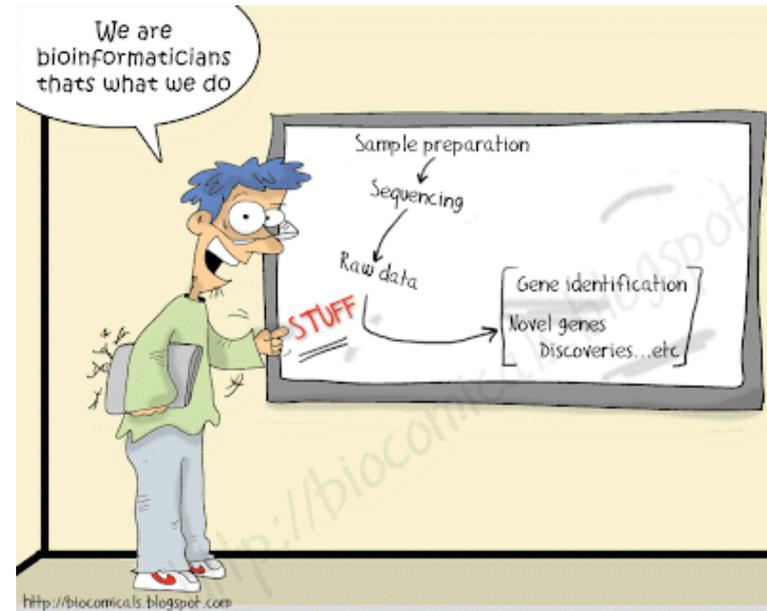


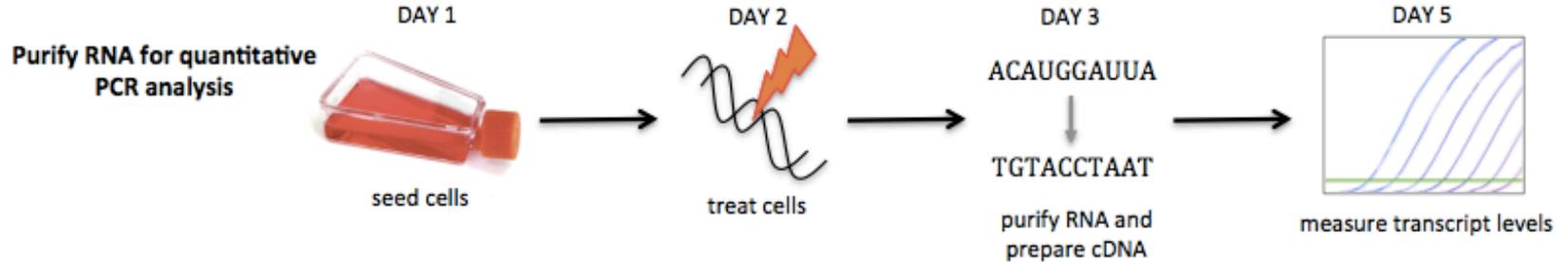
M2D5:

Perform quantitative PCR experiment and explore additional RNA-seq datasets

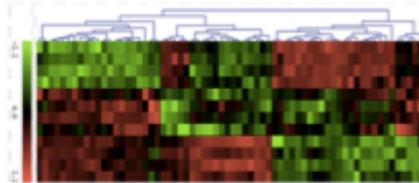
1. Prelab discussion
2. Prepare qPCR reactions
3. Analyze published RNA-seq dataset
4. Begin experimental design



Overview of Mod 2 experiments

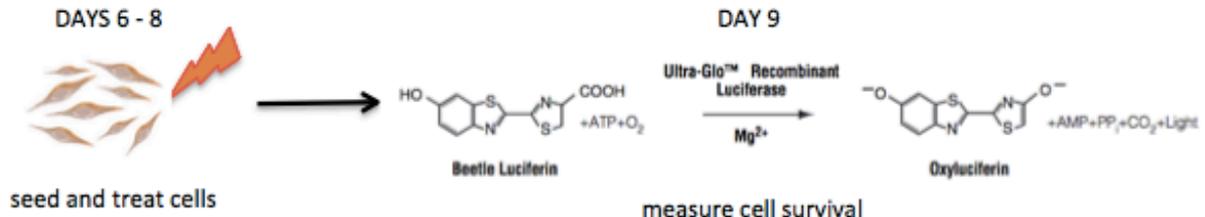


Analyze RNA-seq results



- DAY 4: Evaluate altered gene expression
- DAY 5: Explore public datasets
- DAY 6: Complete RNA-seq data analysis

