M2D2: Induce DNA damage for RNA purification and research cell lines

Announcements/Reminders:
3/17: Mini-presentation due at 10pm
Extra Office Hours:
3/14 & 15: 2-4pm @ 16-317
3/18: Blog post due, 10pm

1. Prelab discussion
2. ½ class to TC to induce DNA damage (Part 1)
3. ½ class research cell lines (Part 2)
4. Switch

Mod2 major assignments

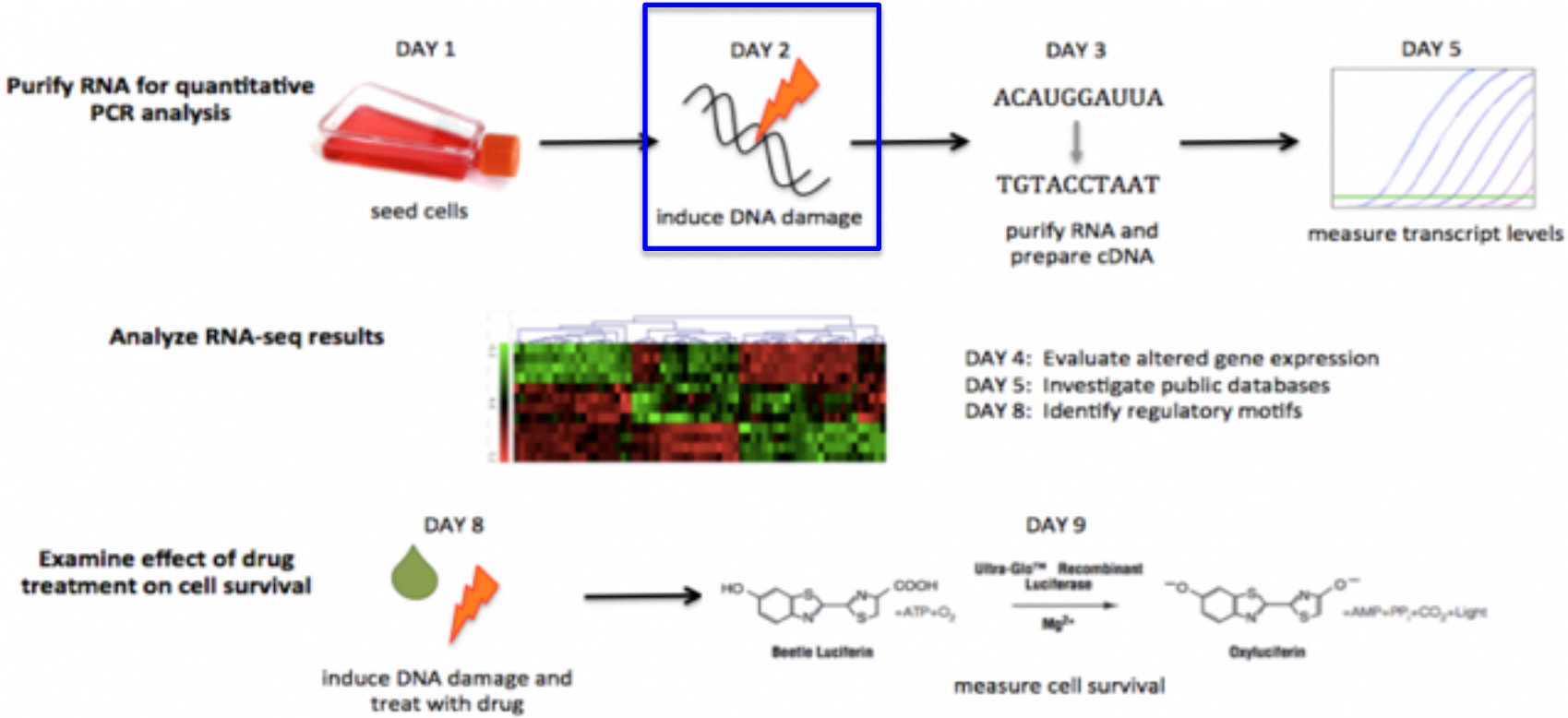
- **Research Article (20%)**
  - Individual, submit on Stellar
  - Due April 21st at 10pm
  - Format: word document
- **Journal Club Presentation (15%)**
  - Individual, presentation during lab
  - Presentation slides due on Stellar 1pm April 4rd or April 6th
  - Format: powerpoint, keynote, or google slides

