

M2D2: Induce DNA damage for RNA purification

03/13/2018

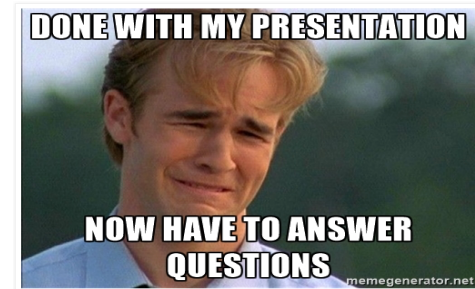
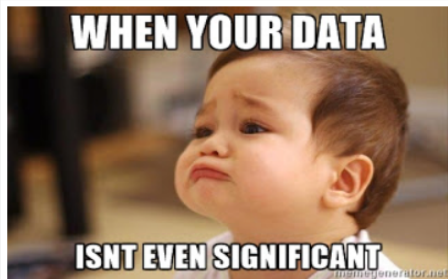
Notes in blue

Mod2 major assignments

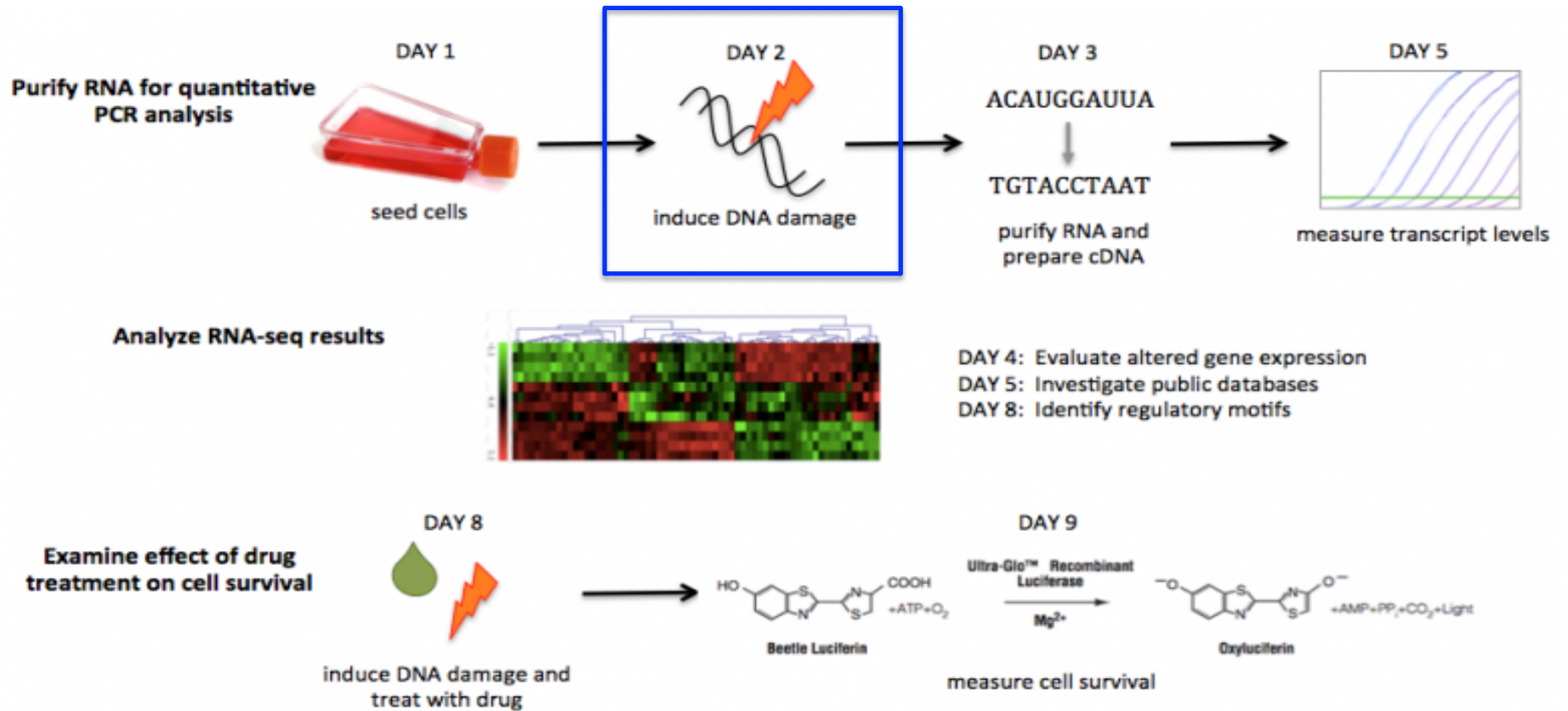
- **Research Article (20%)**
 - individual, submit on Stellar
 - due April 21st at 10pm
 - format: word document
- **Journal Club Presentation (15%)**
 - individual, presentation during lab
 - presentation slides due on Stellar 1pm April 3rd or April 5th
 - format: powerpoint, keynote, or google slides
- *Lab quizzes (5%)*
- *Homework and Notebook (10%)*
- *Blog (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

