M2D2: Prepare Western blot; DNA damage for qPCR assay

03/15/2017

QUIZ!

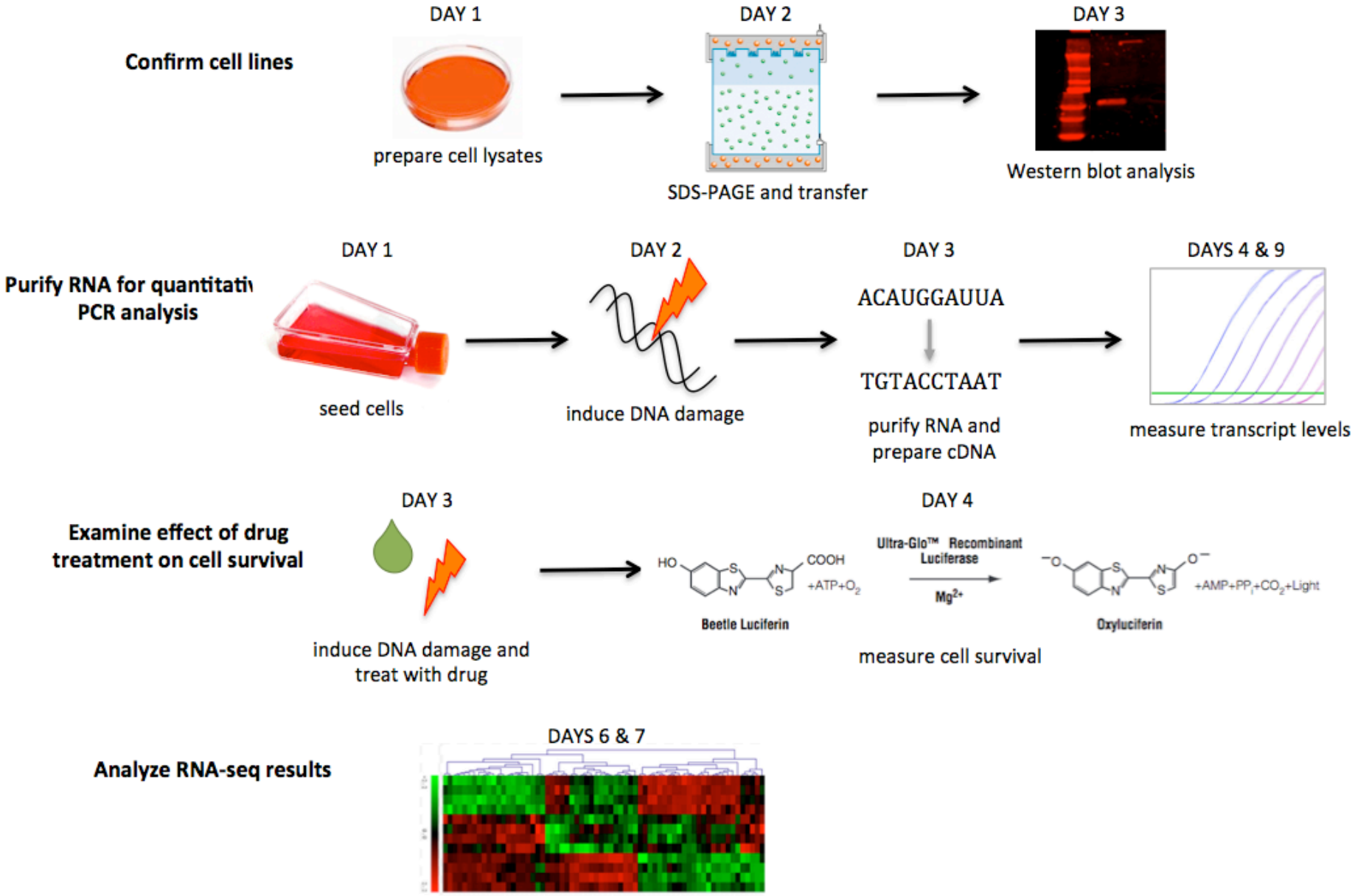
1. Comm lab Workshop: Journal Club
2. Load samples on Western, run, transfer to nitrocellulose
3. Induce DNA damage in DLD-1 and BRCA2 (-/-) cells

