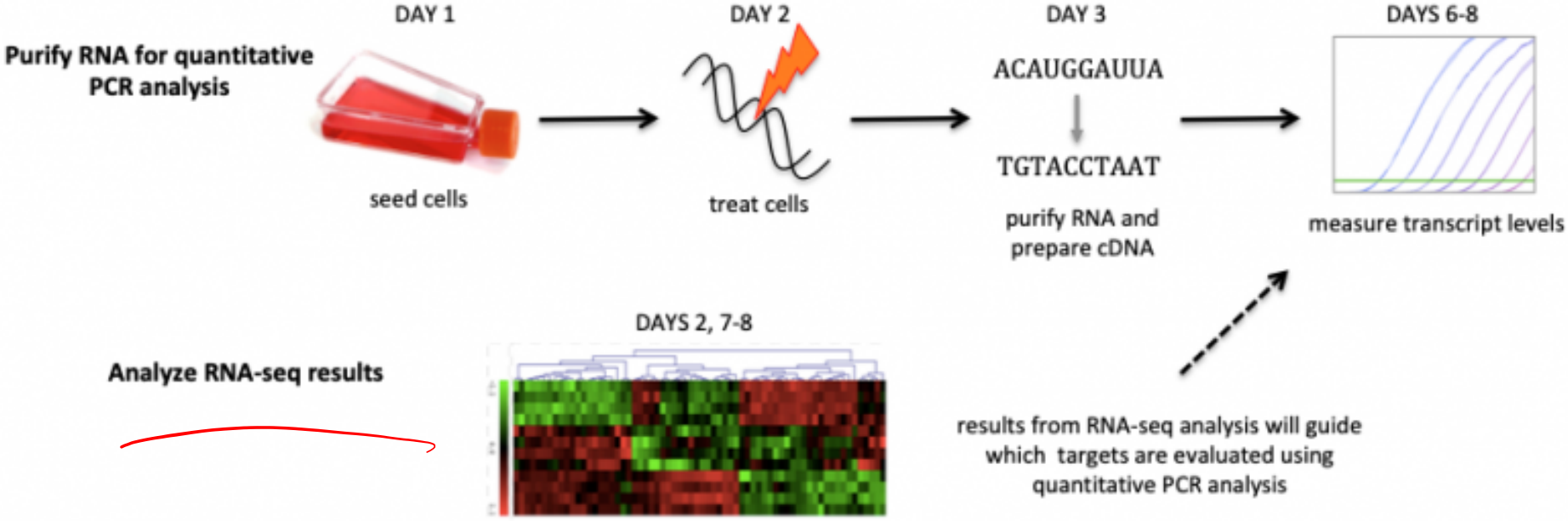
M2D1: Prepare cells for RNA purification

1. Prelab discussion
2. ½ class to TC to seed cells for RNA purification
3. ½ group paper discussion
4. Work on Exercise 1 in Rstudio

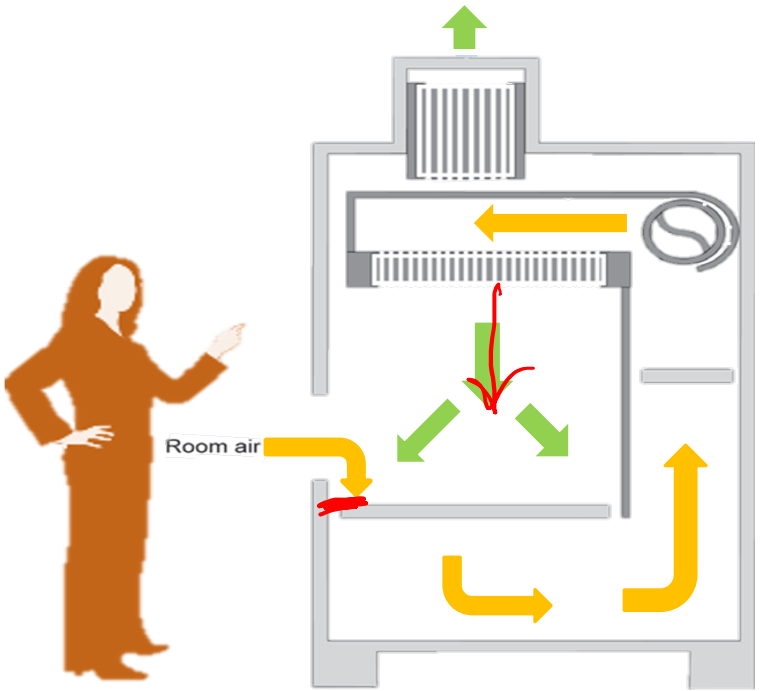
Reminders:
3/9 (Sat): Extra Office Hours, 11am-6pm @ 56-302

