M2D3: Purify RNA and practice RNA-seq data analysis methods

03/15/2018

1. Prelab discussion
2. ½ class to TC to harvest cells for RNA purification followed by cDNA synthesis
3. ½ group start practice RNA-seq analysis

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  - Do not publish MIT logo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

Purify RNA for quantitative PCR analysis

DAY 1

seed cells

DAY 2

induce DNA damage

DAY 3

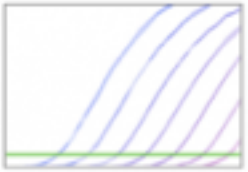
ACAUGGAUUA

↓

TGTACCTAAT

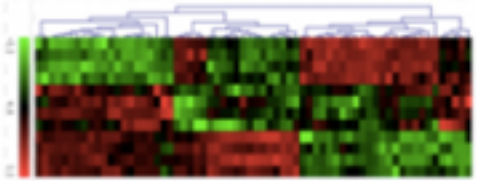
purify RNA and prepare cDNA

DAY 5



measure transcript levels

Analyze RNA-seq results



DAY 4: Evaluate altered gene expression
DAY 5: Investigate public databases
DAY 8: Identify regulatory motifs

Examine effect of drug treatment on cell survival

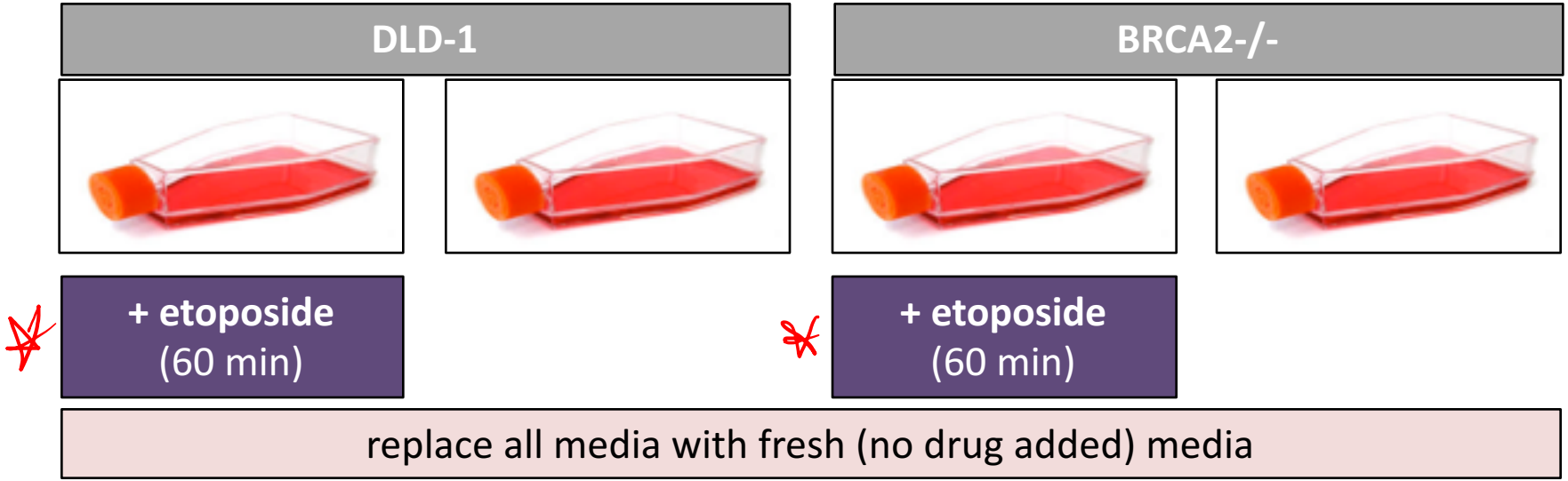
DAY 8

induce DNA damage and treat with drug

DAY 9

measure cell survival

M2D2: (Noreen)Treated cells with etoposide



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

Qiagen 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

