M2D2:
Prepare Western blot;
DNA damage for qPCR assay

03/14/2017

Pi day snow day!

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?

Key assignments of M2

- Journal club presentation
  - 15%
  - individual
  - in class at 1pm on March 23 or April 11

- Research article
  - 20%
  - individual
  - due 10pm on April 22
  - no draft/revision this time around

M2: Experimental overview

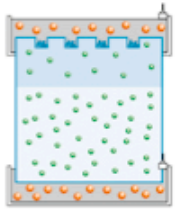
today

Confirm cell lines

DAY 1

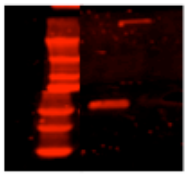
prepare cell lysates

DAY 2



SDS-PAGE and transfer

DAY 3



Western blot analysis

Purify RNA for quantitative PCR analysis

DAY 1

seed cells

DAY 2

induce DNA damage

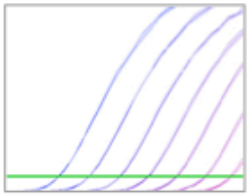
DAY 3

ACAUGGAUUA

TGTACCTAAT

purify RNA and prepare cDNA

DAYS 4 & 9



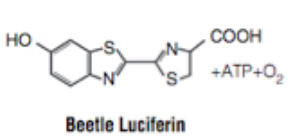
measure transcript levels

Examine effect of drug treatment on cell survival

DAY 3

induce DNA damage and treat with drug

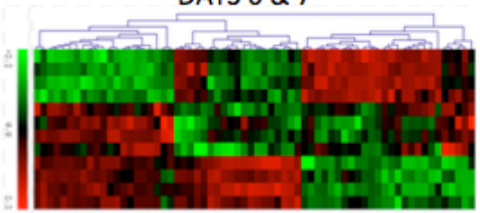
DAY 4



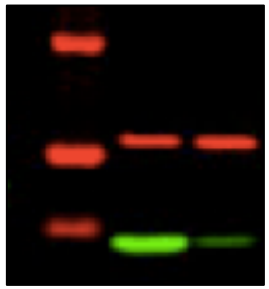
measure cell survival

Analyze RNA-seq results

DAYS 6 & 7



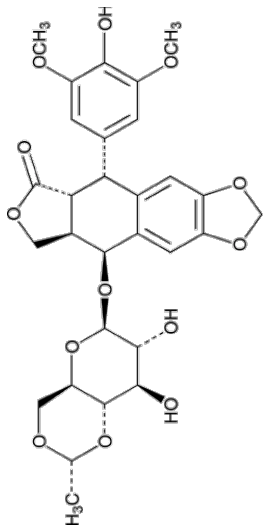
In lab today



1. Verify cell lines by Western blot protein analysis:

"immunofluorescence on PAGE-separated proteins"

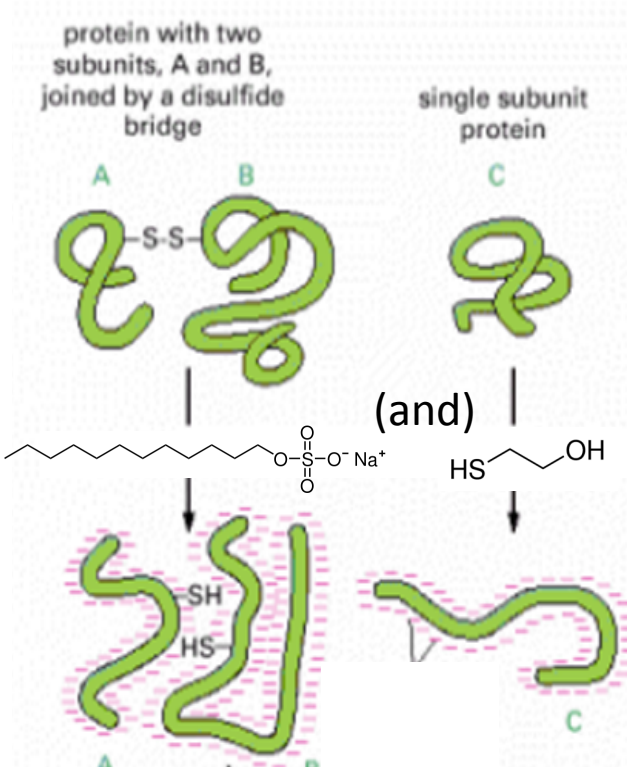
   - Lyse DLD-1 and BRCA2-/- cells
   - Measure protein concentration
   - Separate proteins by SDS-PAGE
   - Transfer proteins onto nitrocellulose membrane
   - Label BRCA2 with primary+secondary antibodies



