M2D2: Induce DNA damage for RNA purification

03/13/2018

Notes in blue

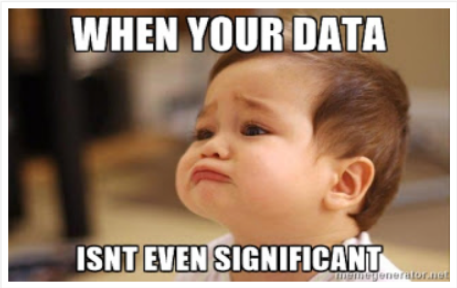
Mod2 major assignments

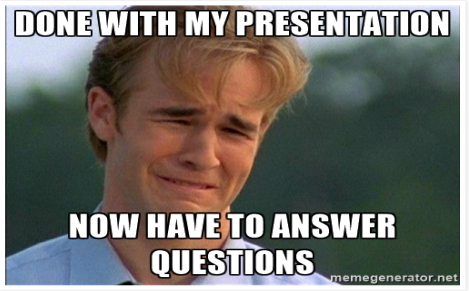
- **Research Article (20%)**
  - individual, submit on Stellar
  - due April 21st at 10pm
  - format: word document
- **Journal Club Presentation (15%)**
  - individual, presentation during lab
  - presentation slides due on Stellar 1pm April 3rd or April 5th
  - format: powerpoint, keynote, or google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

