M2D1:Tissue culture and confirm cell lines

03/10/2017

1. Prelab discussion
2. ½ class to TC to seed cells for drug treatment and RNA purification
3. ½ lyse cells for Western Blot
4. Group paper discussion of Dietlein et al.

by appointment: nllyell@, lesliemm@, jonas_m@

Office hours + Extra

Noreen Lyell

- M 2-5
- in 16-317

Leslie McClain

- T 9:30-11
- in 56-341c

Maxine Jonas

- R 9:30-11
- In 56-322

Friday, 03/10

- 9-11am
- in 56-322

Saturday, 03/11

- 12-5pm
- in 56-302

Homework due M2D2: Sign up for journal club

- Pick 1 of 24 papers, or suggest your own
- Present M2D5 (March 24) or M2D8 (April 12)
- Sign up by adding your name next to paper **[LMM/WF/Rainbow]**
  - first come first serve!
  - you **cannot** switch paper after M2D2 (March 15th)
  - only one T/R and one W/F per article

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

From Prof. Samson's lecture 03/09/17:

What experimental question will you ask in Module 2?

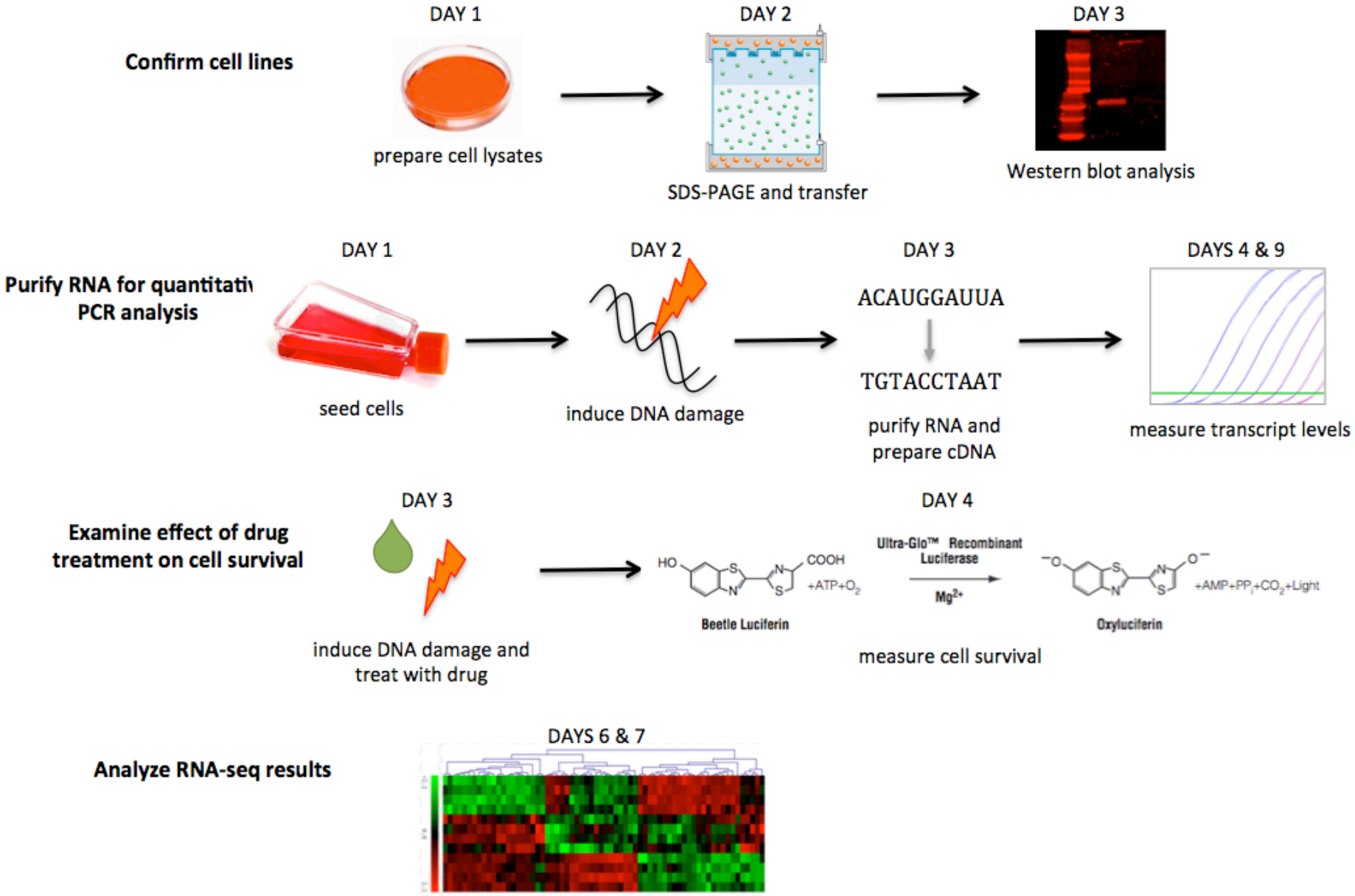
How does DNA repair affect the ability of cancer chemotherapy drugs to kill cancer cells?