M2 major assignments

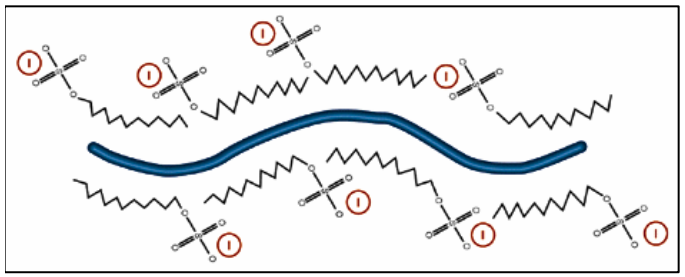
- Lab quizzes (extra credit on homework grade)
- Notebook (5% total)
- Research Article (20%)
  - individual, on Stellar
  - draft due at 10pm on April 22nd
  - word document
- Journal Club Presentation (15%)
  - individual, during lab
  - slides due 1pm March 24th or April 12th
- Blog: http://be20109s17.blogspot.com/ (participation: 5% total)
  - by April 3rd for Mod1
  - by April 15th for Mod2 journal club

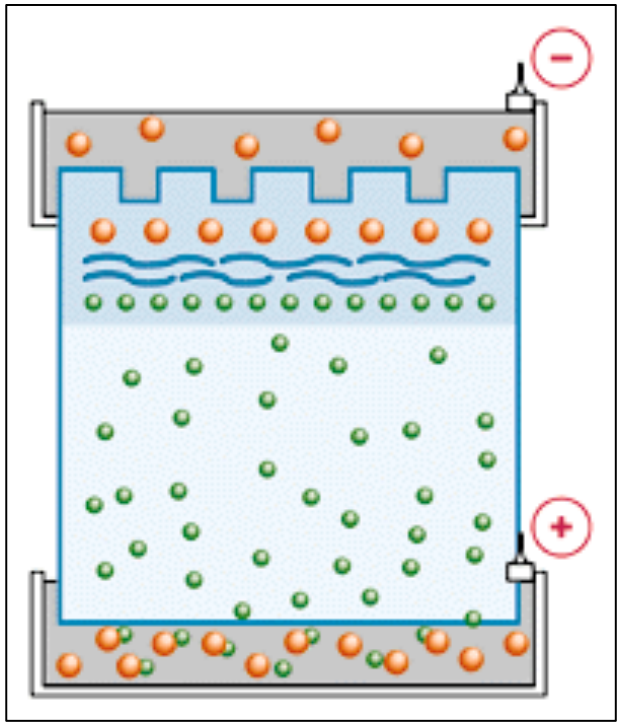
    -by April 23rd for Mod2 overall

M2: Experimental overview



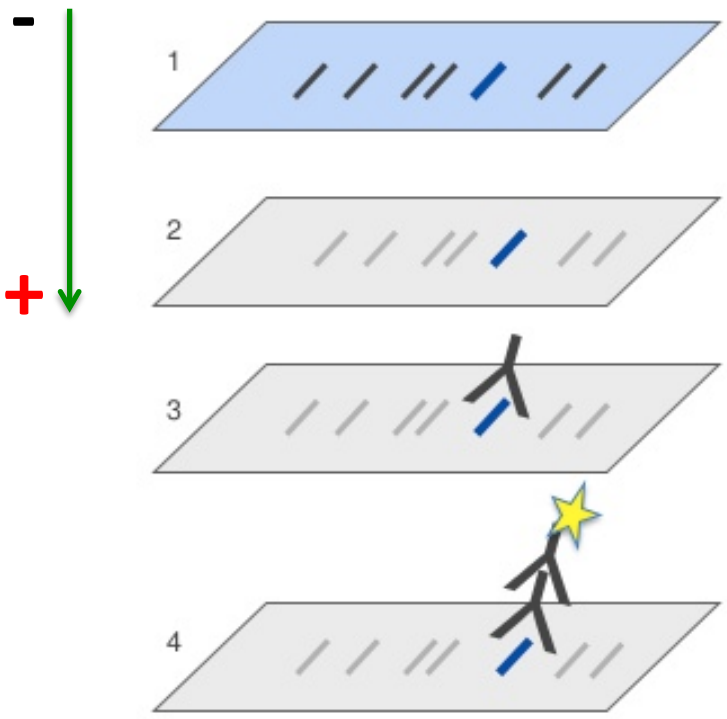
SDS-PAGE separates proteins by size





- Laemmli sample buffer / loading dye:
  - + SDS: detergent, denatures protein and coats with a negative charge
  - + β-mercaptoethanol: breaks disulfide bonds
  - + bromophenol blue: dye that runs 3-5kDa
  - + glycerol: viscosity, helps proteins sink into well
- boiling denatures higher-order structures
- TGS buffer: helps align (stack) the proteins as they enter the gel
  - + Tris-HCl
  - + SDS, protein
  - + glycine

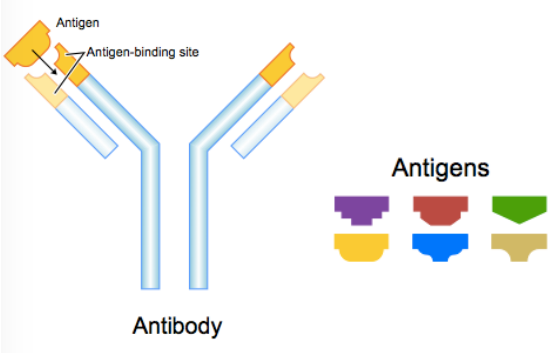
Western blot workflow

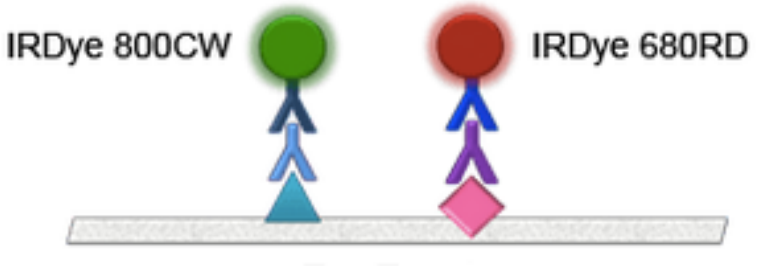


1. Protein separation by SDS-PAGE
   - HiMark stained ladder **31-460kDa**
2. Protein transfer to nitrocellulose membrane
   - **high affinity for proteins**
   - **immobilizes proteins**
3. (Blocking and) probing with primary antibodies specific to
   - **BRCA2**
   - **control-tubulin**
4. Probing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