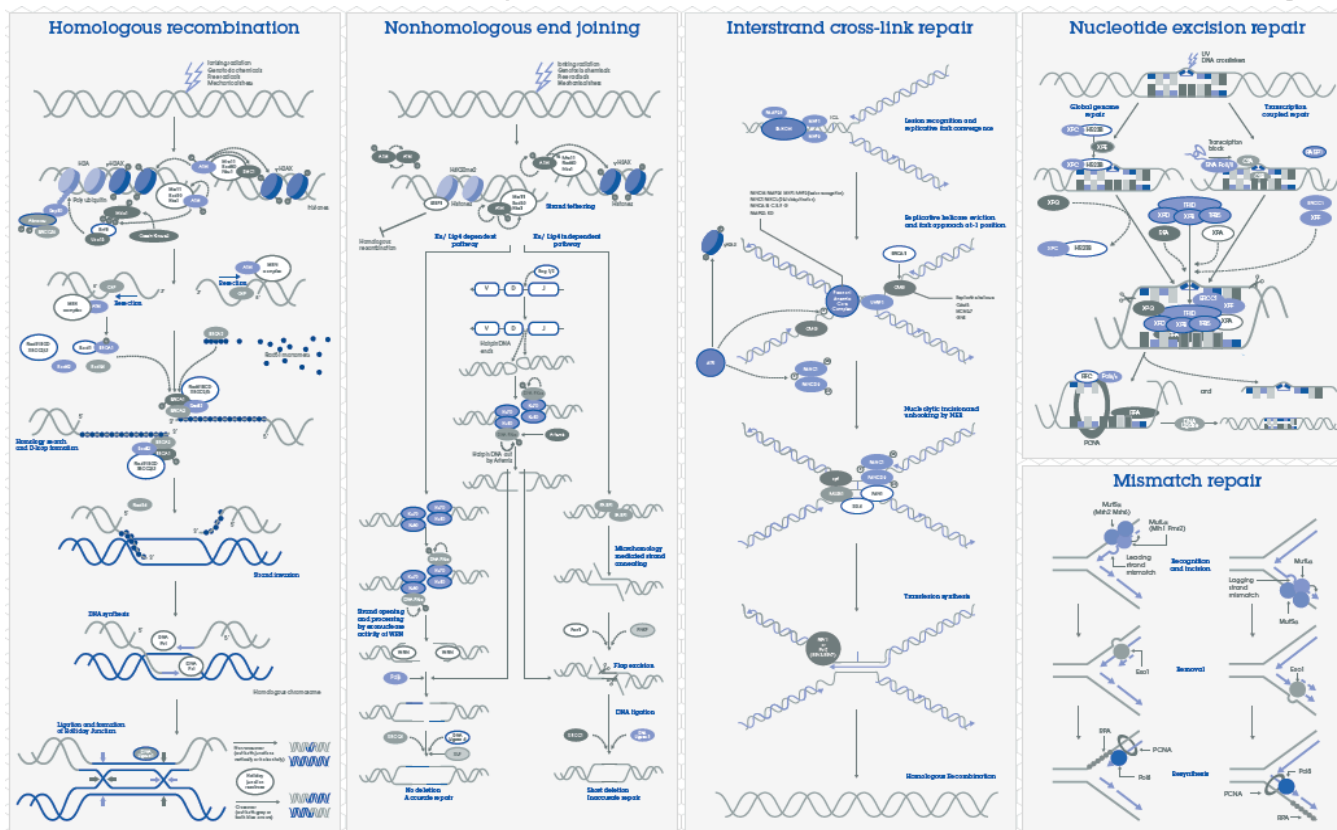
- Possible topics listed on the blog website
- Details about use:
 - Do not publish MIT logo
 - Do not post photographs with names tagged
 - Do not write malicious comments
 - Do not plagiarize



