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 of Dietlein et al.

Office hours

Noreen

- M 2-5pm
- in 16-317

Leslie

- W and F 4-5pm
- in 56-341c or lab

Josephine

- T 4-5pm
- R 10-11am
- in 56-341c or lab

+ Extra

Saturday, 03/10

- **10am-5pm**
- **in 56-302**

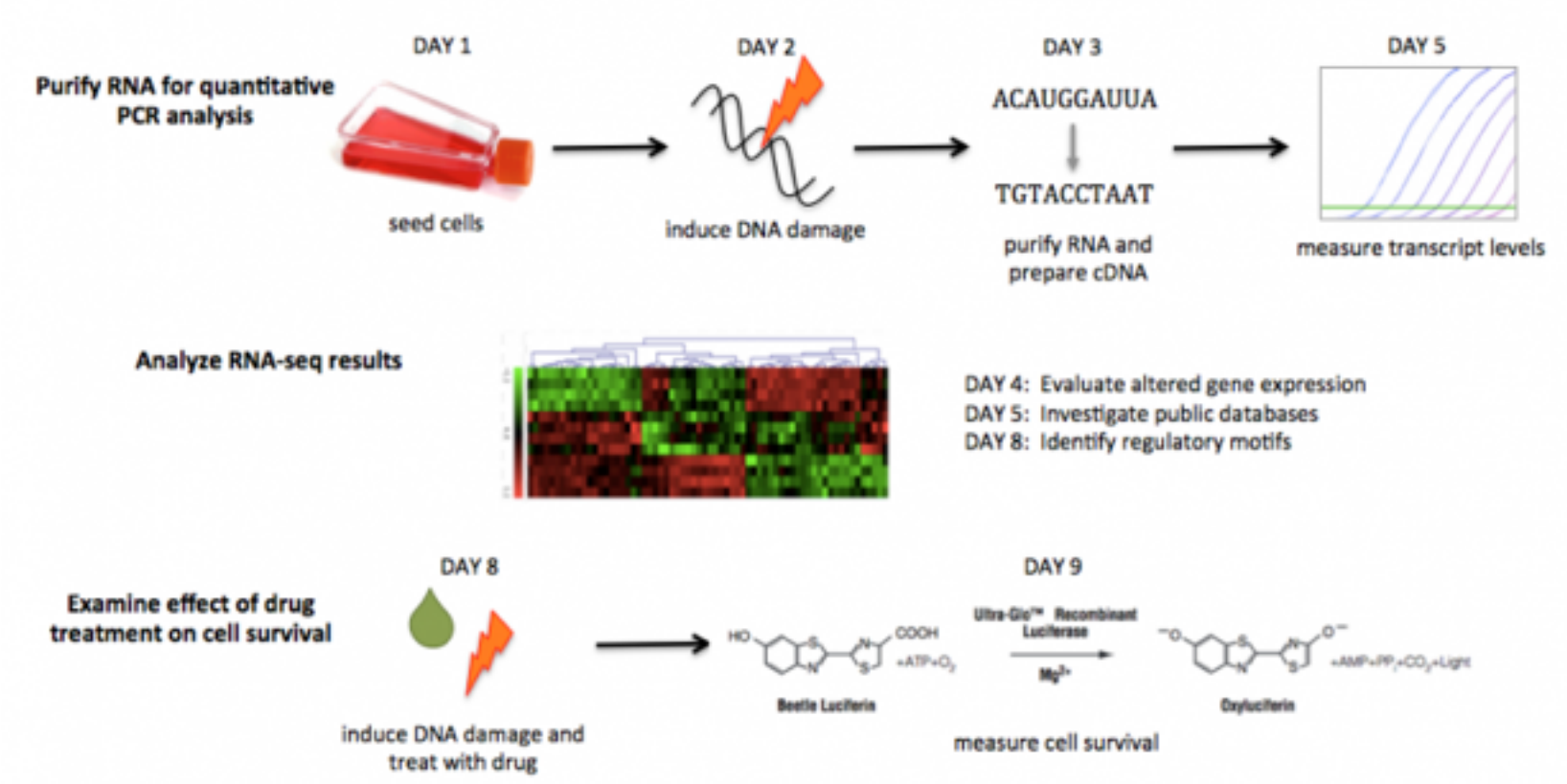
Please email us if you can’t make office hours and we will schedule a time to meet!