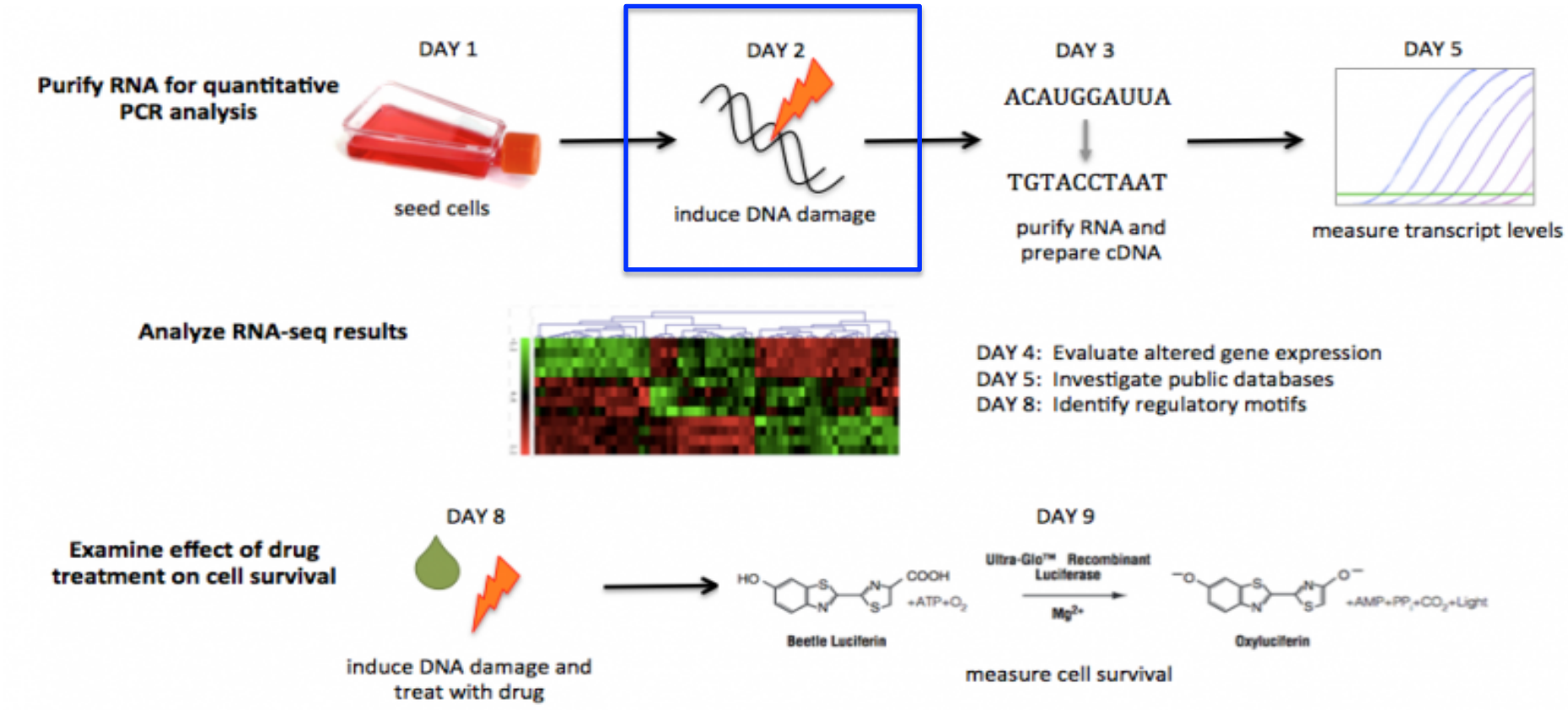
20.109(S18) Class blog

- 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  - Do not publish MIT logo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

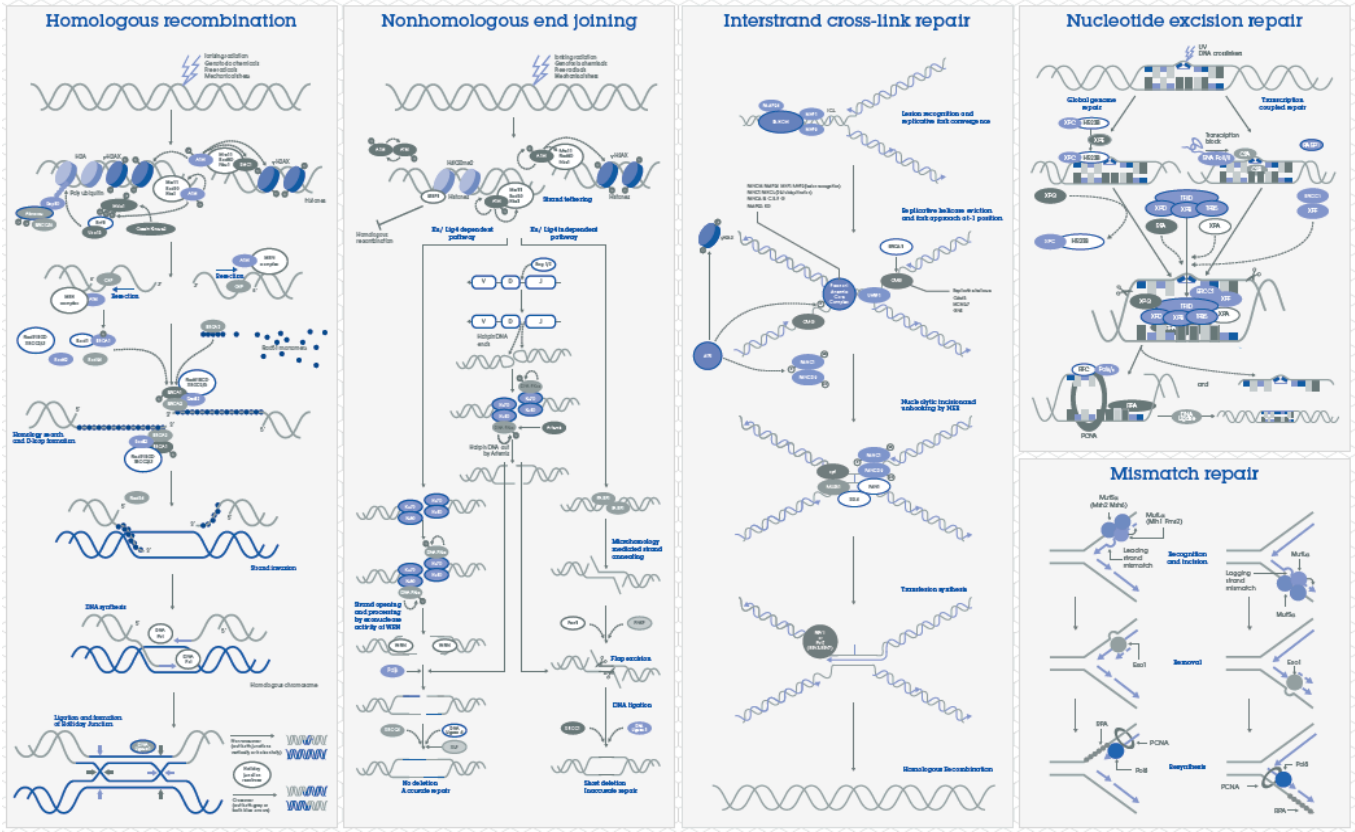




M2: Experimental overview



What genes are differentially expressed in response to DNA damage?

