M2D1: Prepare cells for RNA purification

3/5/20

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

Regular office hours:

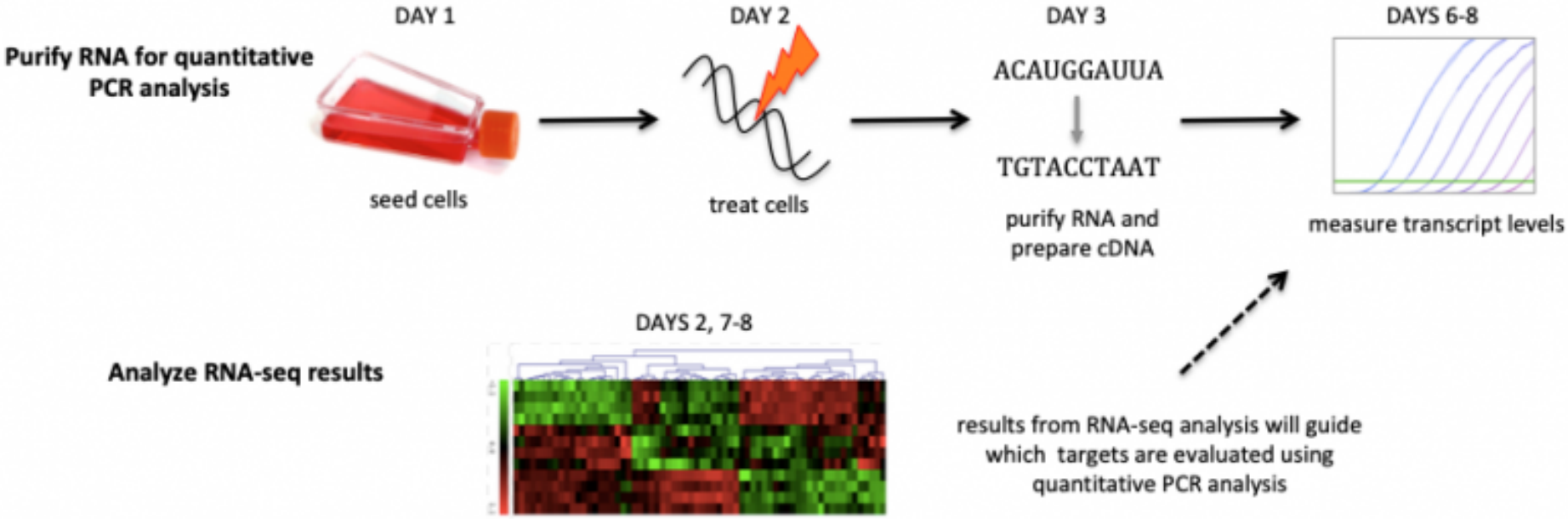
Noreen: Wed 11-1p and Fri 2-5p

Becky: Tues 12-1p, Thurs 9-10a

Leslie: Mon 2-4:30p

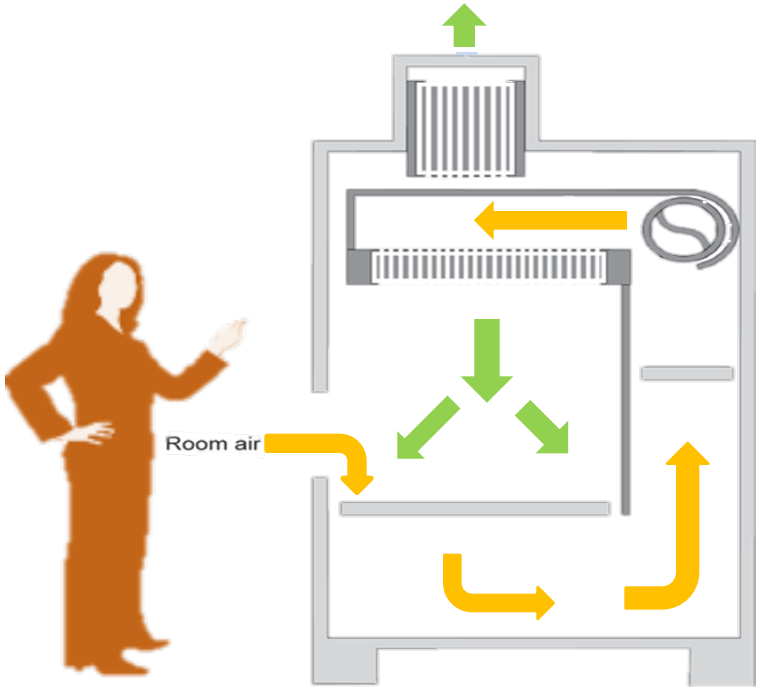
Email us if you can't make office hours and we can schedule time for you!