Sign up for journal club

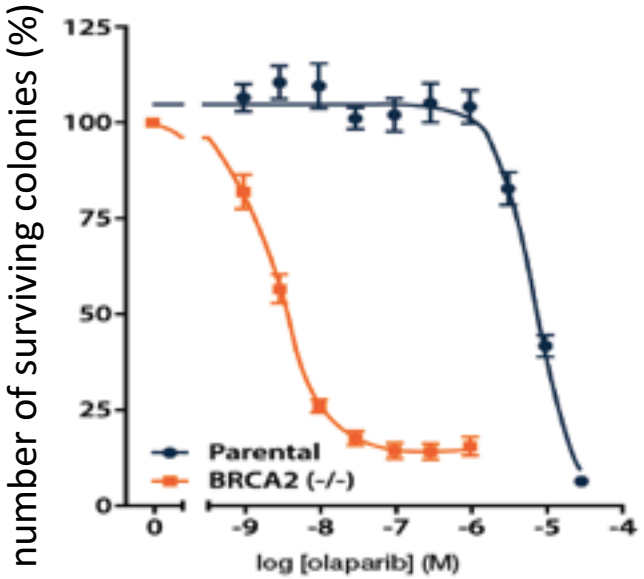
- Pick 1 of 24 papers, or suggest your own
- Present M2D6 (April 4th) or M2D7 (April 6th)
- Sign up by adding your name next to paper **[Josephine Bagnall/WF/Color]**
  - first come first serve!
  - you **cannot** switch paper after M2D4 (March 21st)
  - only one T/R presenter and one W/F presenter per article

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

M2: Experimental overview



Our cell lines: DLD-1 and BRCA2-/-

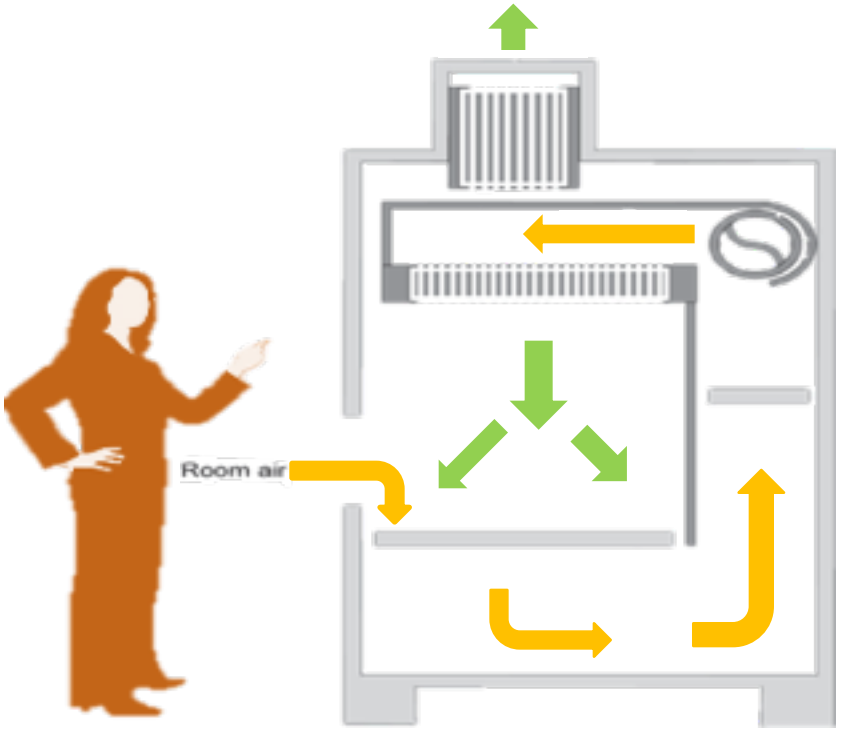


- DLD-1 = parental
  - from the colon of a male with colorectal adenocarcinoma
- BRCA2-/- = mutant
  - disruption of exon 11 from BRCA2 gene
  - deficient in DNA repair (by homologous recombination)

Note: olaparib is a PARP inhibitor (chemotherapy)

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 not 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



Mammalian cell culture medium

What do cells need to survive?

growth, division, viability

- RPMI 1640 (Roswell Park Memorial Institute) — defined
  - (a lot of phosphate)
  - often used to culture lymphoid cells