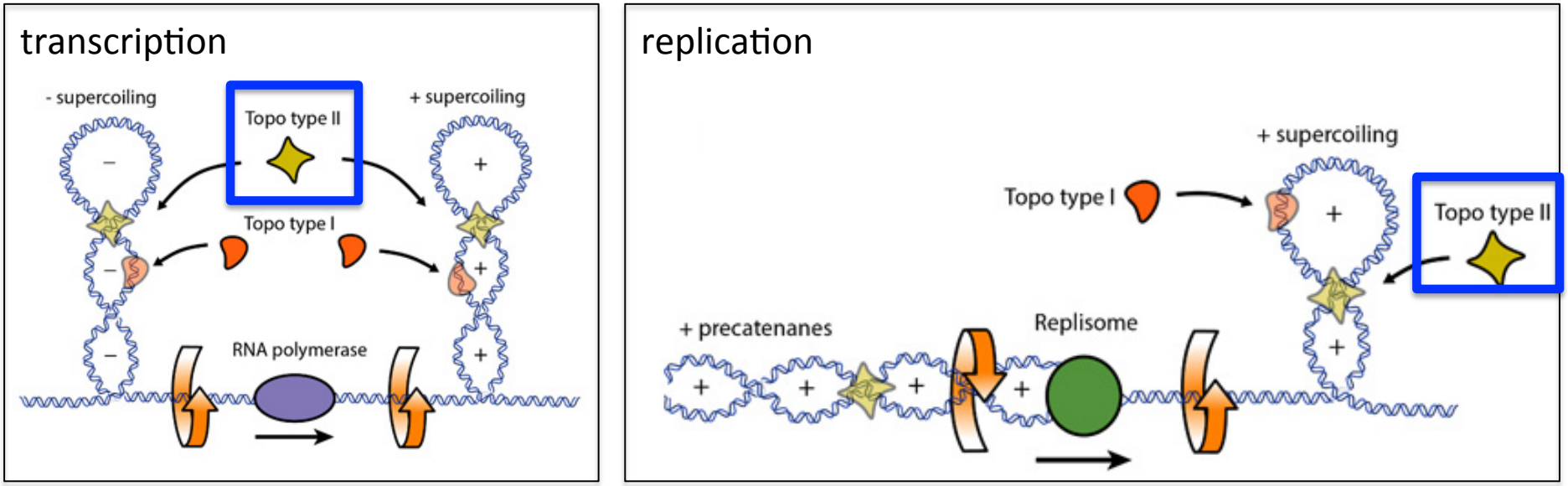


-Much is known about the DNA damage response (DDR), but this a complex signaling cascade that results in many changes in the cell that are not fully understood

