M2D1:
Tissue culture and confirm cell lines

03/09/2017

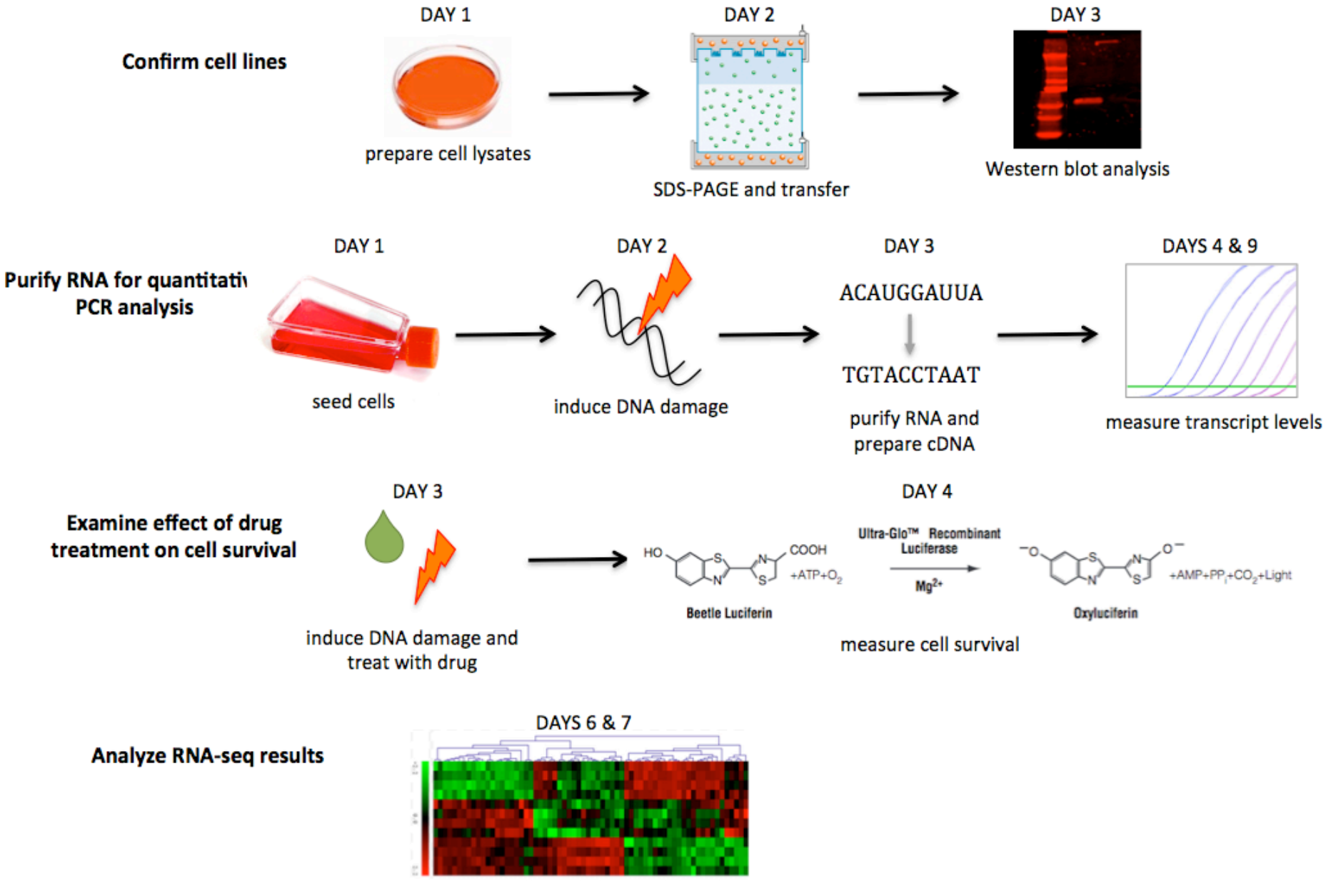
Homework due M2D2: Sign up for journal club

- Pick 1 of 22 papers, or suggest your own
- Present M2D5 (March 23) or M2D8 (April 11)
- Sign up by adding your name next to paper [MJ/WF/Rainbow] [JC/TR/blue]
  - first come first serve!
  - you cannot switch paper after M2D2
  - only one T/R and one W/F per article

