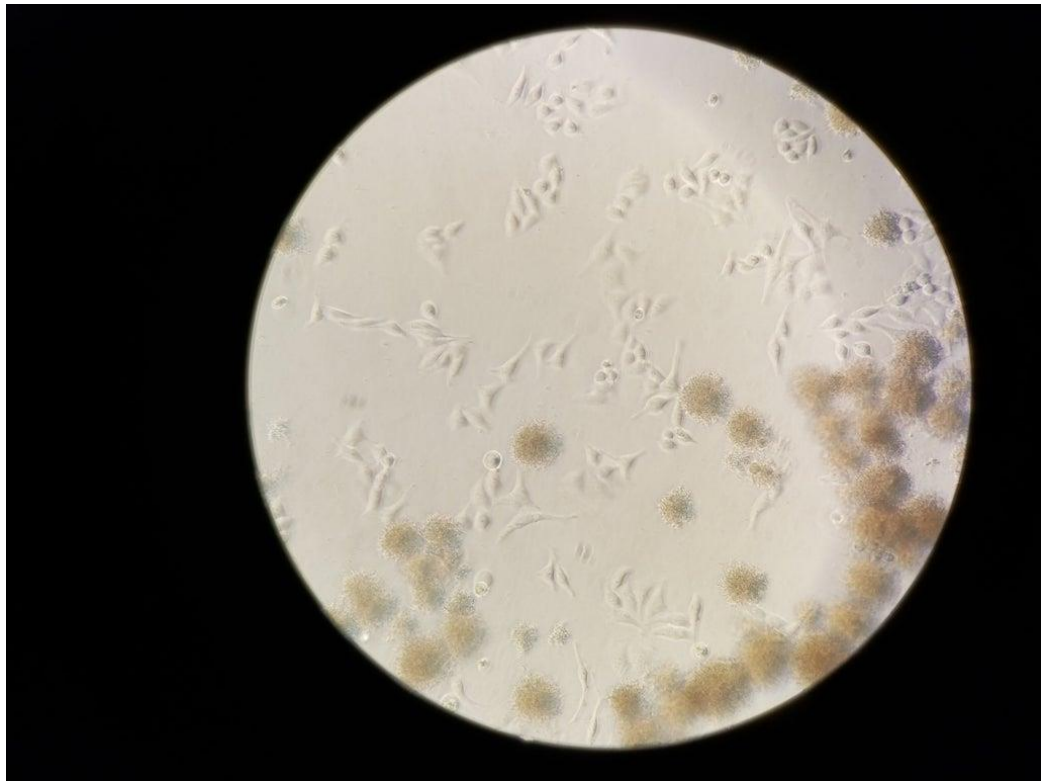
Consider coming to lab today/Monday if you think you'll work with mammalian cells