- *Lab quizzes (5%)*
- *Homework and Notebook (10%)*
- *Blog (with participation, 5%)*
  - *by Sunday, March 18 at 10 pm **(Mod1)***
  - *by Saturday, April 7 at 10 pm*
  - *by Sunday, April 22 at 10 pm*

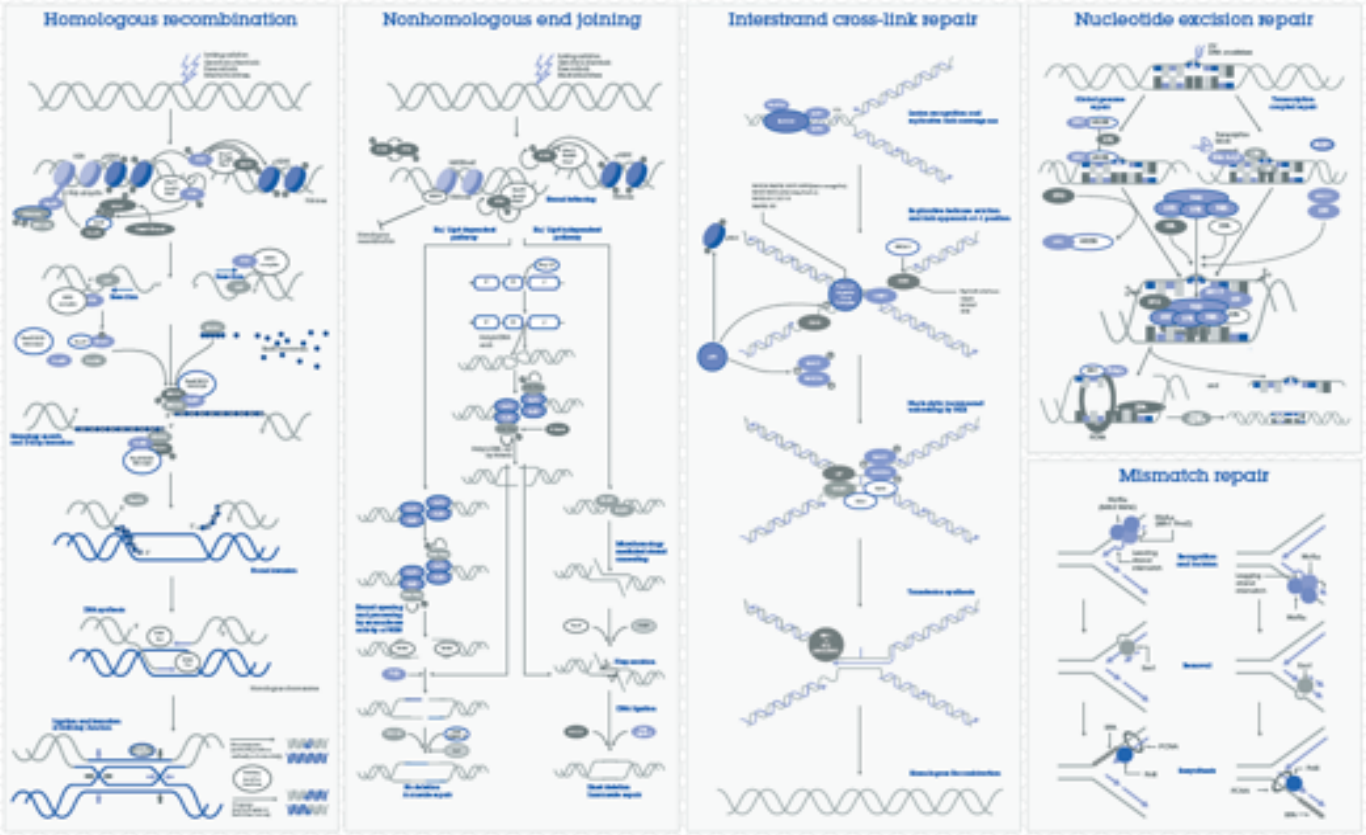
20.109(S18) Class blog

- 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  - Do not publish MIT logo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