M2: Experimental overview



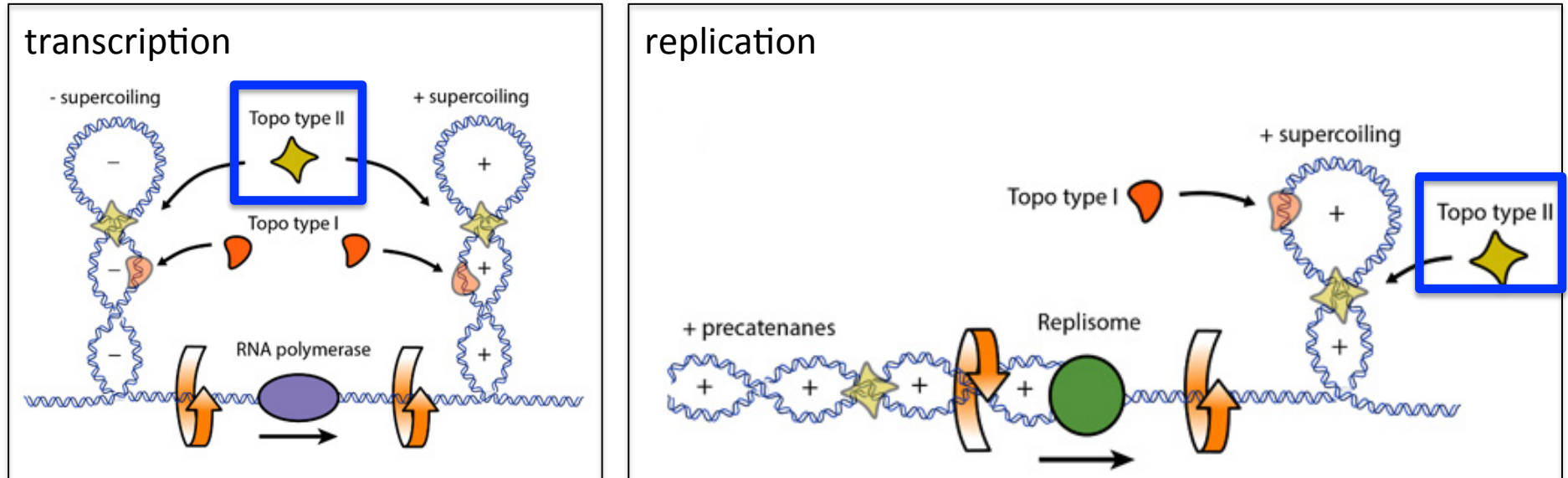
What genes are differentially expressed in response to DNA damage?



