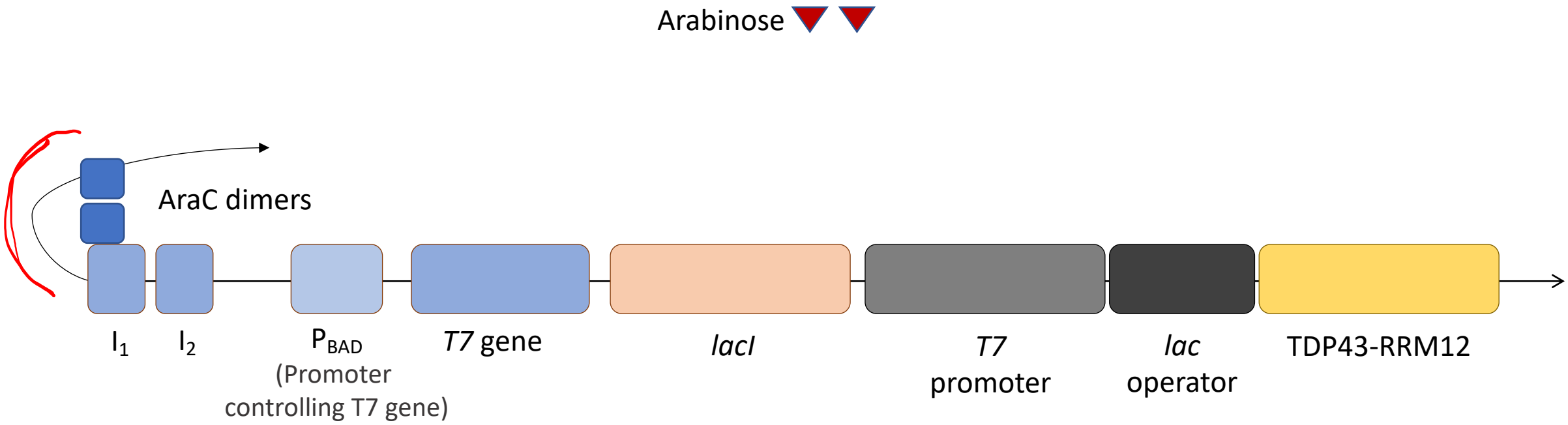
M1D3: Assess purity and concentration of TDP43 protein