M2D2: Induce DNA damage

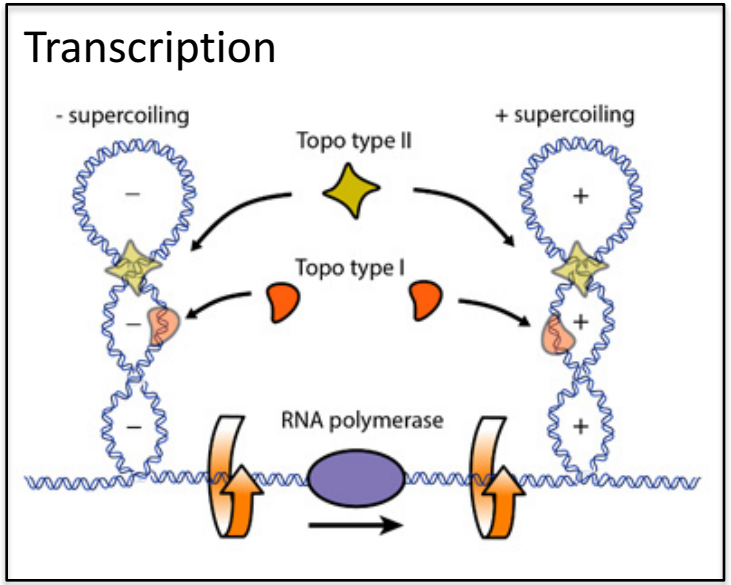


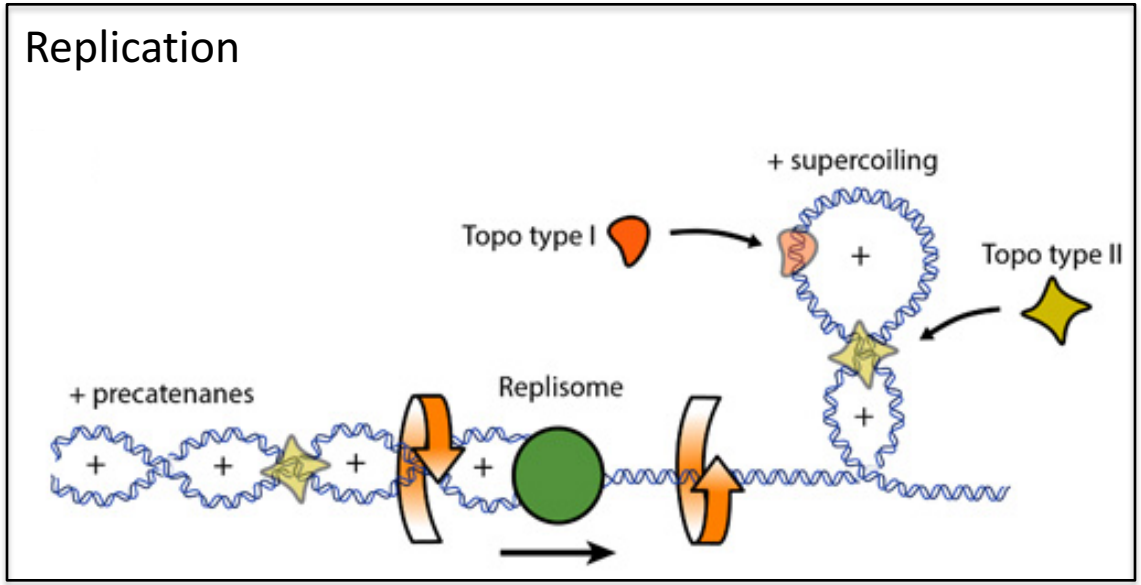
What genes are differentially expressed in response to DNA damage/chemotherapy?



Topoisomerases are involved in winding/unwinding DNA during Transcription and Replication