How does cancer chemotherapy affect gene expression?

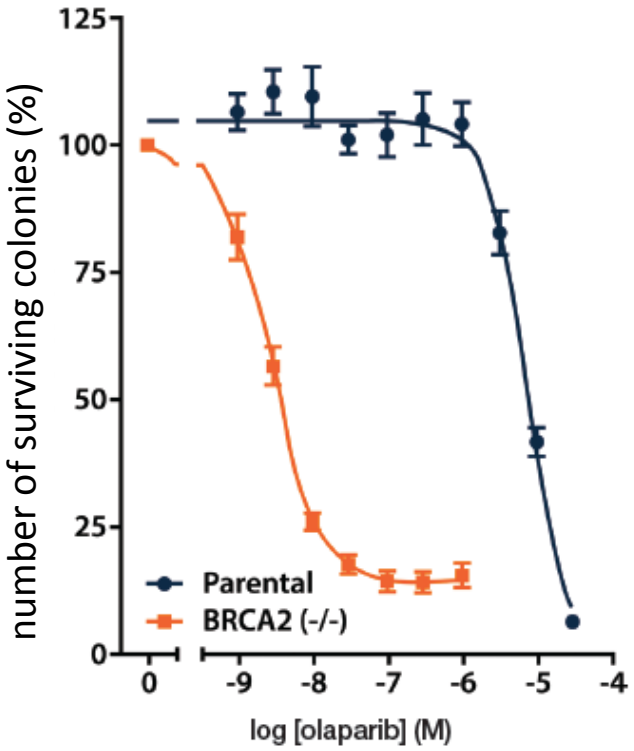
This raises the following questions

- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?

