M2D1: Prepare cells for RNA purification

03/08/2018

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**

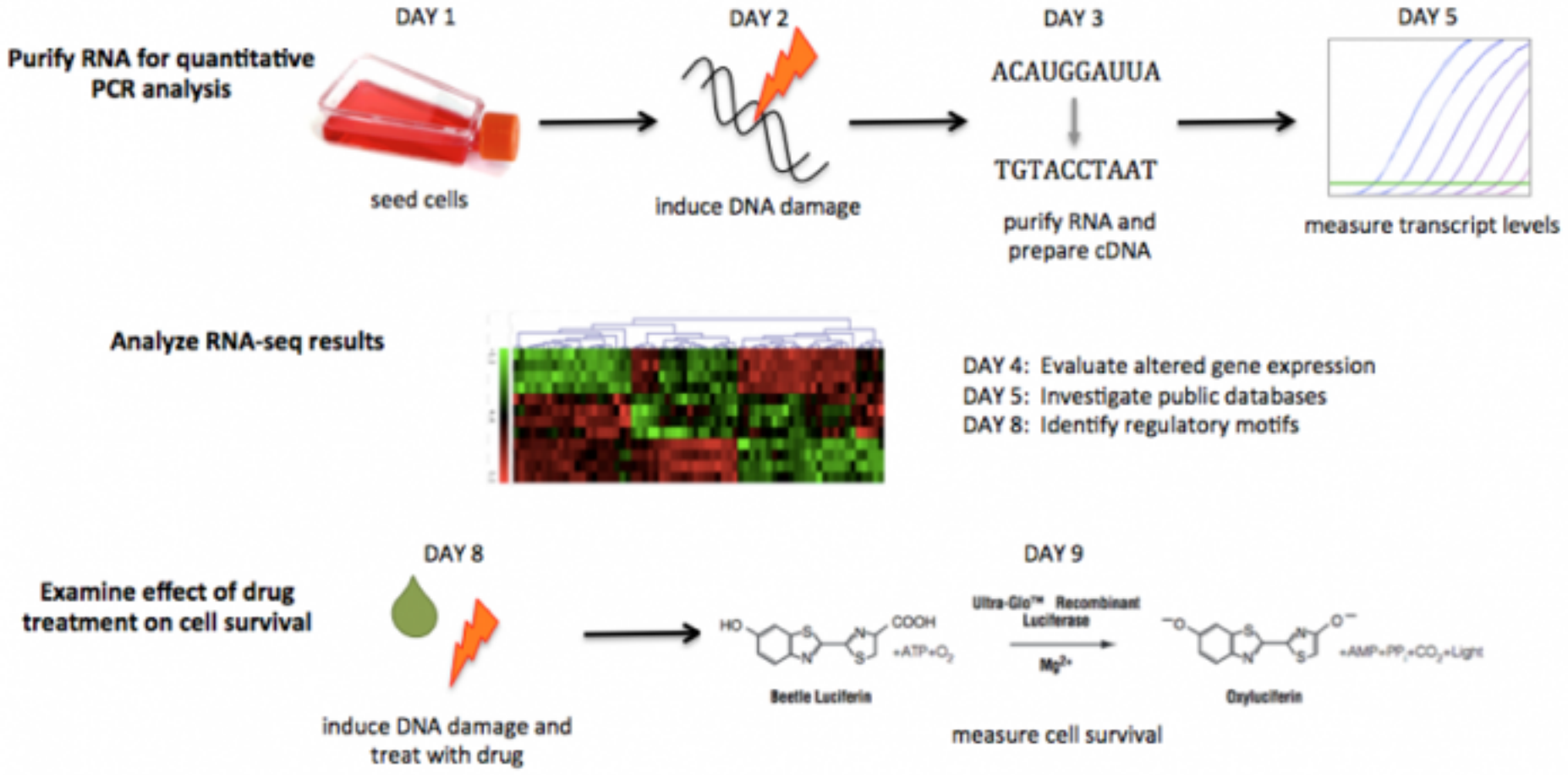
Please email us if you can’t make office hours and we will schedule a time to meet!