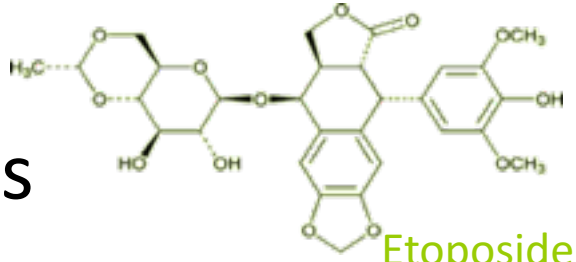
Topo Type II = topoisomerase II enzyme





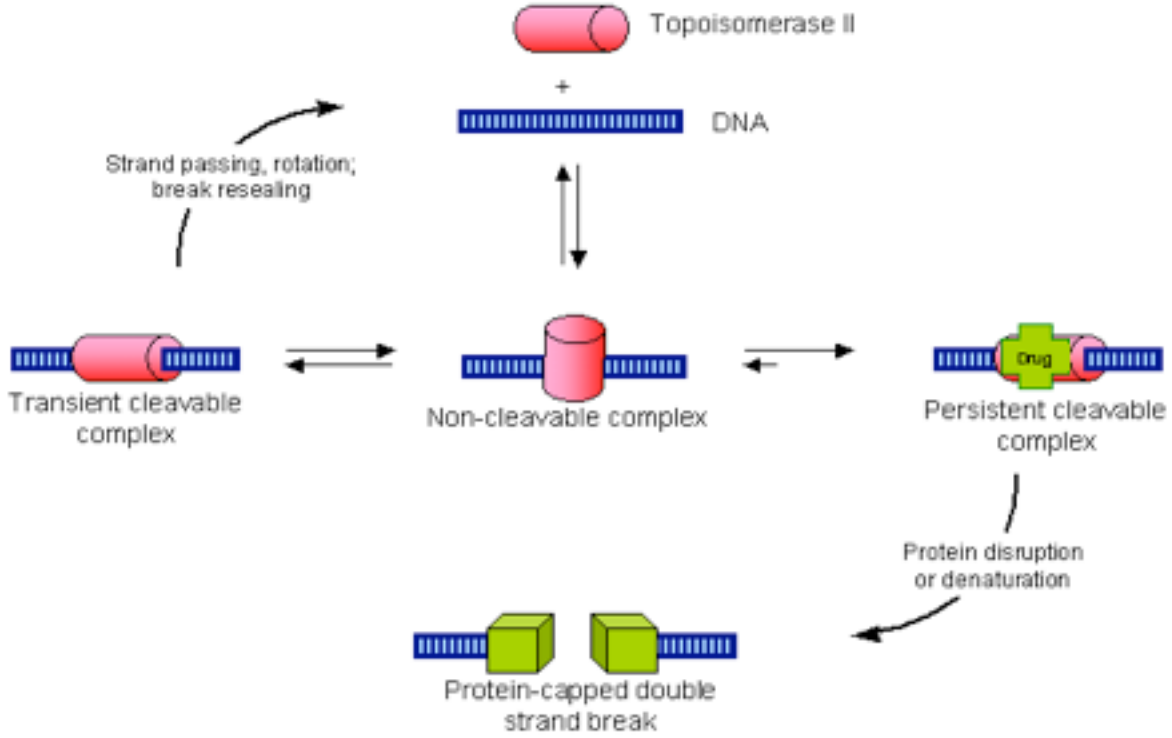
Ma, J. & Wang, M.D. Biophys Rev (2016) 8(Suppl 1): 75. https://doi.org/10.1007/s12551-016-0215-9

