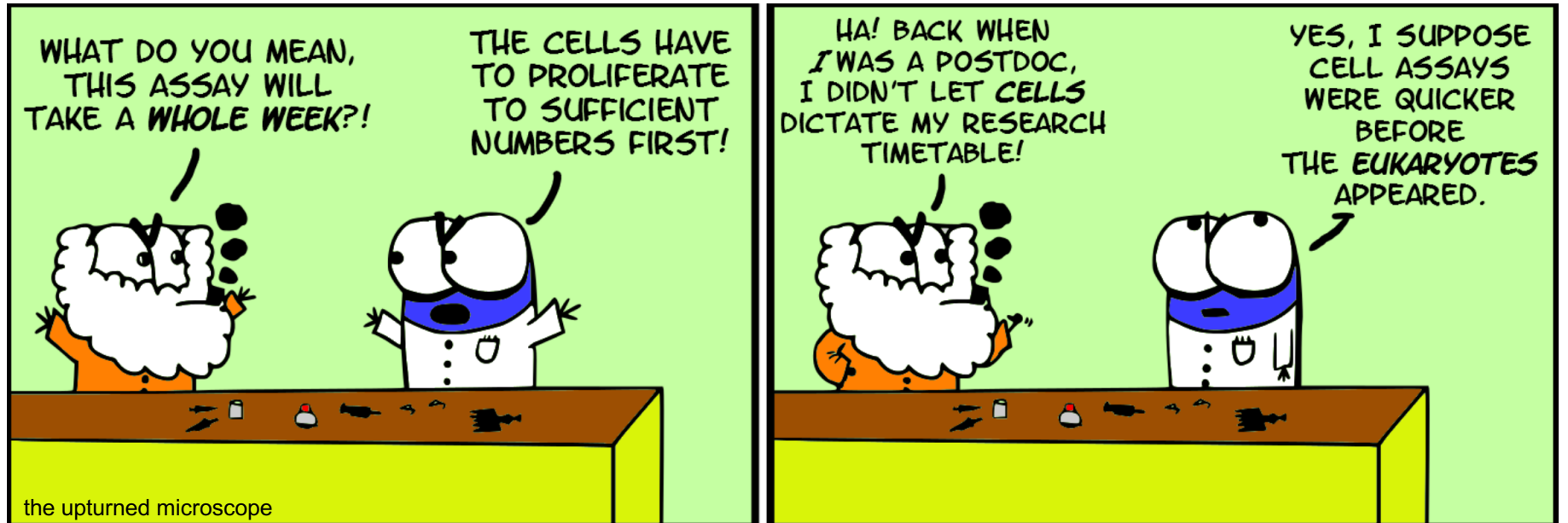


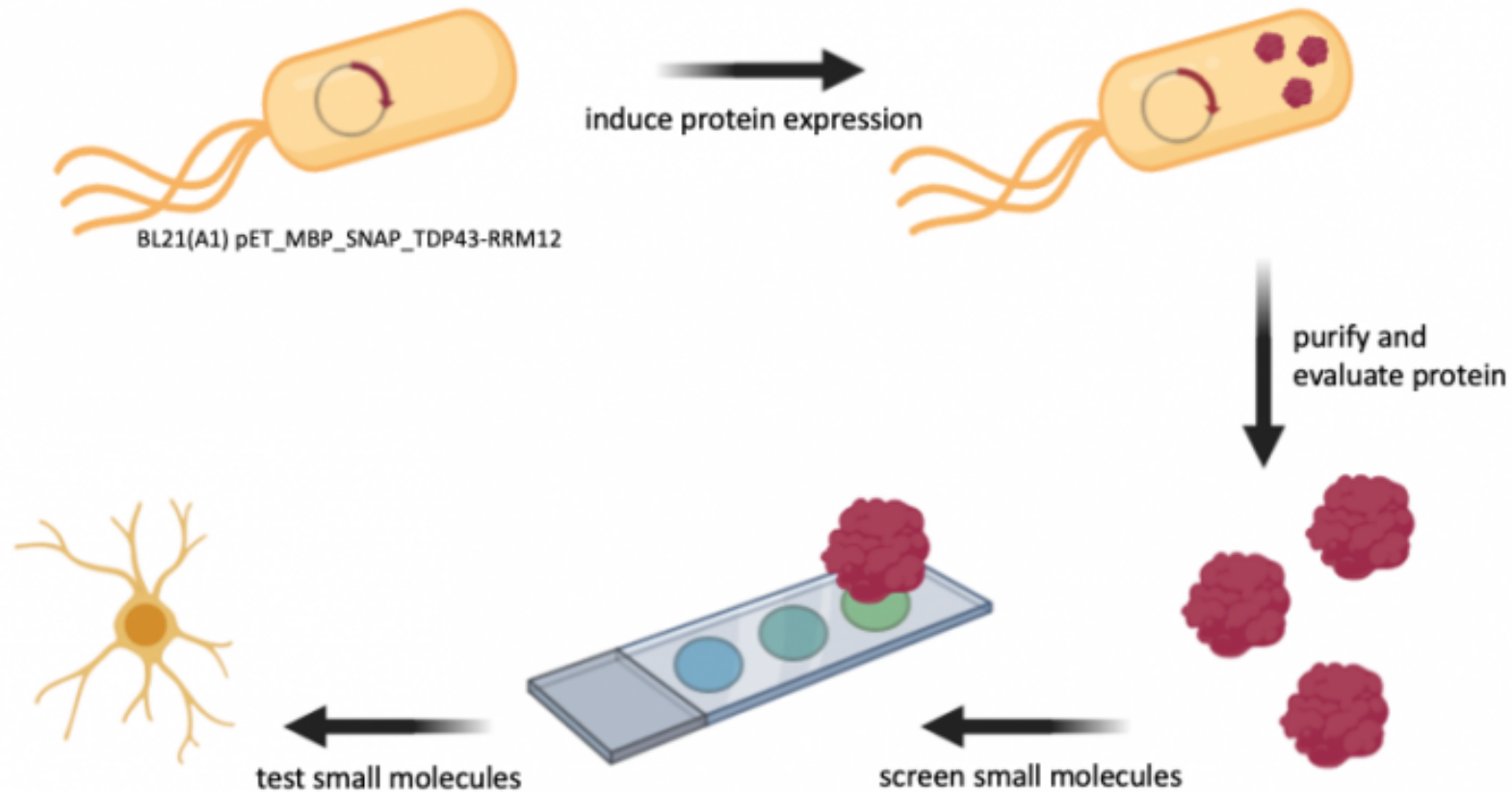
M2D5: Learn best practices for mammalian cell culture

