

M2D9: Mod2 data analysis

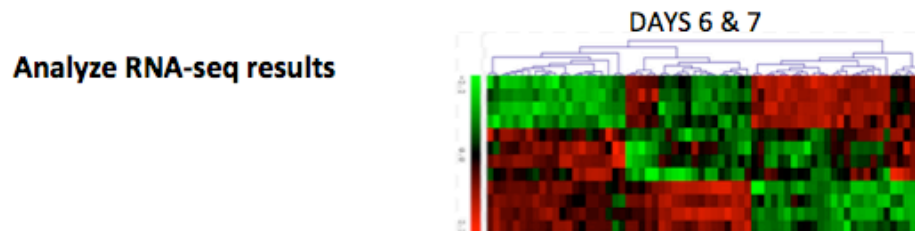
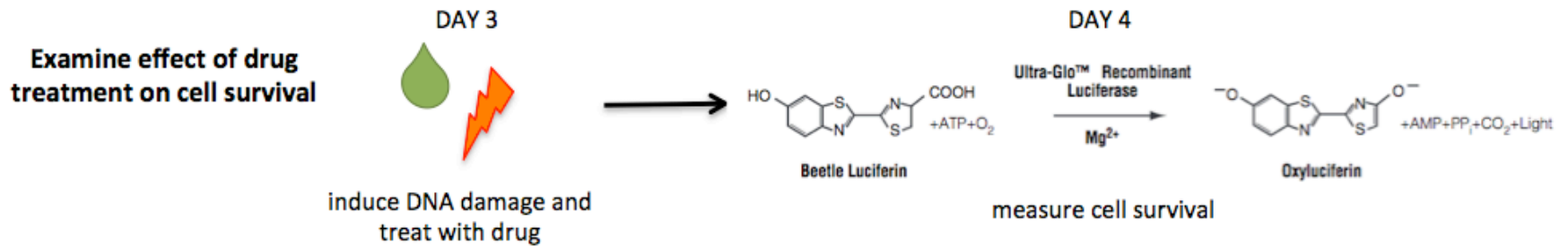
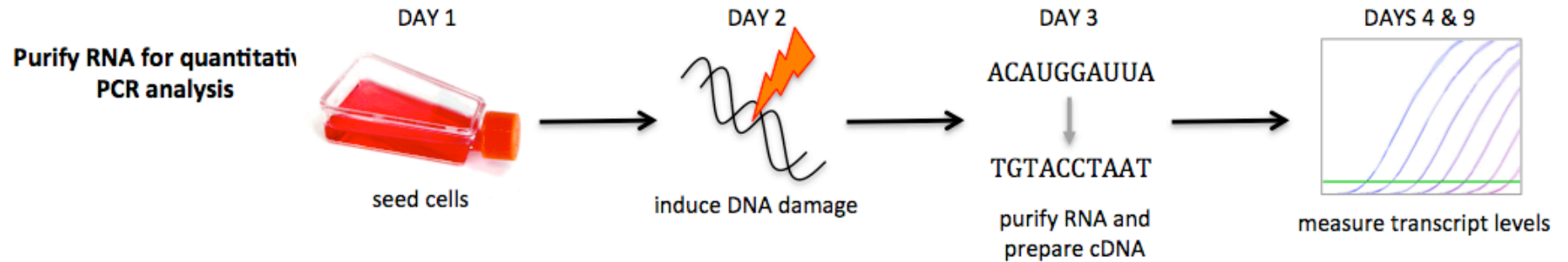
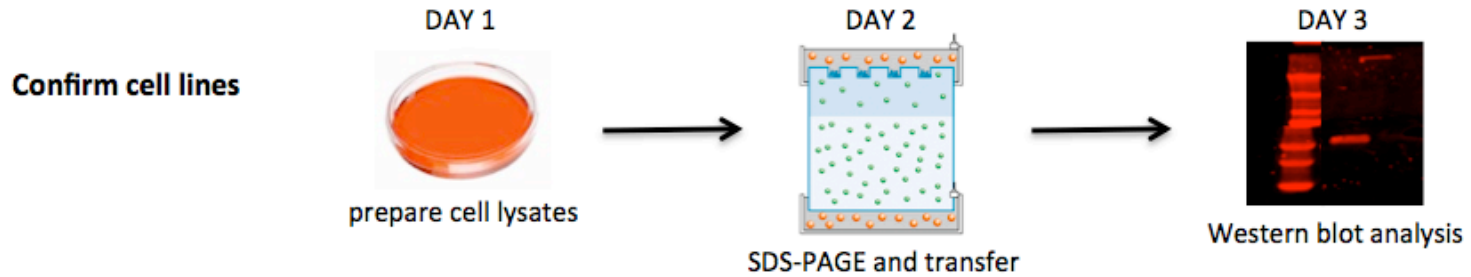
04/14/2017