2. Treat cells with cancer drug etoposide

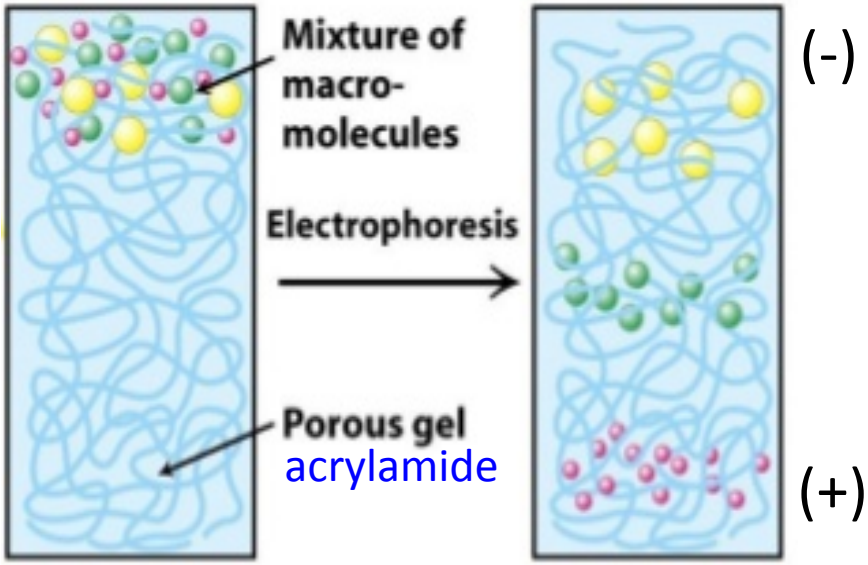
Review: SDS-PAGE

- What gives proteins uniform charge and linear structure?



Laemmli buffer's
SDS + beta-mercaptoethanol

- How are proteins separated?



by size (now proportional to charge)

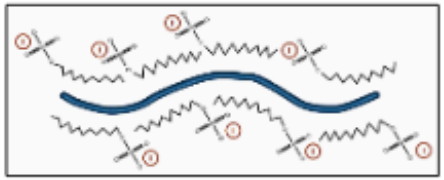
Recall slide from M1:

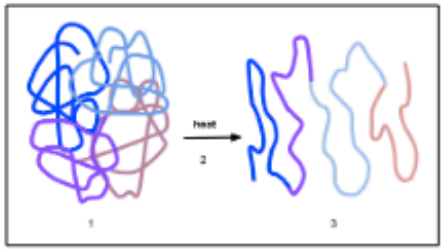
To verify cell lines by Western blot protein analysis