Etoposide is a drug/chemotherapy that causes DNA double strand breaks

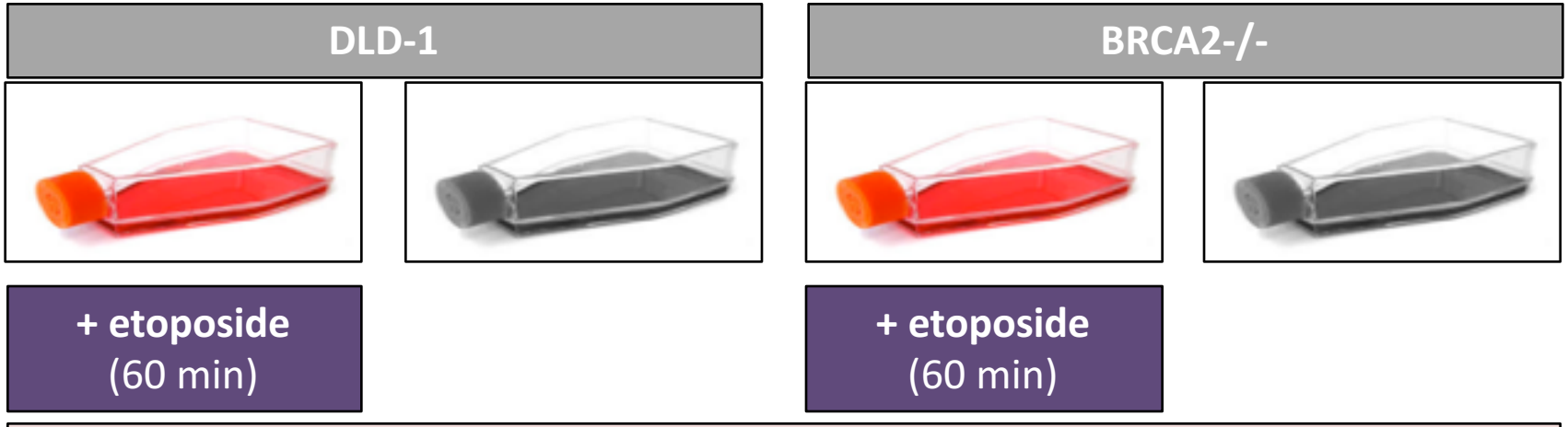


- Mechanism of action: forms ternary complex with DNA and topoisomerase II enzyme, prevents re-ligation of the DNA strands

- Cancer cells (quickly dividing cells) rely on topoisomerase II more than normal cells