· glucose · salts · phenol red (pH indicator)
· amino acids · vitamins

- FBS: fetal bovine serum — undefined

· growth factors · lipids
· cytokines · cholesterol

- antibiotics: — defined
  - penicillin
  - streptomycin

} prevent bacterial growth

Mammalian cell culture terminology

- confluence = density

at ~80%, split cells

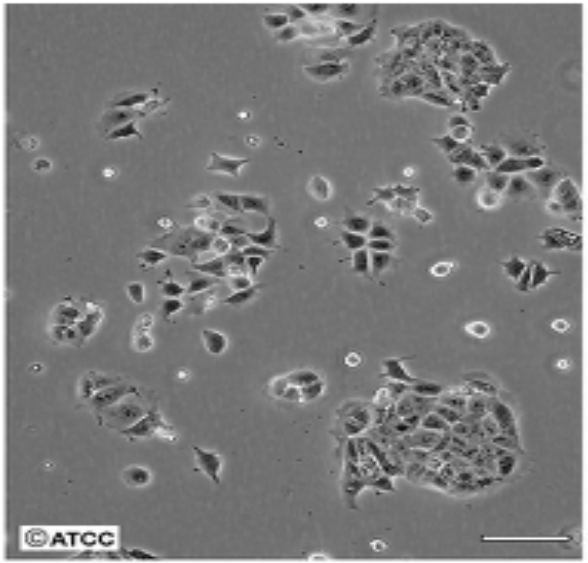
- splitting

Passaging, subculturing

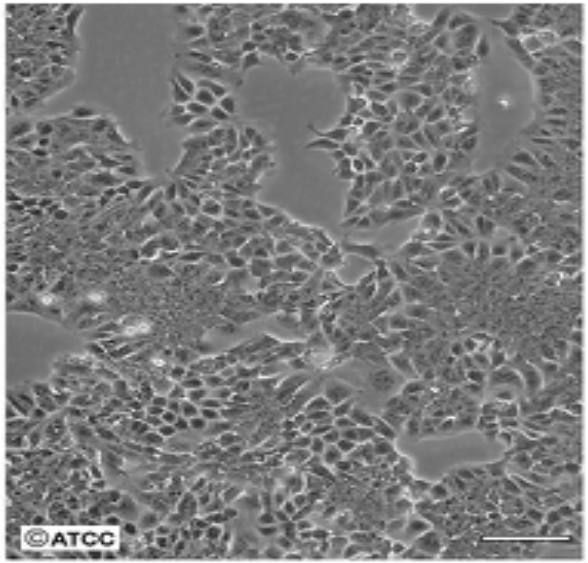
- seeding

~20%-40% on new dish

Low Density



High Density



DLD-1 cells

General steps for splitting cells +WHY?

1. Look at cells, estimate confluence

   estimate growth/viability

2. Rinse with PBS

   wash debris / anti-trypsin agents / serum (FBS)

3. Detach cells with trypsin

   breaks substrate - cell adhesions

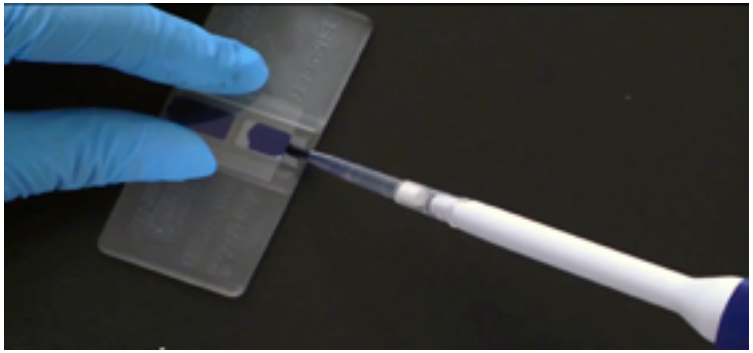
4. Count cells

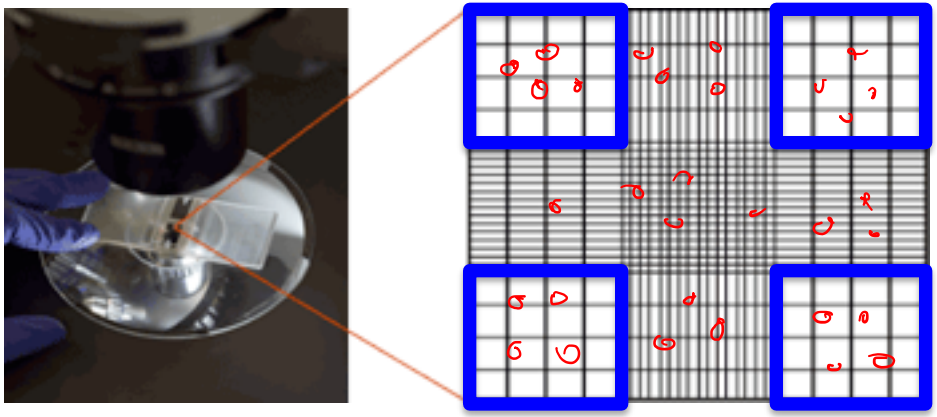
   seed specific # in new flask

5. "Seed" new culture vessel

   room to divide & grow

Counting number of cells





- Hemacytometer
- Trypan blue

  stains dead cells
- # cells / mL = 10,000 x average of 4 corners

4 x 10,000 = 40 000 cells / mL

Today in lab:

1. Tissue Culture (TC)
    - 1st: Red, Orange, Green, Blue
    - 2nd: Pink, Purple, Silver
    - ➢ Protocols printed for TC use, no need to move laptops etc.
    - ➢ Do not wear PPE in or out of TC room
2. Paper discussion of Dietlein *et al.*

- Homework due M2D2
  - Sign up for journal club day (sign up for article next week)
  - Create a single slide from Dietlein *et al.*
- Don't forget about Mod1 assignments!

M2D2 HW: JC Slide

- Slide= Standard 4:3 powerpoint slide
- Title has a message (not just the figure 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