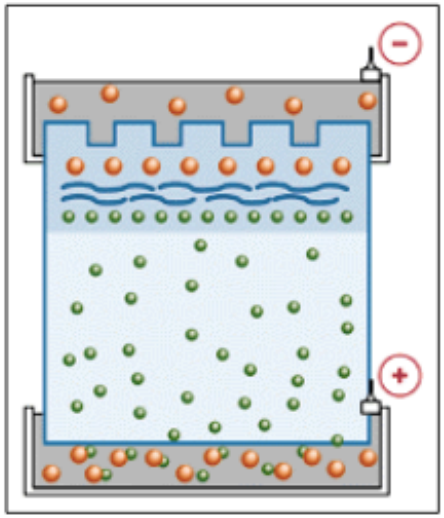
SDS-PAGE separates proteins by size

sodium dodecyl sulfate – polyacrylamide gel electrophoresis

carcinogenic

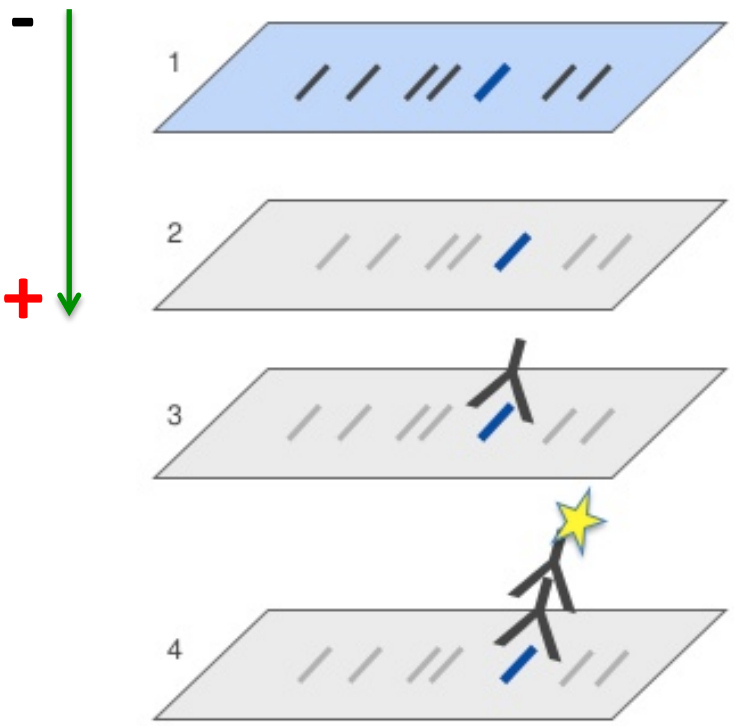






- Laemmli sample buffer / loading dye:
  - + SDS: detergent denatures proteins, coats proteins with negative charges
  - + β-mercaptoethanol reduces disulfide bonds
  - + bromophenol blue to follow front of migration
  - + glycerol
- boiling denatures higher-order structures
- TGS buffer : sandwiched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

Western blot workflow

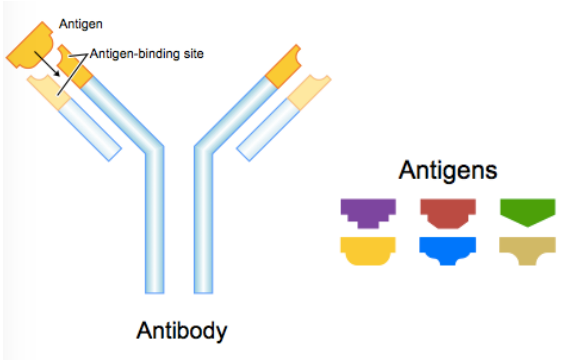


1. Protein separation by SDS-PAGE
   - HiMark stained ladder bands 31-460 kDa
2. Protein transfer to nitrocellulose membrane
   - high affinity for proteins
   - immobilizes proteins
3. (Blocking and) probing with primary antibodies specific to
   - BRCA2
   - tubulin
4. Probing with labeled secondary antibodies specific to primary antibodies