-a major DDR is differential gene expression

RNA Transcription and DNA Replication cause DNA supercoiling

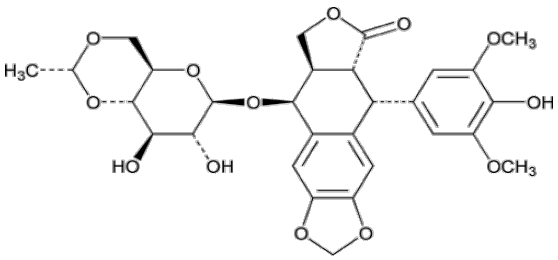
Topo Type II=topoisomerase II enzyme

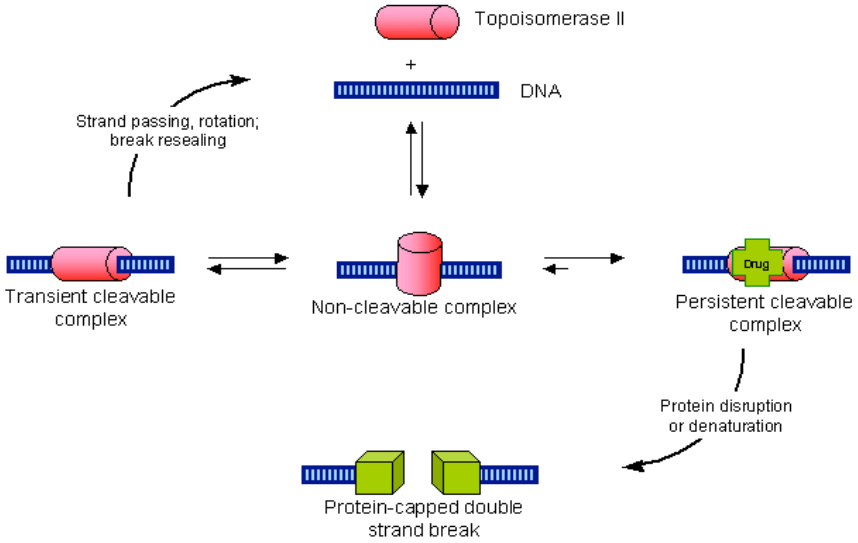


Ma, J. & Wang, M.D. Biophys Rev (2016) 8(Suppl 1): 75. https://doi.org/10.1007/s12551-016-0215-9

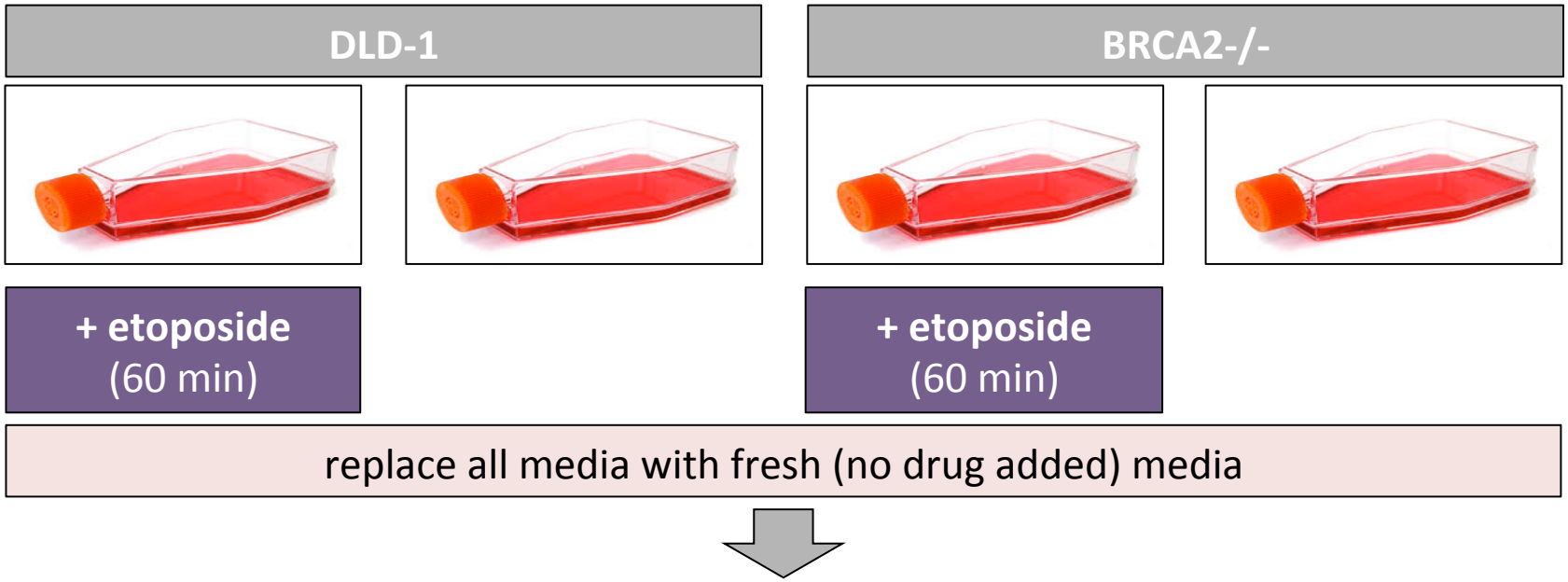
Etoposide is a drug/chemotherapy that causes DNA double strand breaks