Mod2: Experimental overview

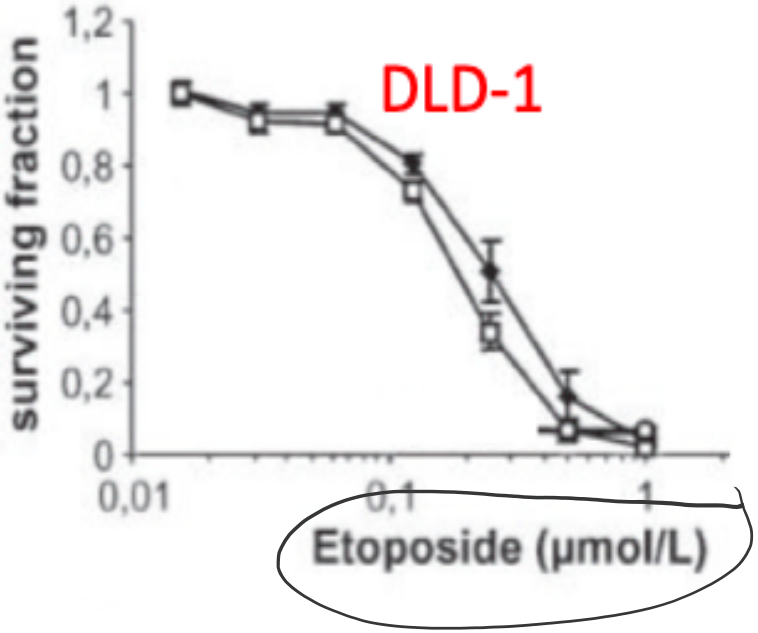


Tissue culture sterile technique

- 70% ethanol is your BFF:
  - 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 damaging agent
chemotherapy

Mammalian cell culture medium

What do DLD-1 cells need to survive?

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

Sugars / amino acids / N.A. vitamins / salts

phenol red = pH indicator

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

cytokines / growth factors / lipids / cholesterols

Not for survival

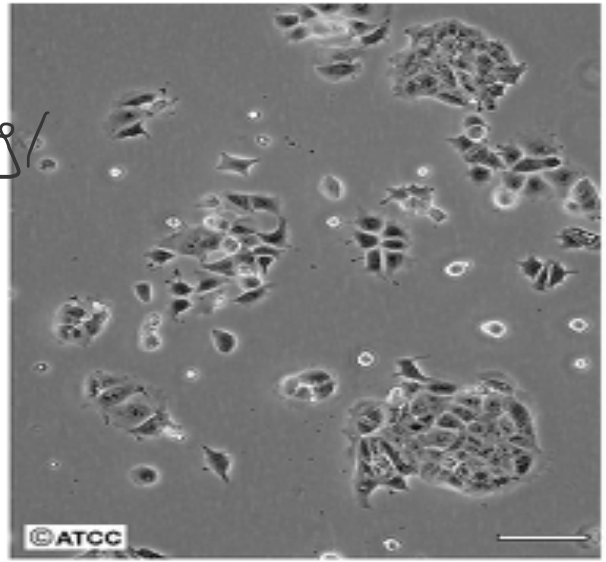
- antibiotics:
  - penicillin
  - streptomycin

] prevent bacterial growth

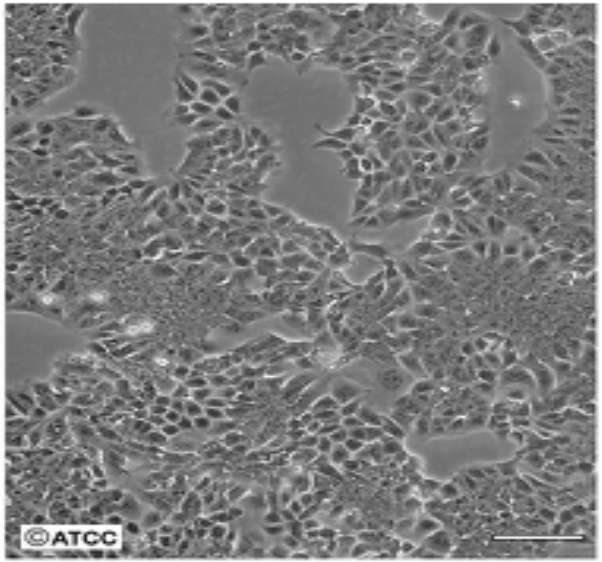
Mammalian cell culture terminology

- confluence / density
  → usually split at 80%.
- splitting / subculturing / passaging
- seeding / moving
  20% culture to new flask

