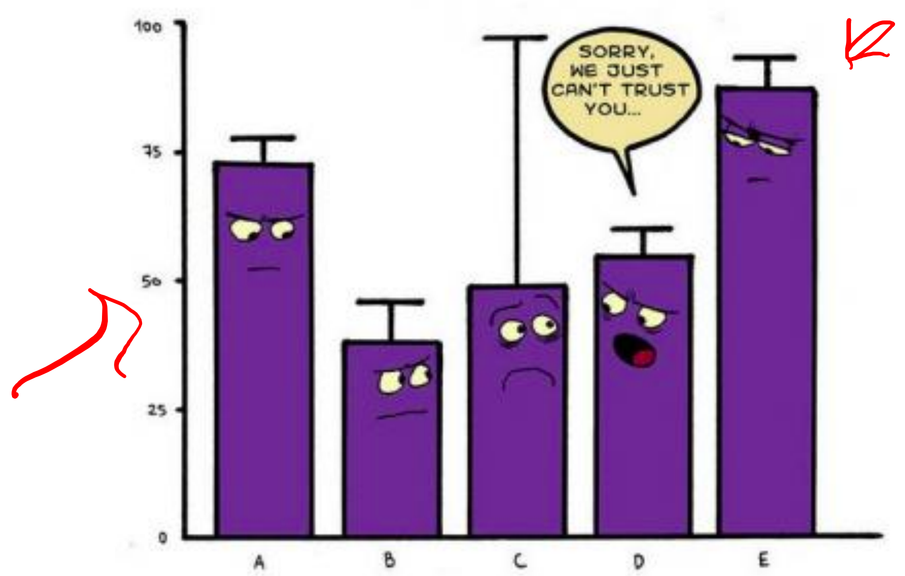
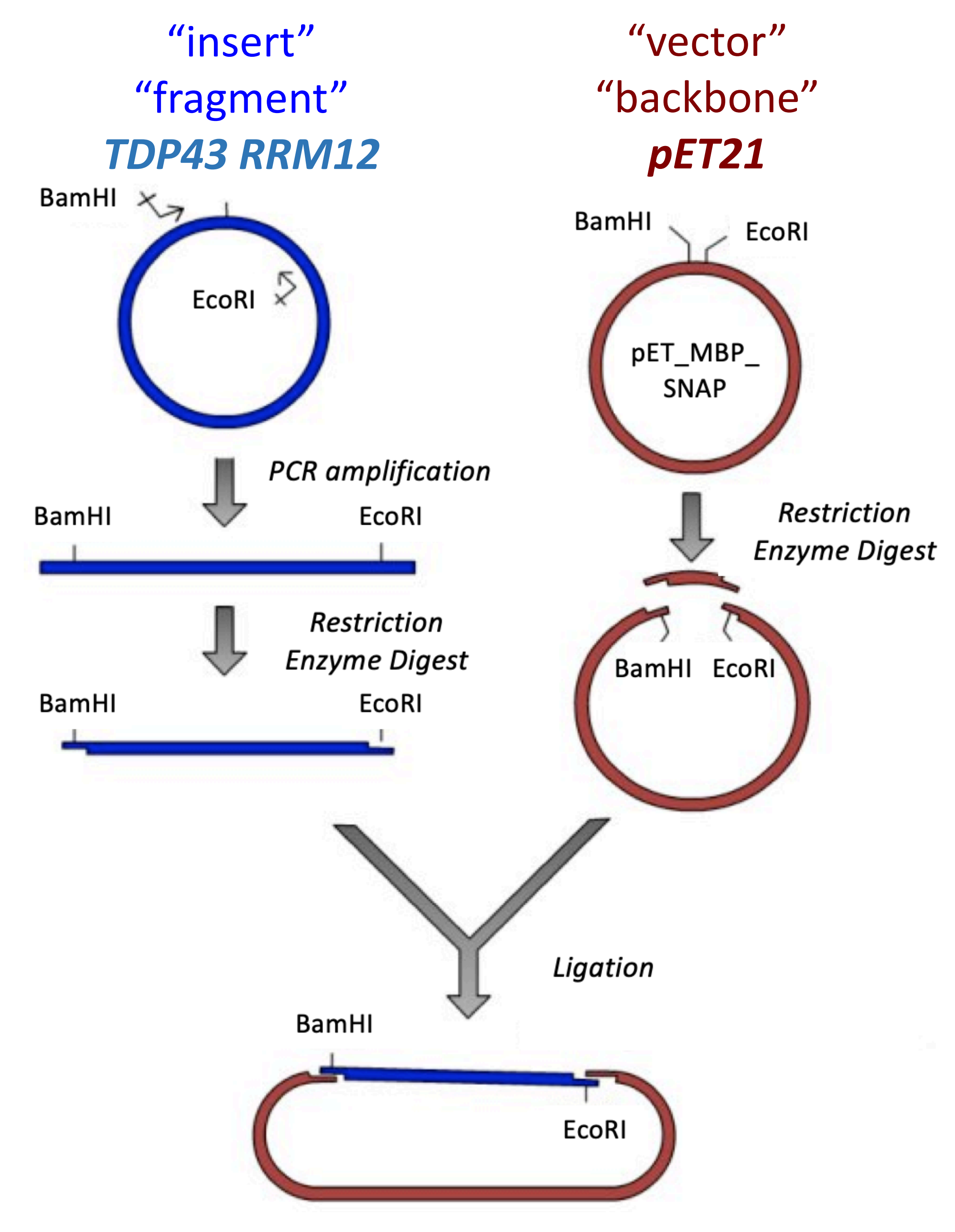


