
HAPPY
ST. PATRICK'S
DAY!


YOU ARE NOW AWARE THAT YOU
CANNOT SAY
IRISH WRISTWATCH

# M2D3:Finish Western blot; Extract RNA for qPCR assay; Treat cells for viability assay

03/17/2017

1. Finish Western Blot
2. Purify RNA from DLD-1/BRCA2(-/-), with and without etoposide; synthesize cDNA from RNA
3. Drug treat cells for viability assay

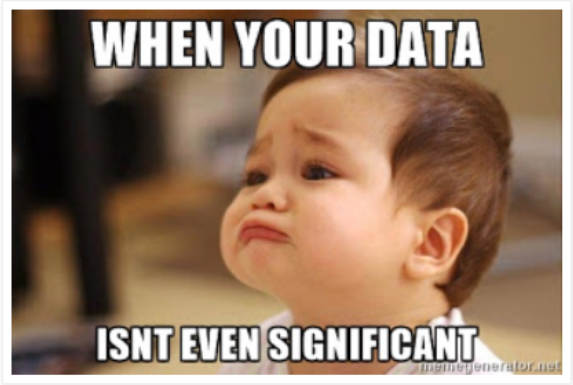
# M2 major assignments

- 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, video recorded
  - power point 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 Journal Club
  - by April 23rd for Mod2

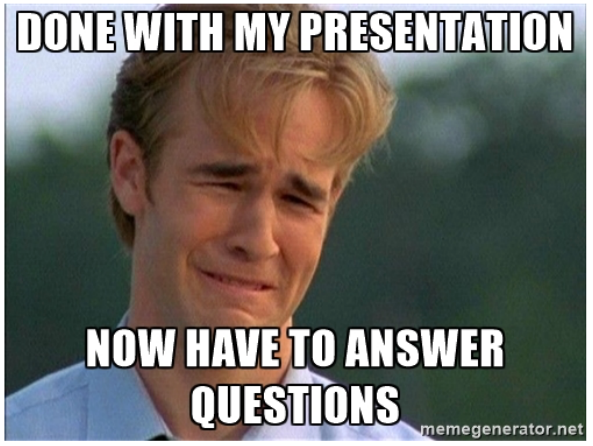
# BE 20.109 (Sp17) Class Blog

Welcome to the 20.109 Class Blog! Our 20.109 Blog is here for MIT's emerging cadre of biological engineers from Course 20. The blog is for your thoughts and work and discoveries in our lab fundamentals class. By capturing your collective experiences in the subject, we hope to learn even more about the work we do -- what's working well and where we need to get better. Please see the first blog post for some important administrative information.

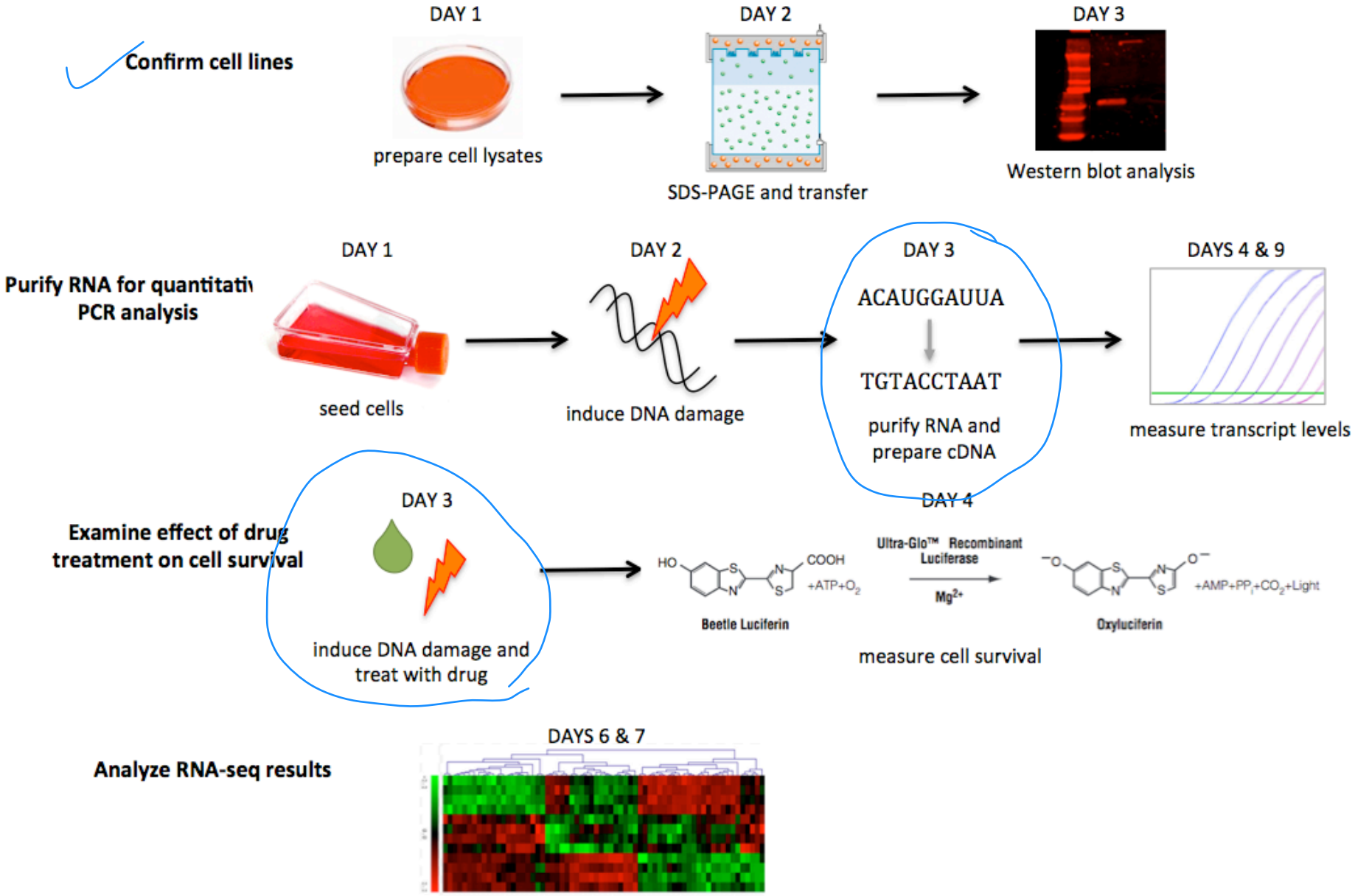
- Possible topics listed on the blog website
- Details about use:
  - Do not publish MIT logo
  - Do not post photographs with names tagged
  - Do not write malicious comments
  - Do not plagiarize