from GE Healthcare

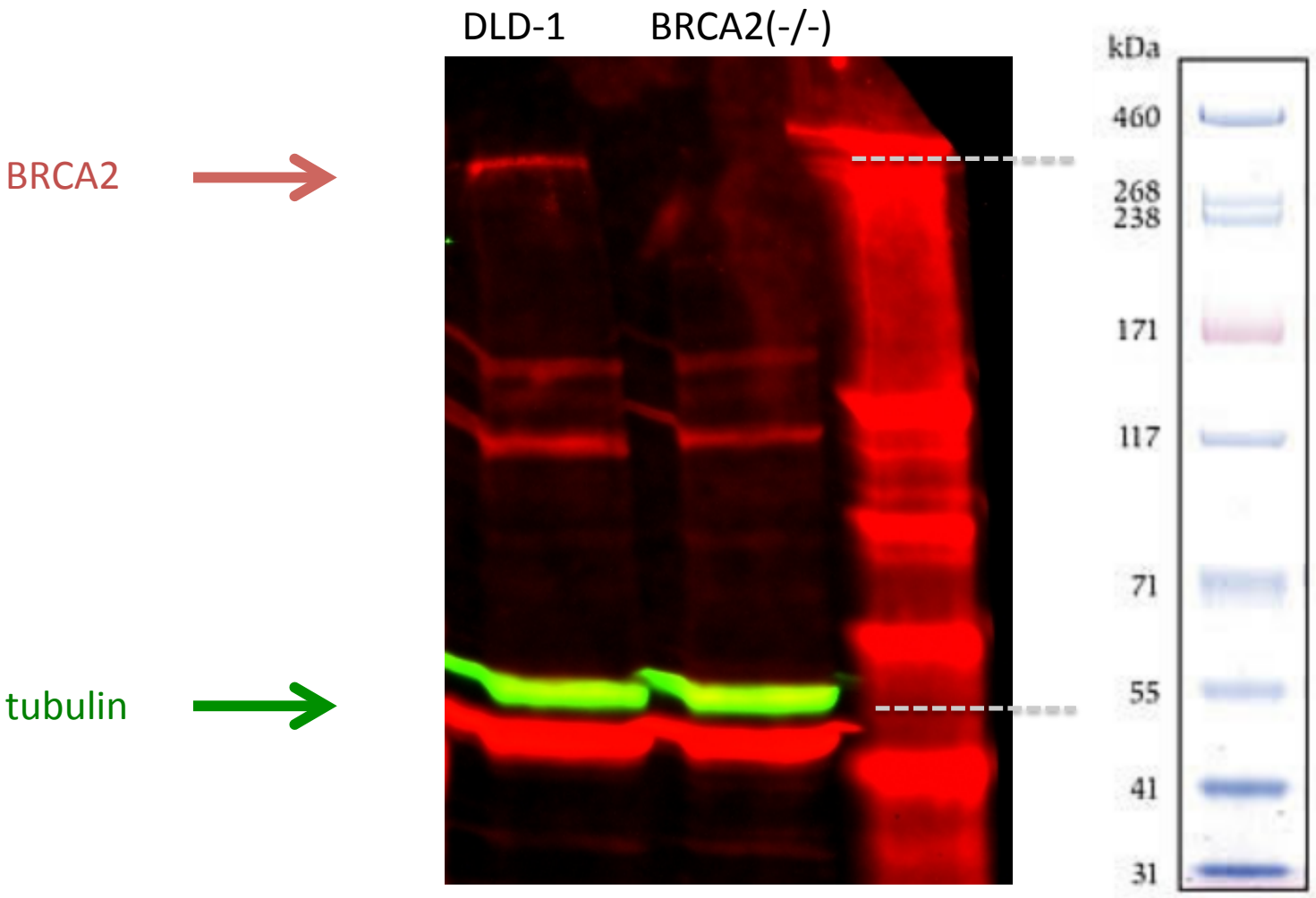
Suite of antibodies for *LI-COR* Western blot