-Much is known about the DNA damage response (DDR), but this a complex signaling cascade that results in many changes in the cell that are not fully understood -a major DDR is differential gene expression

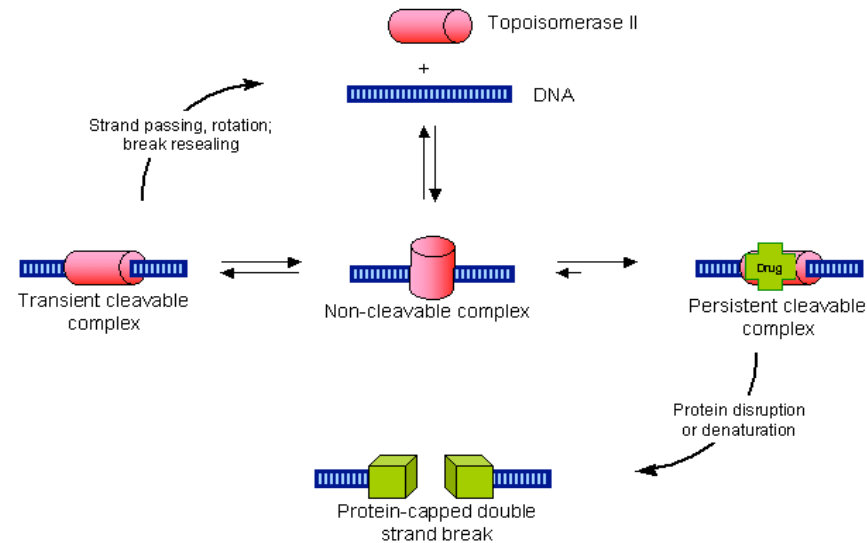
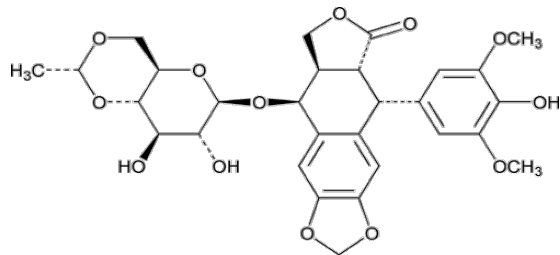
RNA Transcription and DNA Replication cause DNA supercoiling