1. Quiz
2. Prelab Discussion
3. Practice statistics
4. Analyze qPCR data
5. Determine significance of viability assay



CRAFT your STORY

M2: Experimental overview



From Prof. Samson's lecture 03/09/17:

What experimental question will you ask in Module 2? ****hypothesis****

How does DNA repair affect the ability of cancer chemotherapy drugs to kill cancer cells?

How does cancer chemotherapy affect gene expression?

This raises the following questions

- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?

Wrapping up M2!

- Lab notebook:
 - M2**D7** (RNA-Seq data analysis) graded by Rob at 10pm tonight
- Blog posts
 - on journal club due 10pm on Saturday, April 15
 - on M2 in general due 10pm on Sunday, April 23
- M2 research article **20%**
 - due 10pm on Saturday, April 22
 - **extra office hours:** (56-302)

Monday	no office hours	Noreen
Tuesday, April 18	9:30-11:30am	Leslie
	3:00-5:00pm	Noreen
Wednesday, April 19	9:30-11:30am	Leslie
	12:00-2:00pm	Maxine
	2:00-5:00pm	Noreen
Thursday, April 20	9:30-11:00pm	Maxine

Plot error bars as 95% confidence intervals (CI)

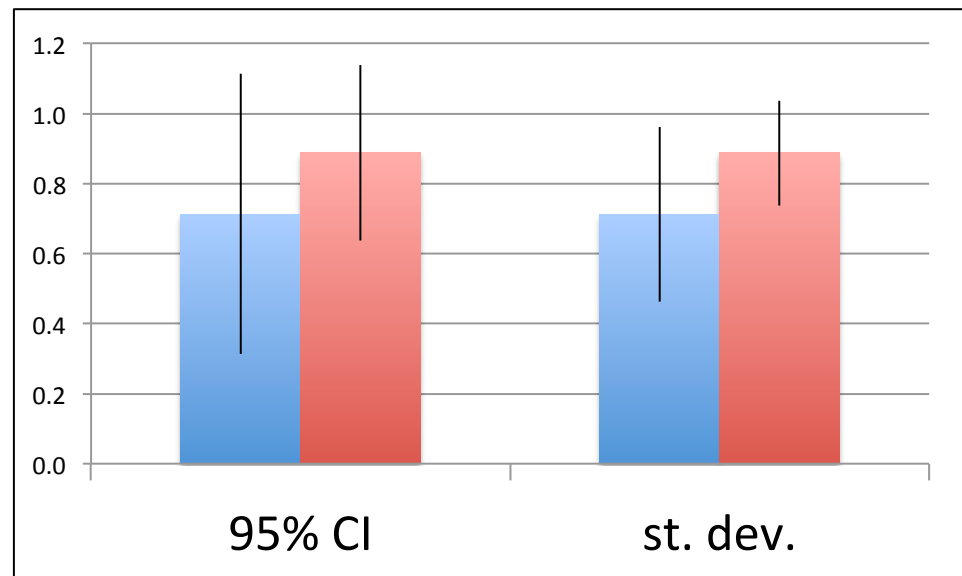
- **95% CI:** the true value of the parameter will be within this interval 95% of the time if the experiment is repeated
- In Excel, find lower and upper bounds:

$$\bar{x} \pm \frac{t_{table} * stdev}{\sqrt{n}}$$

$$t_{table} = TINV(0.05, n - 1)$$

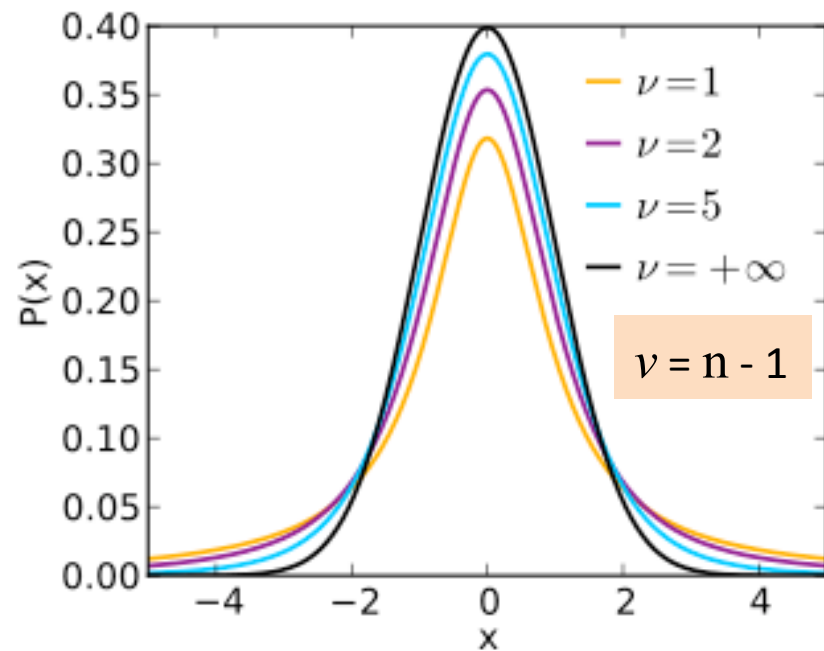
degrees of freedom

0.05 complement of 0.95



Student's t -distribution and t -test

- “Student”
 - pen name of William Sealy Gosset (Guinness brewery in Dublin !)
- t -distribution
 - symmetric and bell-shaped probability distribution, with heavier tails than the normal (Gaussian) distribution
 - to estimate the mean when sample size (n) is small and standard deviation unknown
- t -test
 - the test statistic follows a t -distribution under the null hypothesis
 - used to determine if two sets of data are significantly different



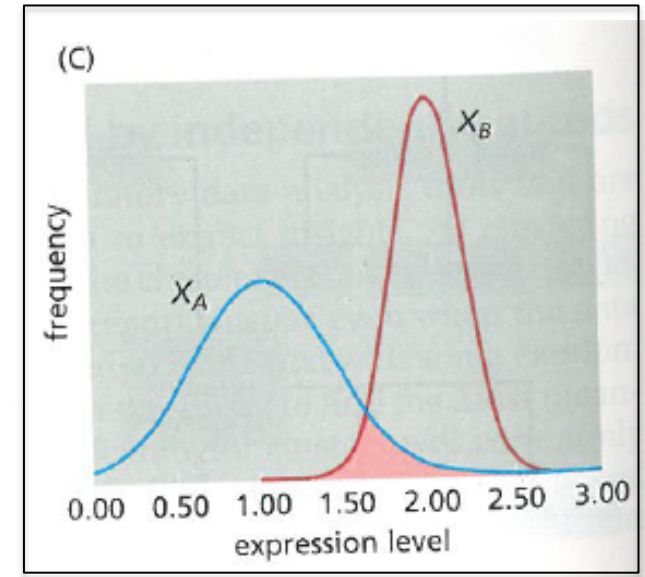
t-test in Excel

- How certain are you that two populations are different?