1. Prelab discussion
2. Concentrate protein solution
3. Visualize protein purity
4. Measure protein concentration

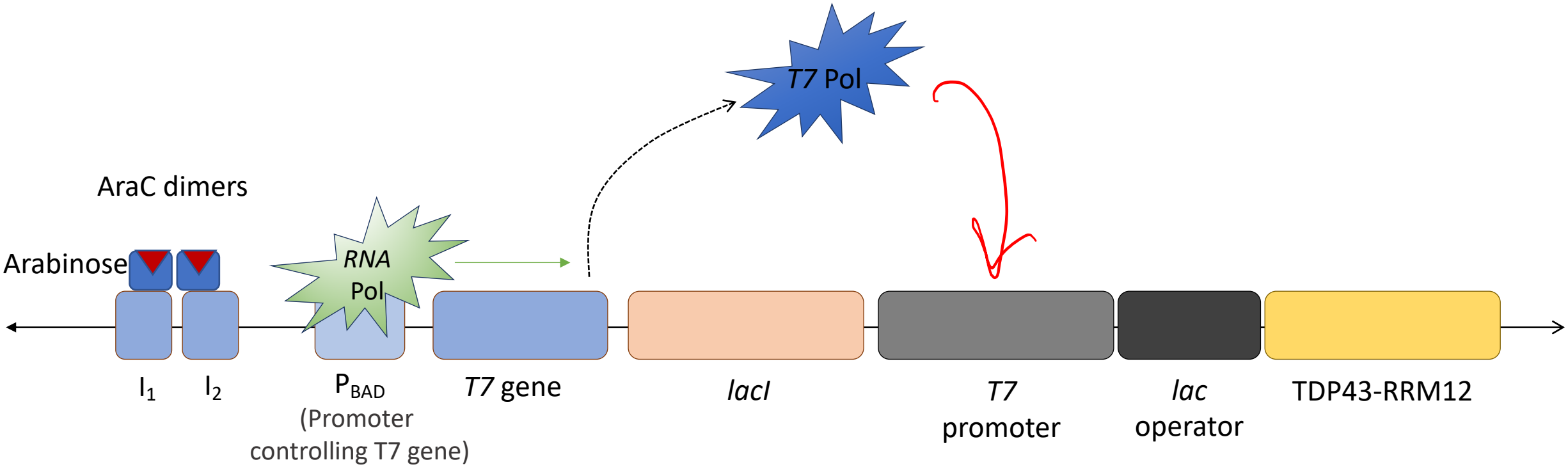
02/13/20

Bacterial induction review: How it begins…



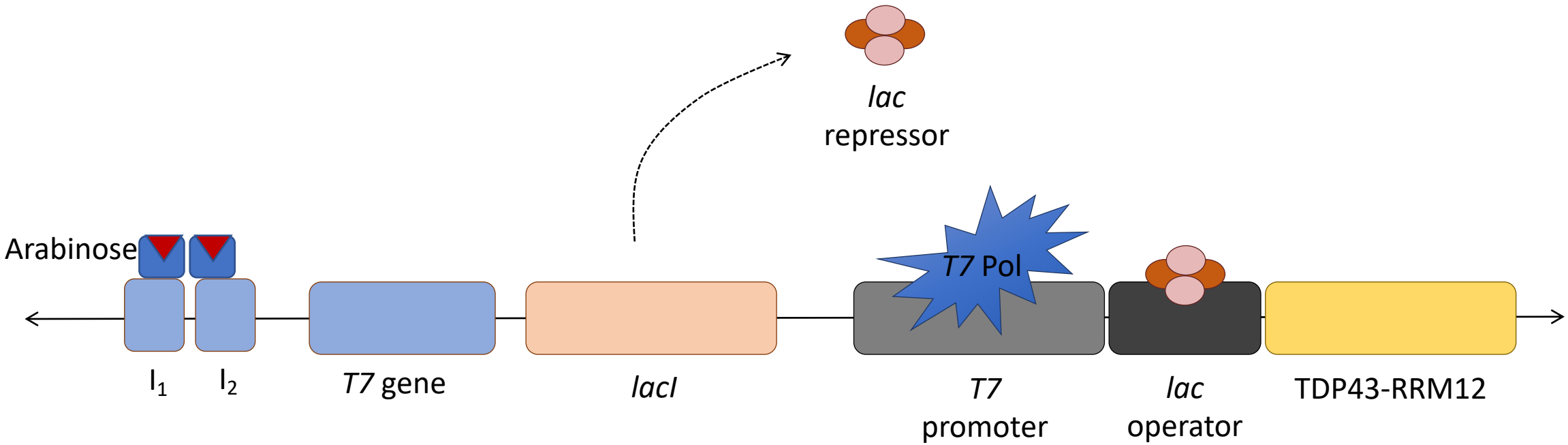
pET_MBP_SNAP_TDP43-RRM12

Bacterial induction review: Arabinose controls T7 expression



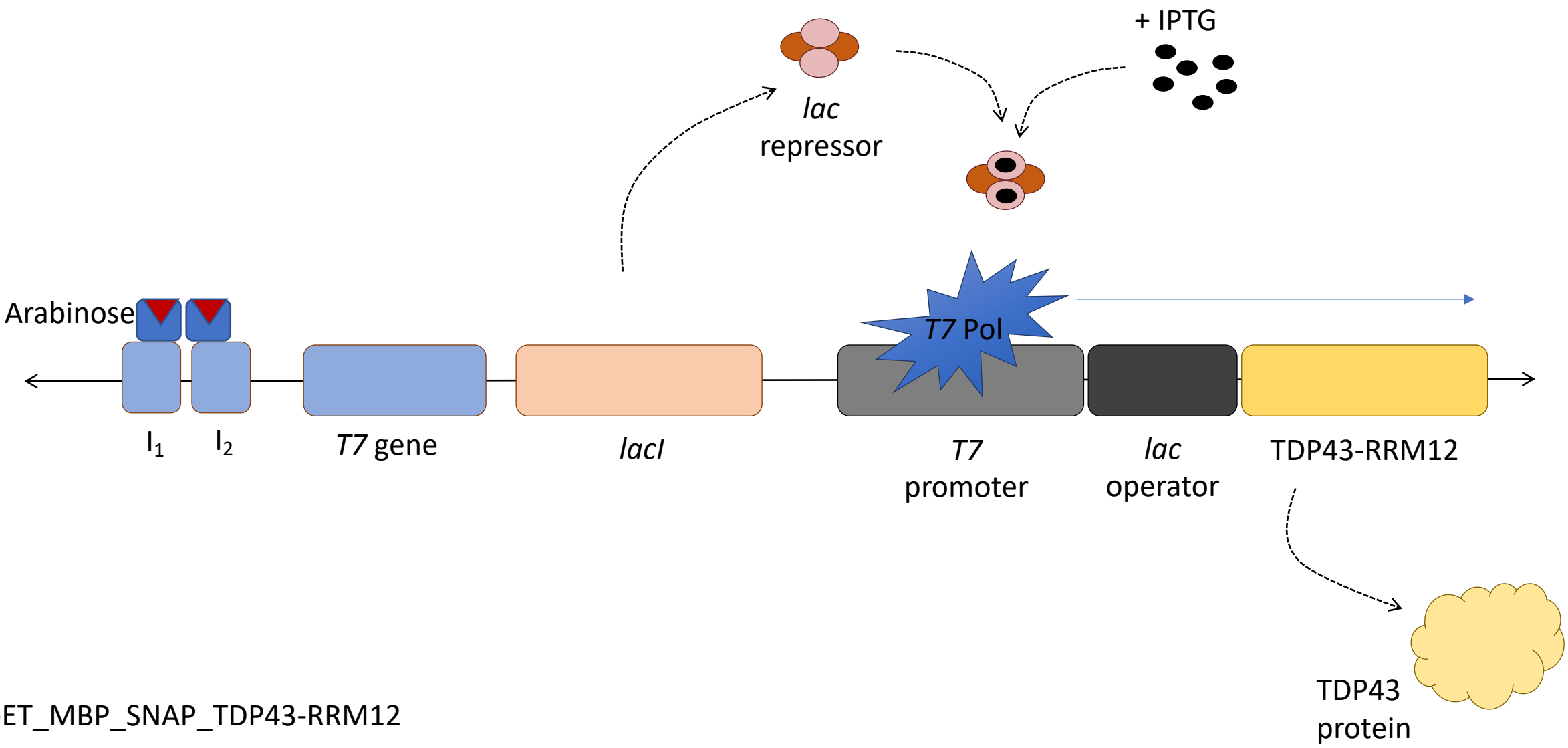