M2D8

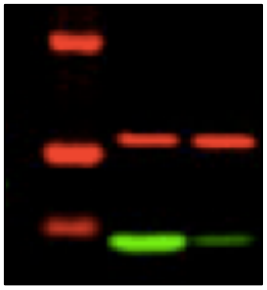
Slot	Day 5 (T/R)	Day 8 (T/R)	Day 5 (W/F)	Day 8 (W/F)
1	Micayla Flores			
2				
3				
4				
5				
6				
7				

M2: Experimental overview

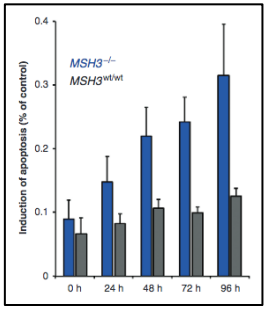


In lab today

1. Learn/practice cell culture



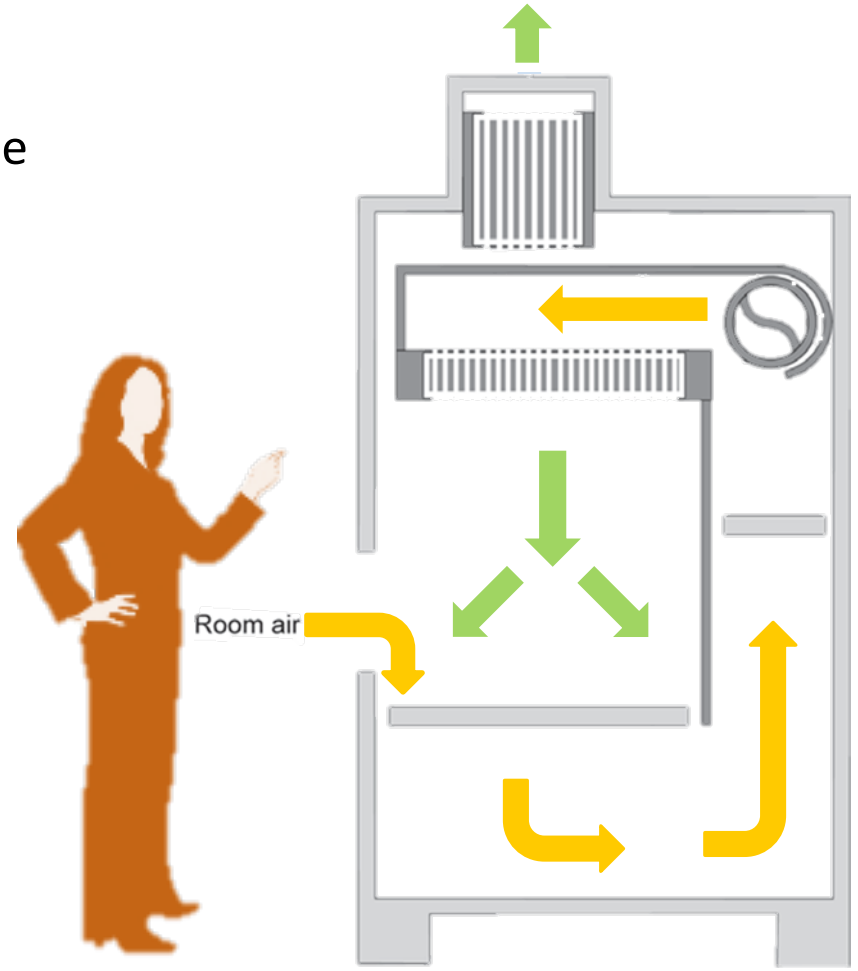
2. Verify cell lines by Western blot protein analysis
   - Lyse DLD-1 and BRCA2-/- cells
   - Measure protein concentration
   - Separate proteins by SDS-PAGE
   - Transfer proteins onto nitrocellulose membrane