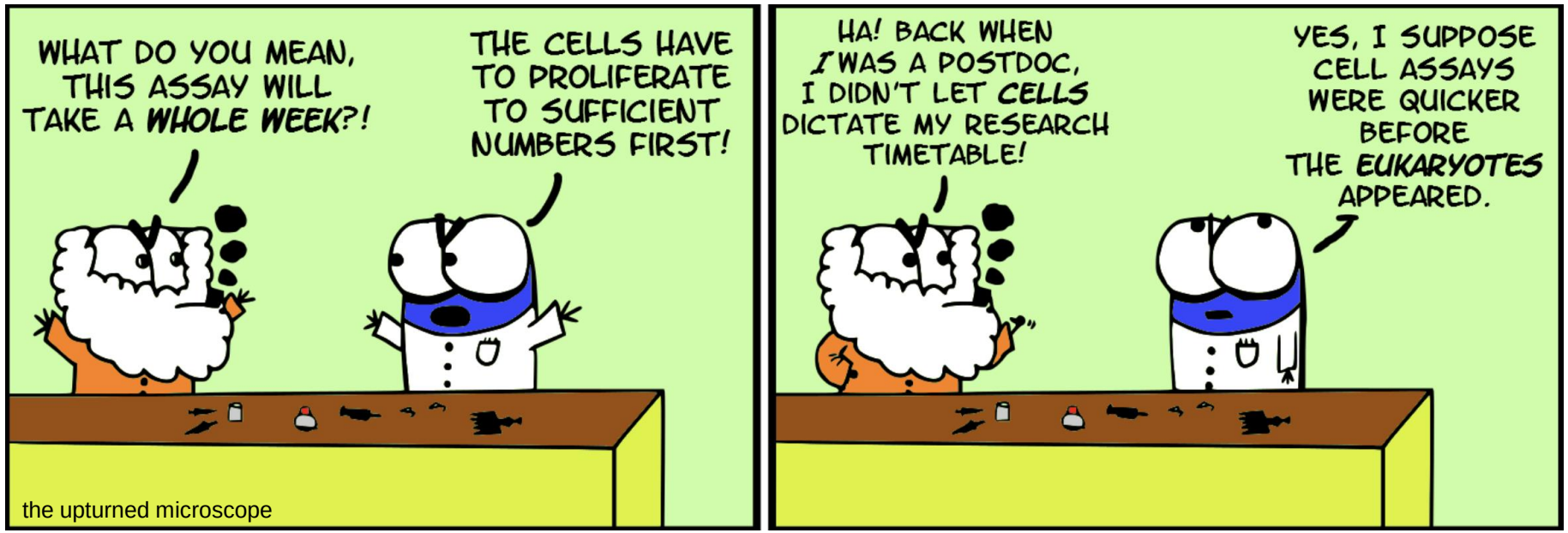
Lab is a safe space for learning what can be pretty anxiety-inducing techniques

- Sterile mammalian cell culture technique
- Working with a benchtop microscope
- Cell viability assays



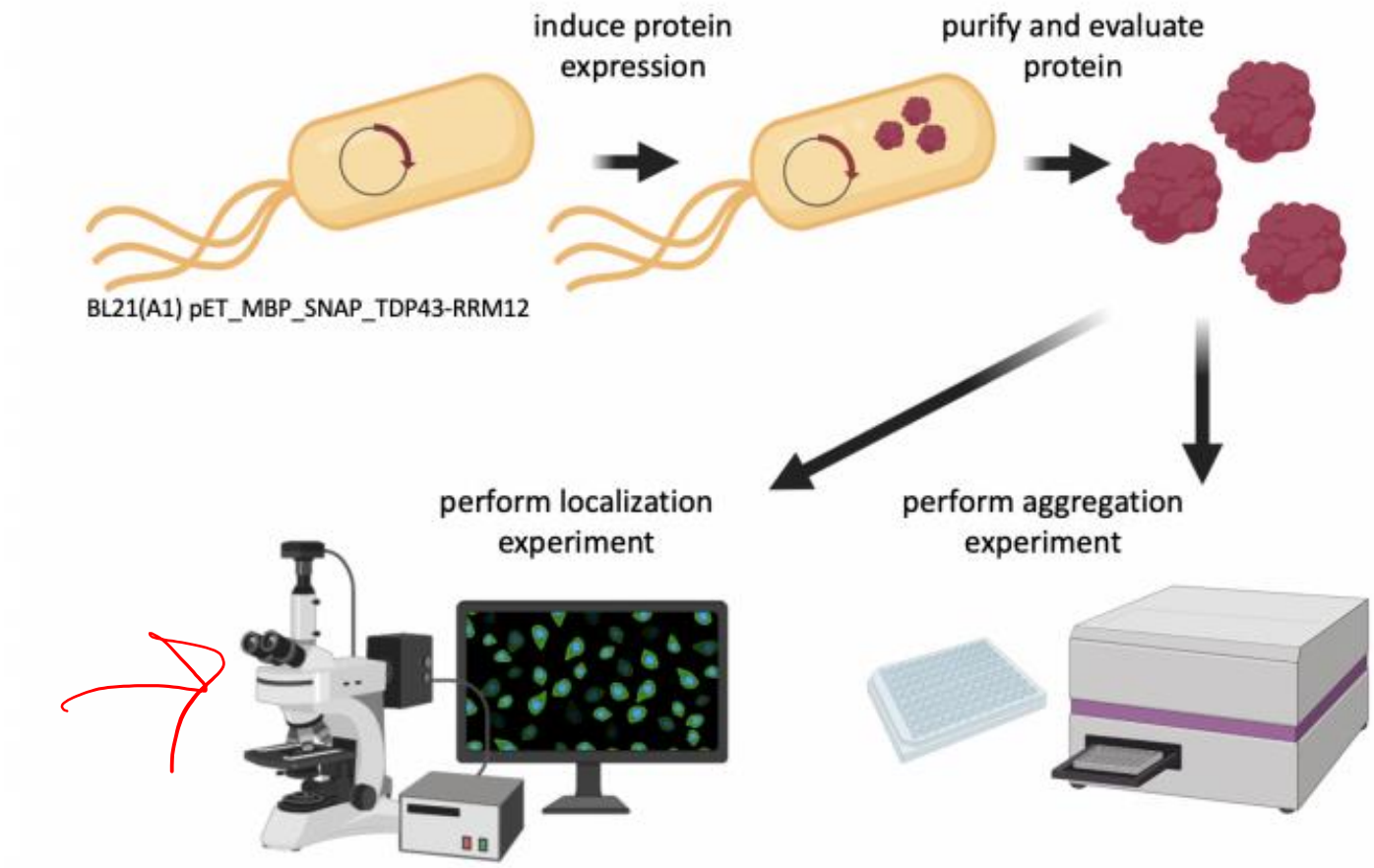
M1D6: Learn best practices for mammalian cell culture and seed CAD cells for TDP43-localization experiment