Sign up for journal club

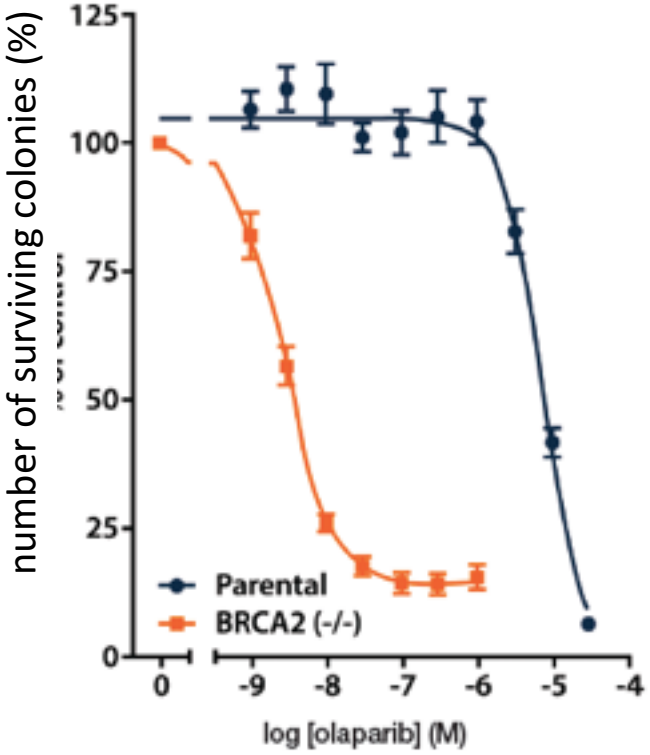
- Pick 1 of 24 papers, or suggest your own
- Present M2D6 (April 3rd) or M2D7 (April 5th)
- Sign up by adding your name next to paper **[LMM/TR/Color]**
  - first come first serve!
  - you **cannot** switch paper after M2D~~3~~4 (~~March 15th~~) March 20th
  - only one T/R presenter and one W/F presenter 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-/-



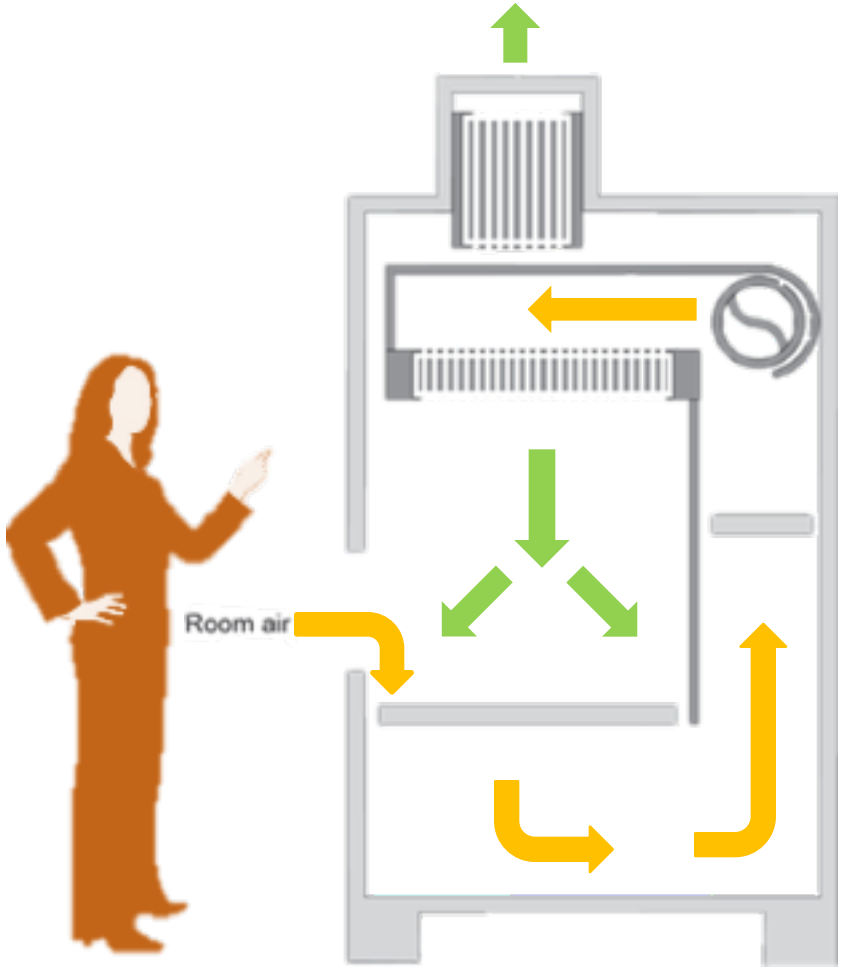
Note: olaparib is a PARP inhibitor (chemotherapy)