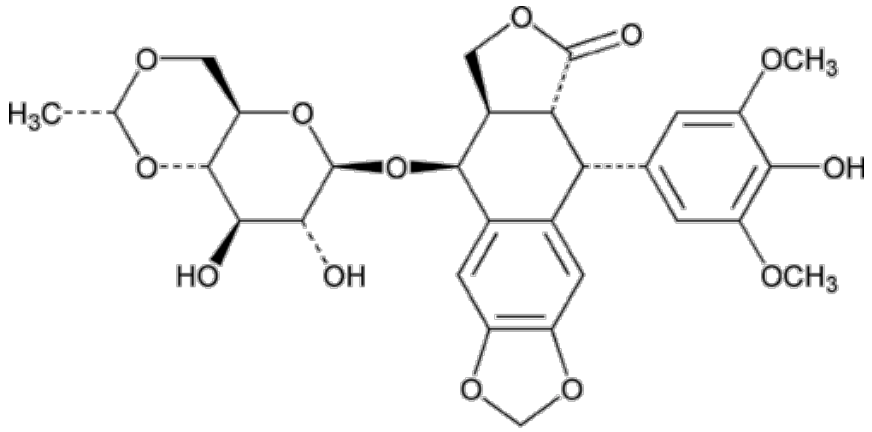


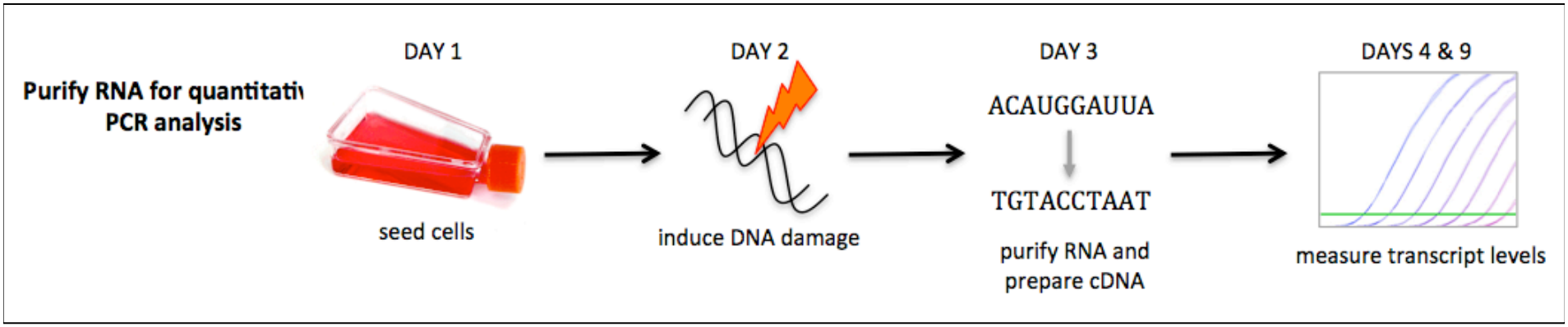
protein of interest	tubulin	BRCA2
primary antibody	mouse anti-human anti-tubulin	rabbit anti-human anti-BRCA2
secondary antibody	goat anti-mouse	donkey anti-rabbit
