

M2D7: Perform quantitative PCR experiment and explore additional RNA-seq dataset

1. Email distribute Quiz, due on Stellar at 10pm
2. Prelab discussion
3. Review qPCR experiment
4. Statistical analysis exercise
5. Continue working on R.studio.cloud Ex3

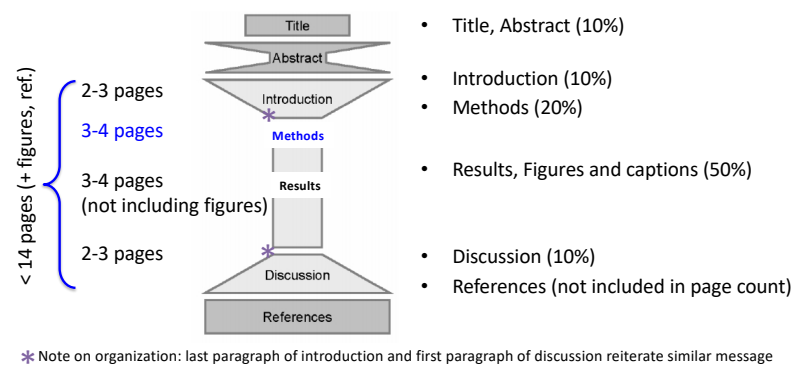
1

Mod2 major assignments

- **Research Article (20%)**
 - individual, submit on Stellar
 - due Monday April 20th at 10pm
 - format: word document, figures can be submitted separately
- **Journal Club Presentation (17.5%)**
 - presentation **slides** due on Stellar April 11th 10pm
 - Presentation **video** due to Dropbox April 11th 10pm (details to follow)
 - format: powerpoint or pdf
- Lab quizzes M2D7, M2D9
- Homework and Notebook (10%)
- Blog (5%), 3 posts for full credit
 - 4/6 at 10 pm, 4/13 at 10 pm, 4/21 at 10 pm, 5/12 at 10pm

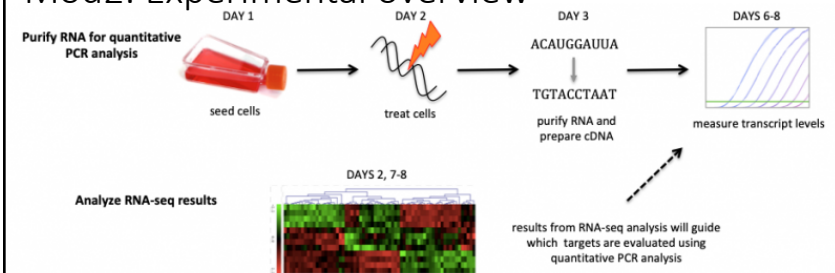
2

Mod2 Research Report (20% of final grade)



3

Mod2: Experimental overview

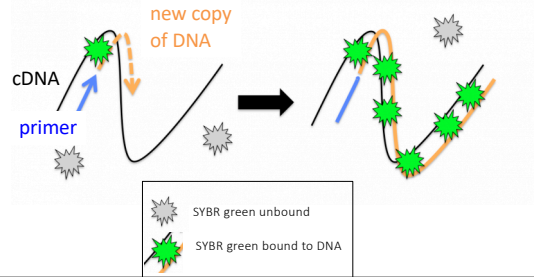


- What genes are differentially expressed in response to DNA damage?
- Why are you using qPCR to measure individual gene expression?
- How does the qPCR data relate to the RNA-seq data?

4

qPCR: quantitative polymerase chain reaction

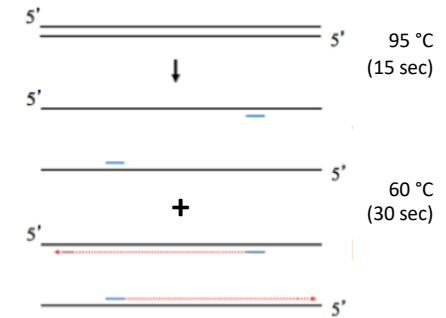
- Monitor PCR product with fluorescence
 - using dye (SYBR green) that is fluorescent (green below) when bound to double stranded DNA; little or no fluorescence when not bound to dsDNA (grey below)
 - signal proportional to initial amount of cDNA (-> mRNA -> _____)



5

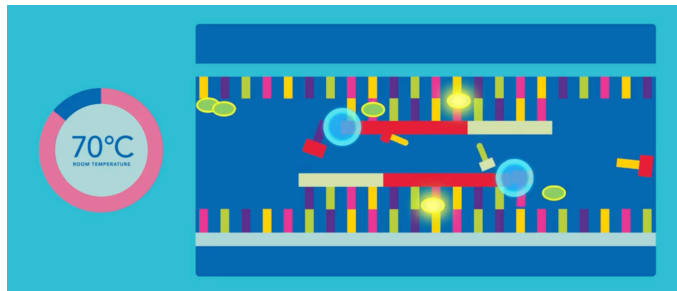
iQ SYBR Green Supermix qPCR reagents and cycling conditions

