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

Announcements/Reminders:
3/17: Mini-presentation due at 10pm
3/18: Blog post due, 10pm

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

M2: Experimental overview

Purify RNA for quantitative PCR analysis

DAY 1

seed cells

DAY 2

induce DNA damage

DAY 3

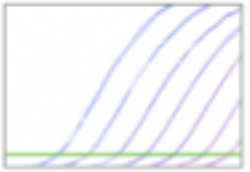
ACAUGGAUUA

↓

TGTACCTAAT

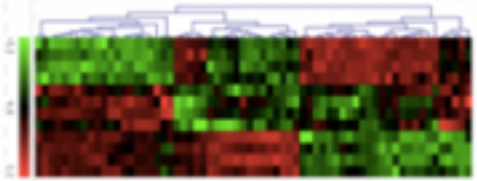
purify RNA and prepare cDNA

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

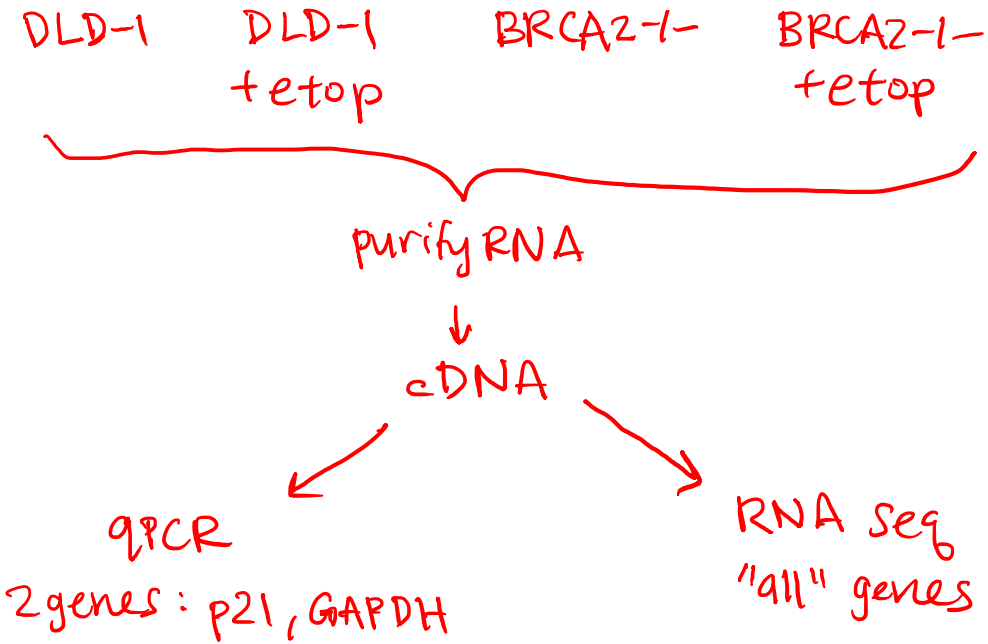
induce DNA damage and treat with drug

DAY 9

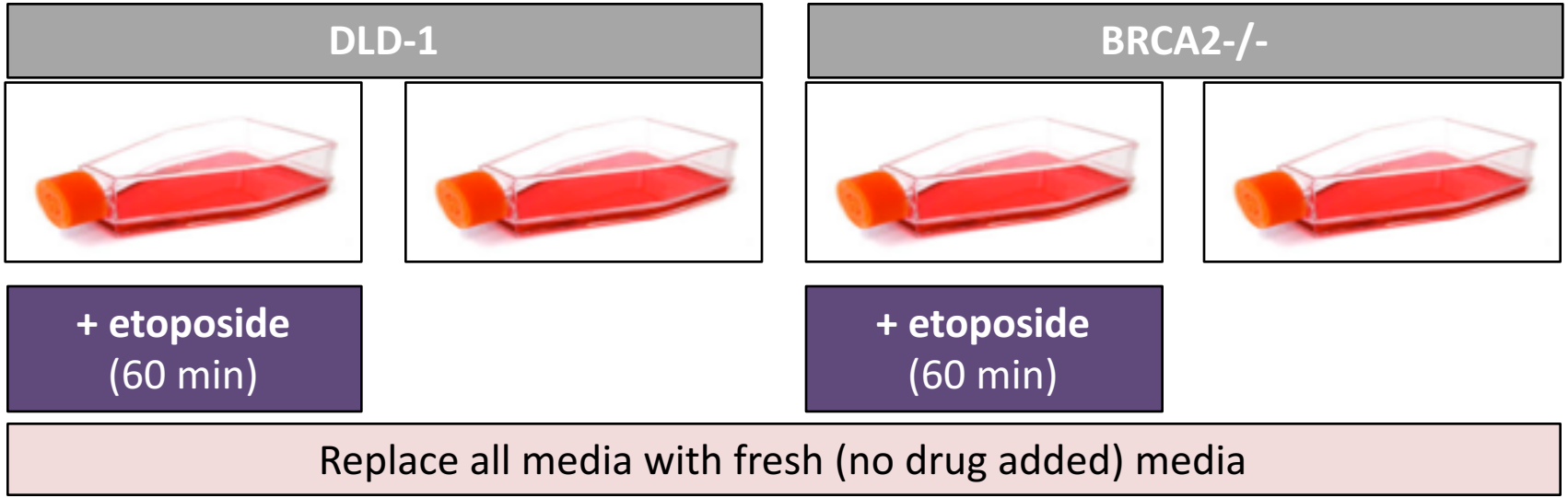
measure cell survival

What genes are differentially expressed in response to DNA damage?