fluorescent dye IR wavelength	800 nm	680 nm
pseudo-color	green	red
molecular weight	~ 50 kDa	~ 380 kDa

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



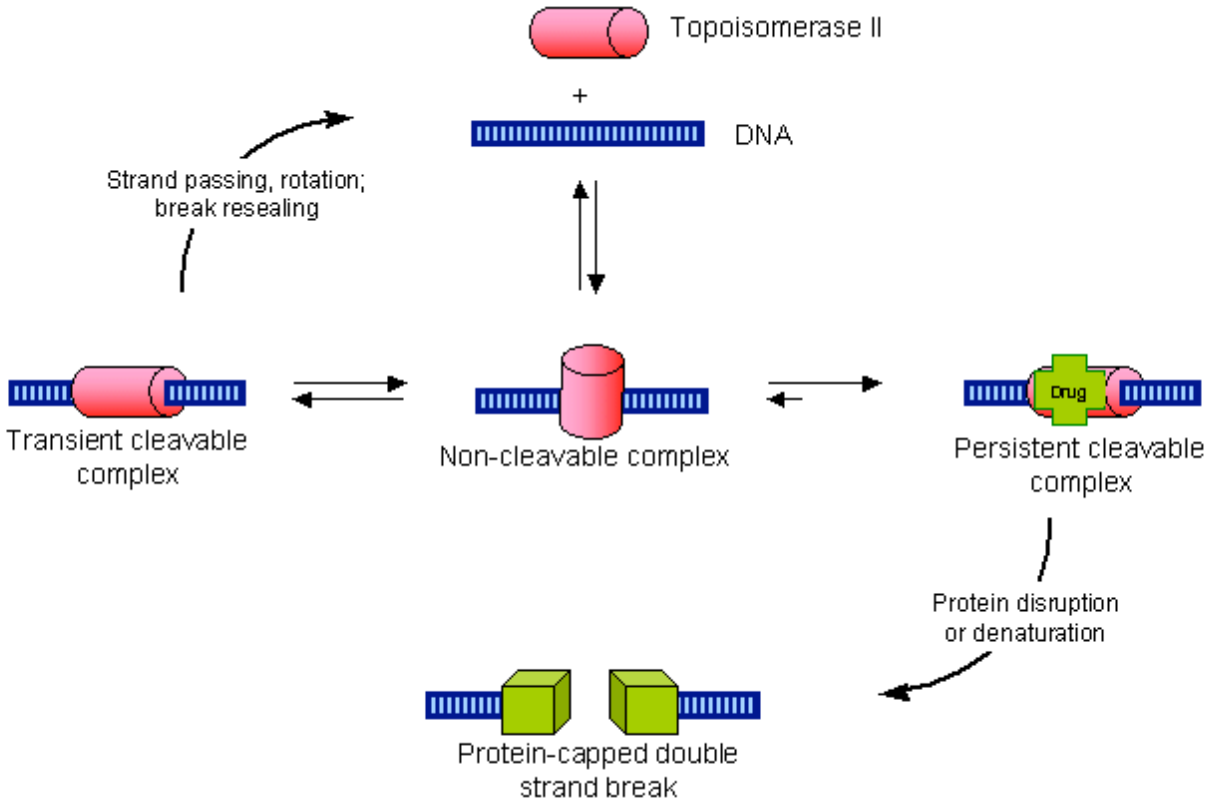
Etoposide is drug (also chemotherapy) that causes double strand breaks