Figure X: Title is the take-home message of the experimental data.
Caption includes all of the details necessary to understand the data presented in the figure…not methods!!

Figure courtesy of Noreen Lyell

Cloning review

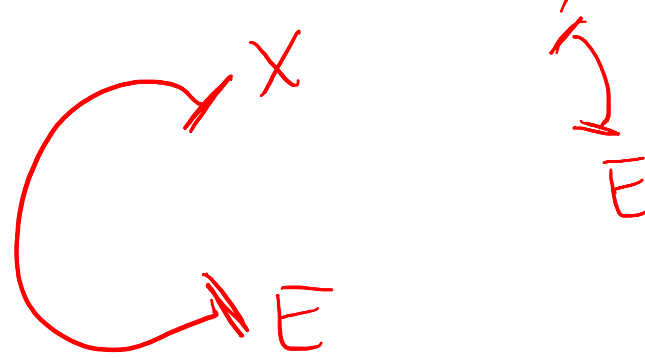
- PCR to amplify insert with restriction enzyme sites
- Digest insert and vector with restriction enzymes to create sticky ends
- Ligate fragment and backbone together
- Diagnostic digest to confirm successful cloning

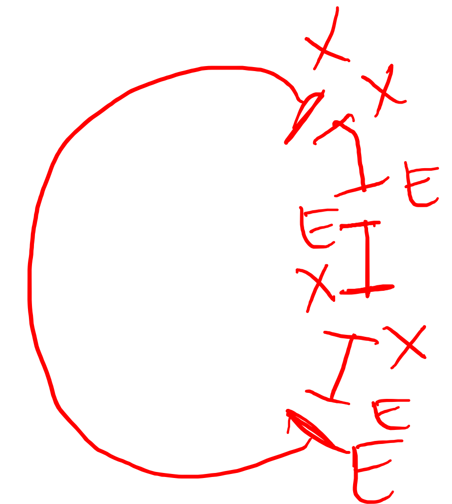


Ligation math! 3:1 molar ratio of insert:backbone

Why do we do confirmation/diagnostic digests?

- Too much insert:





- Too much backbone:

How do we visualize our digest results?

DNA fragments resolved using 1% agarose gel mixed with SYBRsafe DNA stain