$$p = TTEST(array1, array2, 2, 3)$$

2-tailed

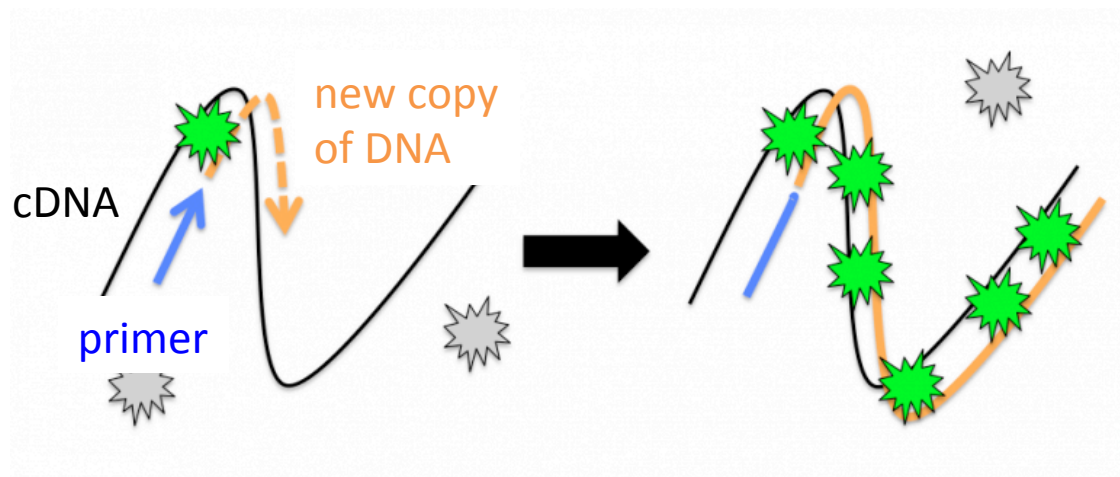
unequal variance (heteroscedastic ☺)



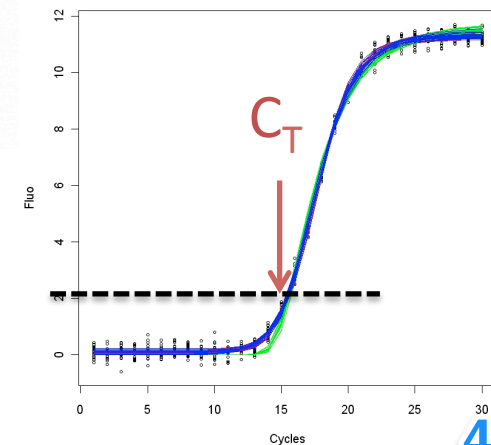
- ✧ The Student's *t*-test only applies to **two** data sets.
Only compare two conditions at a time.
- $p < 0.05$
 - typically considered “statistically significant”
 - the two data sets are different, have a different mean