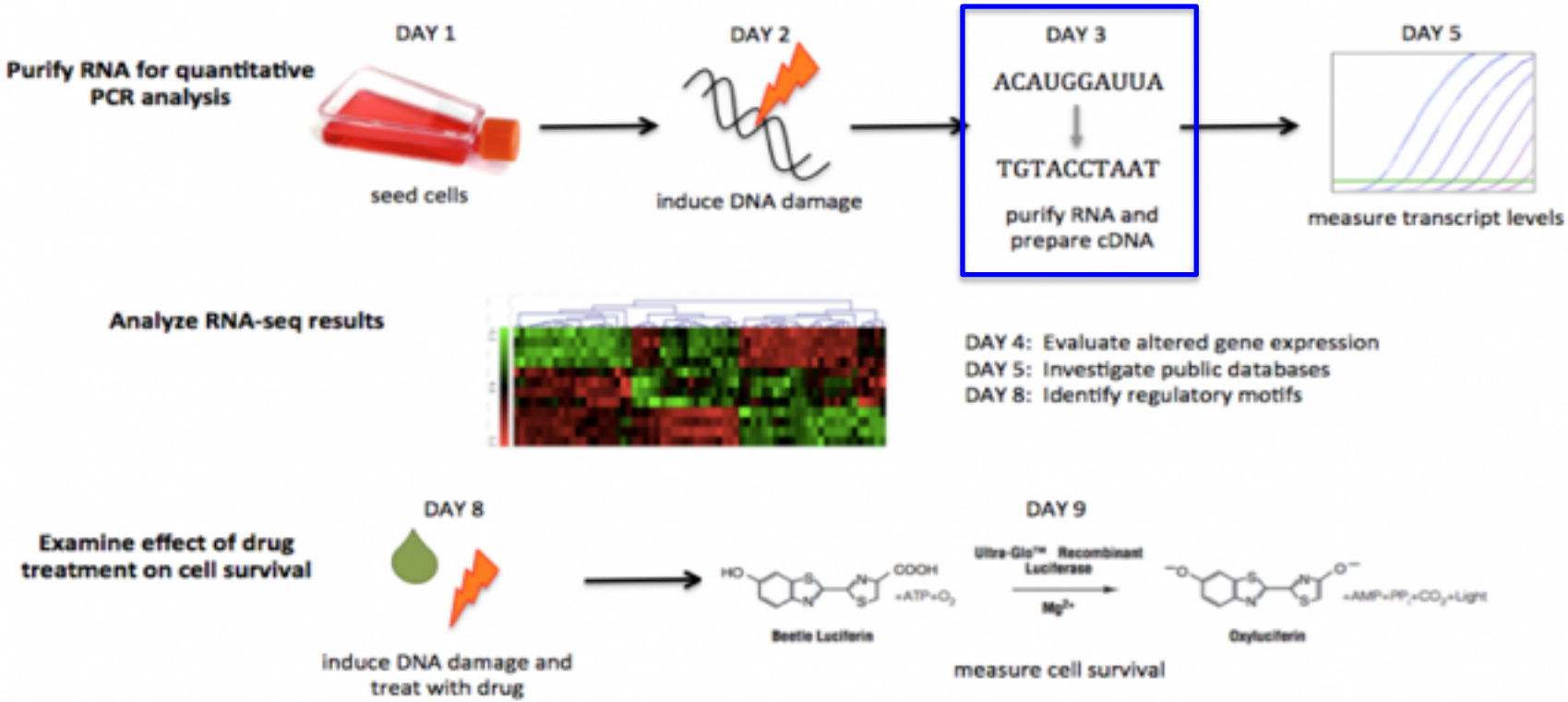


Treat cells with etoposide

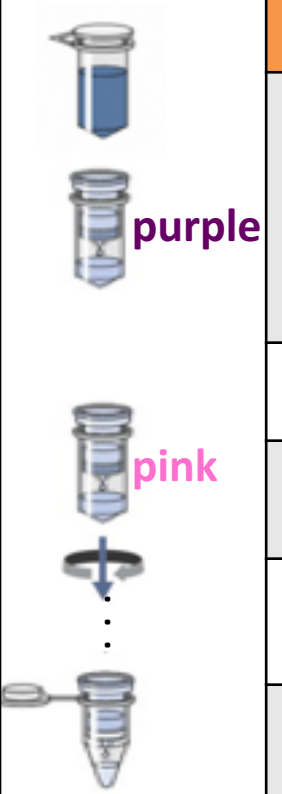


M2D3: extract RNA

M2D3: Purify RNA and prepare cDNA



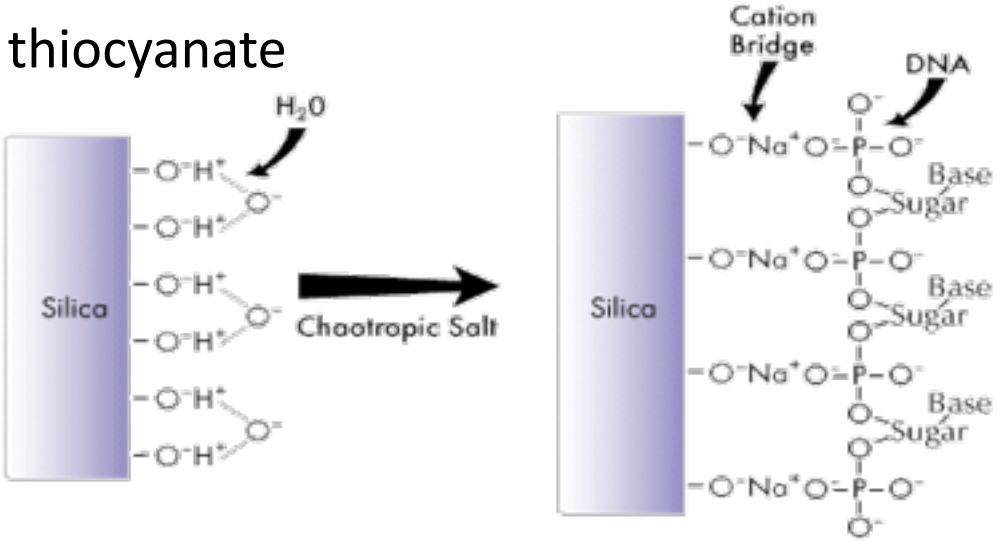
Isolate RNA: QIAshredder + Rneasy kit



Steps	Contents	Purpose
lyse	RLT (with highly denaturing guanidine-thiocyanate salt) + QIAshredder	inactivate RNase, disrupt membranes, helps bind column homogenize (shear high-MW genomic DNA)
prepare	70% ethanol	promote efficient binding to silica
bind	silica membrane in column	retain mRNA
wash	RW1 RPE	remove contaminants ** then, get rid of *all* ethanol
elute	water, RNase-free	high-purity RNA