5. Image fluorescence signal

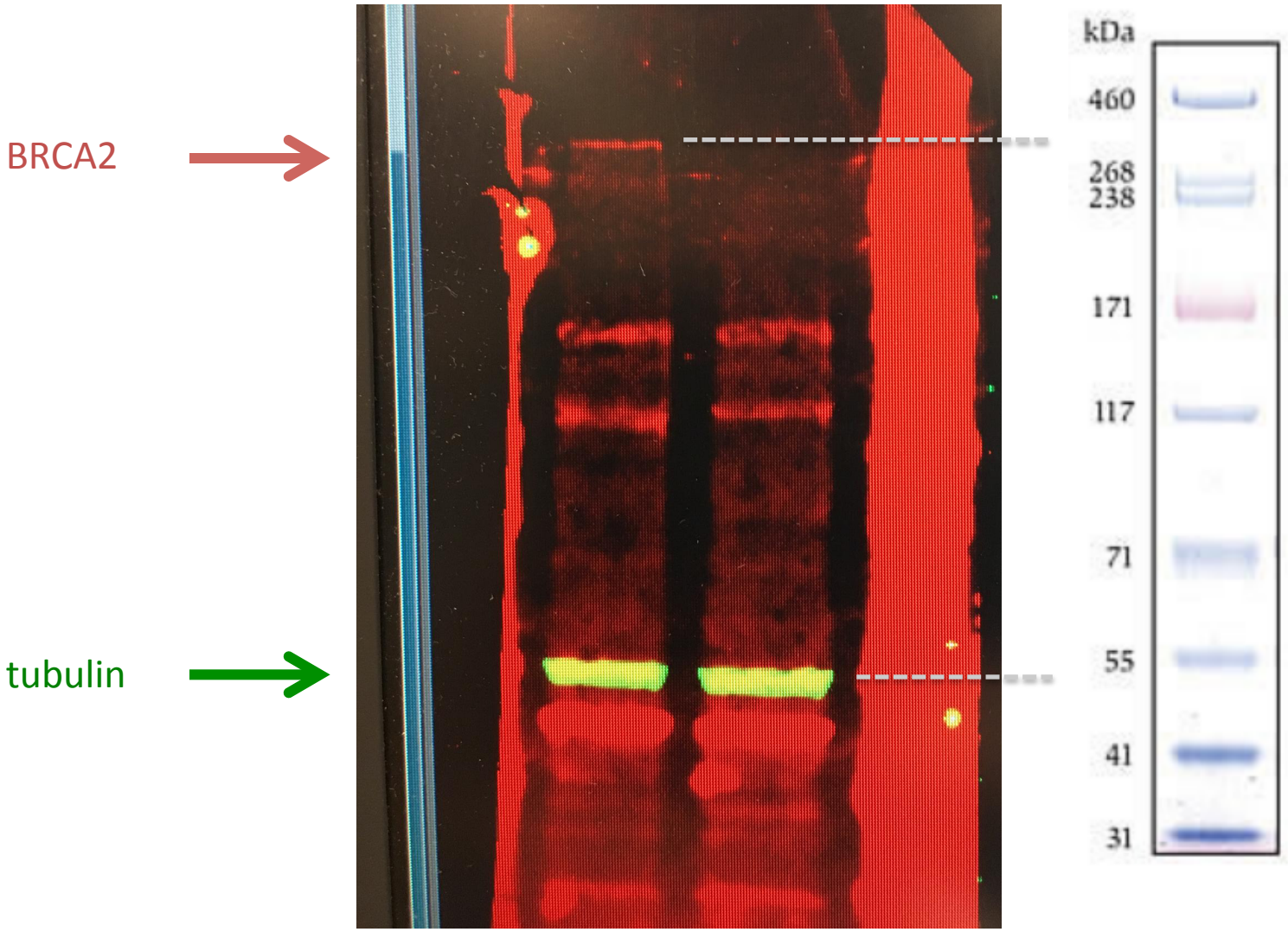
from GE Healthcare

Suite of antibodies for *LI-COR* Western blot



protein of interest	tubulin	BRCA2
primary antibody	mouse anti-human anti-tubulin	rabbit anti-human anti-BRCA2
secondary antibody	goat anti-mouse	donkey anti-rabbit
fluorescent dye IR wavelength	800 nm	680 nm
pseudo-color	green	red
molecular weight	~ 50 kDa	~ 380 kDa

Are BRCA2-/- cells missing BRCA2? (380 kDa)