pET_MBP_SNAP_TDP43-RRM12

Bacterial induction review: Lac repressor



pET_MBP_SNAP_TDP43-RRM12

Bacterial induction review: IPTG removes lac repression



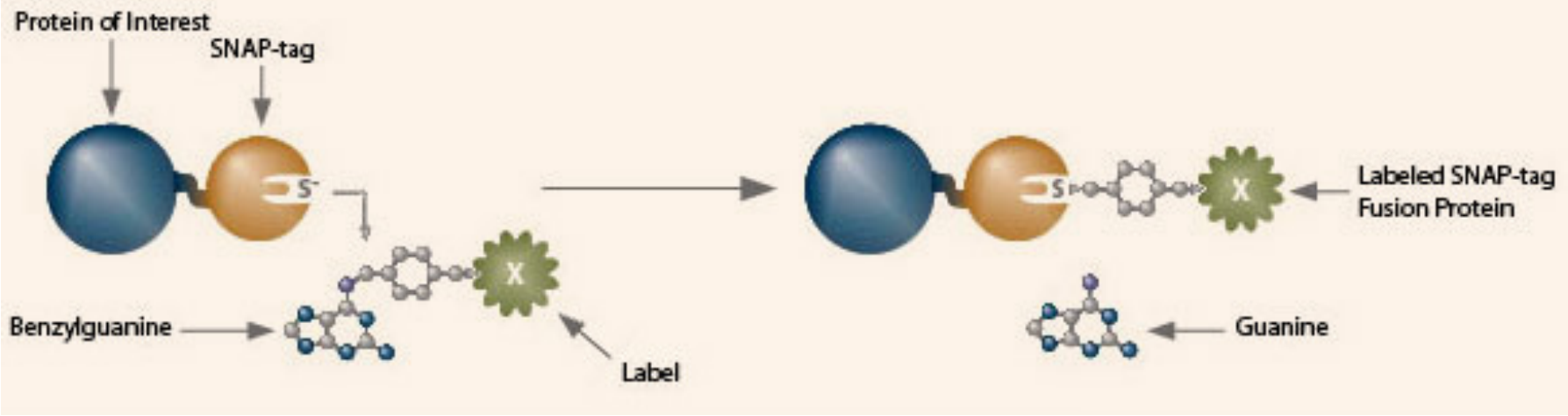
pET_MBP_SNAP_TDP43-RRM12

Protein purification review

- Added lysonase – to what? why? And sonicated – what? why?
- Centrifuged – what? why?