1. Comm lab
2. Prelab discussion
3. Learn about cell culture in the lab



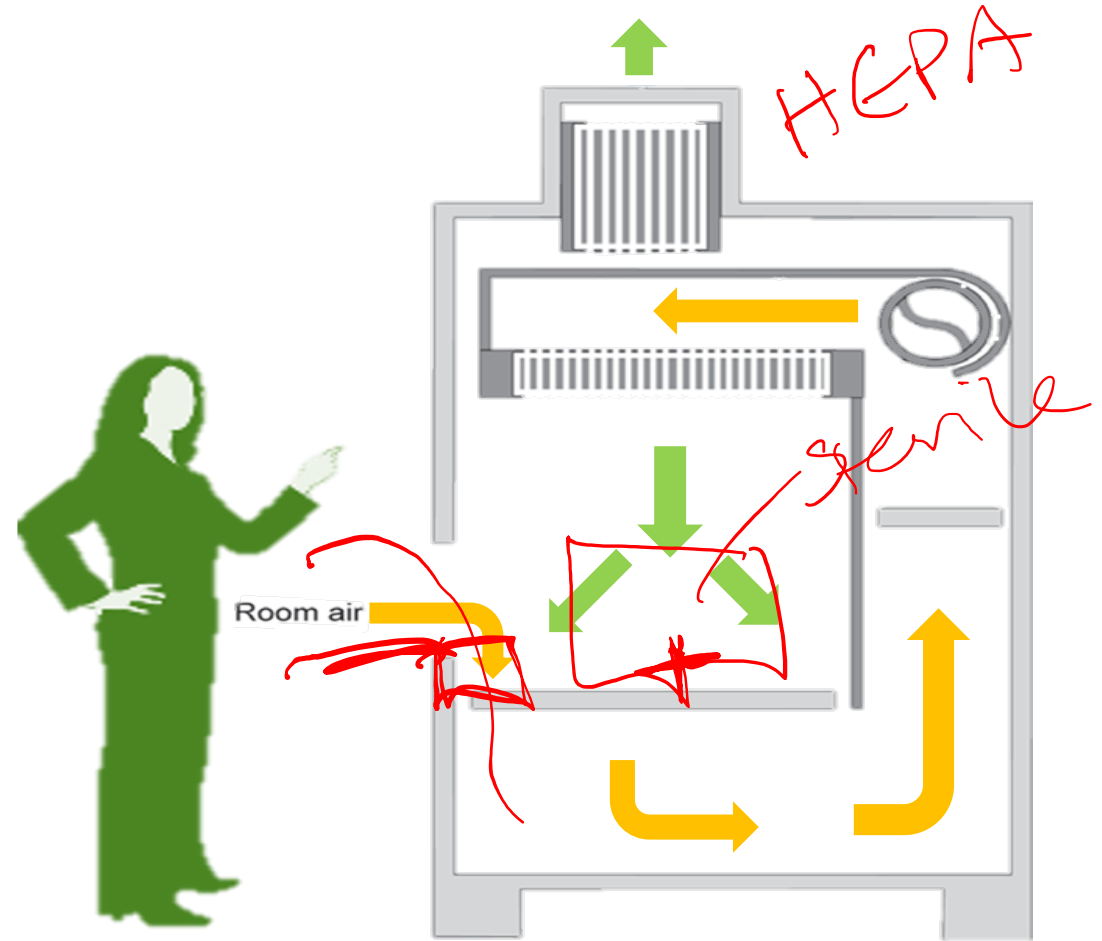
Mod2 Experimental Overview:

Research goal: Identify and characterize small molecule binders to a protein drug target



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 in hood



Mammalian Cell Culture Medium

We are using CAD cells

Food:

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

- **Defined** — know what is there
sugars (glucose), cofactors, salts
amino acids, pH indicator — phenol red

- FBS (fetal bovine serum)

- **Undefined** — Δ depending on stock / lot
lipids, cholesterol, growth factors
cytokines

Non-food:

- antibiotics:

- penicillin
- streptomycin

} prevent bacterial growth / contamination

Media



Mammalian Cell Culture Terminology

⊕ Adherent vs
nonadherent
↓
High Density

Low Density

High Density

- Confluence

density of culture →

- Splitting

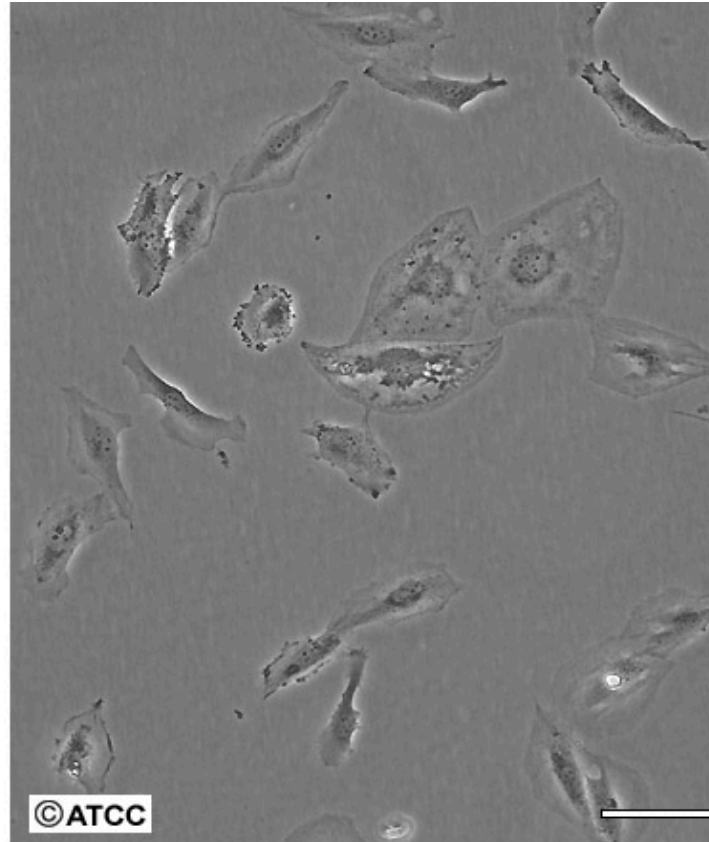
subculturing
Adherent = needs
trypsin

- Seeding

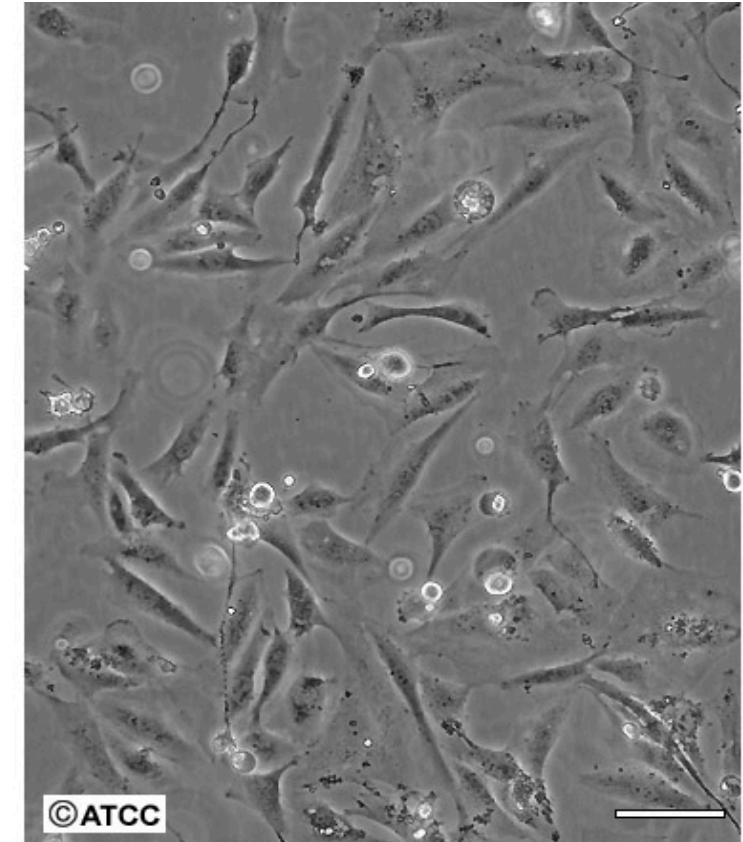
- move cells to