Chaotropic salts help DNA/RNA bind to column

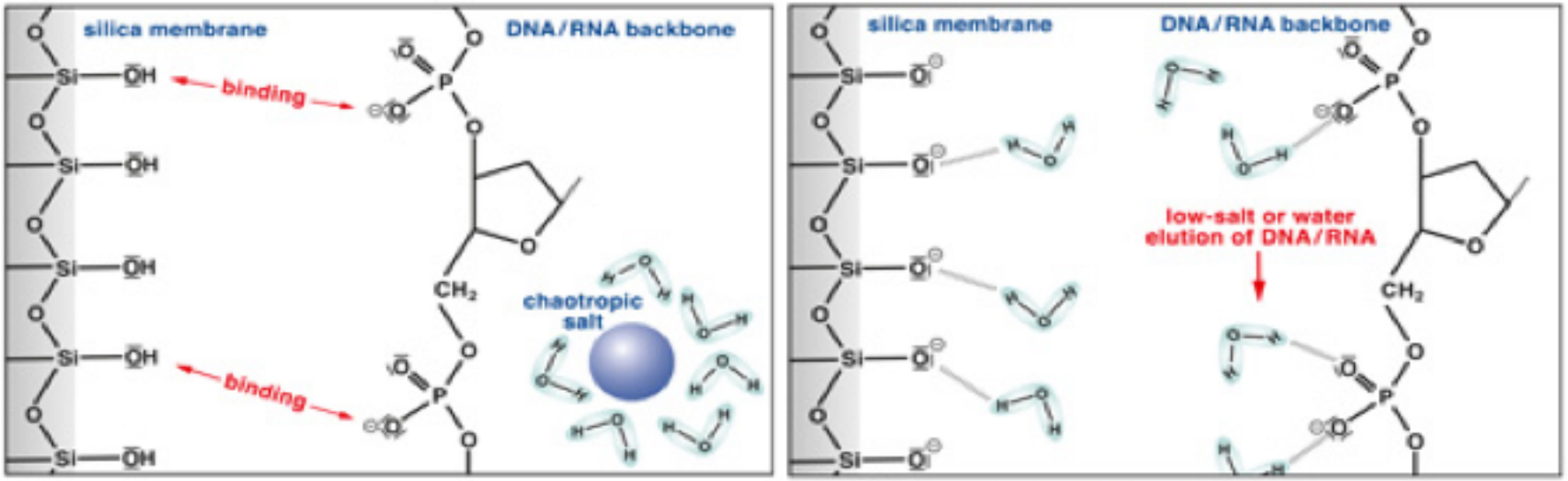
e.g. guanidine thiocyanate



- Washes with RW1 and RPE remove residual contaminants
  - RW1 (with guanidine salt and ethanol)
    - Removes contaminant biomolecules
    - Maintains RNA on column
  - RPE (contains ethanol) – mild washing buffer, removes salts

Water is used to elute nucleic acids

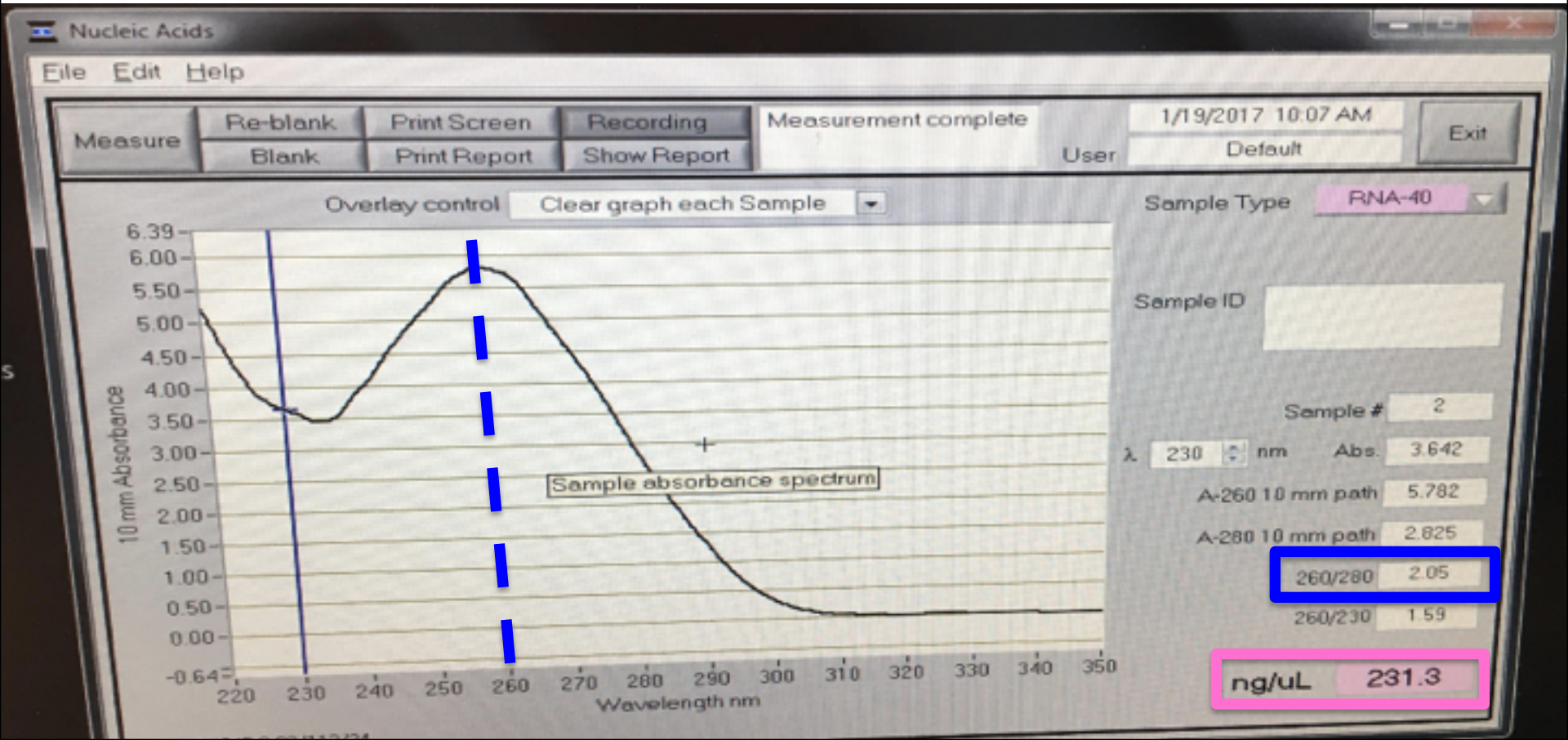
- Water competes RNA off of column
- Collect RNA in new tube!