- Added SnapTag / DTT – to what? Then incubated with nickel resin – why?
- Washed with PBS containing imidazole – what? why?
- Added HRV 3C protease – to what? why?

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



Alexa
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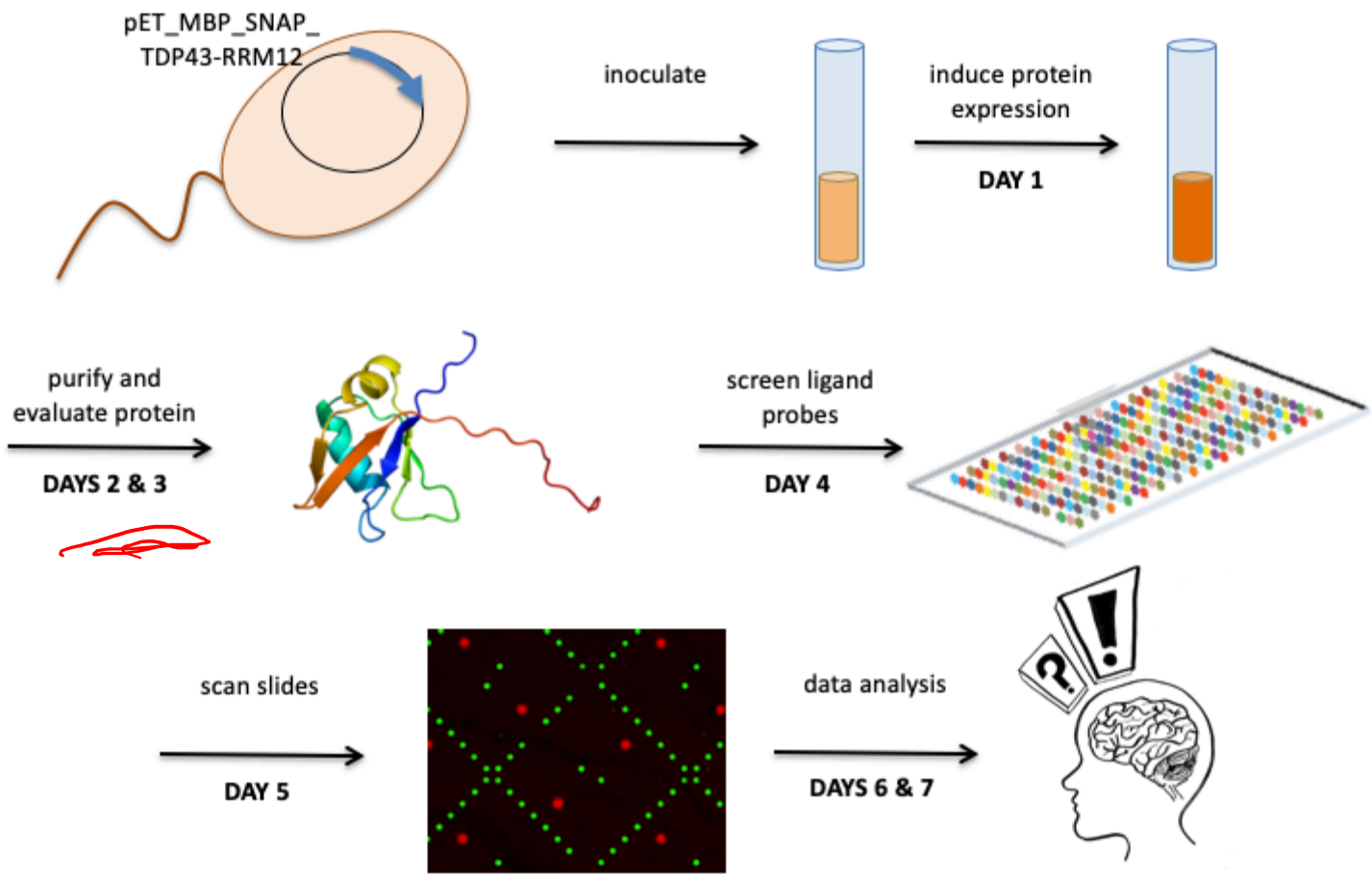
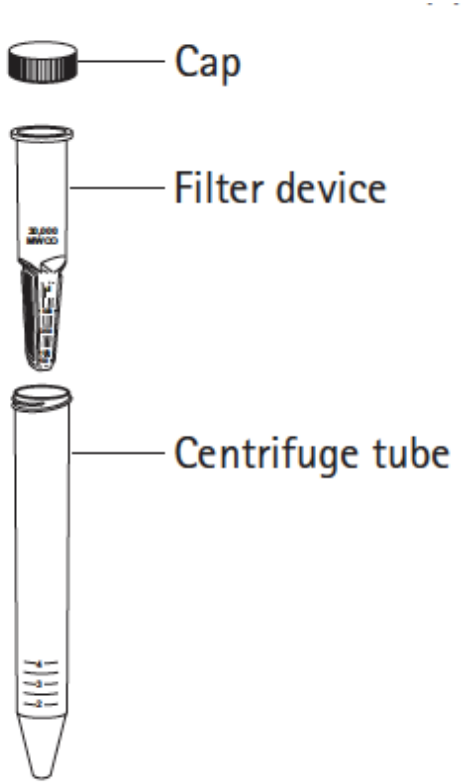
Overview of Mod1 experiments