Mod2: Experimental overview

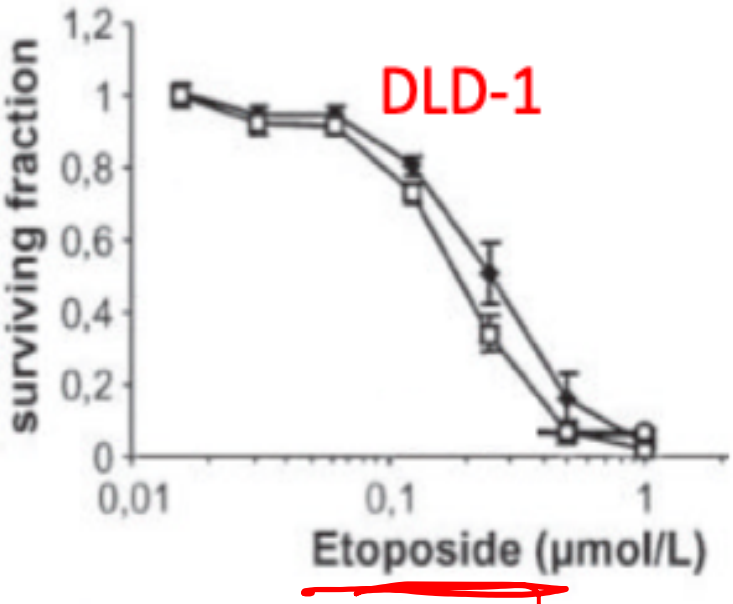


Tissue culture sterile technique

- 70% ethanol is the best!
  - wipe cabinet before and after use
  - wipe everything that enters the cabinet
- Do not disturb air flow:
  - Do no block grille or slots
  - Minimize side-to-side arm movements
  - Work > 6” away from sash
  - Leave blower on
- Do not talk into incubator!
- Only open sterile items in hood



Our cell line: DLD-1



- Origin: human
- From the colon of a male with colorectal adenocarcinoma
- Isolated by D.L. Dexter and associates during a period from 1977-1979

↳ DNA Damage → Chemotherapy

Mammalian cell culture medium

What do DLD-1 cells need to survive?

- **Defined**: RPMI 1640 (Roswell Park Memorial Institute)

salts, sugar, amino acids

pH → phenol red → fuschia → yellow

- **Undefined**: FBS (fetal bovine serum)

serum

growth factors, cytokines, lipids

Not for survival

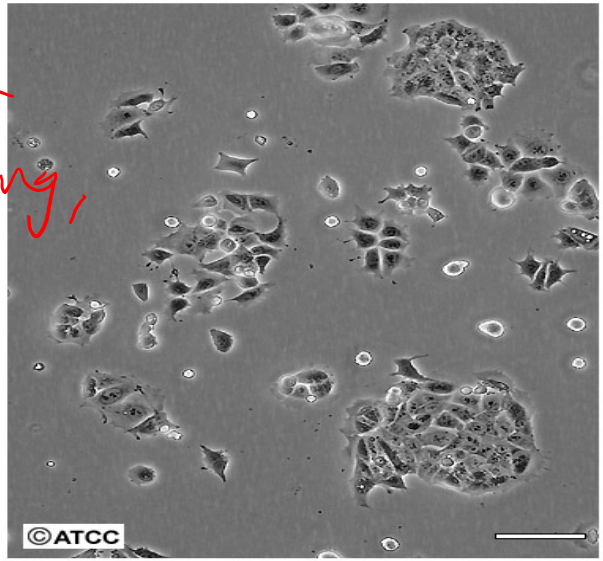
- antibiotics:
  - penicillin
  - streptomycin