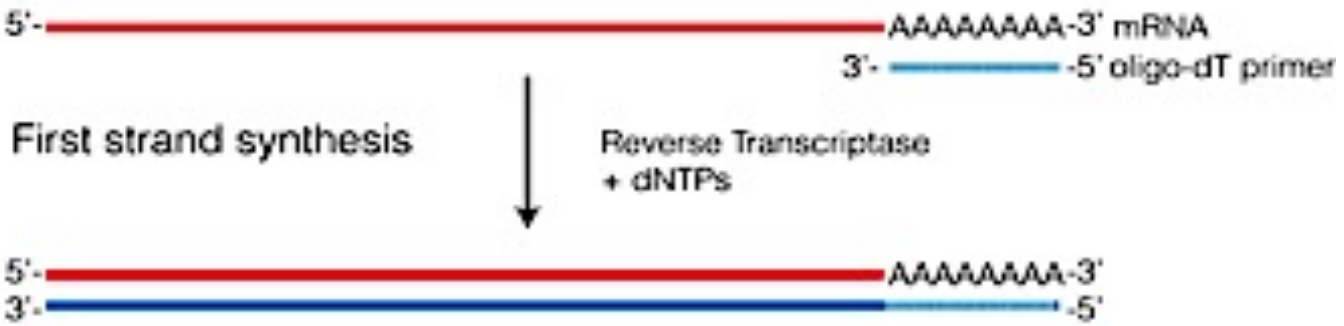
RNA concentration from NanoDrop spectrophotometer

- 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



Utilizing the poly-A tail to synthesize cDNA from purified RNA



- cDNA: Complementary DNA
- Reverse transcription polymerase chain reaction (RT PCR)

Components and procedure of cDNA Synthesis

steps	conditions	reagents added
denature & anneal	65°C 5 min on ice 1 min	1 µg RNA + oligo $(dT)_{20}$ primers + dNTPs
synthesize cDNA	50°C 50 min	**Superscript III** Reverse Transcriptase **$MgCl_2$** **DTT** **RNase OUT** **buffer**
terminate	85°C 5 min	
remove RNA	37°C 20 min	**RNase H**
Purify cDNA	M2D5	

Reminders:

- M2D3 HW: Choose Journal Club paper
  - Review list on M2D7 and edit wiki to select a paper
- Mini presentation due Saturday, March 17th at 10pm.
  - Email video to bioeng20.109@gmail.com
  - Submitting the final version of your video can take time so don't wait until the last minute. Feel free to send us a link so we can download.
  - Don't forget Noreen's extra office hours.