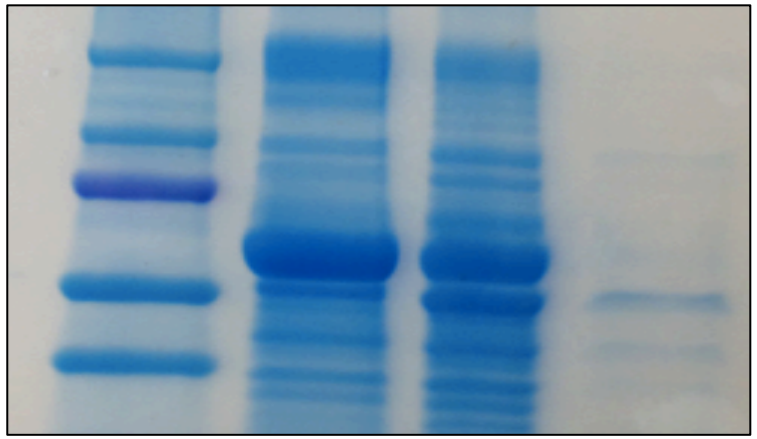
Important notes on concentration procedure!

- Filter device sits within centrifuge tube…add protein to filter device for centrifugation
- Filter device has MW cutoff of 3 kDa …protein is retained in the filter device during centrifugation
- How does this concentrate the protein?

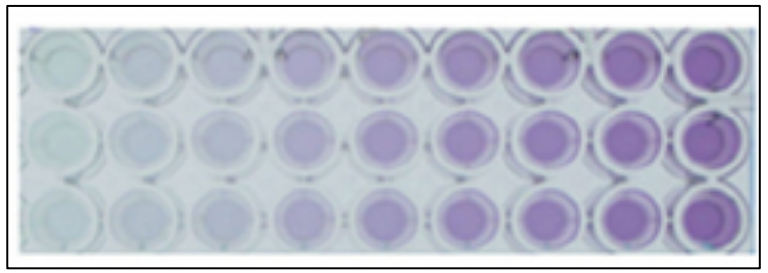


How will you assess purity and concentration?