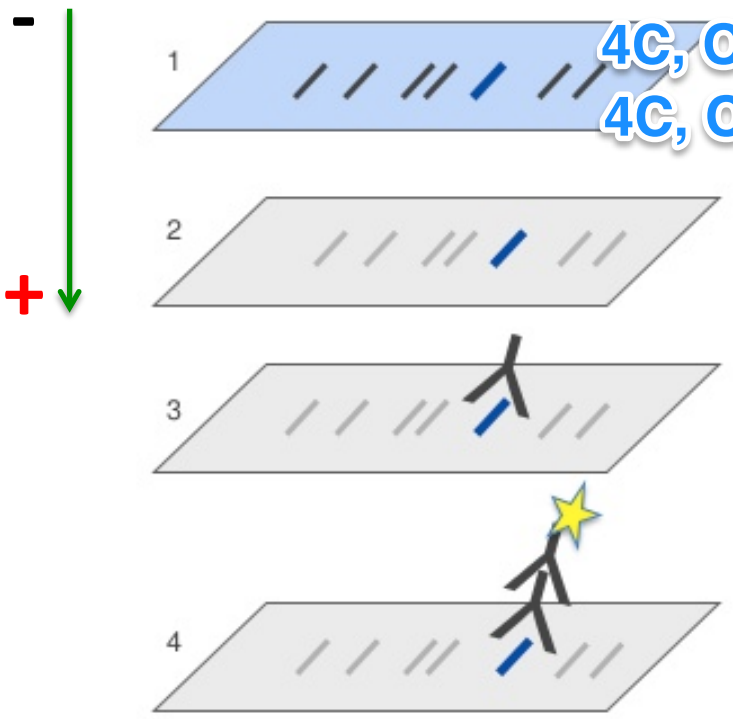




# M2: Experimental overview



# Western blot workflow:

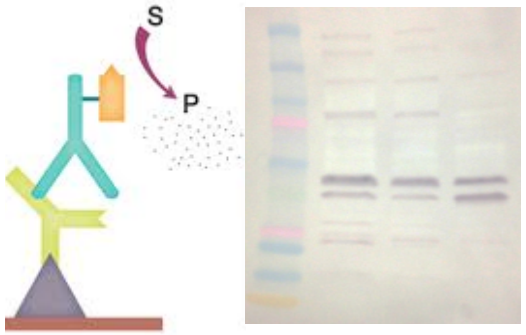
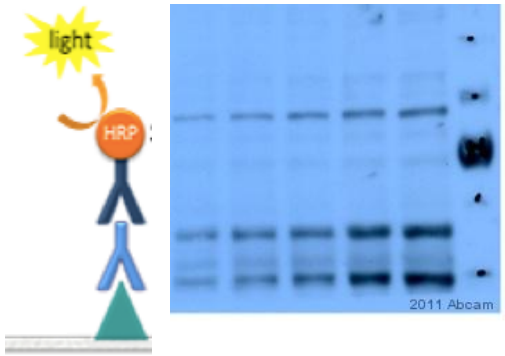
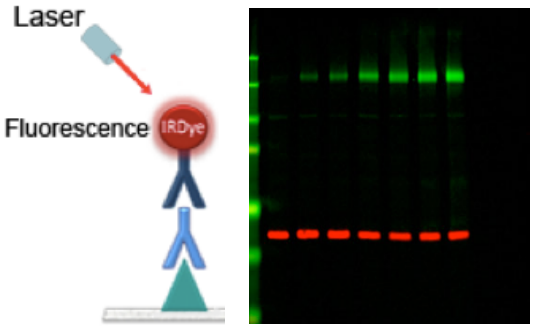



1. Protein separation by SDS-PAGE
2. Protein transfer to nitrocellulose membrane
3. Block membrane **prevent nonspecific binding** **4C, ON**
4. Probe with primary antibodies specific to **4C, ON**
   - BRCA2 **1:1000**
   - tubulin **1:5000**

   **24 hr at 4C**
5. Wash with TBS-T **Tris buffered saline**
   - **tween: mild detergent**
   - to **wash extra primary Ab away**
   - and to **detach weakly interacting Ab**
6. Probe with labeled secondary antibodies specific to primary antibodies **light sensitive**
7. Wash **light sensitive**
8. Image *LI-COR* fluorescence signal

from GE Healthcare

# Western blot detection / visualization

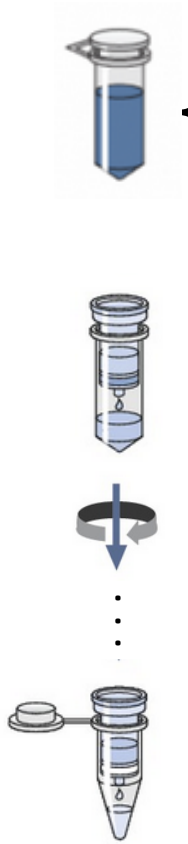
| Colorimetric | Chemi-luminescent | Fluorescent (Li-COR) |
|---|---|---|
|  |  |  |
| Upon incubation with a substrate that reacts with reporter (*e.g.* peroxidase), dye rendered insoluble and colored precipitates on membrane. | Incubation substrate luminesces when exposed to reporter on secondary antibody. | The fluorescently labeled probe is excited by light and the fluorescence emission is detected by a photosensor such as a CCD camera. |
| **Pro**: inexpensive, easy, no equipment required | **Pro**: sensitive, fast, film developer is common | **Pro**: sensitive, stable, able to multiplex |
| **Con**: medium sensitivity | **Con**: requires trial and error, time-dependent snapshot | **Con**: expensive |