Design and perform cell viability experiment



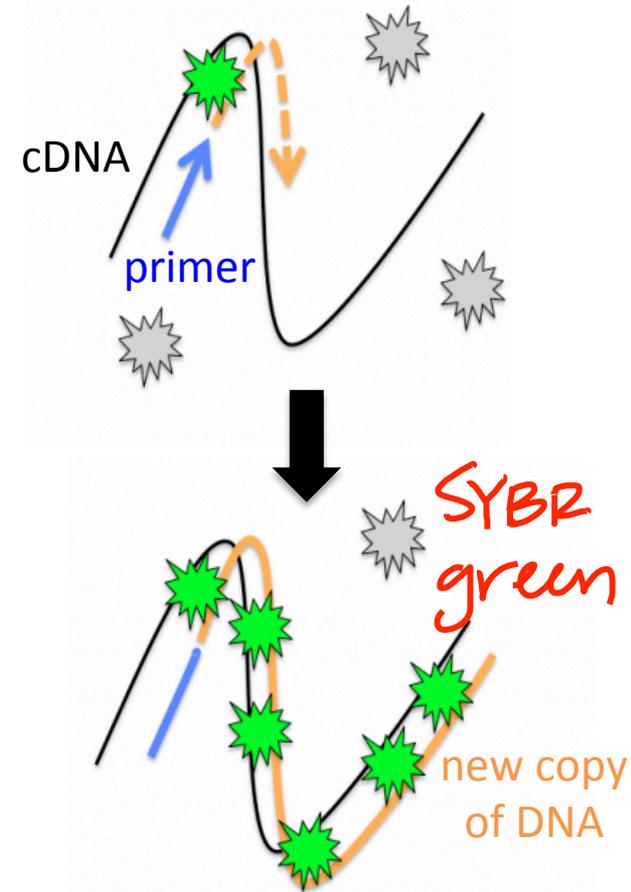
How you will prepare your cDNA for qPCR?

Collect ALL
waste
(flowthrough)
in 50 mL
conical!!

	Steps	Contents / Reagents	Purpose
	prepare	PB buffer	<i>anotropic salts → binding</i>
	bind	column (silica membrane)	
	wash	PE buffer	<i>EtOH</i>
	elute	water	<i>pH = 8</i>

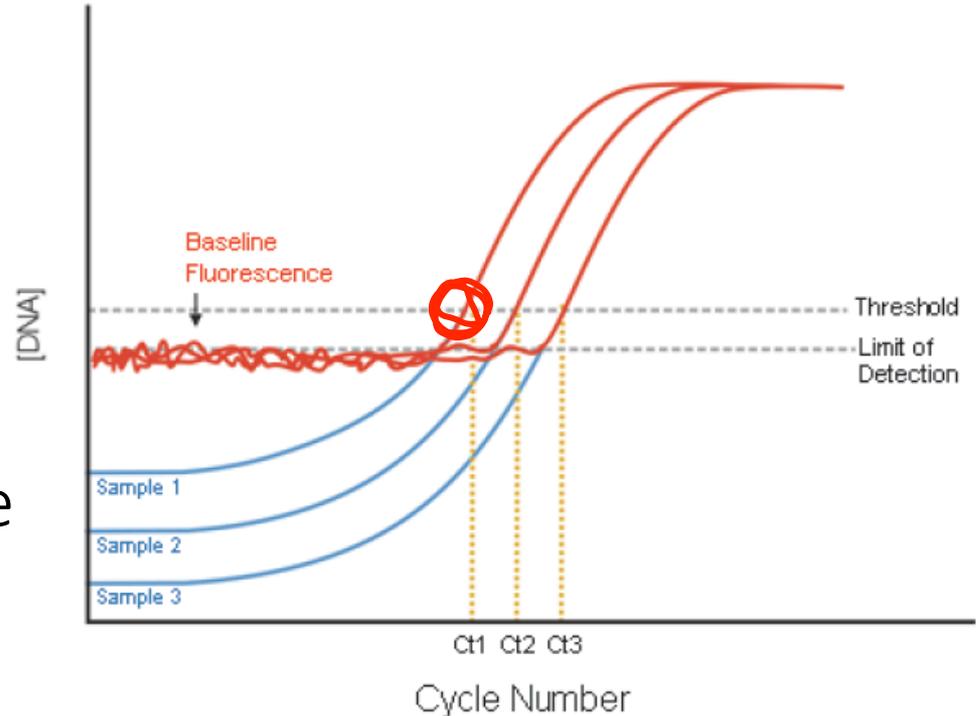
qPCR measures transcript via fluorescent signal accumulation