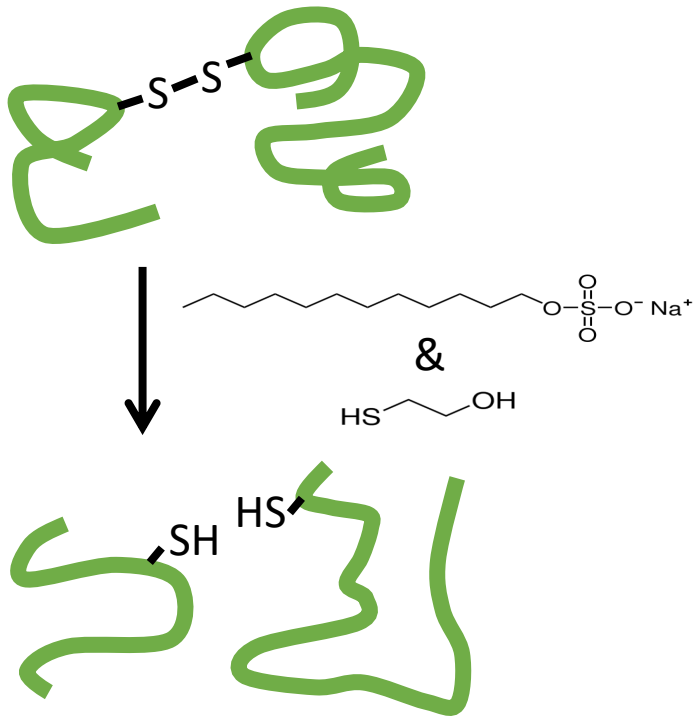
- Check purity using SDS-PAGE
  - visual detection of other proteins in sample
  - Identifies leaky expression of TDP43 from T7 promoter



- Measure concentration using BCA assay
  - Colorimetric assay
  - Calculate concentration from standard curve



Purity: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

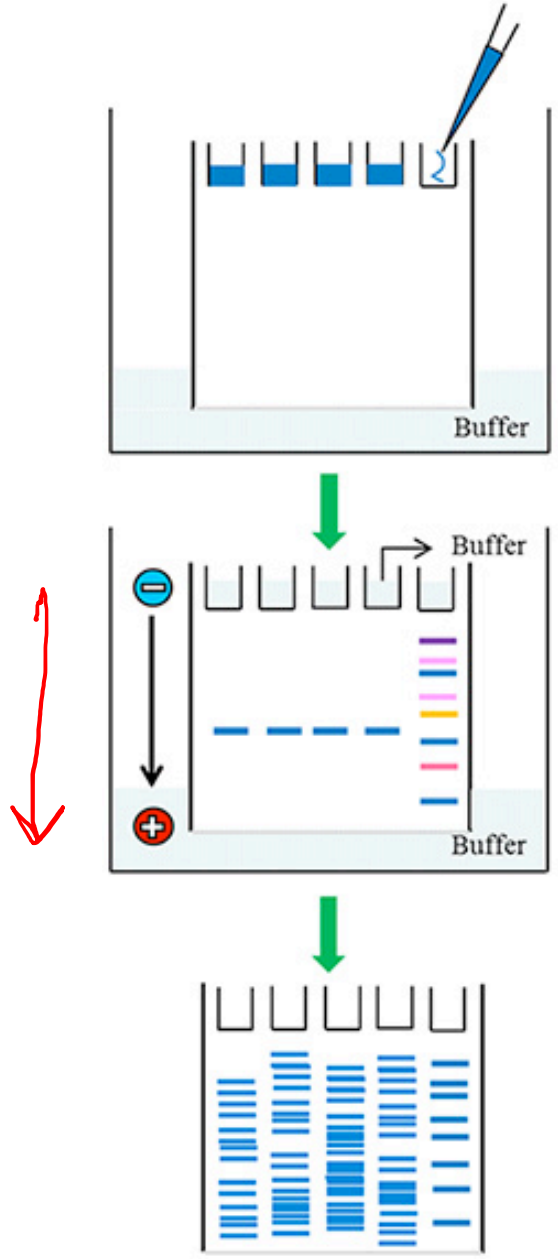


- Laemmli sample buffer / loading dye:
  - SDS Surfactant – denatures proteins (–) charges
  - β-mercaptoethanol (BME) reduces disulfide bonds
  - bromophenol blue track through gel
  - glycerol weight sample
- Boiling: denature further

How are proteins separated?