M2: Experimental overview



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

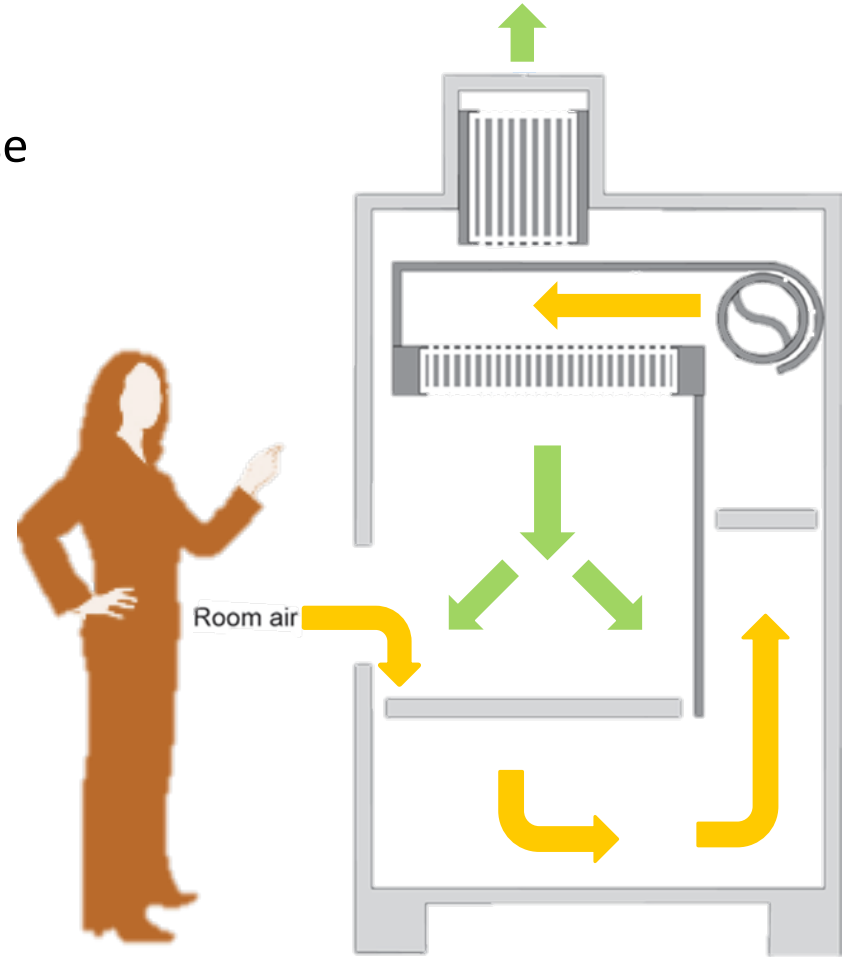


- DLD-1 = wild-type (or parental)
  - from the colon of a male with colorectal adenocarcinoma
- BRCA2-/- = mutant
  - disruption of exon 11 from BRCA2 gene
  - deficient in DNA repair (by homologous recombination)

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 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?