How are we addressing this question?



M2D2: Treated cells with etoposide



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

Insdtructors already extracted RNA from control (no etoposide) flasks

Isolate RNA: QIAshredder + Rneasy kit

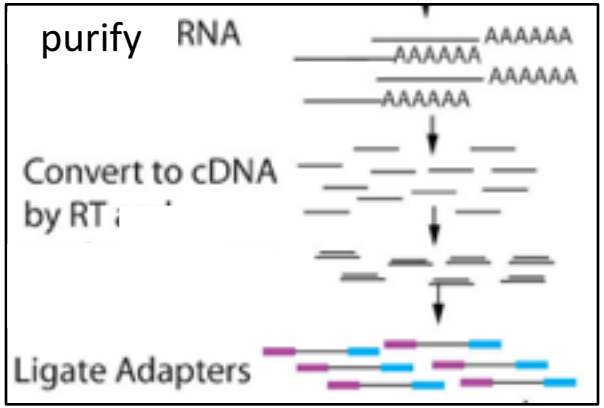
steps	contents	purpose
lyse	RLT (with highly denaturing guanidine-thiocyanate salt) + QIAshredder	inactivate RNase, disrupt membranes, helps bind column homogenize (shear high-MW genomic DNA)
prepare	70% ethanol	promote efficient binding to silica
bind	silica membrane in column	retain mRNA
wash	RW1 RPE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

Components and procedure of cDNA Synthesis

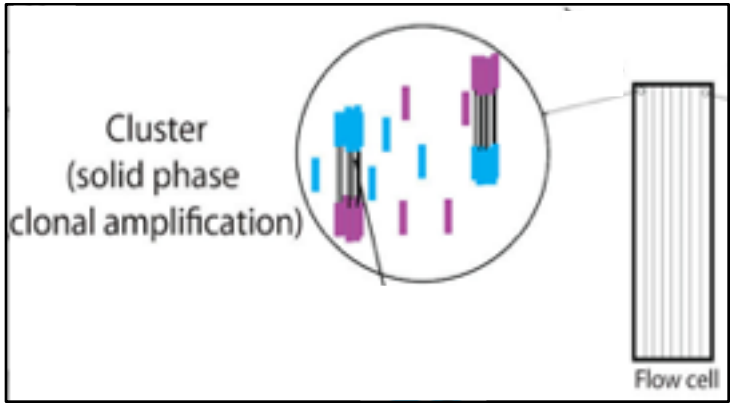
steps	conditions	reagents added
denature & anneal	65°C 5 min on ice 1 min	**1 µg RNA** + oligo $(dT)_{20}$ primer + dNTPs (dATP, dCTP, dGTP and dTTP)
synthesize cDNA	50°C 50 min	Superscript III Reverse Transcriptase $MgCl_2$ DTT RNase OUT (destroys Rnases) buffer added by Instructor
terminate	85°C 5 min	(kills RT enzyme)
remove RNA	37°C 20 min	RNase H
Purify cDNA	M2D5	

Workflow for Illumina HiSeq 2000

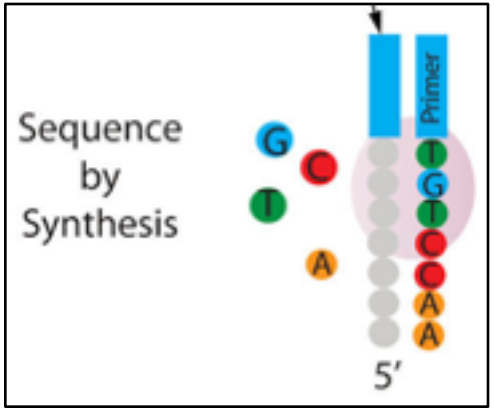
(1) sample prep



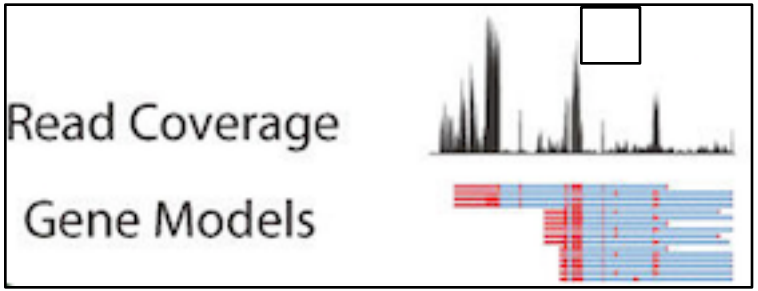
(2) amplification



(3) sequencing



(4) data analysis



Malone JH, Oliver B, BMC Biol. 2011

Sequencing by synthesis (Illumina)

https://www.illumina.com/science/education/sequencing-workflow-accuracy.html

Today in lab

1. Silver, Purple, Pink—TC to harvest cells for RNA purification followed by cDNA synthesis
2. Red, Orange, Green, Blue—start practice RNA-seq analysis

For next time

- M2D4 HW: Methods M2D1-M2D3 (omit practice RNA-seq analysis)
  - NO use of “per manufacturers protocol” for this method homework
- Mini presentation due Saturday March 17th at 10pm.
  - Email video file to bioeng20.109@gmail.com