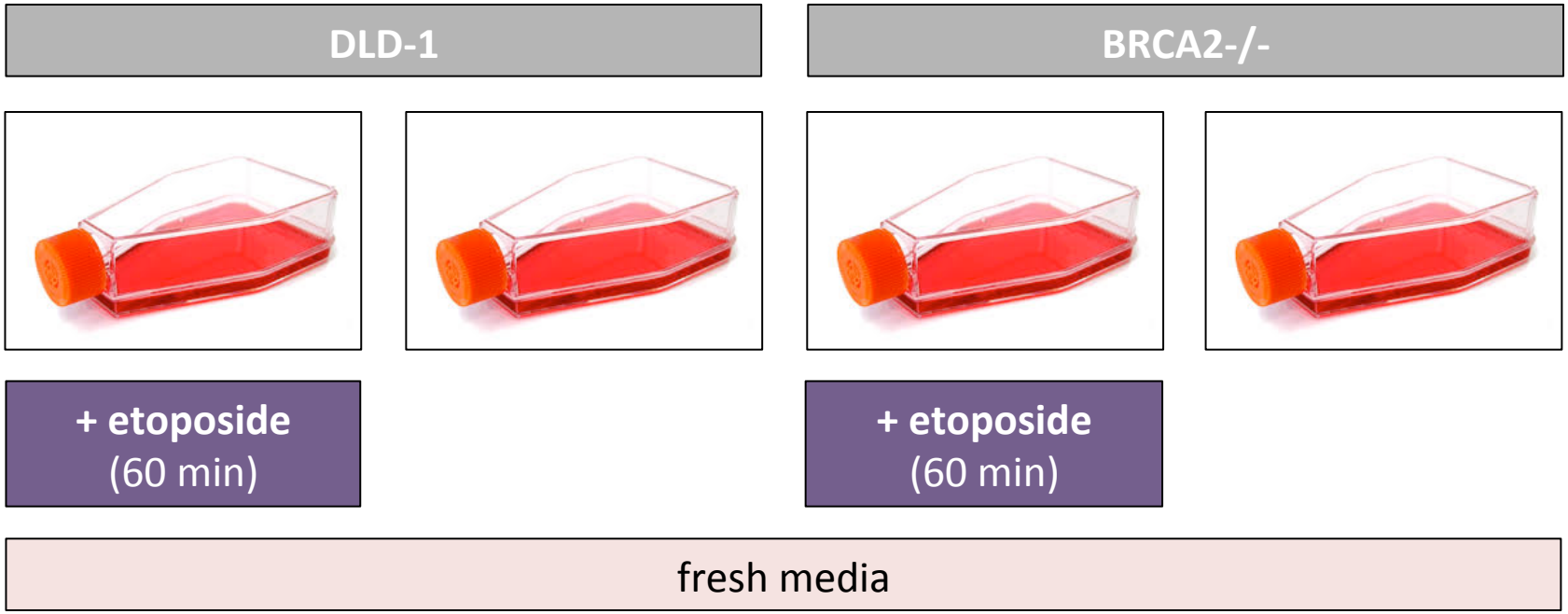


Etoposide's mechanism of action

- forms a ternary complex with DNA and the topoisomerase II enzyme (which aids in DNA unwinding during DNA replication),
- 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

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?

Today in lab:

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

    ➢ Protocols printed for TC use
2. Load SDS-PAGE, set up transfer to nitrocellulose

- Homework due Friday, M2D3
  - Craft a single slide using the data (**your choice**) from the publication by *Dietlein et al*
- Mini-presentation due Saturday