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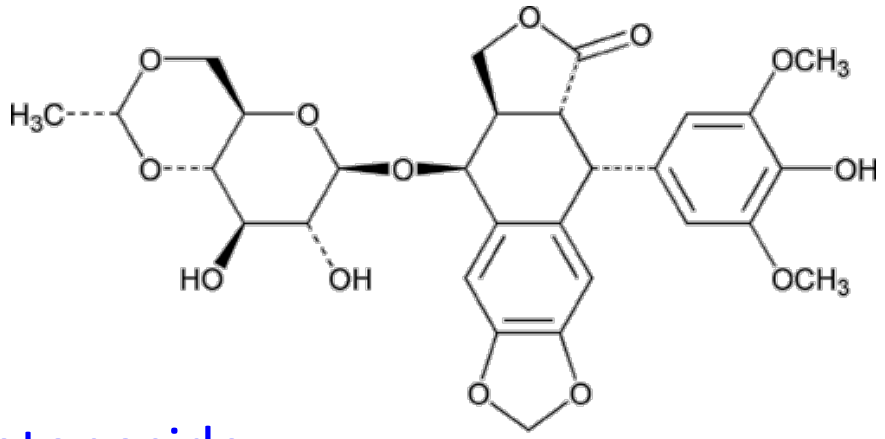
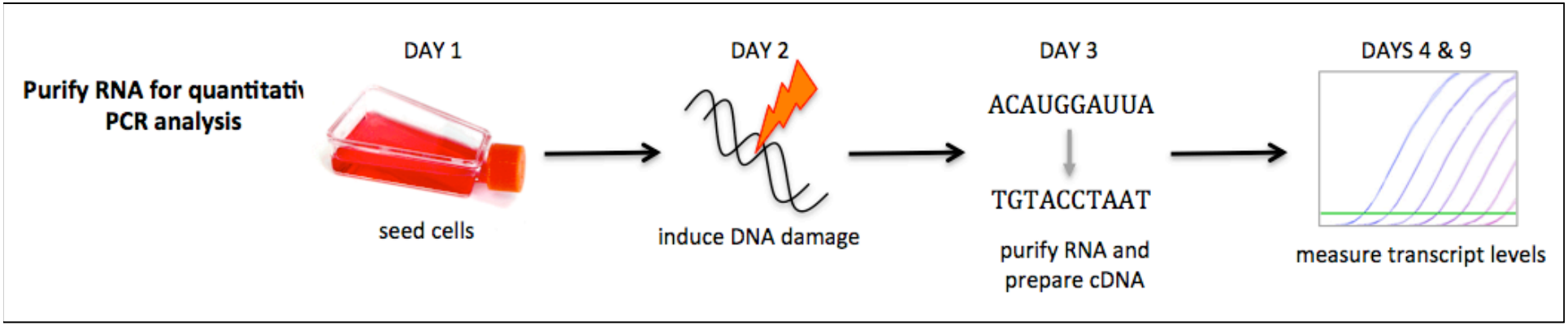
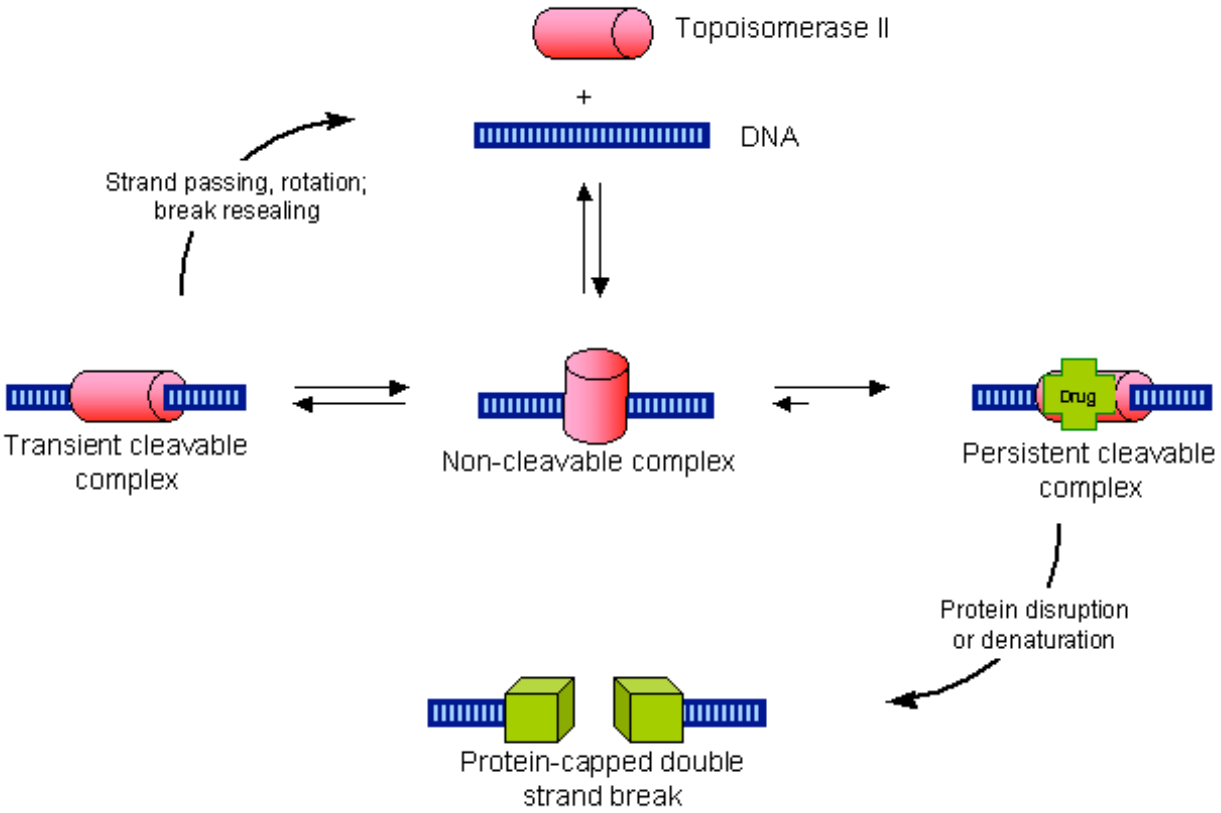