- DLD-1 = ~~wild-type~~ (or 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)

"HR"

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



Mammalian cell culture medium

What do cells need to survive? growth/divison/viability

Defined

- RPMI 1640 (Roswell Park Memorial Institute)
  - (a lot of phosphate)
  - often used to culture lymphoid cells
  - Salts
  - glucose
  - amino acids
  - vitamins
  - phenol red: pH ~~acid~~ indicator

- FBS: fetal bovine serum

UN defined

- growth factors
- cytokines
- lipids
- cholesterol

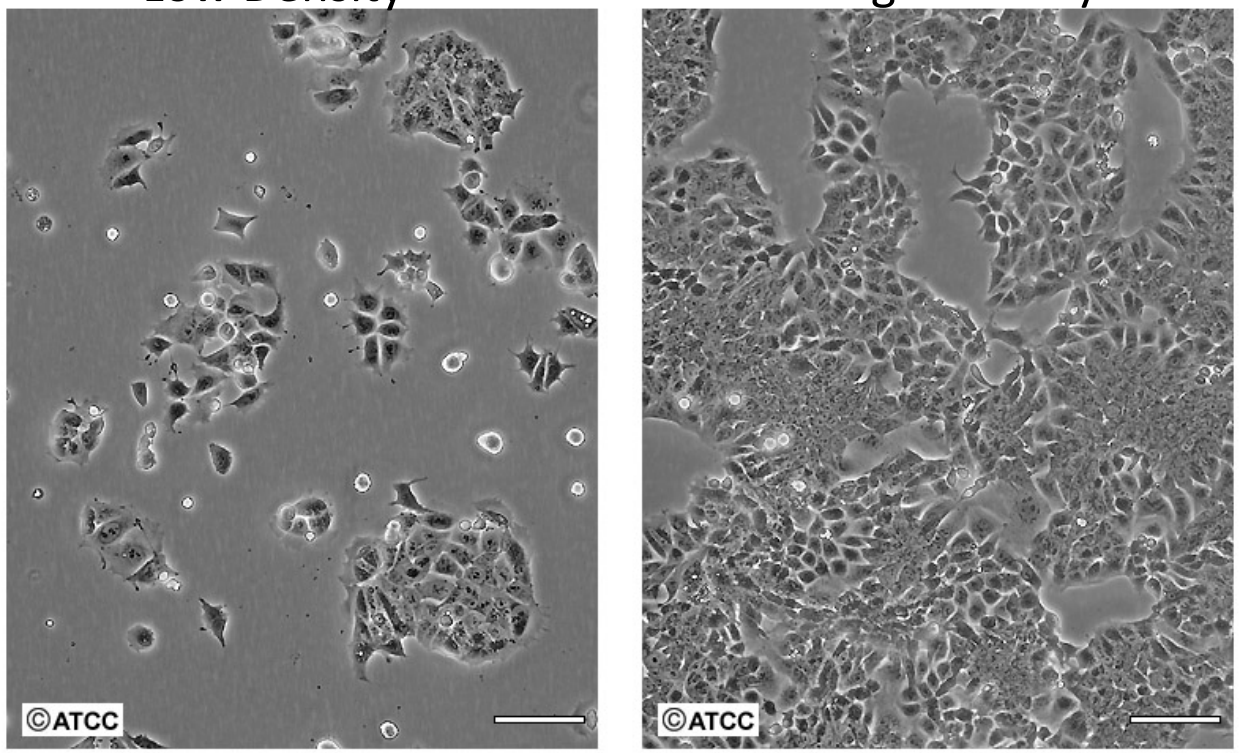
defined

- antibiotics:
  - penicillin
  - streptomycin

] prevent bacterial growth

Mammalian cell culture terminology

DLD-1

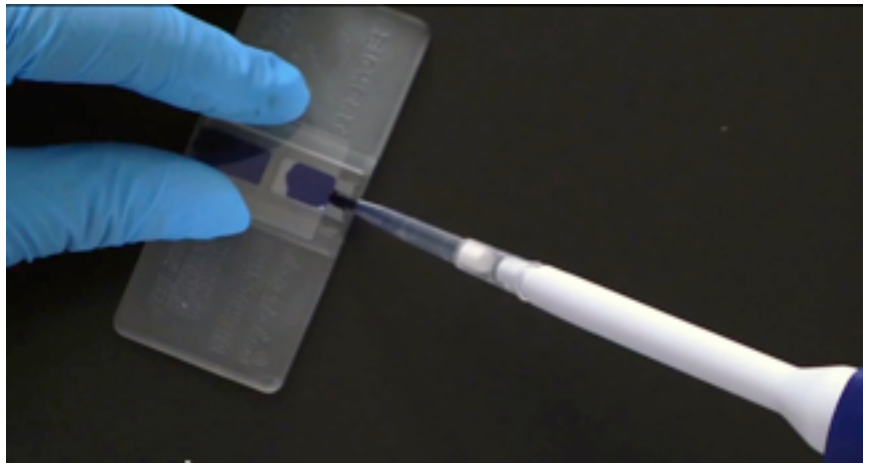


- confluence = density at ~80% split cells
- splitting = subculturing
- seeding = ~20-40% of a confluent culture on new dish

General steps for splitting cells +WHY?