Topo Type II=topoisomerase II enzyme

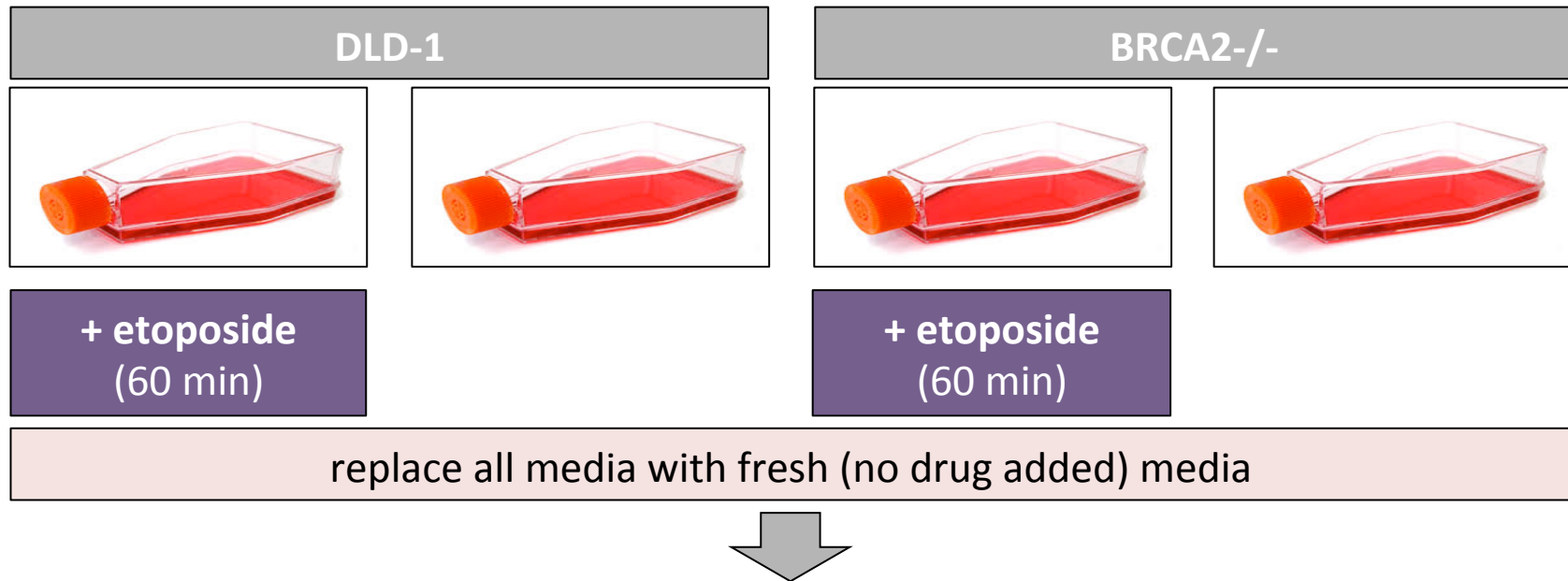


Etoposide is a drug/chemotherapy that causes DNA double strand breaks

- mechanism of action: forms a ternary complex with DNA and topoisomerase II enzyme and prevents re-ligation of the DNA strands = DNA strand break
- cancer cells (quickly dividing cells) rely on topoisomerase II more than normal cells

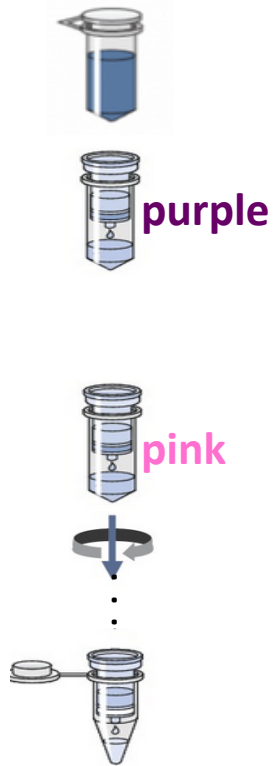


Treat cells with etoposide



M2D3: extract RNA (~48 hours after DNA damage)