# Homework due M2D4: use Western blot

- Figure (with title and caption)
- Results (no more bullet points, paragraph form)
  - one title
  - introductory topic sentence
  - state findings
  - conclude / transition to next result
  - *new paragraph for each new topic*

- (Discussion / interpretation will be separate in M2 research article)

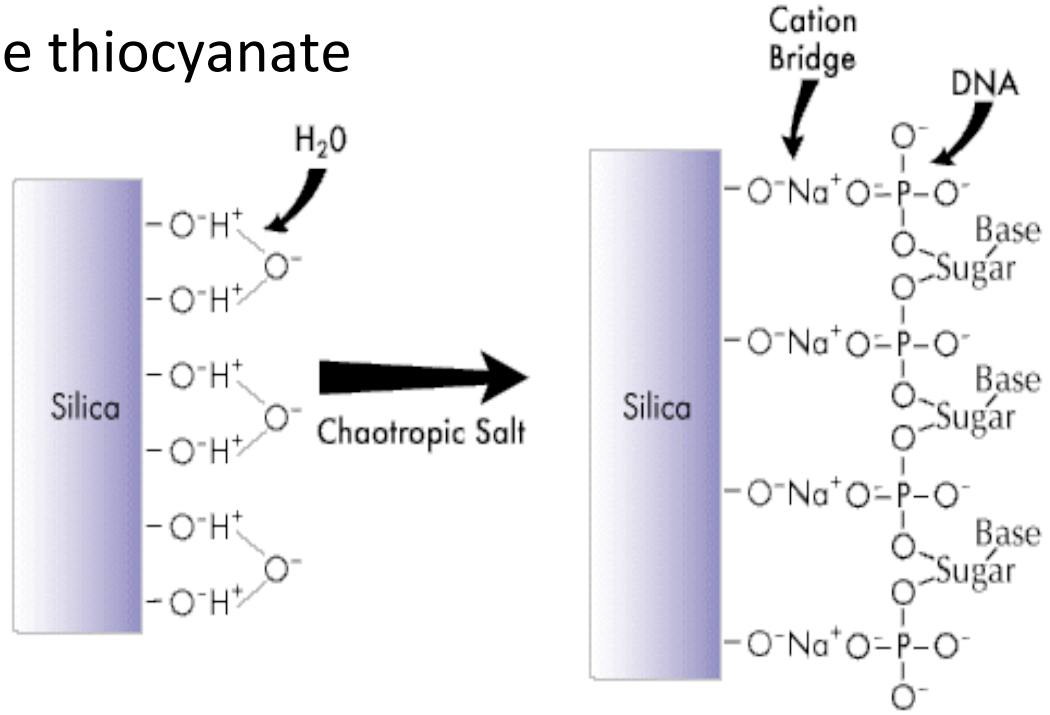
# Isolate RNA: QIAshredder + Rneasy kit



| steps | contents | purpose |
|---|---|---|
| lyse | RLT (with highly denaturing guanidine-thiocyanate salt) **detergents,chaotrophic salt** + QIAshredder | inactivate RNase, disrupt membranes, helps bind column<br>homogenize (shear high-MW genomic DNA) |
| prepare | ethanol **precipitation of RNA(DNA)** | promote efficient binding |
| bind | silica membrane in column | **binding mRNA** |
| wash | RW1<br>RPE | **remove contaminants**<br>** then, get rid of *all* ethanol |
| elute | water, RNase-free | high-purity RNA |