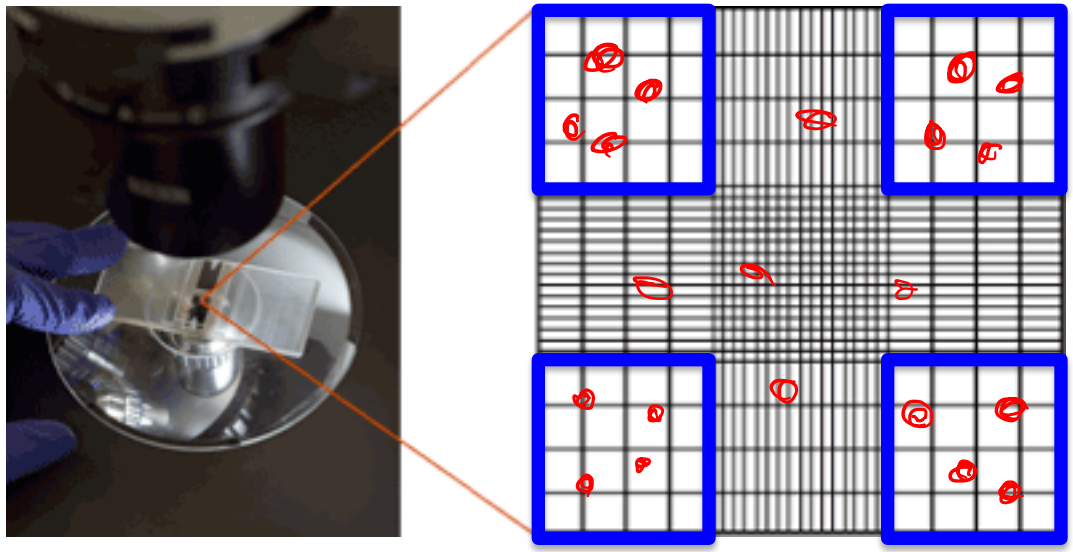
1. Look at cells, estimate confluence
   estimate growth/viability
2. Rinse with PBS
   wash debris/anti trypsin agents/serum (FBS)
3. Detach cells with trypsin
   breaks substrate + cell adhesions
4. Count cells
   Seed specific # in new vessel
5. "Seed" new culture vessel
   room to divide/grow

Calculating number of cells



- Hemacytometer 10ul cell suspension
- Trypan blue – stains dead cells

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



$\frac{16}{4} = 4 \times 10{,}000$ cells/mL =

40,000 cells/mL

Today in lab:

1. Tissue Culture (TC)
   - 1st: Red, Orange, Yellow, & ~~Green~~ Blue
   - 2nd: ~~Blue~~ Green, Pink, Purple, White, & Grey
   - ➢ 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 ~~Wednesday~~ Tuesday, M2D2
  - – Sign up for journal club day and article → wait till next week)
  - – Create a single slide from Dietlein *et al.*
- Don't forget about Mod1 assignments!