defined

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

glucose, vitamins, amino acids, salts

phenol red: pH indictor

undefined

10%

- FBS: fetal bovine serum

growth factor, cytokines, lipids and cholesterols

1%

- antibiotics:
  - penicillin
  - streptomycin

kill bacteria

Mammalian cell culture terminology

- confluence
  density

  split at ~80%

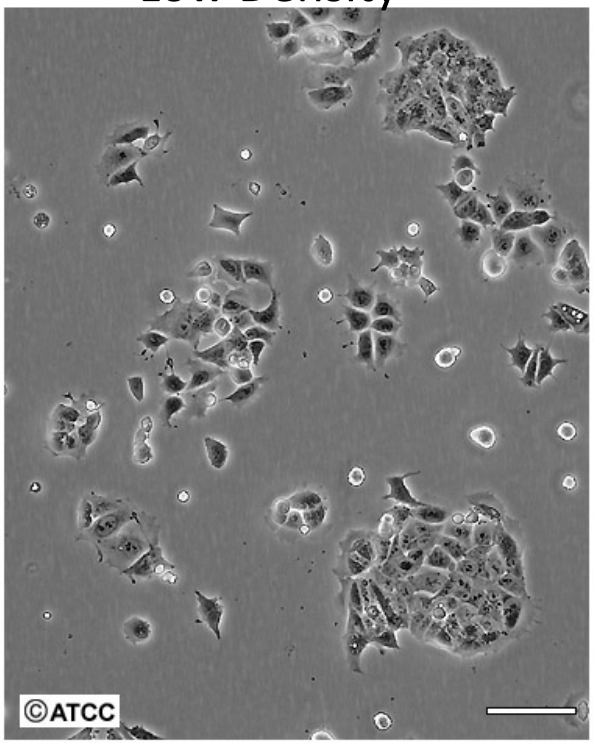
- splitting
  sub culturing

  put cells on new dish

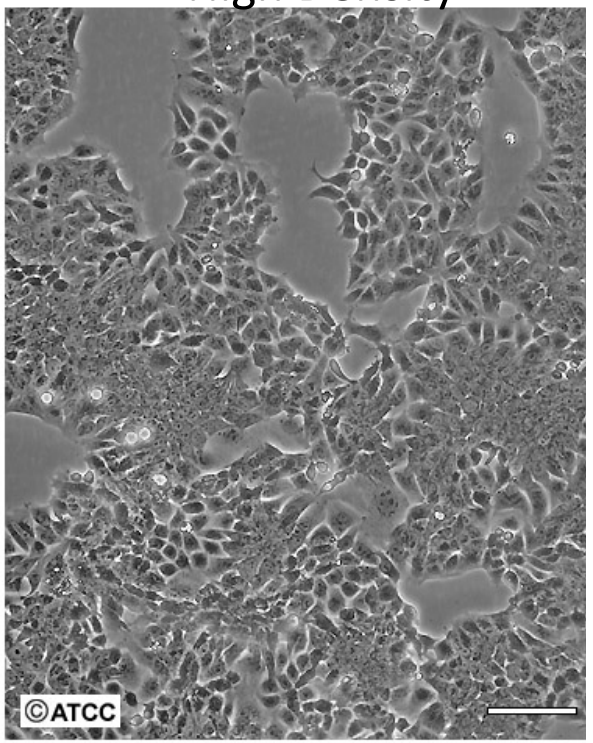
- seeding

~20-40% of confluent culture

Low Density



High Density



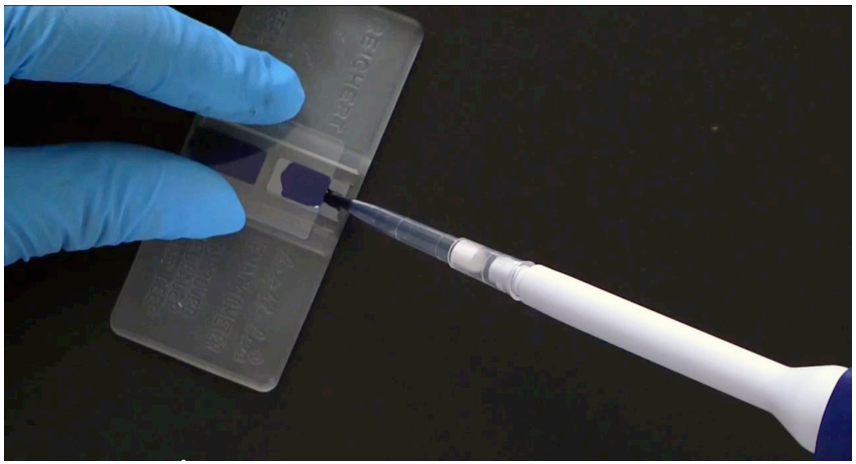
General steps for splitting cells +WHY?

1. Look at cells, estimate confluence
   get an idea of growth rate (time to split?), health
2. Rinse with PBS
   wash media/debris, remove anti-trypsin agents, remove extra protein
3. Detach cells with trypsin
   break substrate cell adhesions
4. Count cells
   seed specific # in new vessel
5. "Seed" new culture vessel room to divide and grow

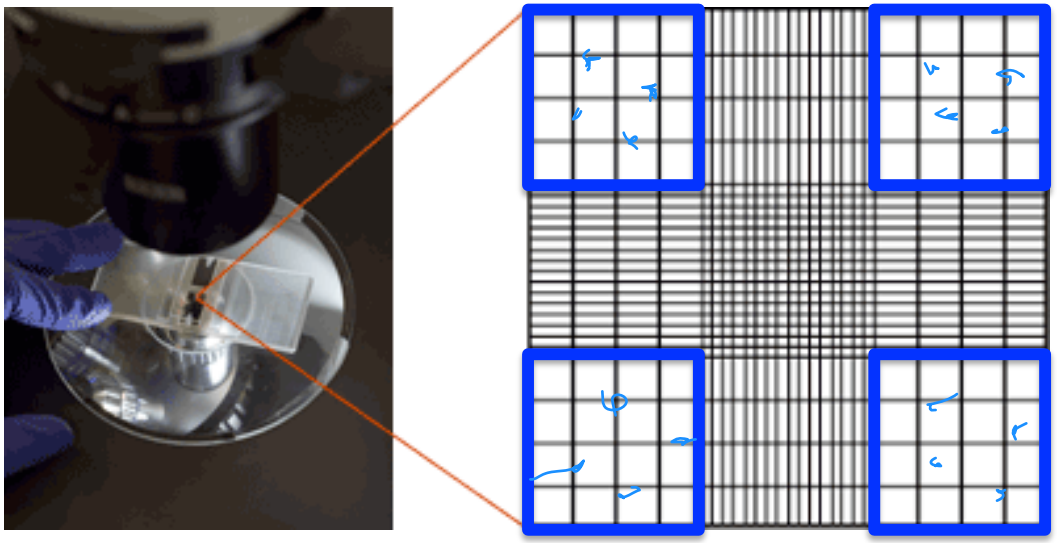
flask

dish

Calculating number of cells

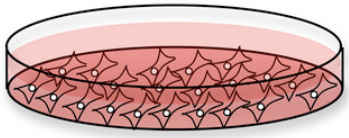


- Hemacytometer
- Trypan blue
  dyes dead cells blue
- # cells / mL = 10,000 x average of 4 corners