2. Treat cells with cancer drug etoposide

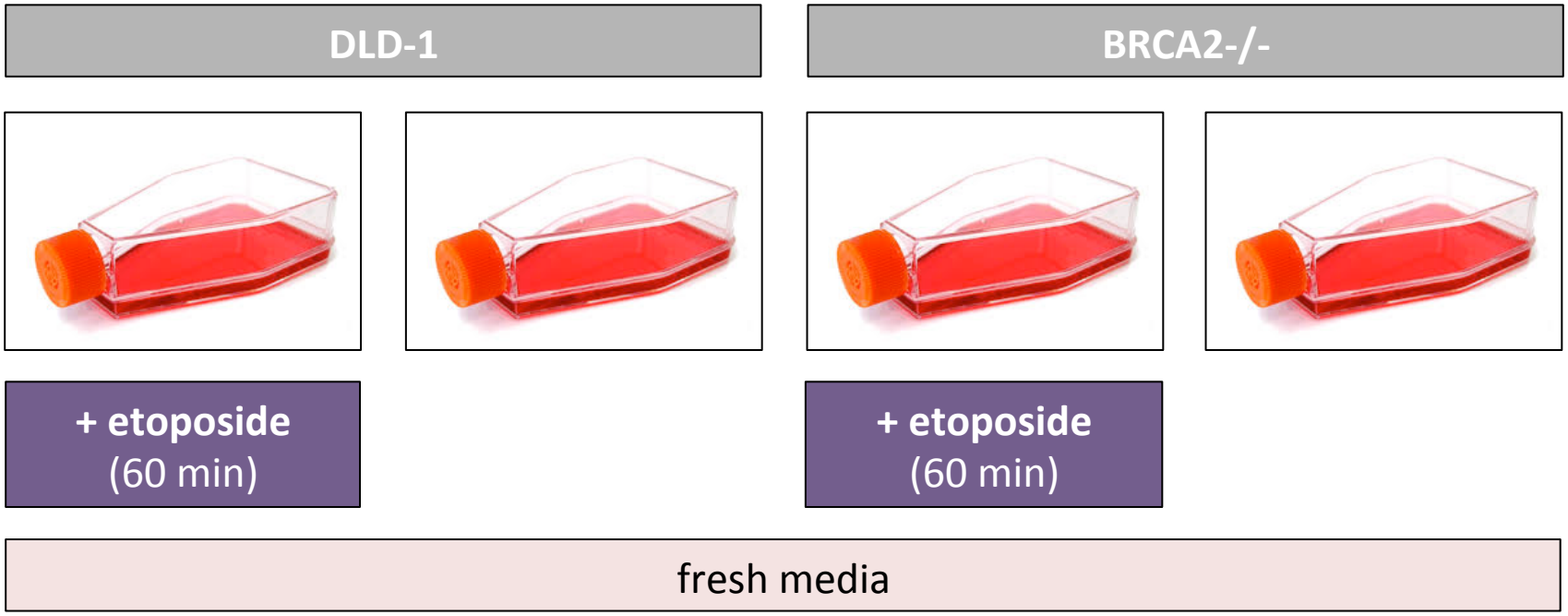


Etoposide's mechanism of action

- forms a ternary complex with DNA and the topoisomerase II enzyme (which aids in DNA unwinding),
- prevents re-ligation of the DNA strands,
- so causes DNA strands to break,
- and (cancer) cells to undergo apoptosis.



Treat cells with etoposide



M2D3: extract RNA

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