Isolate RNA: QIAshredder + Rneasy kit

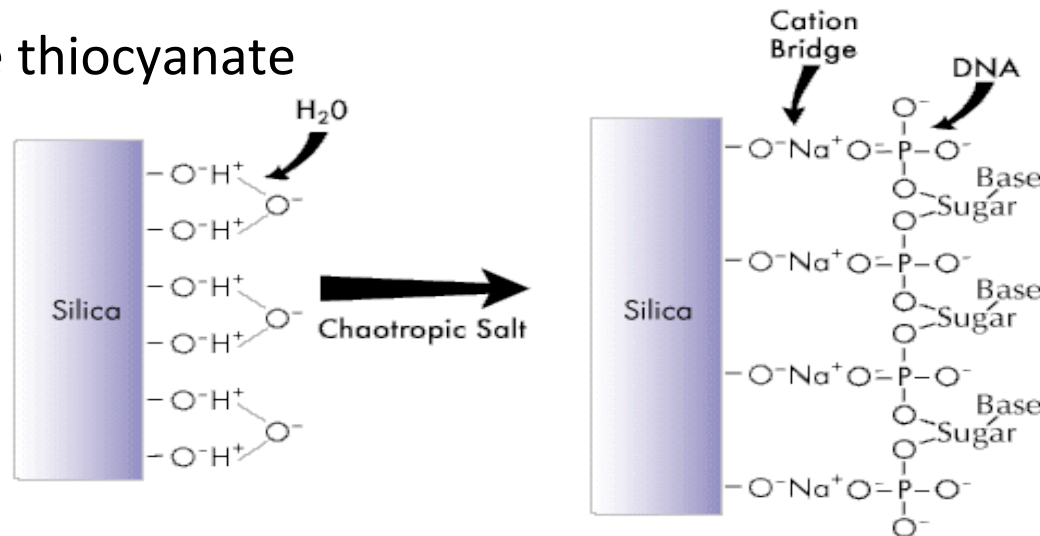


steps	contents	purpose
lyse	RLT (with highly denaturing guanidine-thiocyanate salt) + QIAshredder	inactivate RNase, disrupt membranes, helps bind column homogenize (shear high-MW genomic DNA)
prepare	70% ethanol	promote efficient binding to silica
bind	silica membrane in column	retain mRNA
wash	RW1 RPE	remove contaminates ** after this wash, important to get rid of <u>all</u> ethanol
elute	water, RNase-free	high-purity RNA

RLT buffer: composed of detergents and chaotropic salts (weakens hydrophobic effects)
QIAshredder: polymer that shears high molecular weight components of the cell
EtOH: RNA insoluble in ethanol, RNA precipitates from cell lysate and binds to silica membrane