# Chaotropic salts help DNA/RNA bind to column

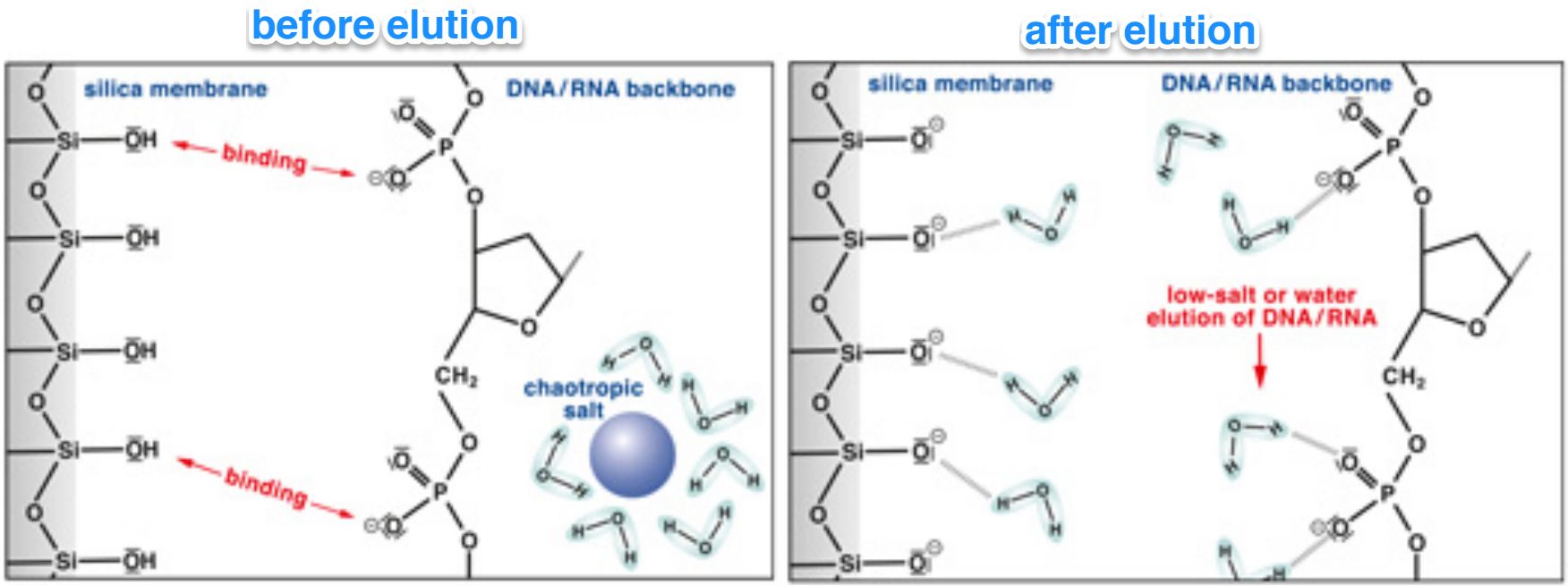
*e.g.* guanidine thiocyanate




- Washes with RW1 and RPE remove residual contaminants
  - RW1 contains a guanidine salt, as well as ethanol, and is used as a stringent washing buffer that efficiently removes biomolecules such as carbohydrates, proteins, fatty acids, etc, that are non-specifically bound to the silica membrane
  - RPE contains ethanol and is a mild washing buffer