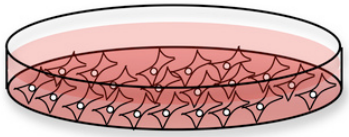
40,000 cells / mL

Confirm cell line:



BRCA2 (-/-)

"DNA repair-deficient cells"



DLD-1

"Normal cells"

breaks all membranes

Mammalian Lysis Buffer, RIPA:

-1% NP40 ; 0.1% SDS;

0.5% sodium deoxycholate

strong detergents

-protease inhibitors

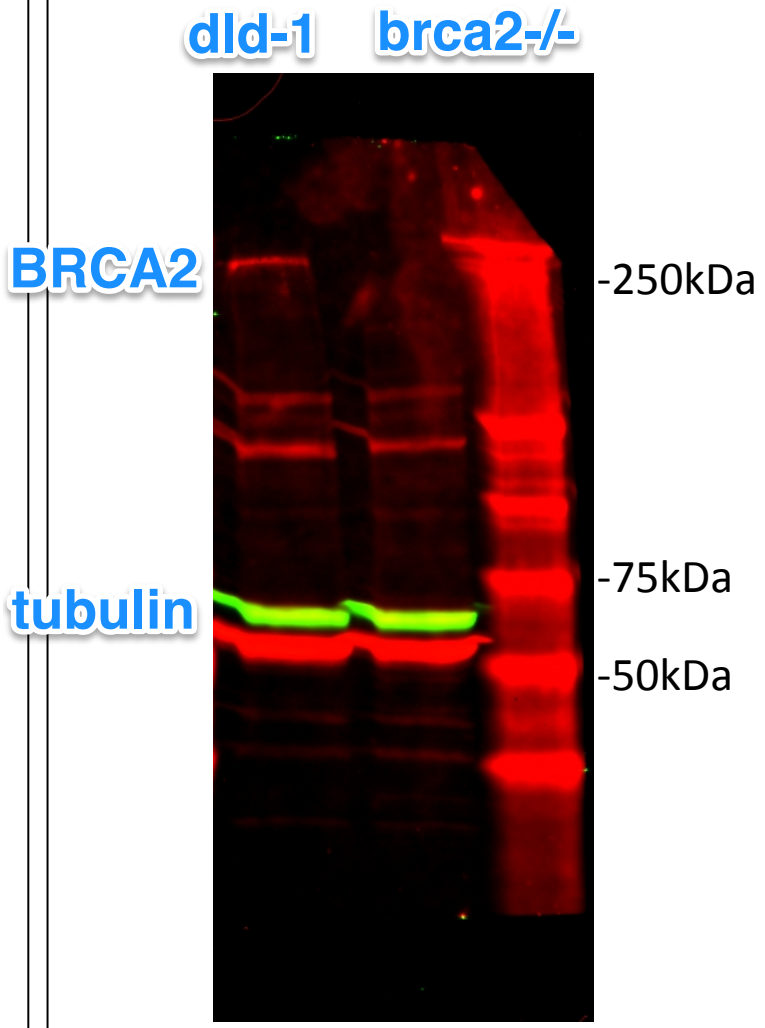
stop protein degradation

-Tris-HCl ph7.4; NaCl

physiolo. pH salts

Cell lysate protein concentration measured using BioRad Protein Assay

LI-COR Western blot



Today in lab:

1. Tissue Culture (TC)
    - 1st: Yellow, Green, Blue
    - 2nd: Red, Pink, Purple

    ➢ Protocols printed for TC use, no need to move laptops etc.

    ➢ Do not wear PPE in or out of TC room
2. Prepare WB samples from DLD-1 and BRCA2 (-/-) cells
3. Paper discussion of Dietlein *et al.*

- Homework due Wednesday, M2D2
  - Sign up for journal club day and article

- Don't forget about Mod1 assignments ☺