Analyze quantitative PCR data

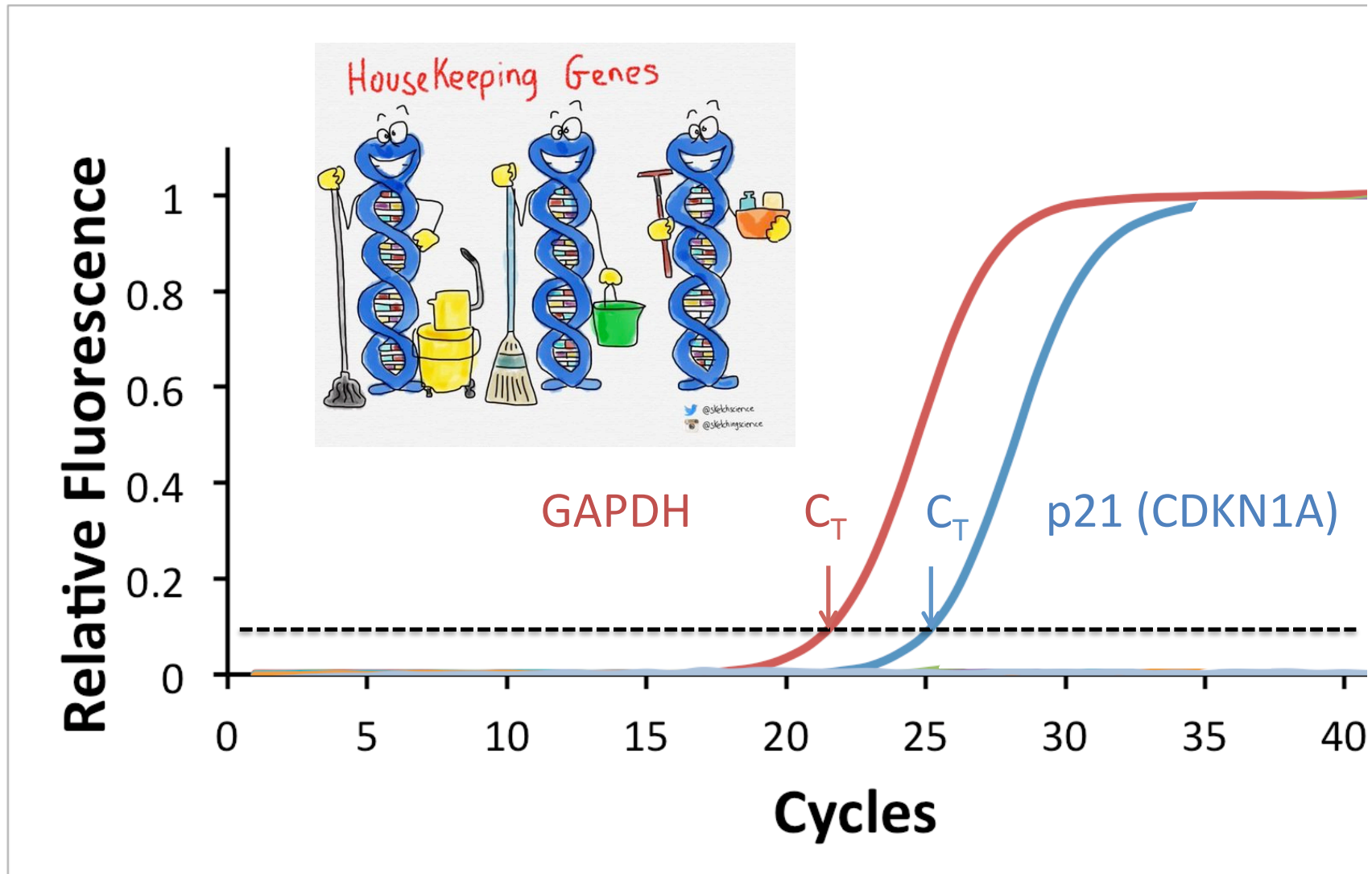
- Monitor PCR as it occurs
 - using dye that is fluorescent when DNA is **double-stranded**
 - **signal** proportional to initial amount of cDNA (= original RNA)



- Plot fluorescence vs. cycle number
- Extract C_T threshold cycle



C_T value \sim amount of cDNA template present at the start of the amplification reaction



What do we calculate from these C_T values?

$$\Delta C_T = C_T (\text{p21}) - C_T (\text{GAPDH}) \quad \text{+exponentially transform}$$

	DLD-1	DLD-1 + etoposide	BRCA2-/-	BRCA2-/- + etoposide
1	$2^{-\Delta C_t}$	$2^{-\Delta C_t}$	$2^{-\Delta C_t}$	$2^{-\Delta C_t}$
2	$2^{-\Delta C_t}$	$2^{-\Delta C_t}$	$2^{-\Delta C_t}$	$2^{-\Delta C_t}$
3	$2^{-\Delta C_t}$	$2^{-\Delta C_t}$	$2^{-\Delta C_t}$	$2^{-\Delta C_t}$
average	plot the average			