# Water is used to elute nucleic acids

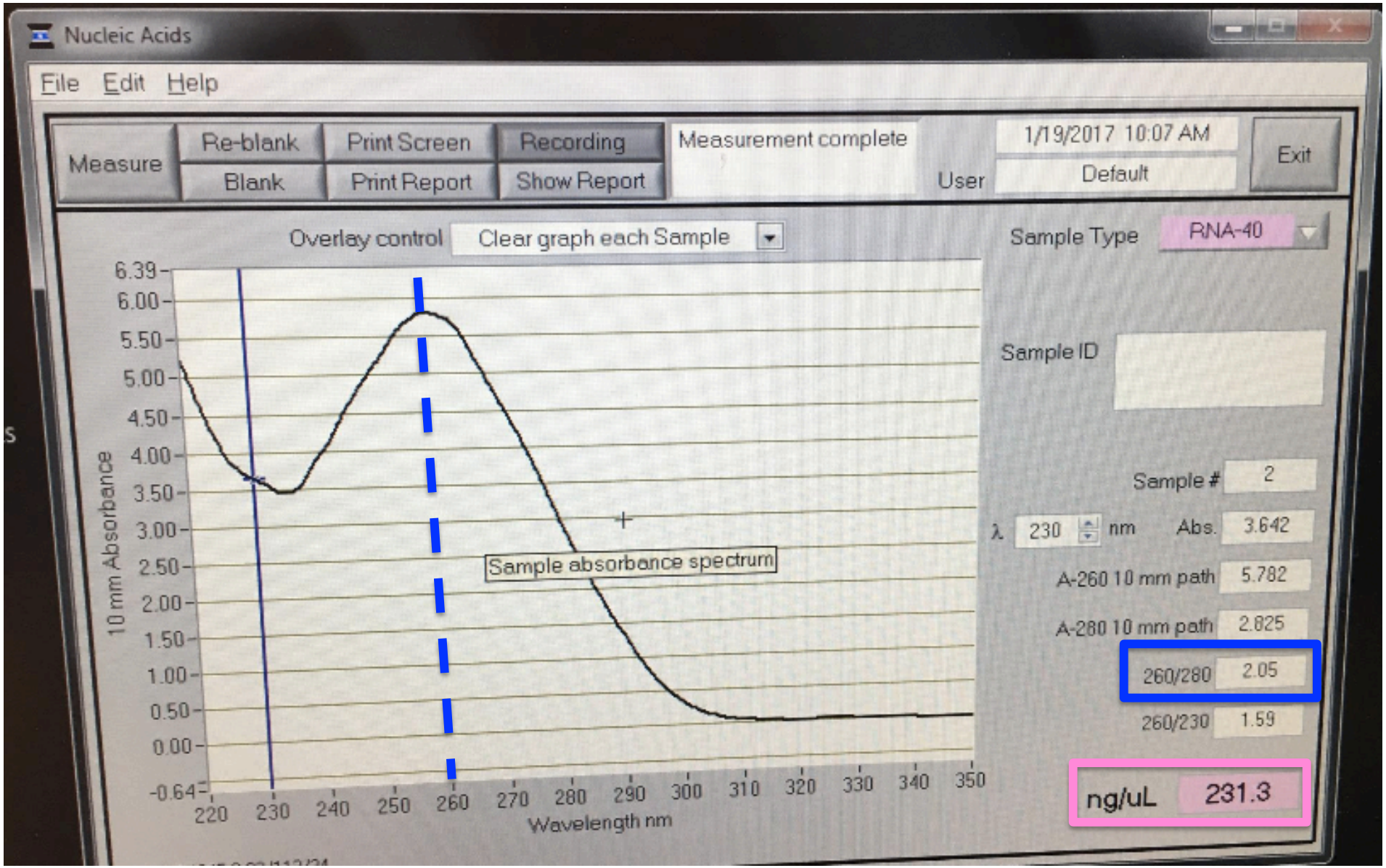
- Water competes RNA off of column



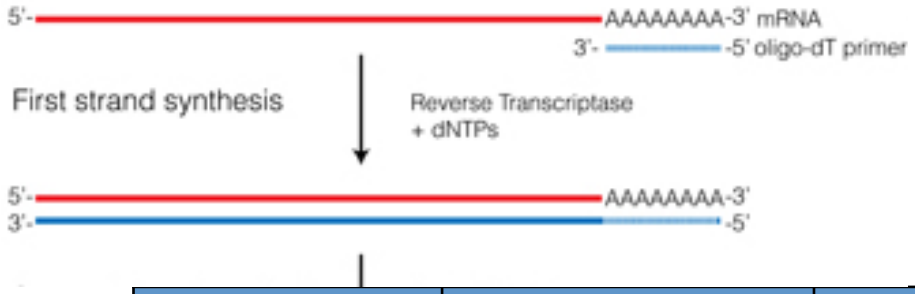
# RNA concentration from NanoDrop spectrophotometer

- $A_{260}/A_{280}$
  - nucleic acids absorb at **260** nm
  - proteins absorb at **280** nm
  - ratio ~ 1.8 "pure" DNA
  - ratio ~ 2.0 "pure" RNA
  - note: $A_{230}$ from contaminants (phenol, guanidine, carbohydrates,..)

# RNA concentration from NanoDrop



# Synthesize cDNA from purified RNA

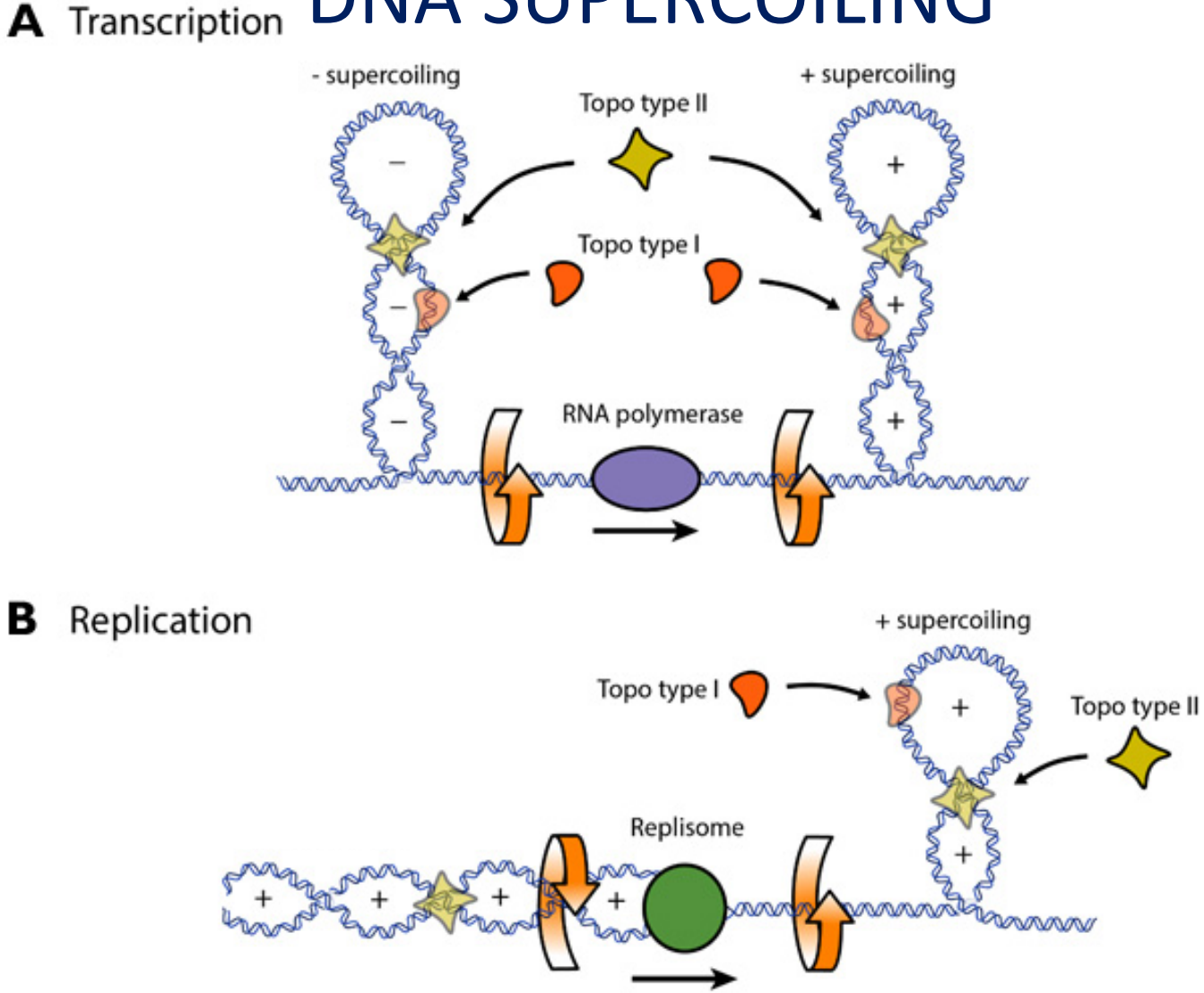



- cDNA: **complementary DNA**
- RT-PCR: **reverse transcriptase PCR**

| steps | conditions | ingredients added |
|---|---|---|
| denature | 65°C 5 min<br>on ice 1 min | 1 µg RNA + primers + dNTPs |
| anneal | oligo $(dT)_{20}$ | oligo $(dT)_{20}$ |
| synthesize | 50°C 50 min | SuperScript III RT, RNaseOUT, $MgCl_2$, DTT, buffer |
| terminate | 85°C 5 min | |
| remove RNA | 37°C 20 min | RNase H **chews RNA** |
| PCR amplify | M2D4 | |