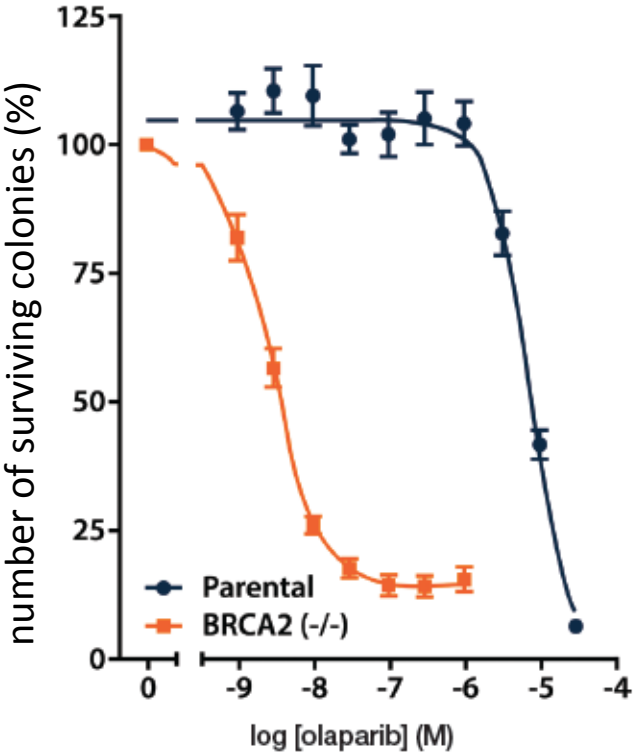
3. Paper discussion

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 in hood



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



- DLD-1 = wild-type
  - 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

Note: olaparib is a PARP inhibitor

Mammalian cell culture medium

Food:

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

glucose, amino acids, salts, vitamins

phenol red: pH indicator

- FBS: fetal bovine serum 10%

BSA and other proteins

growth factors, cytokines, lipids, cholesterol

"undefined"

Non-food:

- antibiotics:
  - penicillin 1%
  - streptomycin

Mammalian cell culture terminology

- confluence

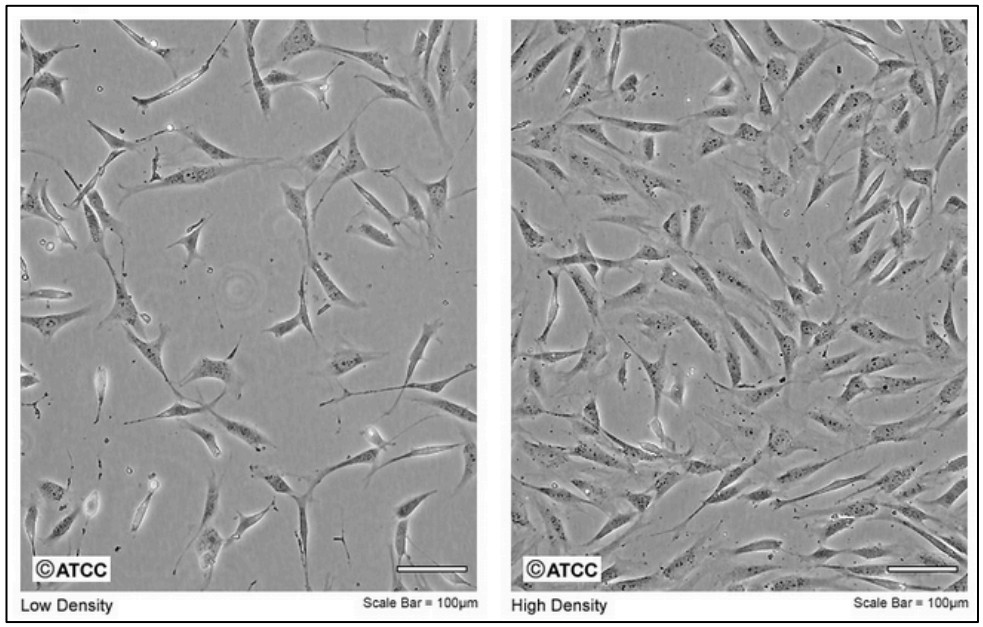
  80% when split

- splitting

  "sub-culturing"