- mechanism of action: forms 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



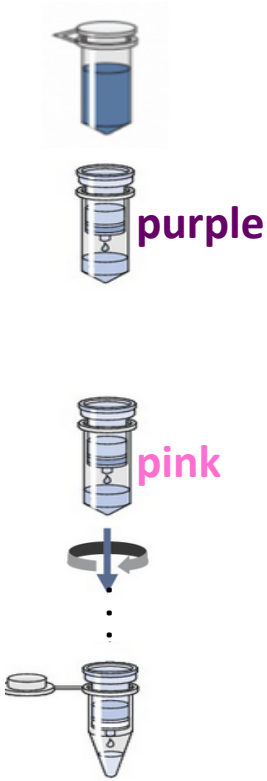


Treat cells with etoposide



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

Isolate RNA: QIAshredder + Rneasy kit

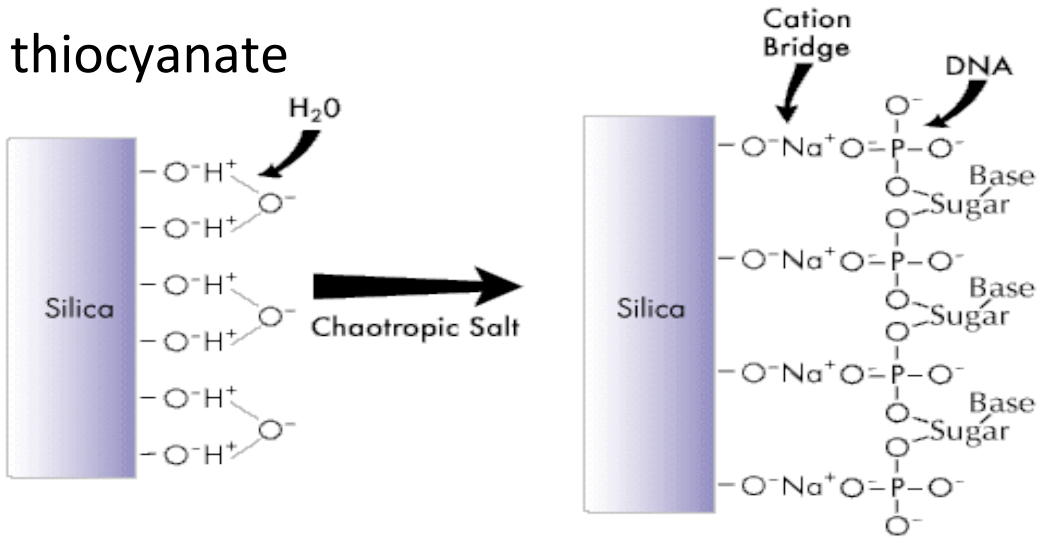


steps	contents	purpose
lyse	RLT (with highly denaturing guanidine-thiocyanate salt) + QIAshredder	inactivate RNase, disrupt membranes, helps bind column homogenize (shear high-MW genomic DNA)
prepare	70% ethanol	promote efficient binding to silica
bind	silica membrane in column	retain mRNA
wash	RW1 RPE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

RLT buffer: composed of detergents and chaotropic (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

