M2D2: Perform protein purification protocol

1. Prelab discussion #2

2. Protein purification

3. Prelab discussion #2

4. Assess RE 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!

Module 2 Roadmap

Determine putative PF3D7_20109-F21 binders via high throughput screening (SMM)



Use purified protein to validate binding of small molecules identified in SMM

Bacterial induction: T7 expression



pET-28b(+)_PF3D7_20109-F21

Bacterial induction: Lac repressor



pET-28b(+)_PF3D7_20109-F21

Bacterial induction: IPTG removes lac repression



How do we induce protein expression?



Why do we induce protein expression at $OD_{600} = 0.6$?

How will you purify PF3D7_20109-F21?

First, need to lyse cells to release proteins:

- B-PER bacterial extraction reagent
- Lysonase
- Protease Inhibitor Cocktail



Strep tag binds protein to Streptactin resin to allow affinity purification of protein of interest



• Streptactin chelated onto agarose resin



 Strep tag fused onto protein of interest binds streptactin causing protein to 'stick' to resin / column

Biotin competes for binding to Streptactin



- High concentration of biotin included in elution buffer
- Biotin has a stronger affinity for streptactin and thus outcompetes the Strep-tag

Purification process (and where you will save samples)



For today...

- 1. Purify your protein for validation assay
 - 1. During a centrifugation step, electrophorese your RE digests
- 2. At 3:30pm, Kylie Gallagher will lecture on the RE digest and interpreting your results

For M2D4...

- 1. Create a journal club slide and script for 1 figure from your paper
 - Use the Comm Lab slides and homework checklist on the wiki for guidance

For homework, make a Journal Club presentation slide

Craft 1-2 slides using your JC article to present the data from a figure.

- Your slide(s) should show the data and highlight the key finding(s).
- The information should be clear and large enough to read.
- Keep text to a minimum. (NO figure captions on slide!)
- The title should state the take-home message of the data that are shown.

EXAMPLE SLIDE: Football coaches are the highest paid academic employees at doctoral-granting universities

\$1,057,305

- Data represent expression of Y using method A
- Possibly something about the control(s), if applicable
- Perhaps an important note about the data that is not already stated in the title
- Transition to next slide...