Low Density DLD-1, ~30%



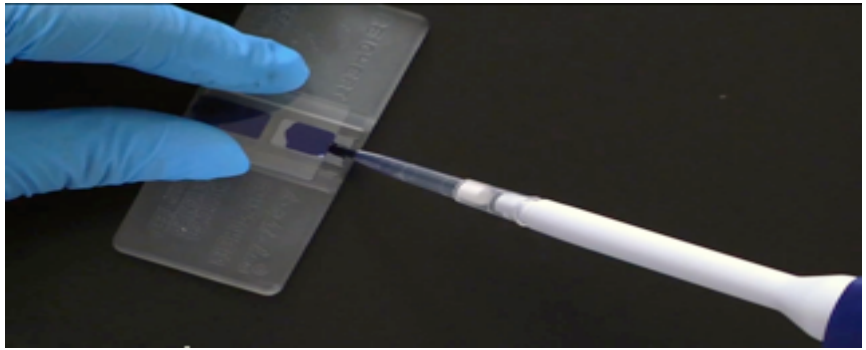
High Density DLD-1, ~80%



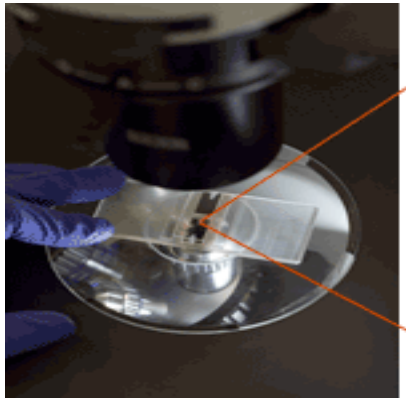
General steps for splitting cells +WHY?

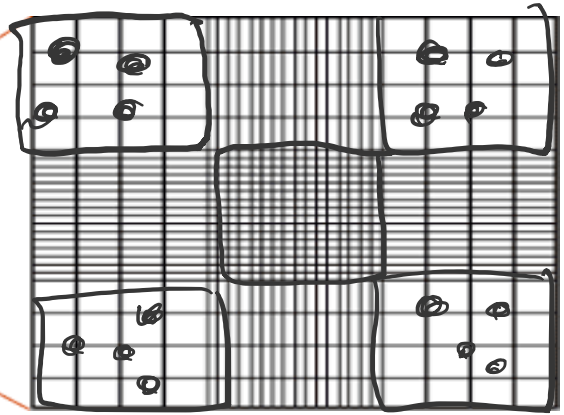
1. Look at cells, estimate confluence
   estimate growth/viability
2. Rinse with PBS
   wash debris / anti-trypsin / remove serum/FBS
3. Detach cells with trypsin
   break substrate-cell adhesions
4. Count cells
   seed specific # in new flask
5. Seed new culture vessel
   room / media to grow / divide

Calculating number of cells with a Hemacytometer



- $ Hemacytometer, holds 10uL on each side
- Trypan blue: stains dead cells

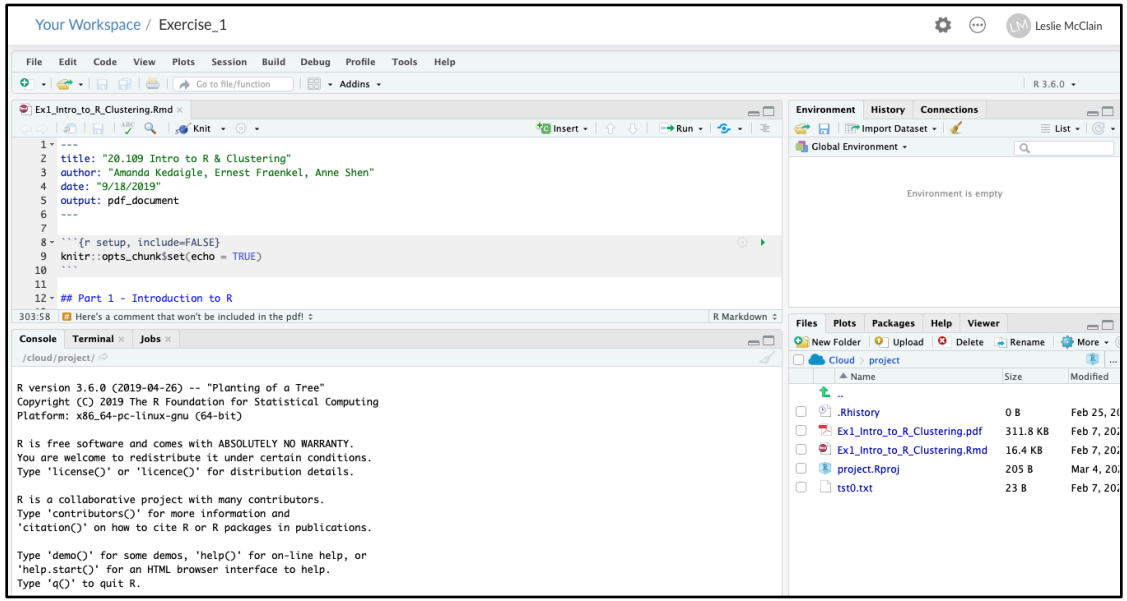




cells / mL = 10,000 x average of 4 corners

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
- Interface called Rstudio.cloud, online workspace

Documenting R analysis in your Benchling notebook

- Each lab day with a R exercise, Kevin 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, yellow, green, blue
    - 2nd: green, pink, purple, white, silver
    - ➢ 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

- **Homework due Tuesday, 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
- Present M2D4 (March 17th) or M2D5 (March 19th)
- Sign up by adding your name next to paper [LMM/TR/Color]
  - first come first serve!
  - you cannot switch paper after M2D3 (March 12th)
  - 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