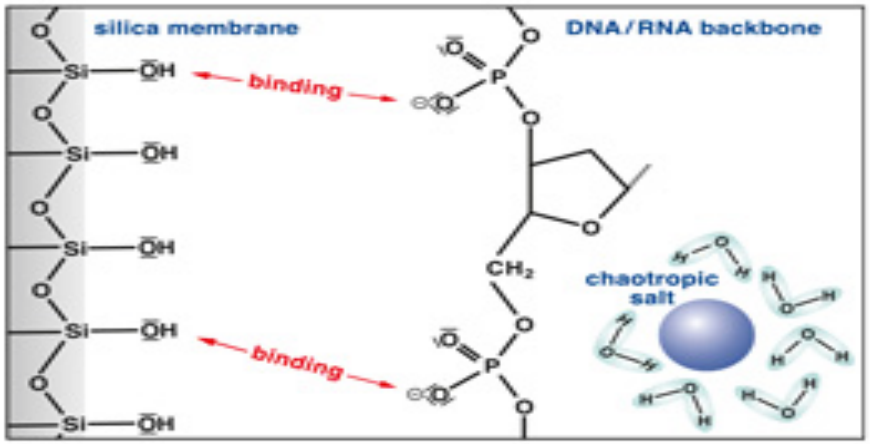


- Washes with RW1 and RPE remove residual contaminants
  - RW1 contains a guanidine salt, as well as ethanol, and is used as a stringent washing buffer that efficiently removes biomolecules such as carbohydrates, proteins, fatty acids, etc, that are non-specifically bound to the silica membrane
  - RPE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