bacterial growth prevention

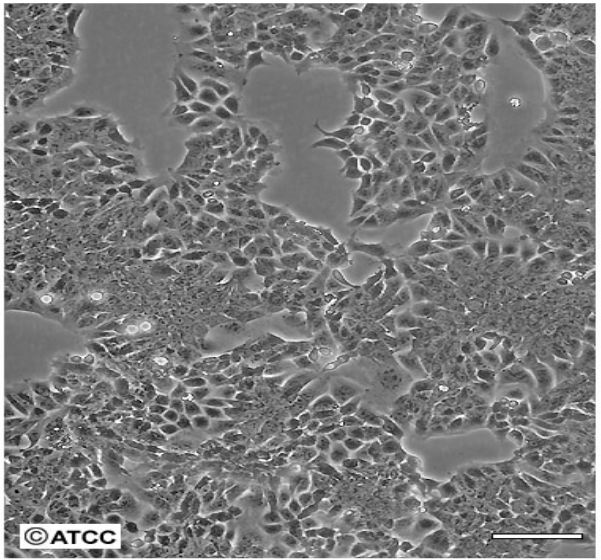
Mammalian cell culture terminology

- confluence/density
  split @ 80% confluence
- splitting/subculturing, passaging
- seeding/plating

Low Density DLD-1, ~30%



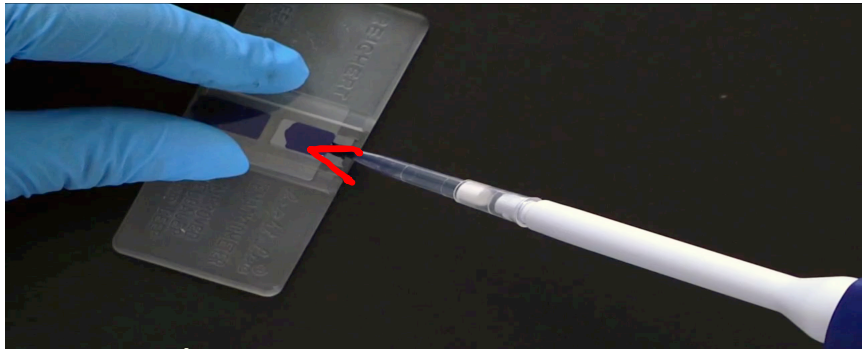
High Density DLD-1, ~80%



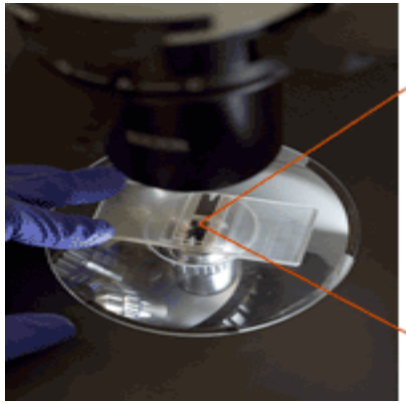
General steps for splitting cells +WHY?

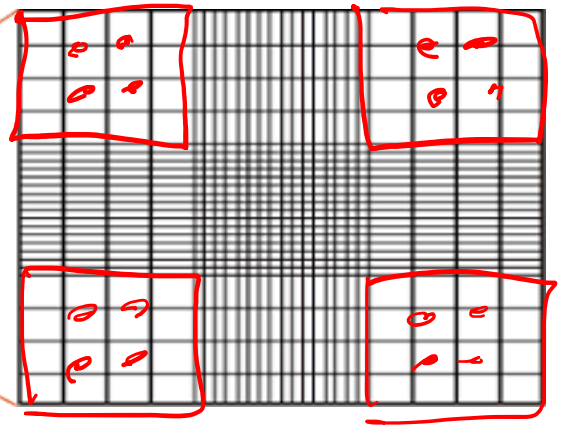
1. Look at cells, estimate confluence
   health? ready to split?
2. Rinse with PBS
   get rid of debris, protein, anti-trypsin factors
3. Detach cells with trypsin
   clear adhesions b/w cells & substrate
4. Count cells
5. Seed new culture vessel

Calculating number of cells with a Hemacytometer



- Hemacytometer, holds 10uL on each side
- Trypan blue:



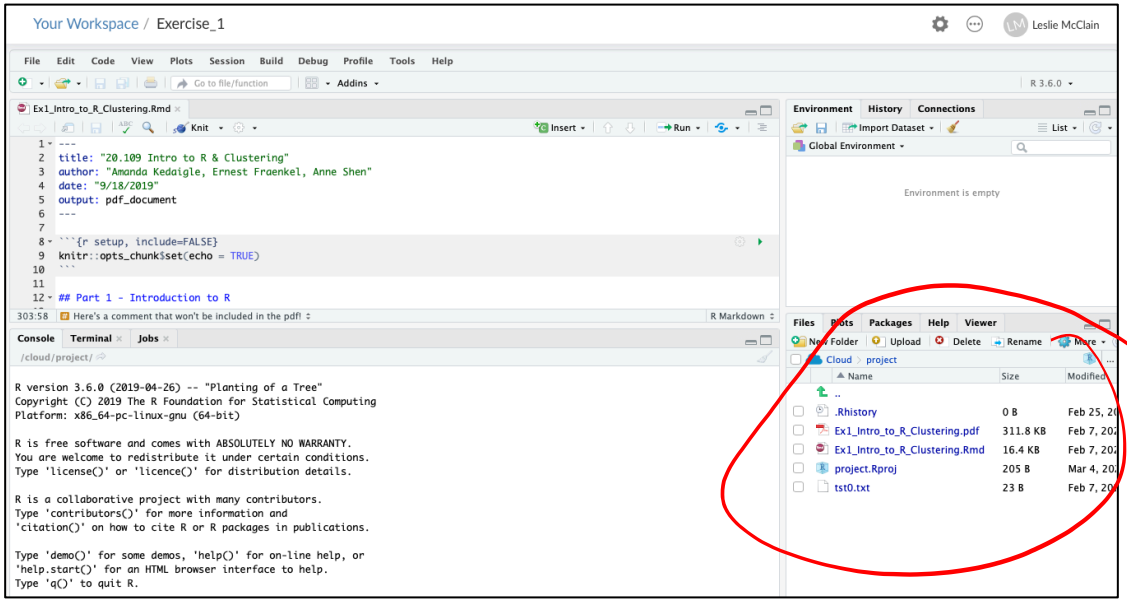


cells / mL = 10,000 x average of 4 corners

16/4 x 10,000

40,000 cells/mL

R programming language



- R is a language and free software environment
- R is popular for analysis of complex biological data

Rmd.

- Interface called Rstudio.cloud, online workspace

https://rstudio.cloud/spaces/7339/join?access_code=C2B0D5OQ1MHPiDhX9vuTKkDn0aGpQGq0SYA9cKYn

Documenting R analysis in your Benchling notebook

- Each lab day with a R exercise, Joe will check your progress in Benchling before you leave
- Today for Exercise 1 include:
    - ❑Plot of 100 random numbers
    - ❑Plot line in yellow of 100 random numbers
    - ❑Histogram of 100 random numbers
    - ❑Scatterplot of two animal populations per city
    - ❑Scatterplot of two animals per city without NAs
    - ❑Include 1-2 sentences that describes the differences in the scatterplots
- Feel free to make other notes in Benchling you think are important. The above list is the minimum.

Today in lab:

1. Tissue Culture (TC)
    - 1st: red, orange, white, silver, blue
    - 2nd: yellow, green, pink, purple
    - Protocols printed for TC use, no need to move laptops etc.
    - Do not wear PPE in or out of TC room
2. Group discussion of Wei *et al.,* see wiki for guidelines
3. Practice data analysis in R studio Cloud

For M2D2

- Sign up for a Journal Club day and paper to present
- Turn in single Journal Club slide from Wei et al.

Don't forget about Mod1 assignments!

- Draft data summary due Sunday March 8th at 10pm (team)
- Mini presentation due Sunday March 15th at 10pm (individual)

Sign up for journal club

- Pick 1 of 25 papers, or suggest your own (must be approved by instructors)
- Present M2D4 (March 18th) or M2D5 (March 20th)
- Sign up by adding your name next to paper [BCM/WF/Color]
  - first come first serve!
  - you cannot switch paper after M2D3 (March 13th)
  - only one T/R presenter and one W/F presenter per article

Slot	Day 4 (T/R)	Day 5 (T/R)	Day 4 (W/F)	Day 5 (W/F)
1				
2				
3				
4				
5				
6				
7				
8				
9				

M2D2 HW: Journal Club Slide

- Slide= Standard 4:3 powerpoint slide
- Title has a message (not just the figure / paper title)
- Don’t put too much on one slide, (1 slide=1 message)
- Don’t fill slide with text
- Don’t include the caption from paper or a citation
- Figures from paper can be cropped or modified
- Read homework description for additional tips