- seeding

  ~ 20-40% of confluent culture

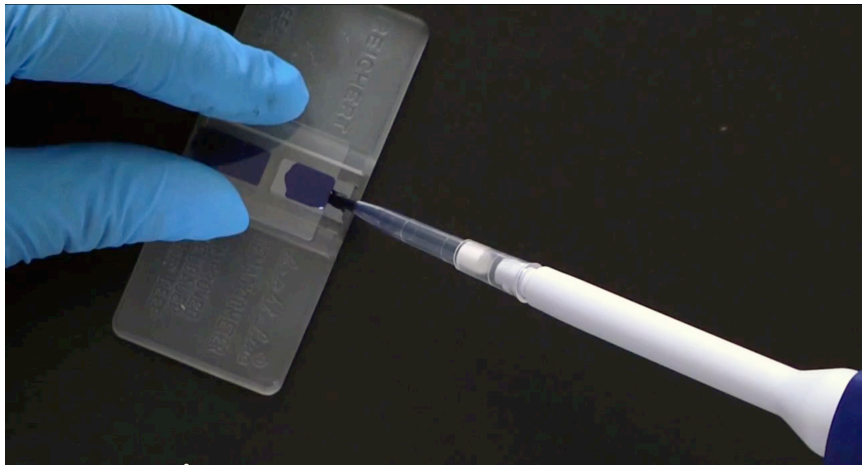


Seeding your DLD-1 and BRCA2-/- cells

why?

1. Rinse with PBS
   - get rid of debris, dead cells
   - eliminate some anti-trypsin agents (released)
2. Detach with trypsin
   - serine protease
   - breaks surface-cells adhesions
3. Calculate number of cells
   - consistency, reproducibility
4. Seed new culture dish

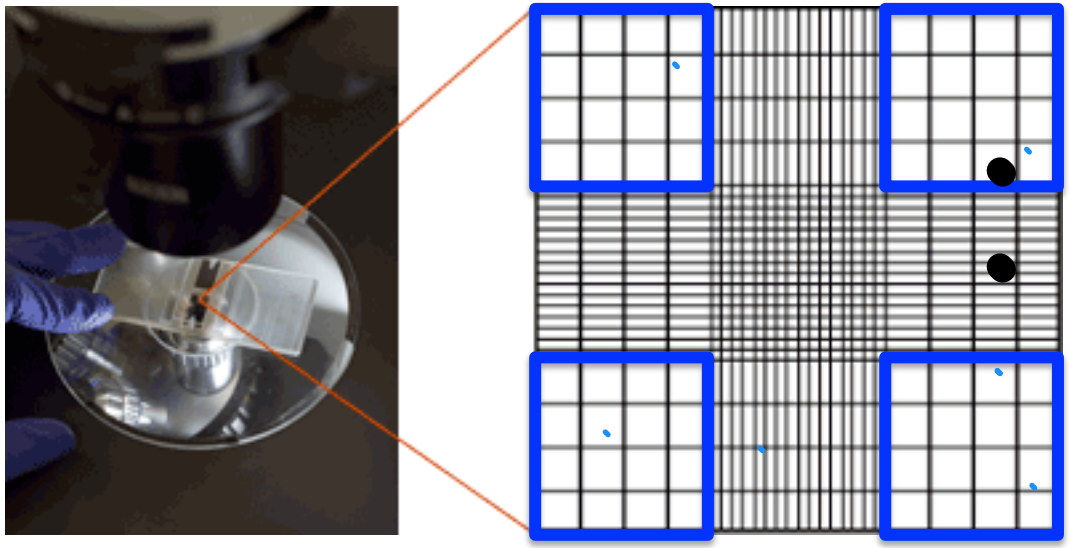
Calculating number of cells



- Hemacytometer
- Trypan blue

dye stains dead cells

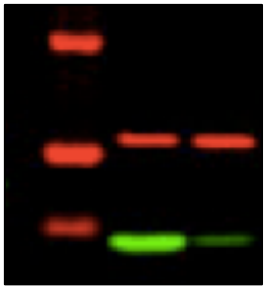
- # cells / mL = 10,000 x average of 4 corners