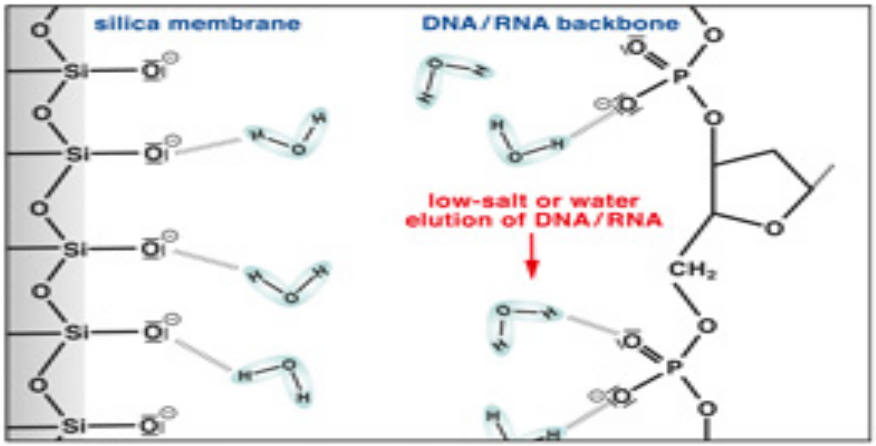
Water is used to elute nucleic acids

- Water competes RNA off of column

before elution



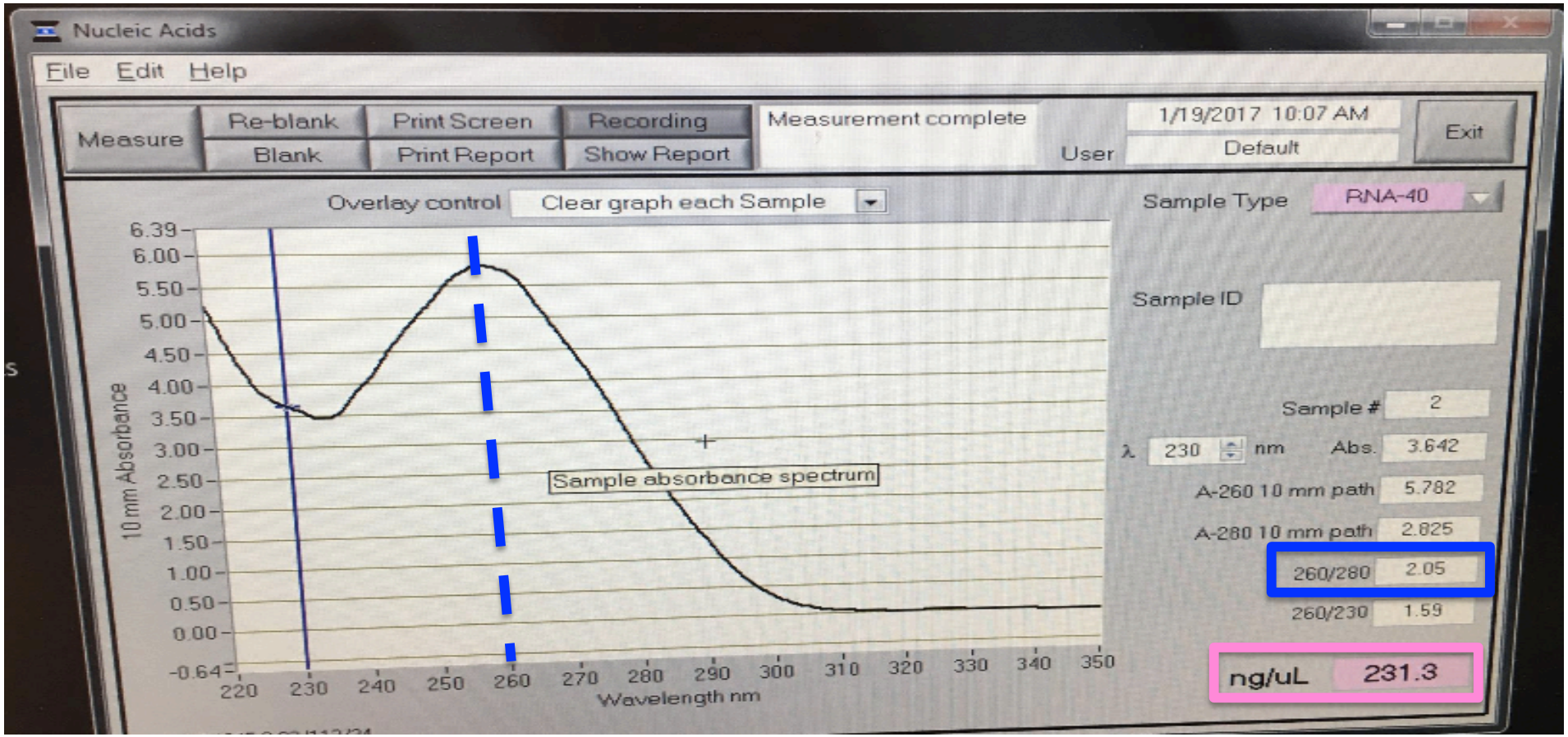
after elution



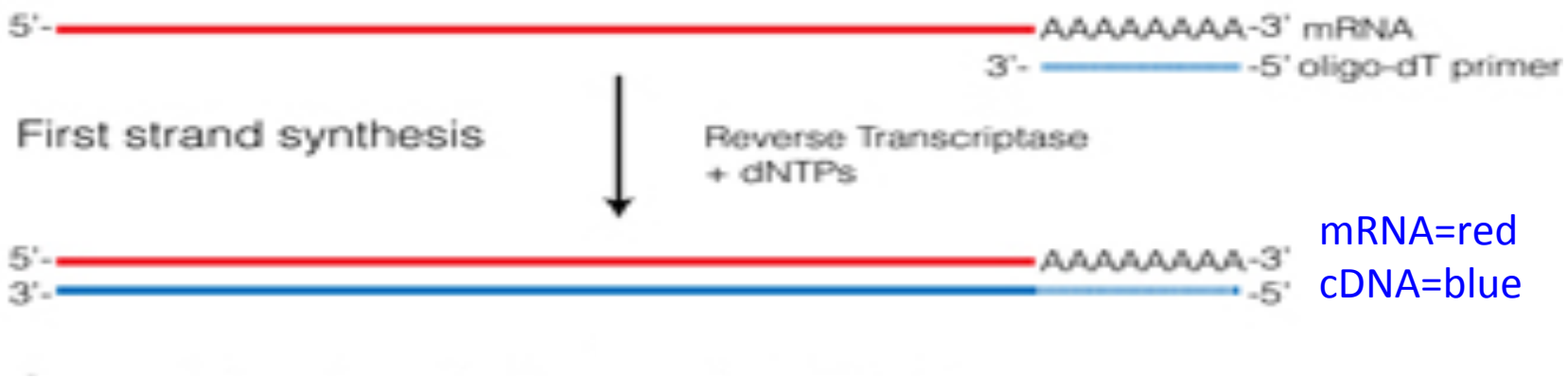
RNA concentration from NanoDrop spectrophotometer

- 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