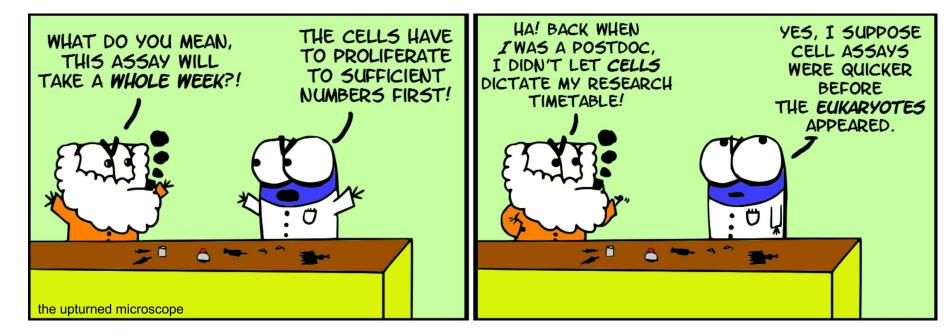
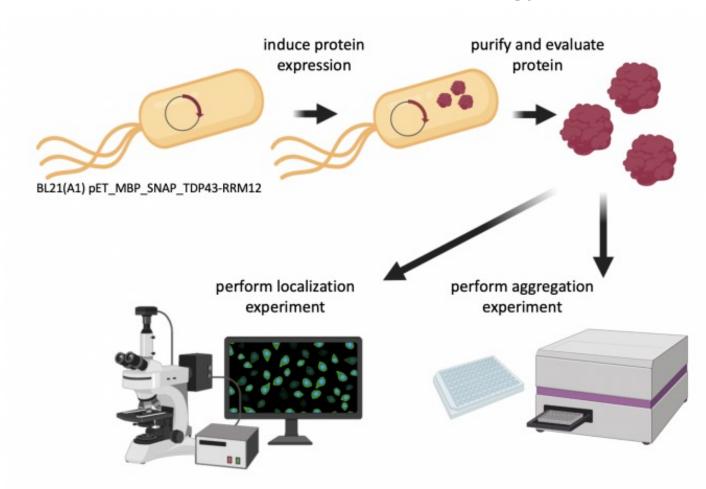
M1D6: Learn best practices for mammalian cell culture and seed CAD cells for TDP43-localization experiment

- 1. Prelab discussion
- 2. Learn about cell culture in the lab
- 3. Research CAD cell line



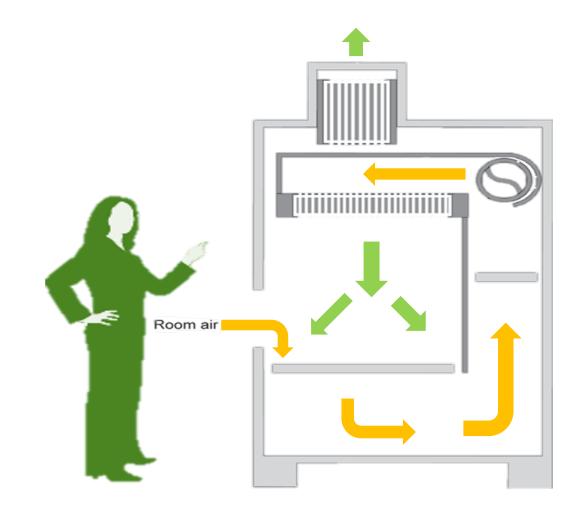
Overview of Mod 1 experiments

Research goal: Use functional assays to characterize ligands identified as binders to TDP43 from SMM technology



Tissue culture sterile technique

- 70% ethanol everything:
 - Wipe cabinet before and after use
 - Wipe everything that enters the cabinet
 - Do not spray cells with EtOH
- Do not disturb air flow:
 - Do not block grille or slots
 - Minimize side-to-side arm movements
 - Work > 6" away from sash
 - Leave blower on always
- Do not talk into incubator!
- · Only open sterile media and pipettes in hood



Mammalian Cell Culture Medium



Food:

- DMEM/F12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12)
 - Defined



- FBS (fetal bovine serum)
 - Undefined



Non-food:

- antibiotics:
 - penicillin
 - streptomycin

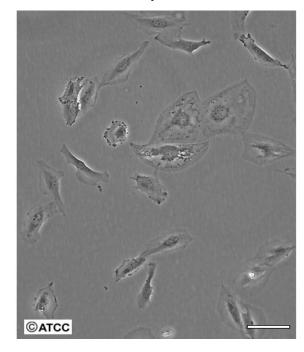
Mammalian Cell Culture Terminology

Adherent vs Suspension

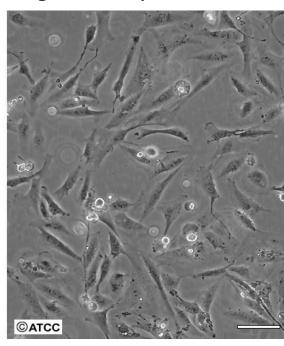
Confluence

Splitting

Low Density

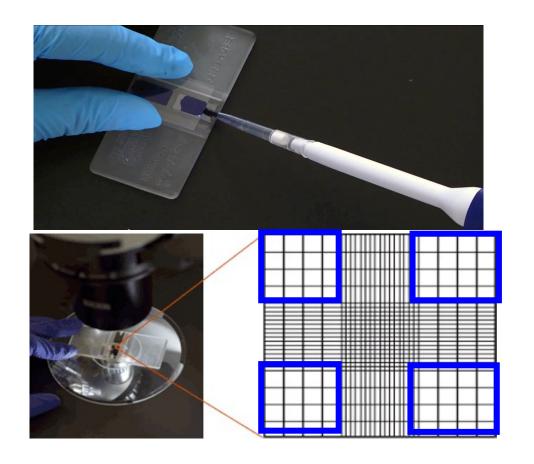


High Density



Seeding

Counting cells





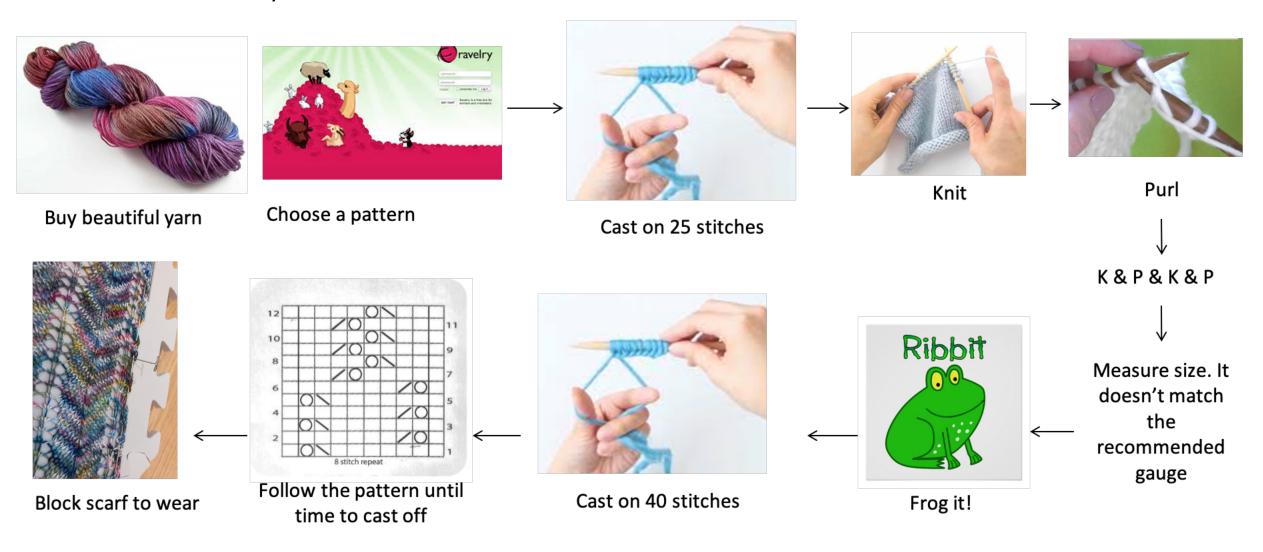
Hemocytometer

Trypan blue

cells / mL = 10,000 x average of 4 corners

Notes on experimental schematics...

How does Becky knit a scarf?



What should be in the Title and Caption?

Title: State what is shown / represented in the schematic

Caption:

- Explain the flow of information using concise / clear language
- Expand on text shown in figure labels to eliminate excess wordiness / clutter from the figure
- Define all abbreviations / jargon / labels / symbols

Revised example:

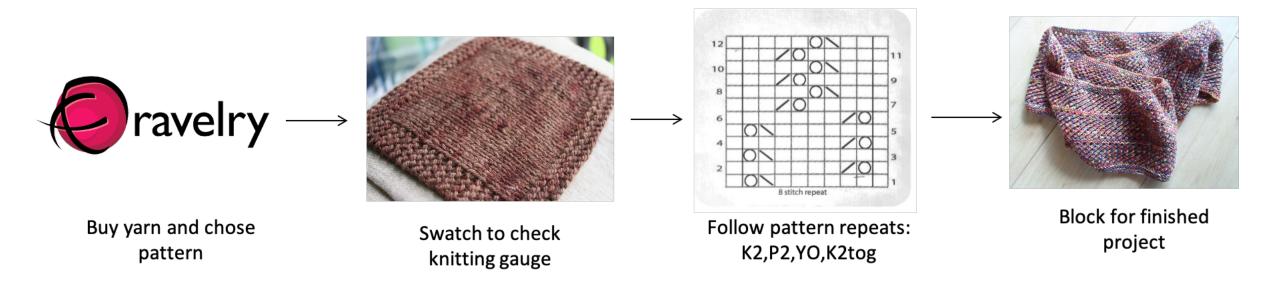


Figure 1: Becky's knitting process produces a functional scarf. Becky follows a specific protocol to knit a scarf. She choses her yarn and checks the pattern before following the written pattern and blocking to complete the project. K2= knit two, P2= purl 2, YO= yarn over, K2tog= knit two together

For today:

- 1. Practice cell culture and seed cells for localization assay White, Gray, Purple, Teal teams to TC first!
- 2. Research CAD cell line
- 3. Work on Data Summary in downtime
 - Homework for M1D7
 - 2. Background and Motivation section

For M1D7:

- With your lab partner create an experimental overview schematic of the purification process for TDP43-RRM12
 - This is a figure and should have a title and caption

Protein purification Data Slide

What you turned in today!

 If you had difficulty identifying a band in your protein elution and would like additional data, we have a Western blot of the elution and flow through

 Used an anti-His primary antibody with an HRP-conjugated secondary antibody for detection