- Laemmli buffer and boiling results in denature and (−) charged proteins
- SDS-PAGE separates proteins by size
- Electrophoresis completed in TGS buffer
  - Tris-HCl
  - SDS
  - Glycine

salts
pH



Be mindful when loading protein samples

Consider the order of your samples:

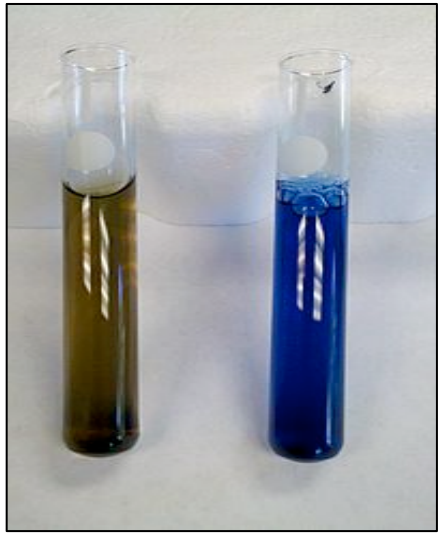
- Samples:
  - Un-induced / induced cell lysates
  - Induced cell pellet
  - Induced lysate flowthrough
  - First wash flowthrough
  - Concentrated TDP43-RRM12
  - Stained and unstained ladders

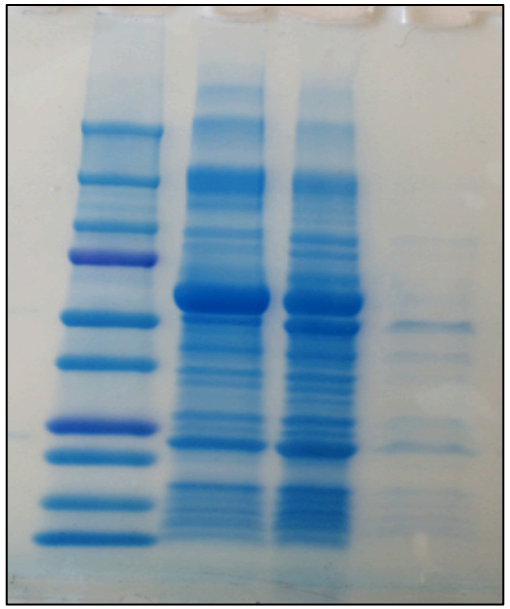
- Figure will be included in your Data summary!

How are proteins visualized?

Coomassie brilliant blue G-250 dye used to stain gel after electrophoresis

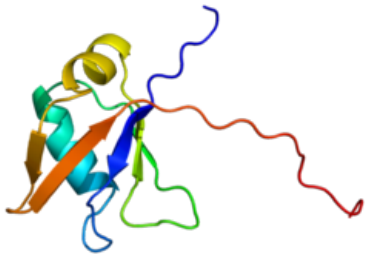
- Red if unbound (cationic form)
- Blue if bound to protein (anionic form)
- Hydrophobic and electrostatic interactions with basic residues
  - Arg (also His, Lys, Phe, Trp)





Concentration: Bicinchoninic acid (BCA) protein assay

Step 1: Chelation of copper with protein, reduction of copper sulfate to copper ion



+ Cu^{2+} $\xrightarrow[\text{Temperature-dependent}]{OH^-}$ Cu^{1+}

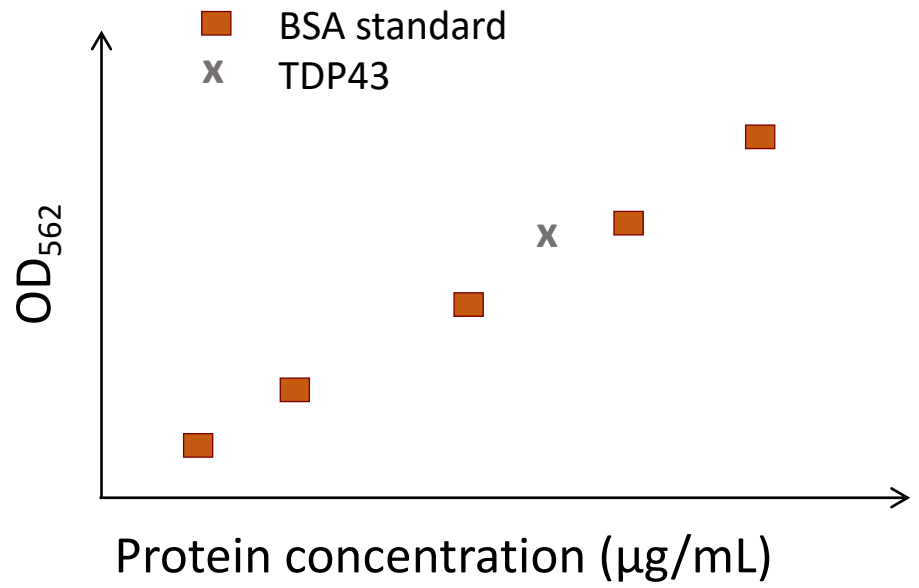
Step 2: BCA complexes with cuprous cation