Chaotropic salts help DNA/RNA bind to column

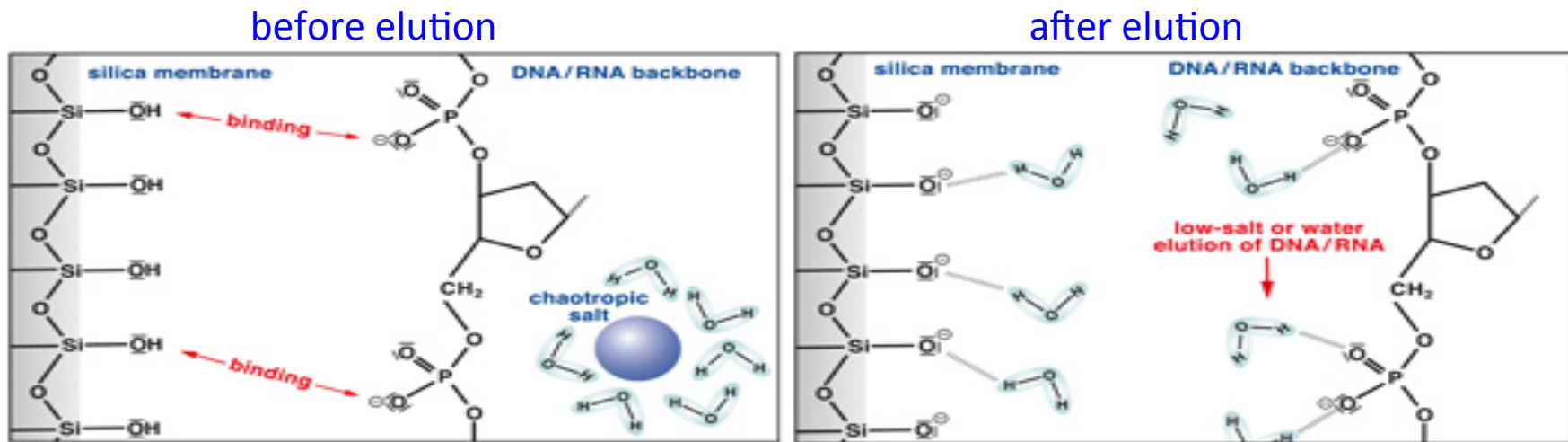
e.g. guanidine thiocyanate



- 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

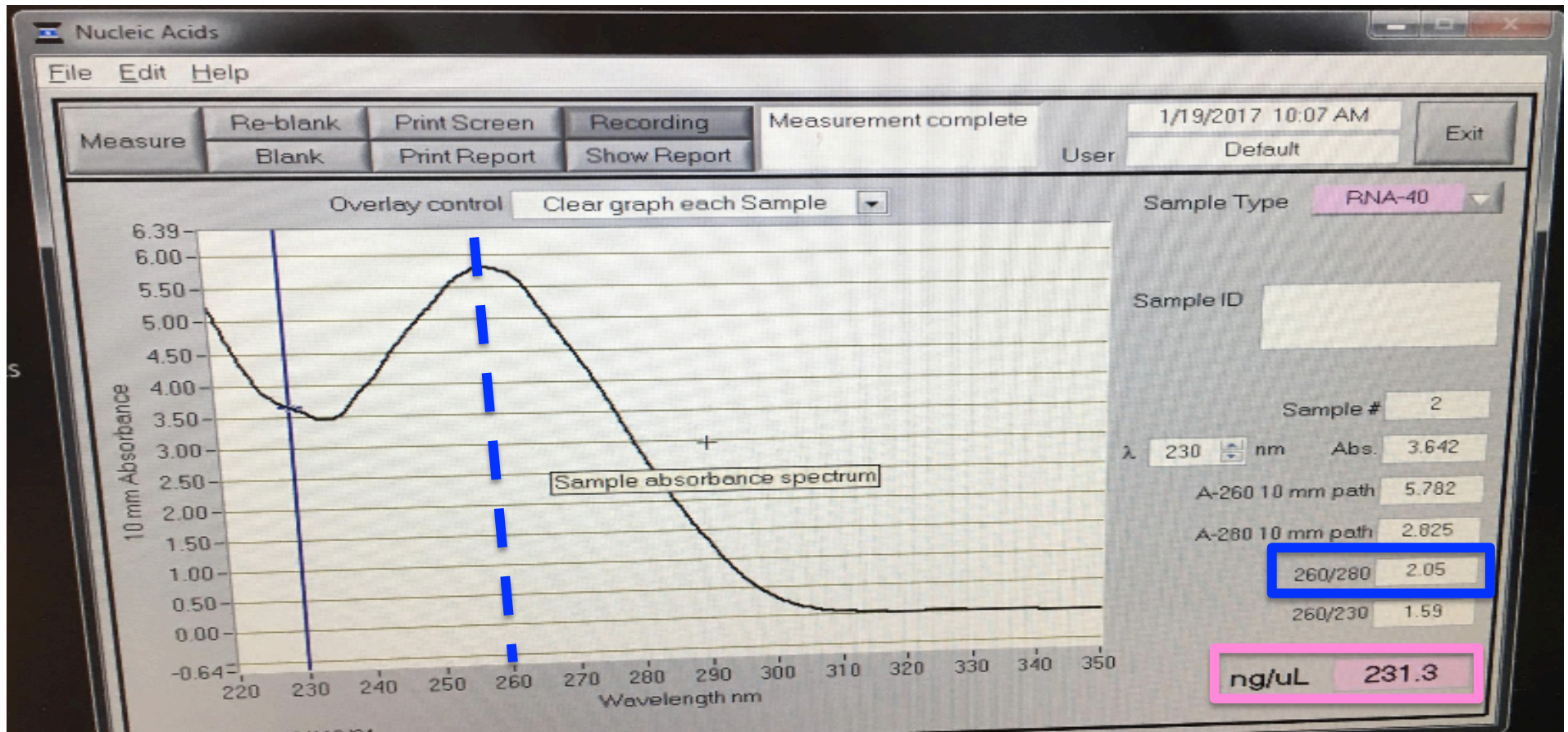


RNA concentration from NanoDrop spectrophotometer

- 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



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



- cDNA: complementary DNA
- RT-PCR: reverse transcription-polymerase chain reaction (not real time 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 primer + dNTPs (dATP, dCTP, dGTP and dTTP)
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 a Journal Club paper
 - review list on M2D7 and edit wiki to select a paper
- Mini presentation due Saturday March 17th at 10pm.
 - Email video file to bioeng20.109@gmail.com
 - Submitting the final version of your video can take time so don't wait till the last minute. Feel free to send us a link so we can download.
- Noreen will have extra office hours Wed.(3/14) and Thurs.(3/15) 2-4pm in 16-317