30,000 cells/mL

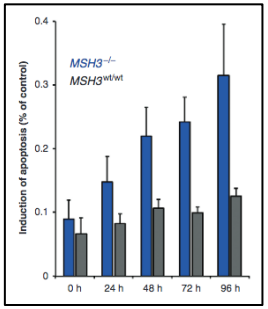
In lab today

1. Learn/practice cell culture



2. Verify cell lines by Western blot protein analysis

"immunofluorescence on PAGE-separated proteins"



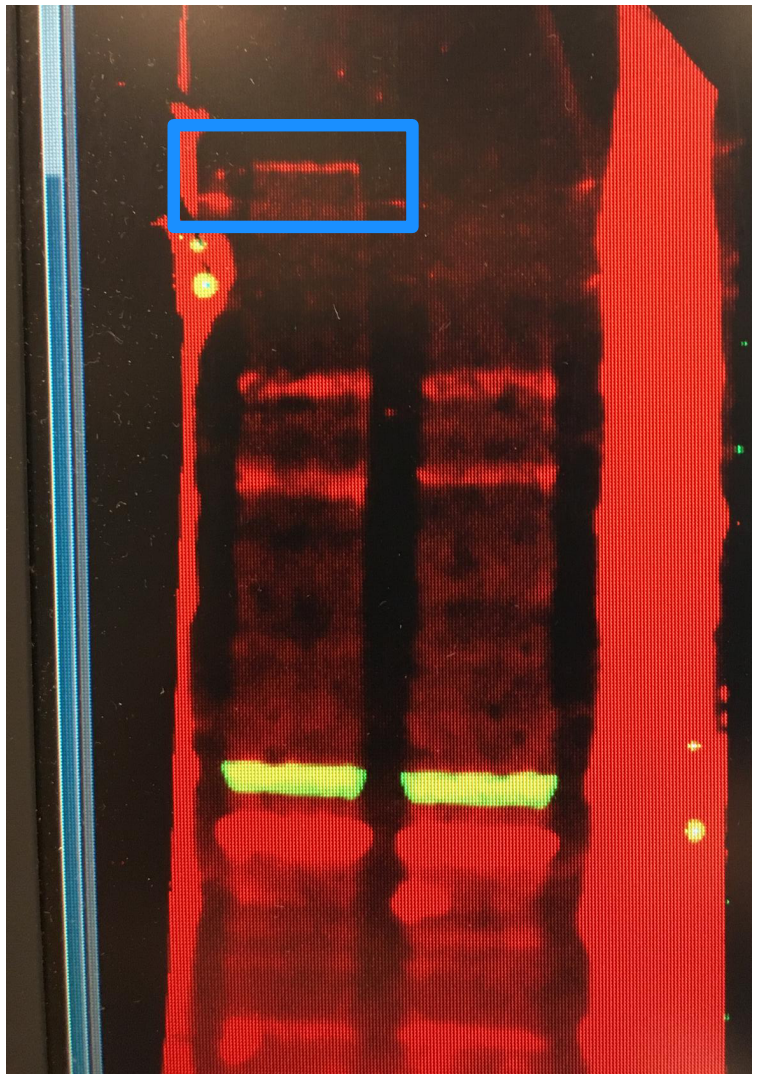
3. Paper discussion

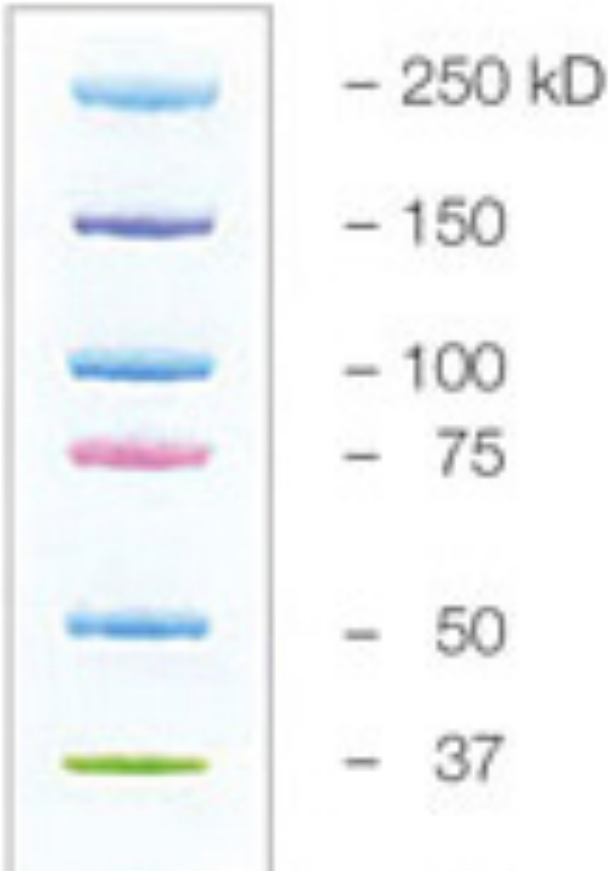
Verify BRCA2-/- are missing BRCA2 (380 kDa)