**Prof. Samson's lecture 3/16**

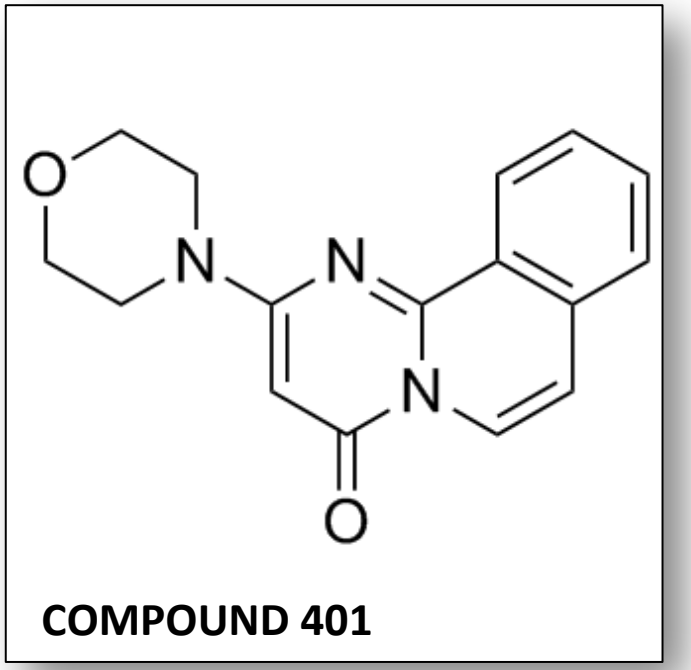
# RNA Transcription and DNA Replication cause DNA SUPERCOILING



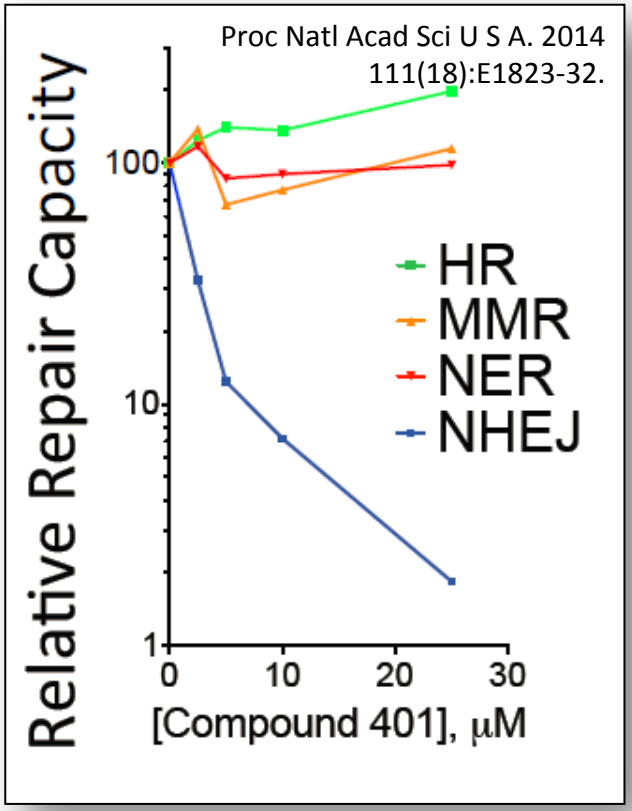

https://www.google.com/search?q=transcription+and+supercoiling&rlz=1T4GGHP_enUS635US636&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiht7nTmcrSAhUC8IMKHR31CPoQ_AUICSgC&biw=1451&bih=689#imgrc=pBDGWOKz0axQVM:

Prof. Samson's lecture 3/16

# COMPOUND 401 – "Selective" Inhibitor of DNA-PK and NHEJ



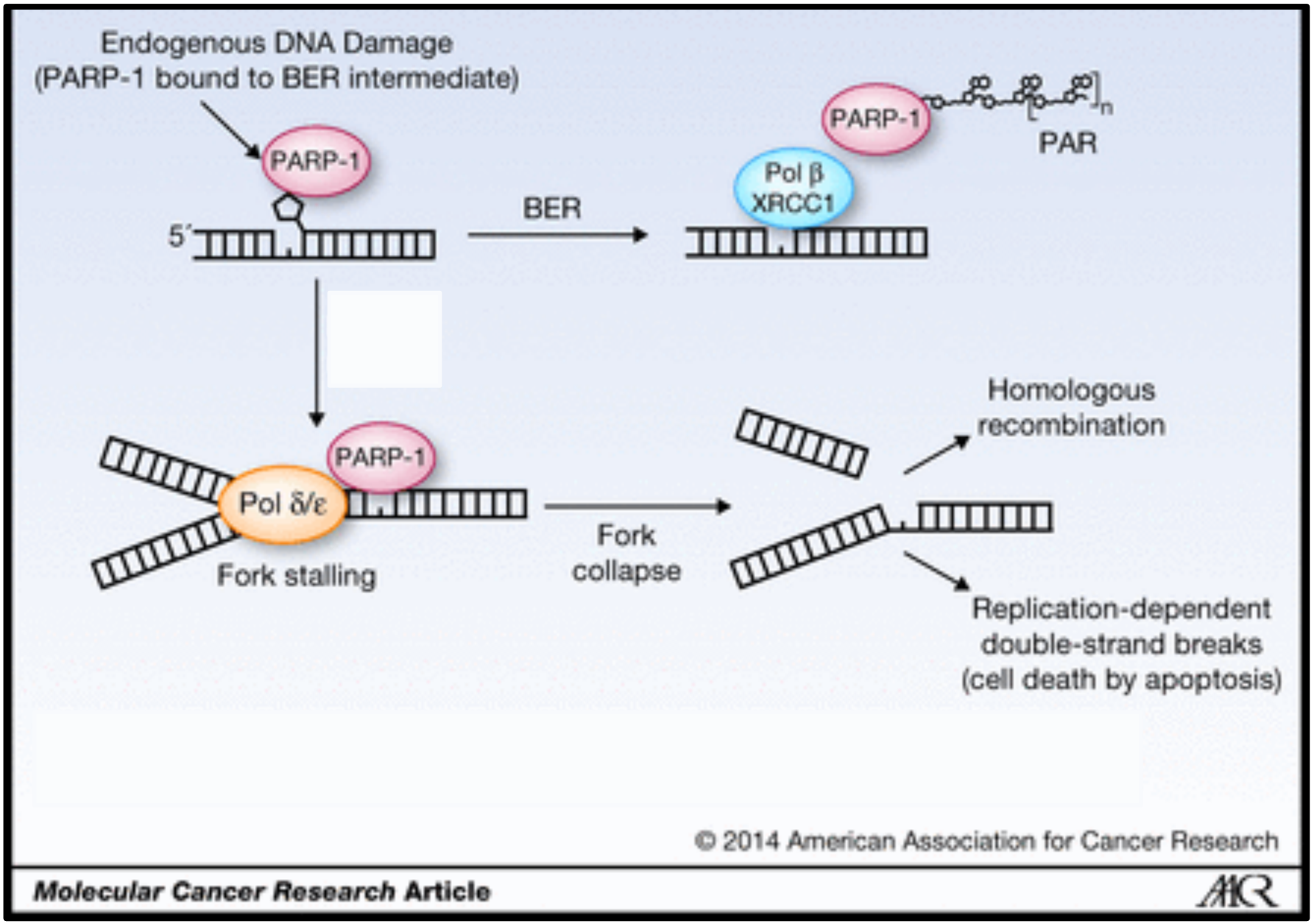

**COMPOUND 401**




## Biological Activity of COMPOUND 104

Reversible and selective inhibitor of DNA-dependent protein kinase (DNA-PK) and mammalian target of rapamycin (mTOR) ($IC_{50}$ values are 0.28 and 5.3 μM respectively). Displays little affinity for other commonly studied kinases including PI 3-K, ATM and ATR ($IC_{50}$ values are all > 100 μM).

**Prof. Samson's lecture 3/16**




http://mcr.aacrjournals.org/content/12/8/1128

# Treat cells to examine viability

- etoposide:

chemo-therapeutic drug

- compound 401:

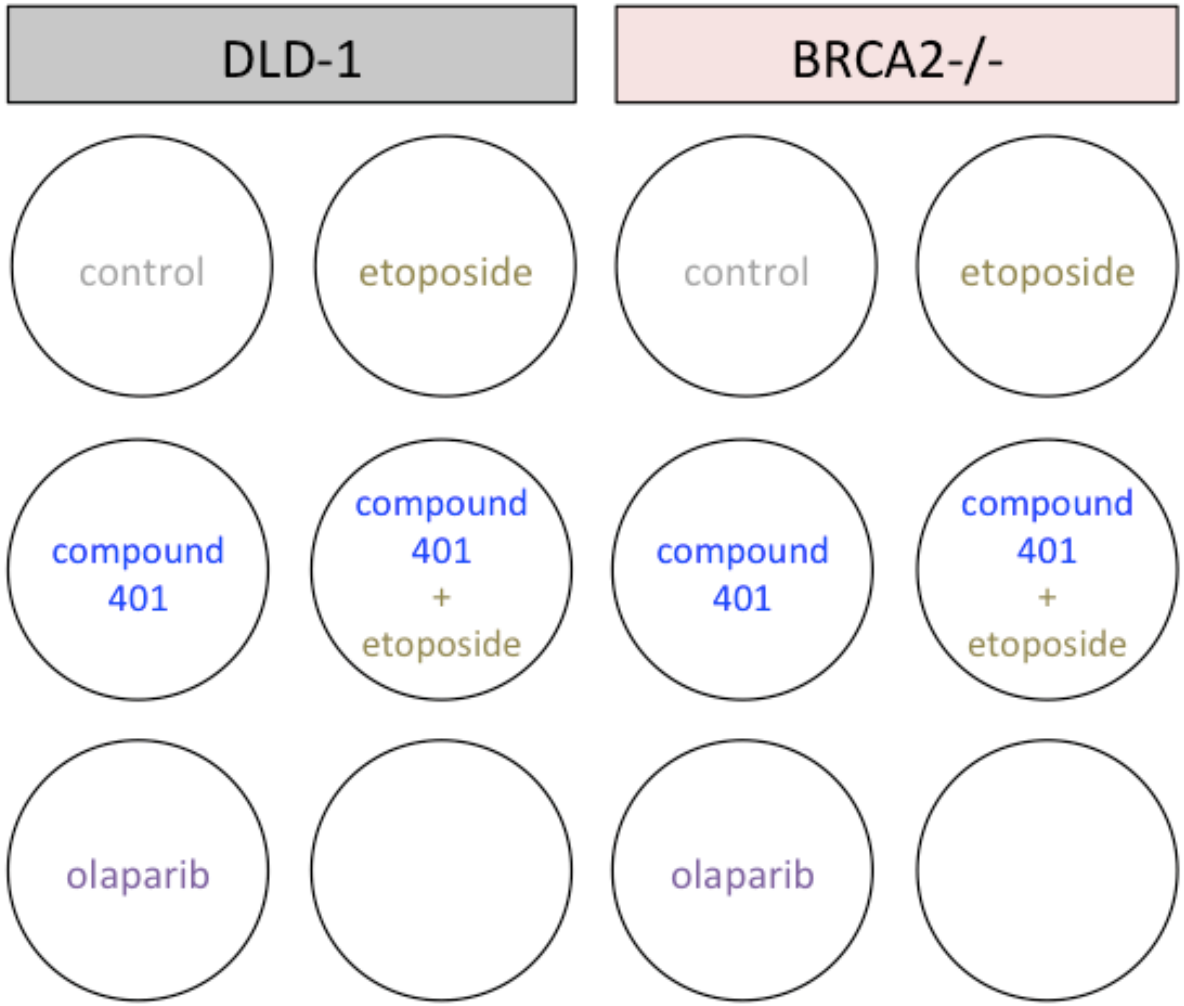
inhibitor of DNA-PK

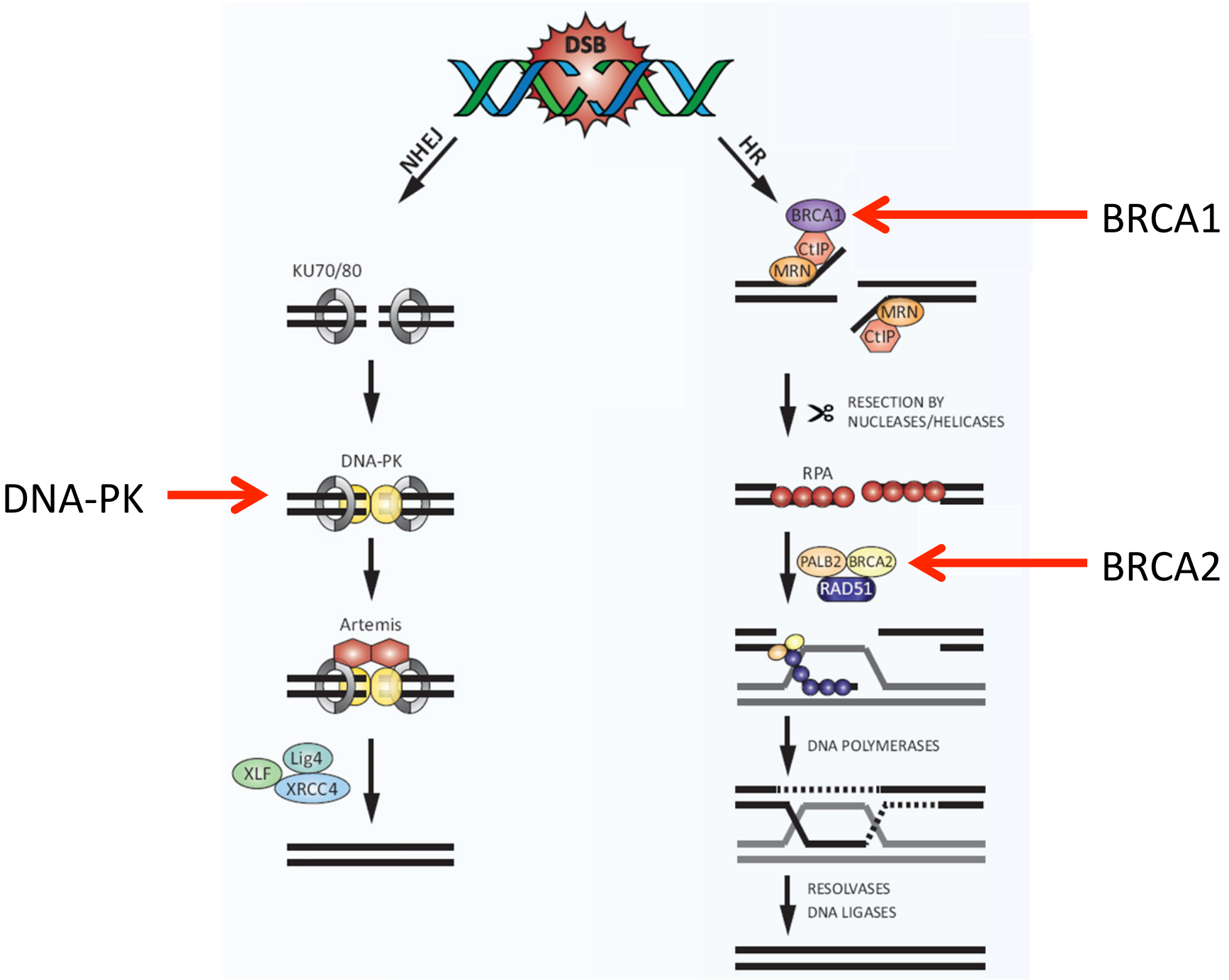
NHEJ

- olaparib:

PARP inhibitor (poly ADP ribose polymerase)

BER




DSB
NHEJ
HR
KU70/80
DNA-PK
Artemis
Lig4
XLF
XRCC4
BRCA1
CtIP
MRN
MRN
CtIP
RESECTION BY
NUCLEASES/HELICASES
RPA
PALB2
BRCA2
RAD51
DNA POLYMERASES
RESOLVASES
DNA LIGASES
DNA-PK
BRCA1
BRCA2

# Today in lab:

1. Teams choose a lead to complete:
   a. Wash western blot and start incubation with secondary antibody (light sensitive)
   b. Collect T75s for RNA extraction followed by cDNA synthesis
2. Drug treat DLD-1 and BRCA2(-/-) for viability assay
   a. One hour etoposide treatment (induce DSBs)
   b. Incubation with HR and NHEJ inhibitors
3. Complete Western Blot and image on LiCor scanner

- Homework due Wednesday, M2D4
  - Western Blot Figure, caption and accompanying results section (paragraph)
- Mini-presentation due Saturday
- Start reading your Journal Club paper NOW