new flask

- count # cells

- part of subculture
or set up for experiment



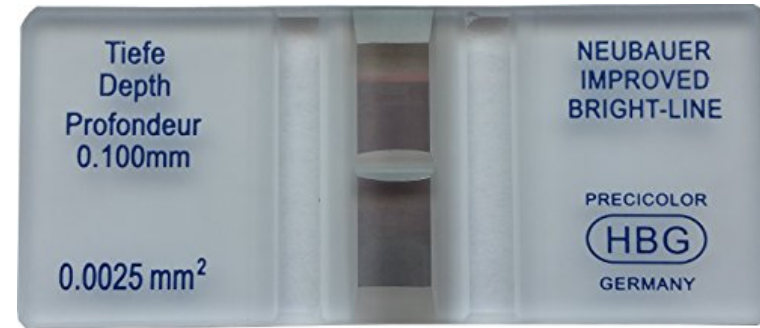
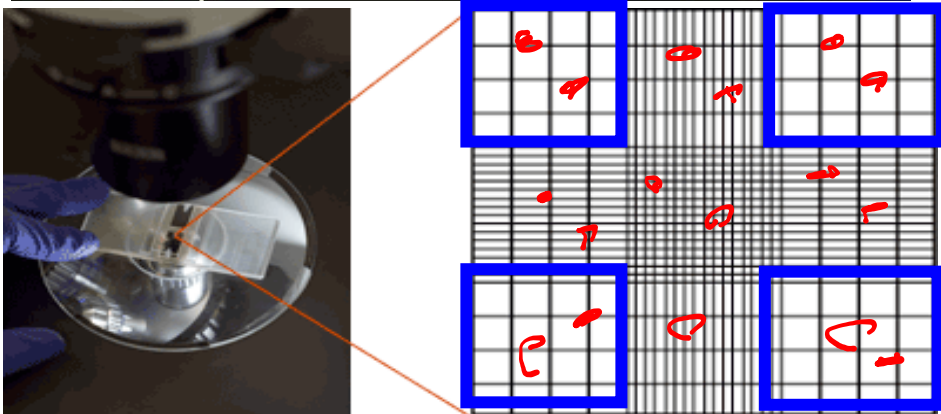
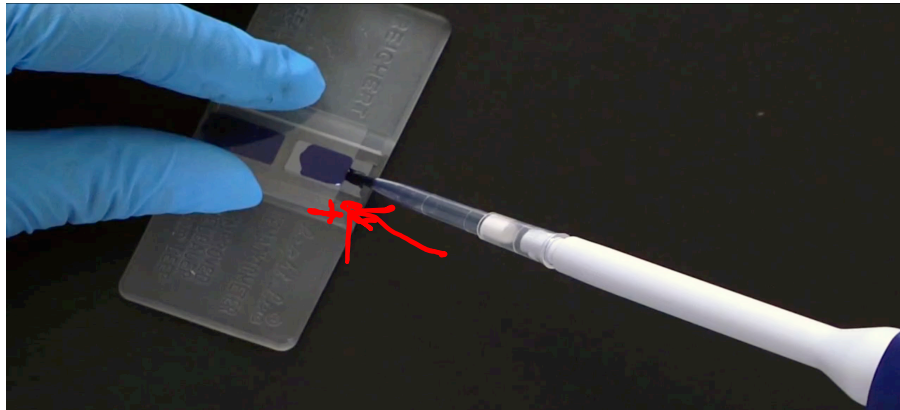
10-20%



80-90%

- split cells
70% dense

Counting cells



- Hemocytometer

- Trypan blue - dead

10^4

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

$$8/4 = 2 \times 10^4 = 20,000 \frac{\text{cells}}{\text{mL}}$$

For today:

1. Complete the wiki on cell culture
2. Finish up any SMM work/ think about data for the research article
** If you can't get the jupyter notebook to work and you are over it, talk to me
AND/OR
3. Take off early and remind yourself what happiness feels like (or study)

For M2D6:

Peer review of methods

** If you did not turn in methods homework, talk to me

Redacted methods will be emailed to you. Please review according to the wiki and turn in your comments on Stellar.