wt DLD-1 mt BRCA2-/-

BRCA2

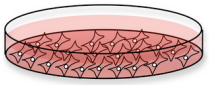
tubulin
(~50 kDa)
control



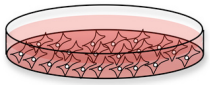


Verify cell lines by Western blot protein analysis

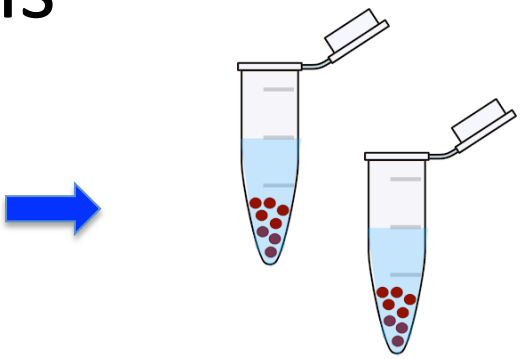
– Lyse DLD-1 and BRCA2 cells



"normal cells"
DLD-1



"DNA repair-deficient cells"
BRCA2-/-



RIPA buffer for mammalian cell lysis:

- detergents;
  - 1% NP-40 (nonyl phenoxypolyethoxylethanol)
  - 0.1% SDS (sodium dodecyl sulfate)
  - 0.5% sodium deoxycholate

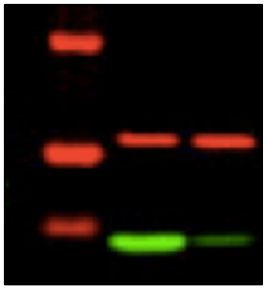
- protease inhibitors — prevent protein degradation keep proteins intacts
- Tris-HCl, pH 7.4 + NaCl