- Primers designed to amplify specific target sequence / gene
- Dye binds dsDNA and results in fluorescent signal
- Signal measured over several cycles of amplification

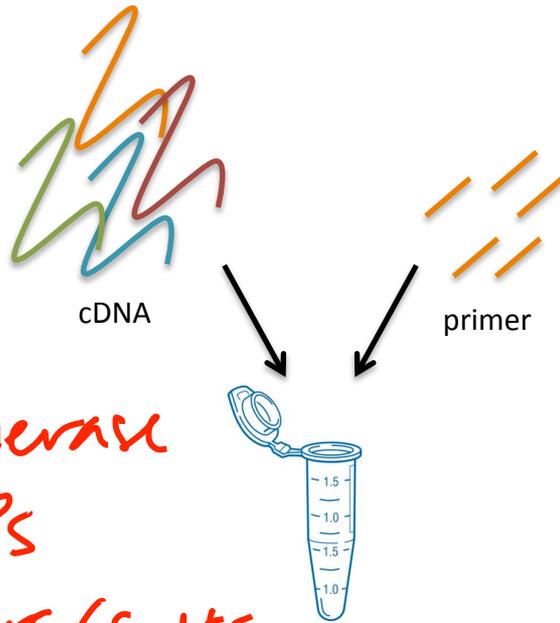


Transcript abundance in original sample related to the C_T value

- C_T = cycle number at which fluorescent signal reaches threshold value
- Determined by baseline fluorescence in the 'run'