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



Overview of Mod 1 experiments

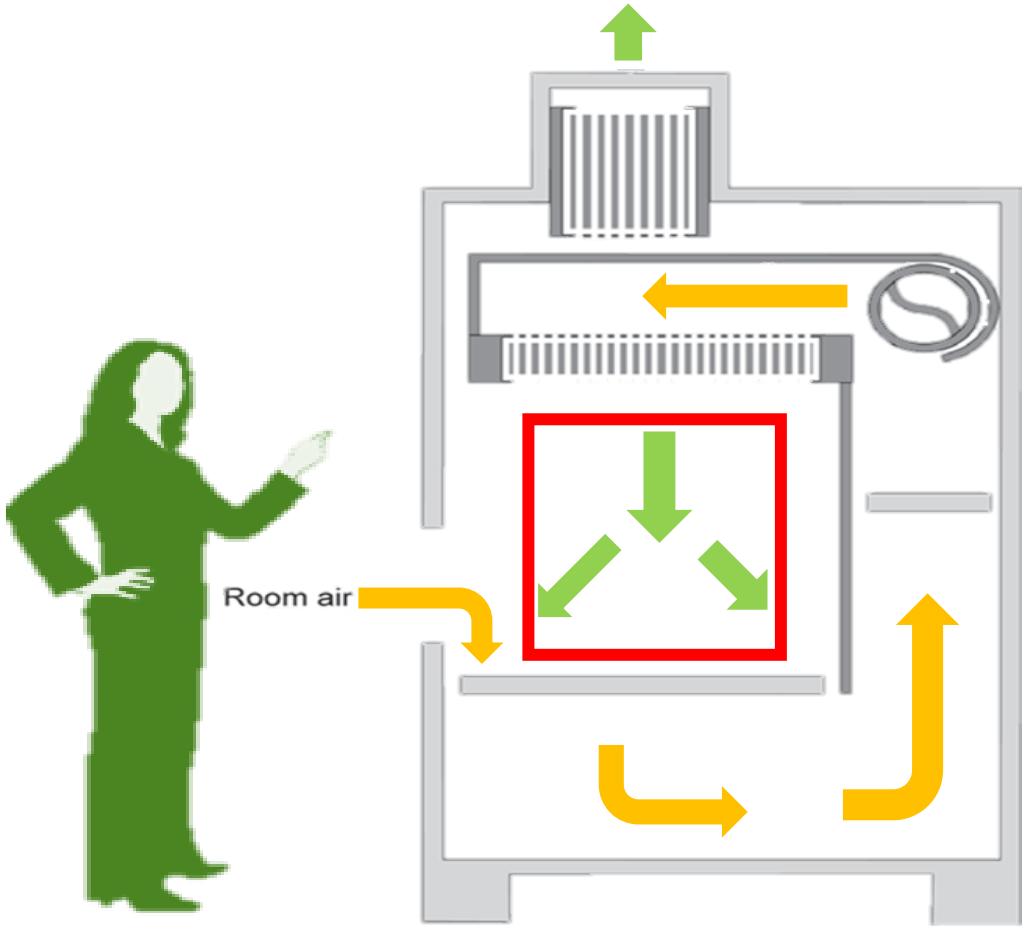
Research goal: Use functional assays to characterize ligands identified as binders to TDP43 from SMM technology