  physiological pH and ionic strength

In lab today

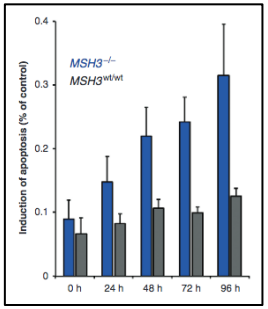
1. Learn/practice cell culture

   - red, yellow, purple and silver teams



2. Verify cell lines by Western blot protein analysis

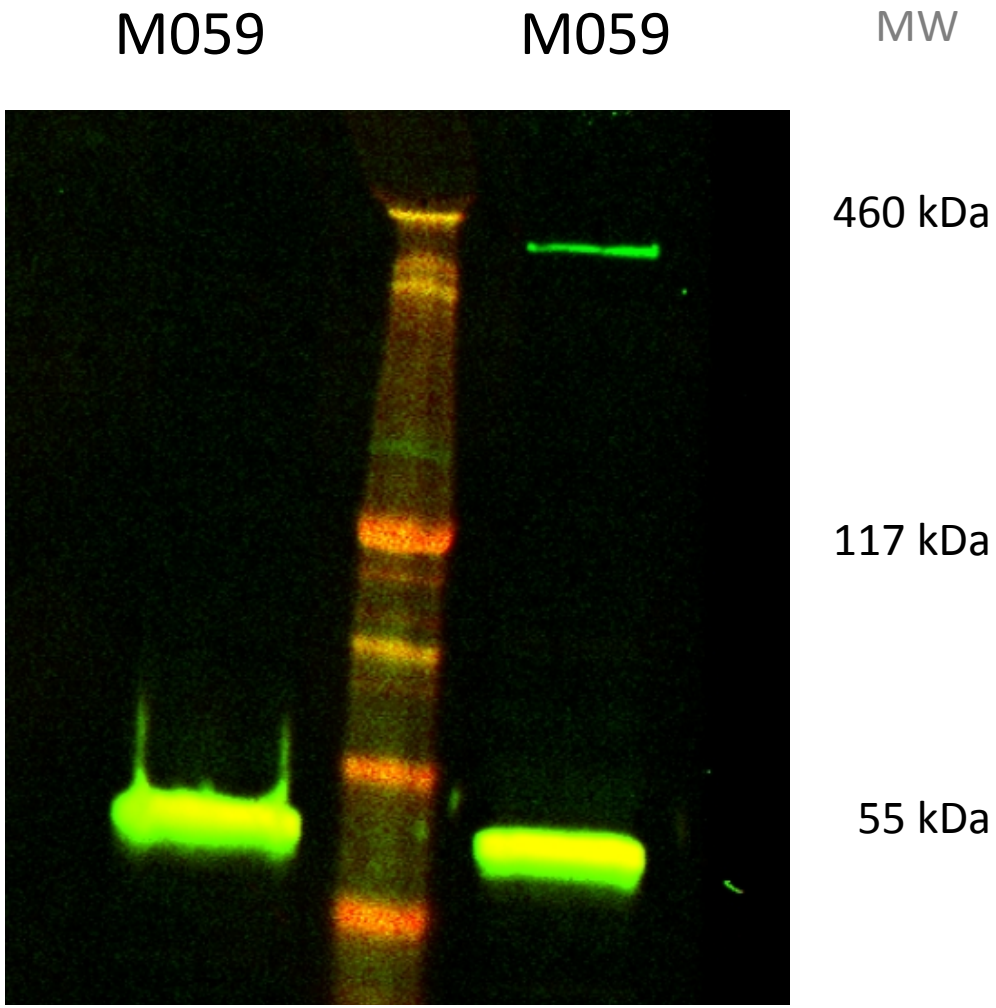
   - green, blue, and pink teams



3. Paper discussion

   - 4pm?

2. Verify BRCA2-/- are missing BRCA2 by *LI-COR* Western blot

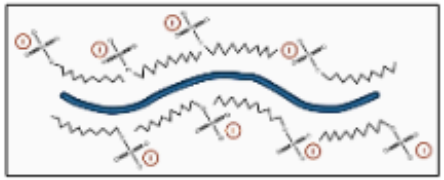


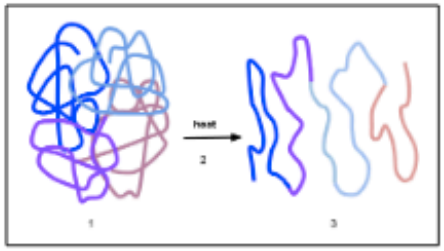
1. Verify cell lines by Western blot protein analysis