Preparing your qPCR reactions



A = DLD-1, GAPDH

B = DLD-1 +etoposide, GAPDH

C = BRCA2-/-, GAPDH

D = BRCA2-/- +etoposide, GAPDH

E = DLD-1, p21

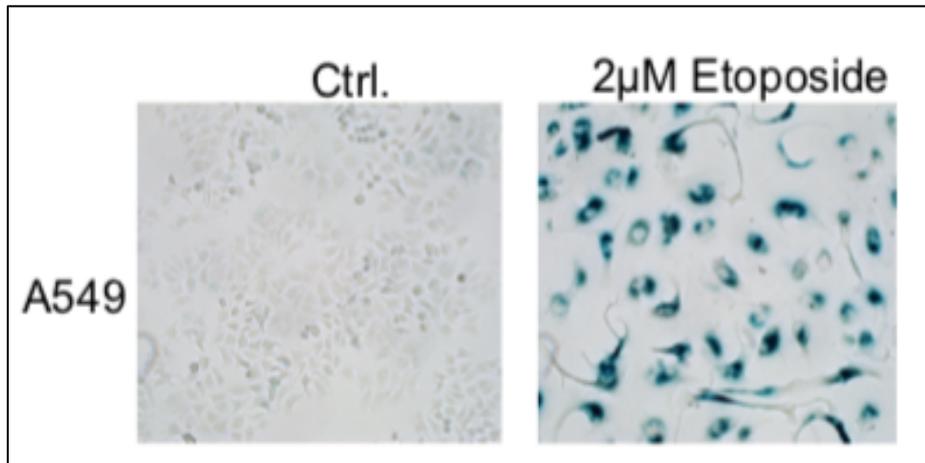
F = DLD-1 +etoposide, p21

G = BRCA2-/-, p21

H = BRCA2-/- +etoposide, p21

Flexing your RNA-seq data analysis muscles

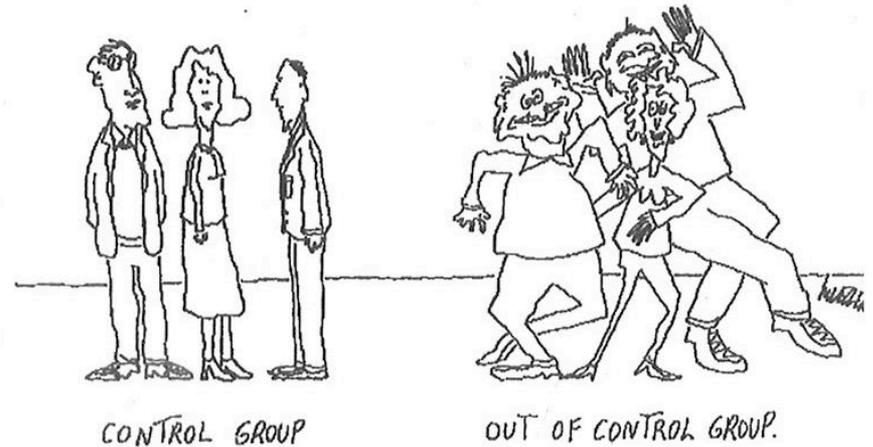
How do the DLD-1 & BRCA2^{-/-} results compare to those for a different cell line treated with etoposide?



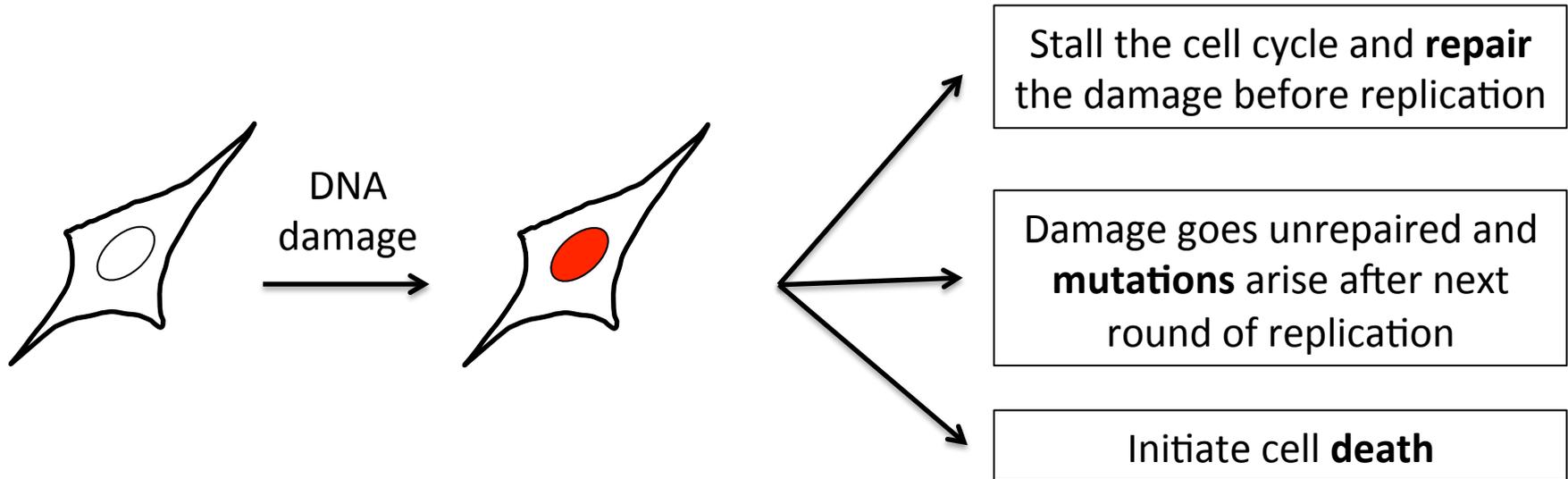
- A549 cells = adenocarcinomic alveolar basal epithelial cells
- Model used in lung cancer research

Designing your cell viability experiment

1. What is your research question?
2. What variable(s) will you test?
3. What control(s) will you include?



Considerations for cell viability experiment



How do these responses to DNA damage relate to cell viability results?

For today...

- Submit experimental design (Part #3) to Noreen!!!
 - Email to nllyell@mit.edu by 10 pm

For M2D6...

- Outline Introduction of your Research article
 - Write the first paragraph in full sentences
- Outline the figures for your Research article

Notes on outlining your figures...

- Do not need to include drafts of actual figures!
- Rather, for each figure you intend to include:
 - 1 sentence describing the figure you will include
 - 1 sentence motivating that figure
 - 1 sentence transitioning to the next figure

Lastly, don't forget about Journal Club!!!

“Reading, or rather decoding, this paper was probably one of the most difficult parts of the process for me. As interesting as the subject matter was, there was a lot of terminology and methods that were used that I was unfamiliar with, and the paper was quite long.”