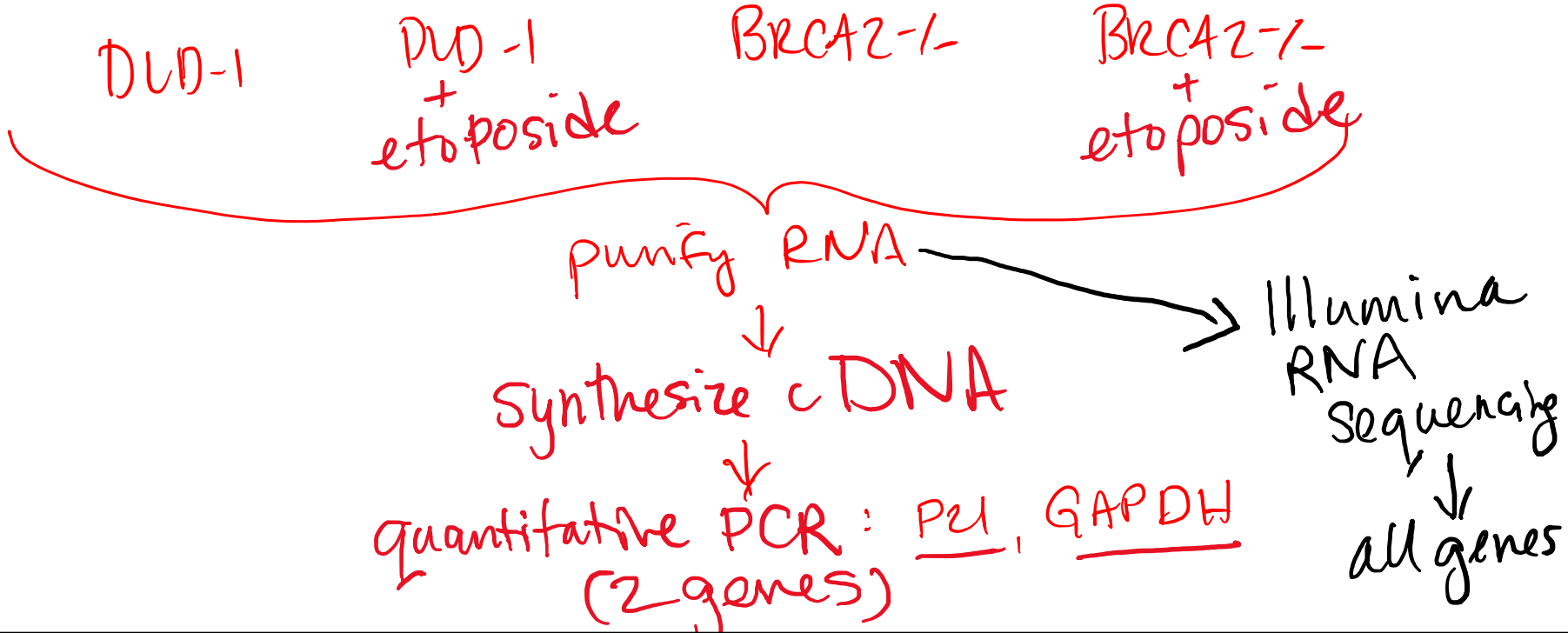
RLT buffer: composed of detergents and chaotropic salts(weakens hydrophobic effects)
Qiashredder: polymer that shears high molecular weight components of the cell
EtOH: RNA insoluble in ethanol, RNA precipitates from cell lysate and binds to silica membrane

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	kills RT enzyme
remove RNA	37°C 20 min	RNase H
Purify cDNA	M2D5	

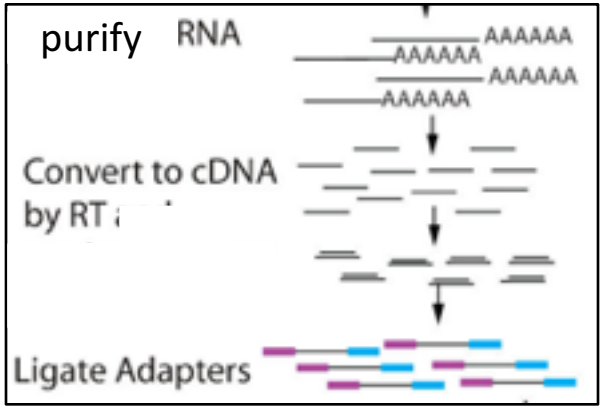
What genes are differentially expressed in response to DNA damage?

How are we addressing this question?

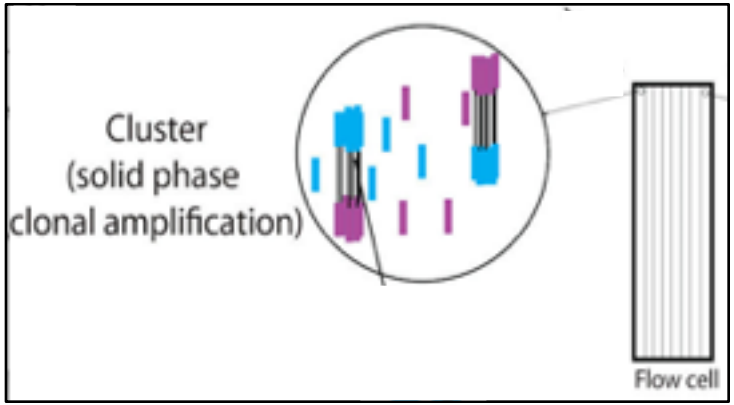


Workflow for Illumina HiSeq 2000

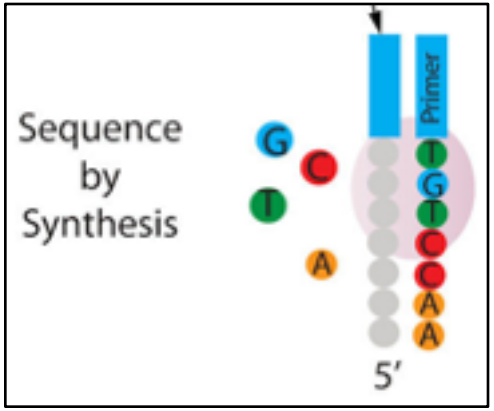
(1) sample prep



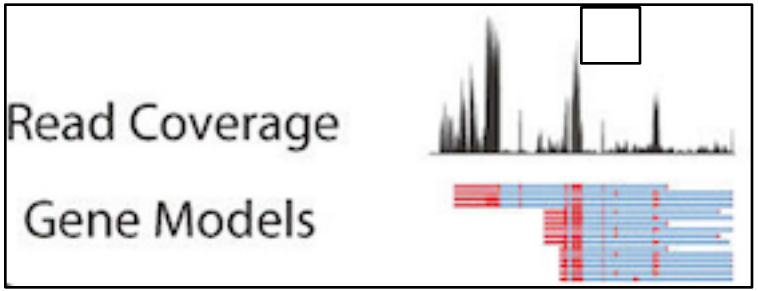
(2) amplification



(3) sequencing



(4) data analysis



Malone JH, Oliver B, BMC Biol. 2011

Reminders:

- M2D4 HW: Methods M2D1-M2D3 (omit practice RNA-seq analysis)
  - NO use of “per manufacturers protocol” for this methods homework
- 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.