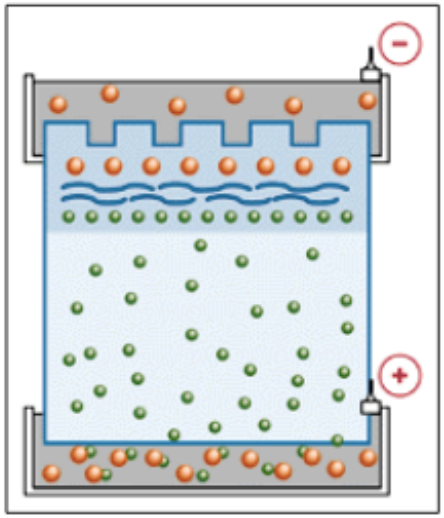
SDS-PAGE separates proteins by size

sodium dodecyl sulfate – polyacrylamide gel electrophoresis

carcinogenic

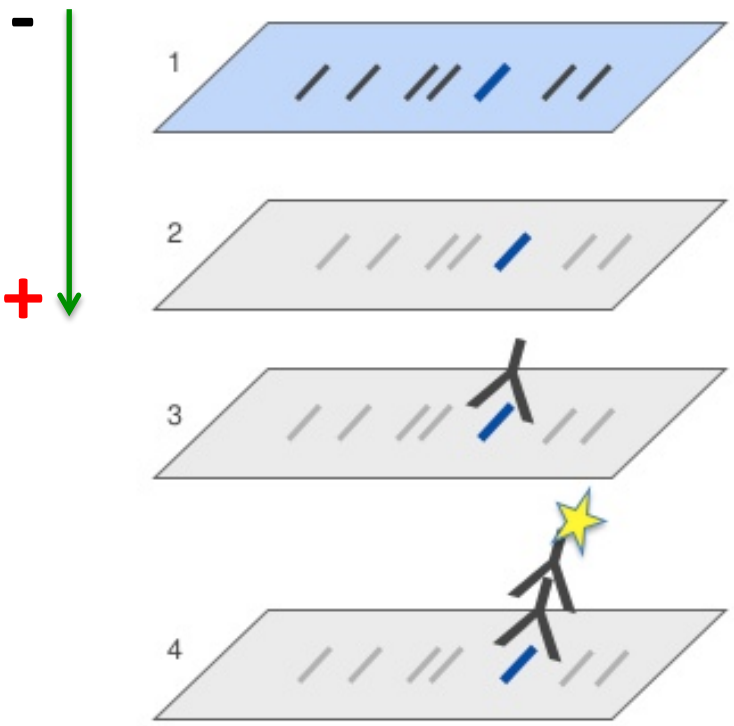






- Laemmli sample buffer / loading dye:
  - + SDS: detergent denatures proteins, coats proteins with negative charges
  - + β-mercaptoethanol reduces disulfide bonds
  - + bromophenol blue to follow front of migration
  - + glycerol
- boiling denatures higher-order structures
- TGS buffer : sandwiched proteins form tight bands
  - + Tris-HCl
  - + SDS
  - + glycine

Western blot workflow

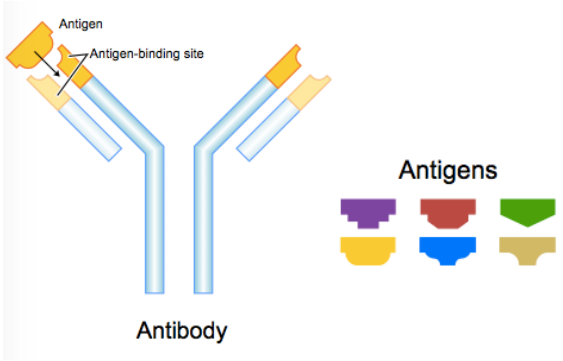


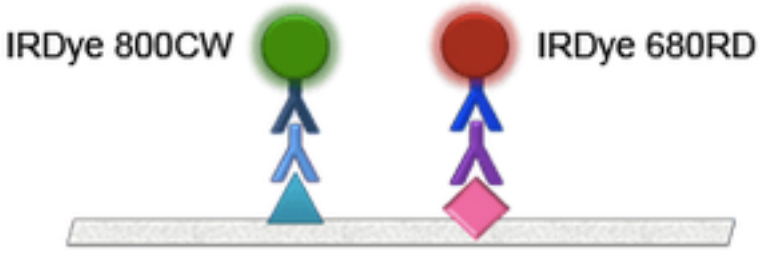
1. Protein separation by SDS-PAGE
   - HiMark stained ladder
2. Protein transfer to nitrocellulose membrane
   -
   -
3. (Blocking and) probing with primary antibodies specific to
   -
   -
4. Probing with labeled secondary antibodies specific to primary antibodies

5. Image fluorescence signal

from GE Healthcare

Suite of antibodies for *LI-COR* Western blot





protein of interest	DNA-PKcs	tubulin
primary antibody	mouse anti-human anti-DNA-PK	rabbit anti-human anti-tubulin
secondary antibody	goat anti-mouse	donkey anti-rabbit
fluorescent dye IR wavelength	800 nm	680 nm
pseudo-color	green	red
molecular weight	~ 465 kDa	~ 50 kDa

Key assignments of M2

- Journal club presentation
  - 10%
  - individual
  - in class at 1pm on Friday, March 18 or April 8

- Research article
  - 25%
  - individual
  - due 5pm on Monday, April 18
  - no draft/revision this time around