Tissue culture sterile technique

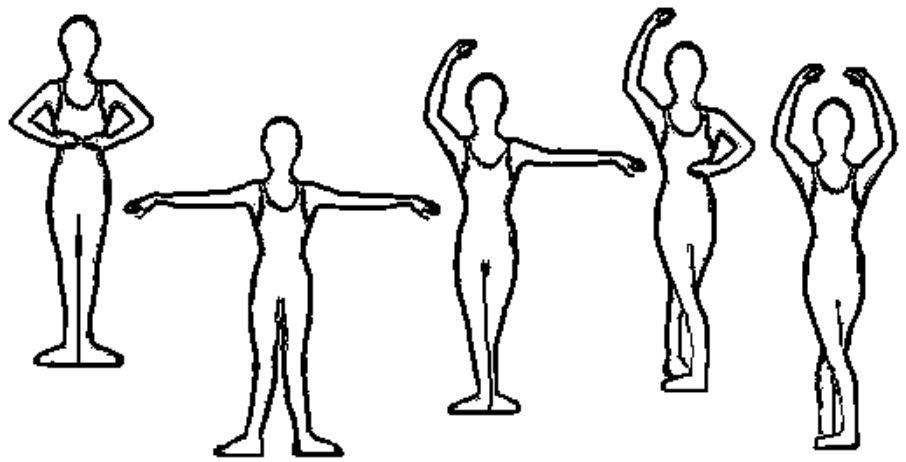
- **70% ethanol** everything:
  - Wipe cabinet before and after use
  - Wipe everything that enters the cabinet
  - Do not spray cells with EtOH
- **Do not disturb air flow:**
  - Do not block grille or slots
  - Minimize side-to-side arm movements
  - Work > 6” away from sash
  - Leave blower *on always*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

Area of highest sterility



Sterile Technique, condensed

1) Ethanol everything (minus anything containing cells)
    1) Gloved hands, unopened plastic pipettes, autoclaved containers, media bottles…. EVERYTHING
2) Move *slowly*
3) Don’t block the grate



Mammalian Cell Culture Medium – CAD formulation

Food:

- DMEM/F12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12)
  - Defined
    - Salts, H20, Sugar, Amino Acids
    - Phenol Red (Purple – Old, Orangey-Red – OK, Yellow – Bad)
- FBS (fetal bovine serum)
  - Undefined
    - Lot Dependent
    - Lipids, cytokines, growth factors, nucleic acids

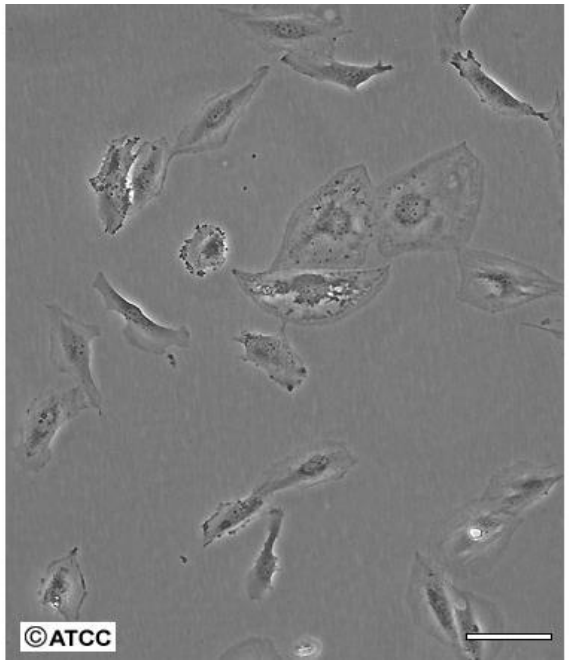
Non-food:

- antibiotics:
  - penicillin
  - streptomycin

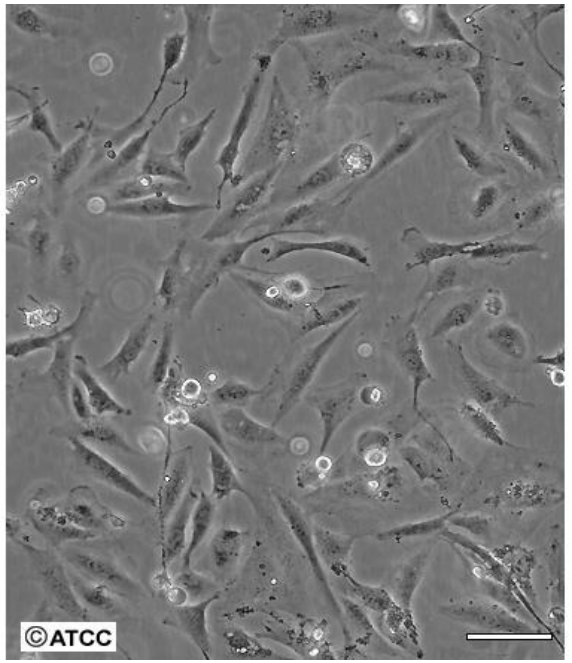
Mammalian Cell Culture Terminology

- Adherent vs Suspension
  - Adherent – Cells that like to stick to flask/coverslip surface. Flattened.
  - Suspension – Spheroid cells that float in solution
- Confluence
  - % surface covered in cells
- Splitting
  - Taking one culture's cells and passing to more flasks to either expand or maintain cell culture
- Seeding
  - Splitting with a known number of cells to achieve a desired confluence/density

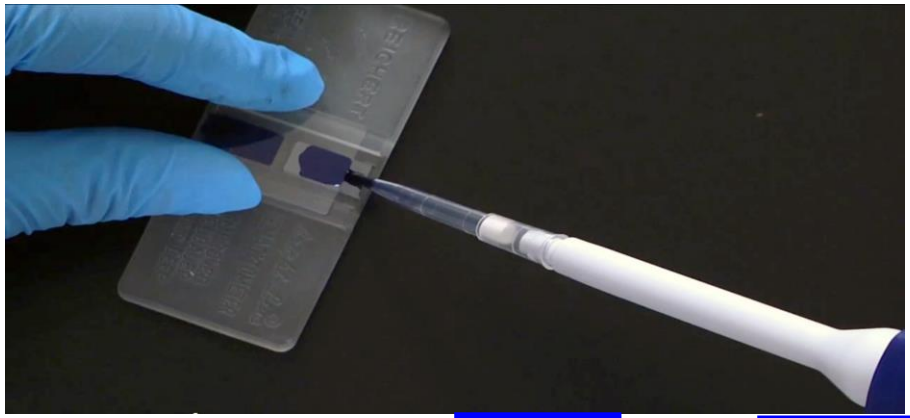
Low Density

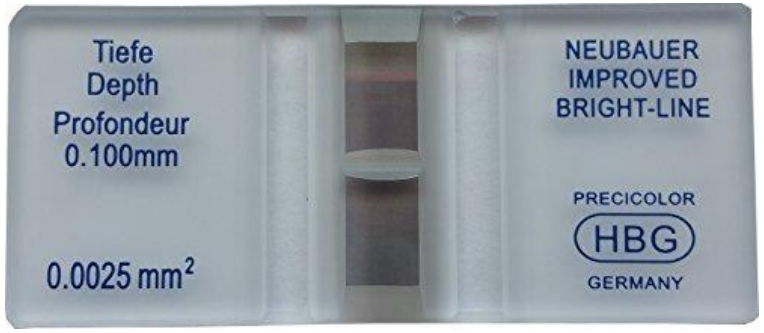


High Density



Counting cells





- Hemocytometer
- Trypan blue

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

Trypan Blue is an Exclusion Dye

1) TB is toxic, viable cells pump the dye out
2) Count clear cells with visible light halos