Cu^{1+} + 2 BCA $\longrightarrow$ BCA-Cu^{1+} complex (A = 562 nm, violet)

BCA/Cu^{1+} absorbance proportional to protein concentration

Standard curve generated using serial dilutions of bovine serum albumin (BSA)

- Use fresh tips between tubes
- Mix well between dilutions
- Be mindful of volumes



For today…

- Keep an eye on the time
- Feedback provided on figure homework during class
  - Can email revised version to Becky by 10p!
- Use downtime to finish M1D1 exercises or edit M1D2 homework

For M1D4…

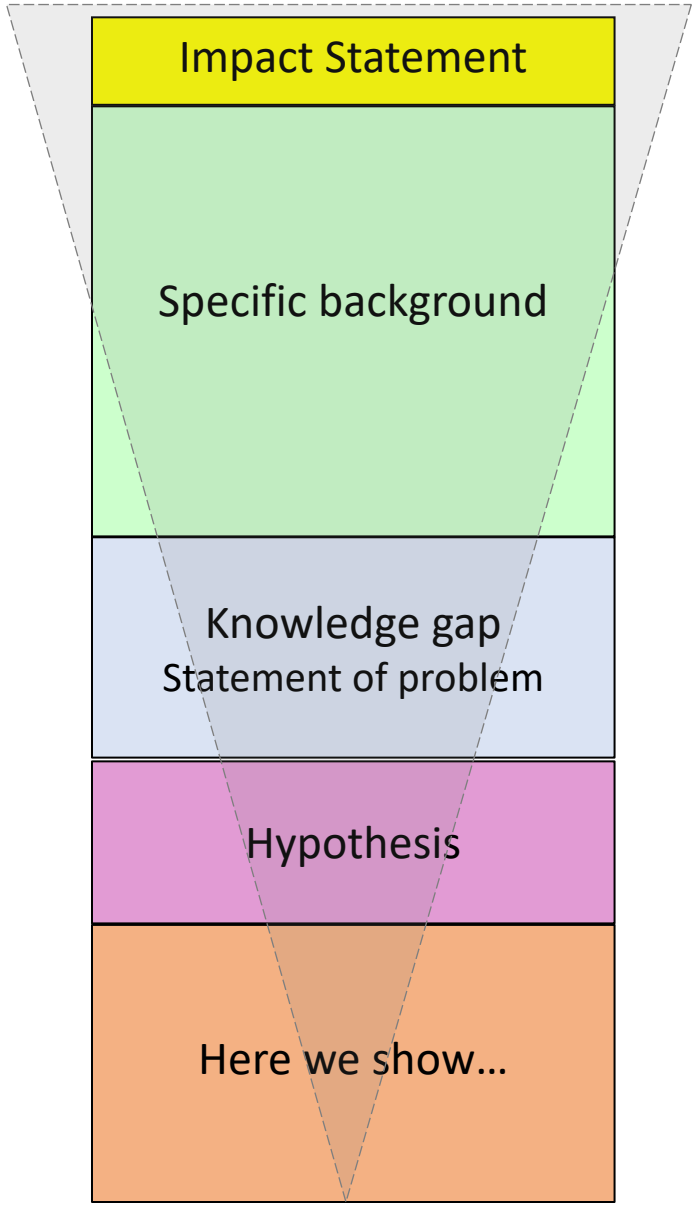
- Draft schematic of TDP43-RRM12 construct
  - ALL figures must include a TITLE and a CAPTION
- Write topic sentences for Data summary introduction

Notes on topic sentences…

- Topic sentence = First sentence of each paragraph

- Should ‘funnel’ from big picture topic to your specific research question / project
  - Provide only the background needed to understand research / problem / goal
  - Clearly state what is not currently known
  - Address how you will fill knowledge gap
  - Provide preview of your results

- Include references!! And summary!!



How should you introduce your story?

1st paragraph: what is the big picture / problem?

2nd paragraph: what is currently known?

3rd (or 4th) paragraph: what is your research question?

4th (or 3rd) paragraph: how will you address your question?

5th paragraph: here we show…