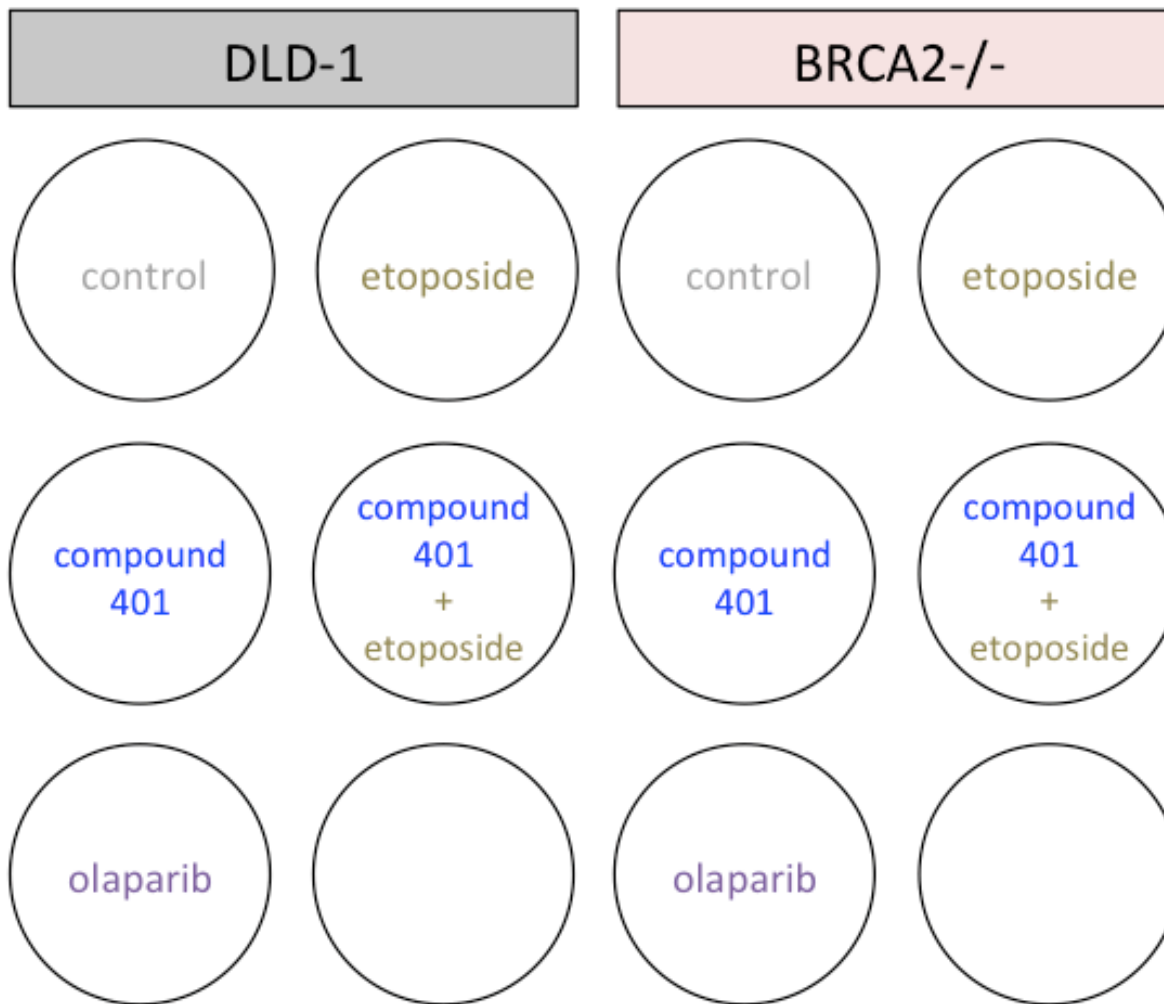
- Compare p21 mRNA levels in 4 conditions, 3 replicates

Analyze cell viability data

- etoposide:
creates double-stranded
breaks

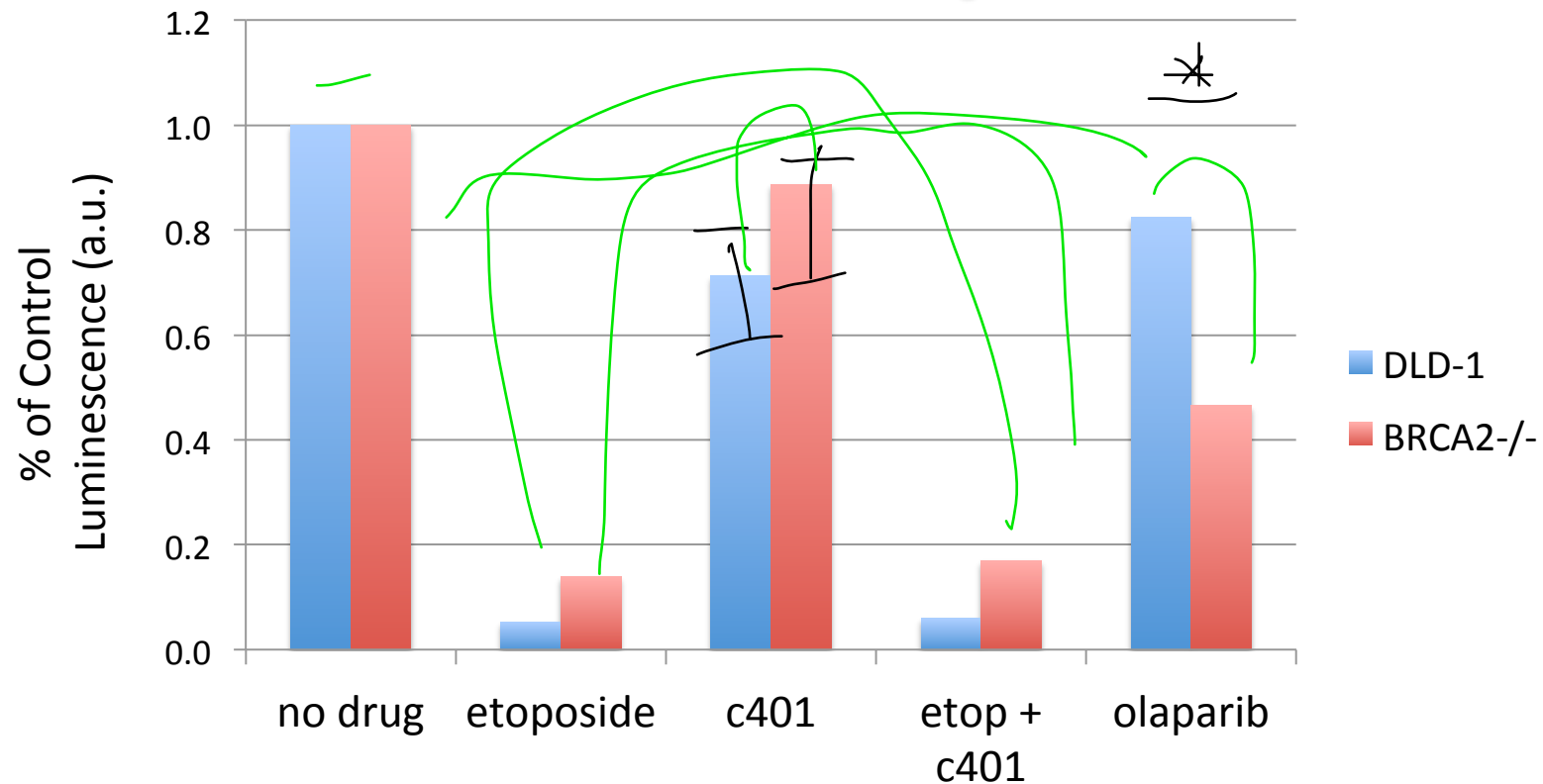
- compound 401:
inhibitor of DNA-PK
NHEJ

- olaparib:
inhibitor of PARP
BER



Analyze cell viability data, with error bars and find the statistical significance

use TR and WF data (delete TR blue first excel)



Today in lab

1. Practice statistics
2. Analyze qPCR data
3. Analyze CellTiter Glo cell viability data
4. Polish lab notebook M2D7

❖ Review Prof. Samson's M2L8 lecture for ideas

- In Discussion / Future Directions, propose 2 years of research
 - to further elucidate DLD-1 / and BRCA2-/- genotype
 - and/or to continue answer “How does DNA repair affect the ability of chemotherapeutic drugs to kill cancer cells?” and “How does chemotherapy affect gene expression?”