PCR ingredients
cDNA mix (template)
buffer and water
sequence-specific primers
iTaq DNA polymerase
dNTPs



6

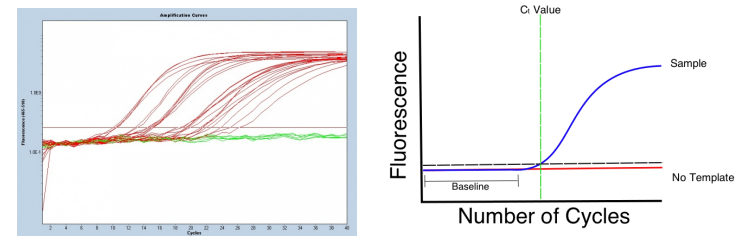
<https://www.youtube.com/watch?v=GCzH2Wcvd8E>



7

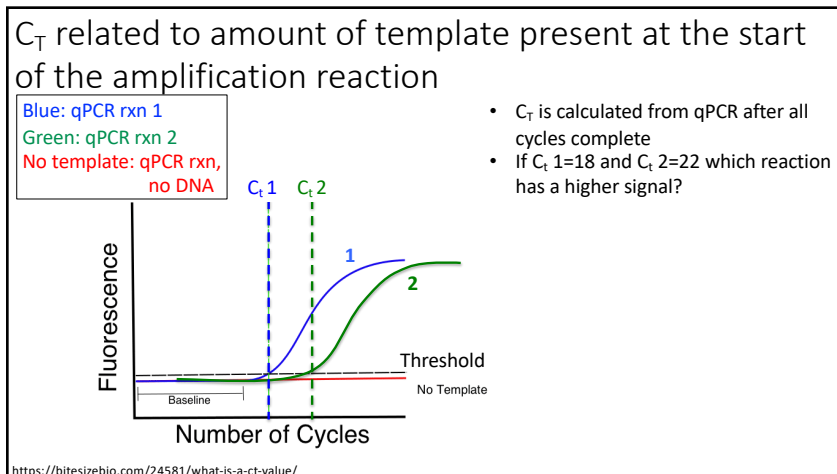
qPCR data output is the threshold cycle (C_T)

- Plotted as Fluorescence vs. cycle number
- C_T threshold cycle
 - fluorescent signal significantly above the background fluorescence
 - relative measure of the initial number of copies of cDNA



<https://bitesizebio.com/24581/what-is-a-ct-value/>

8

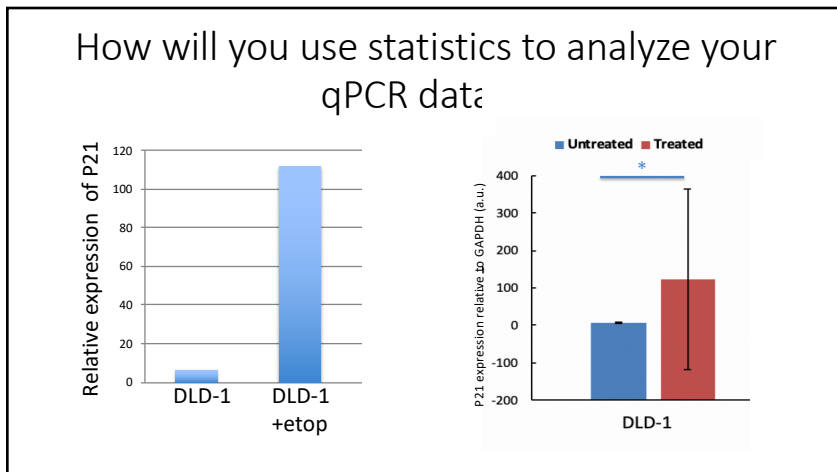


9

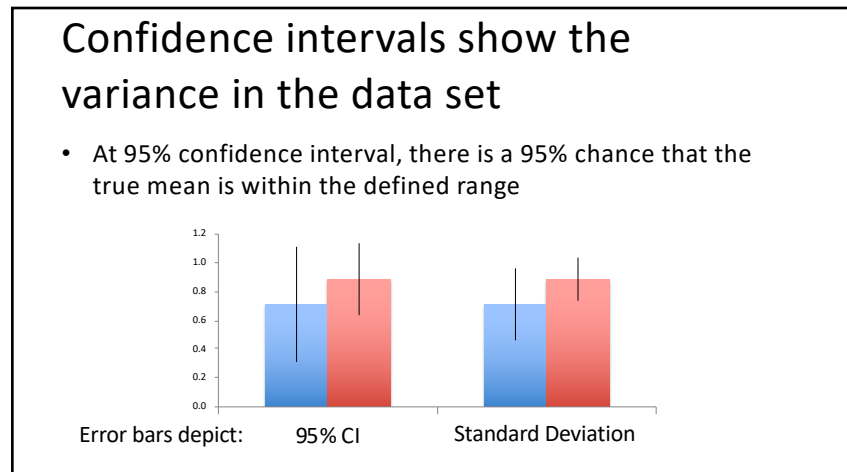
Practically-- Calculating ΔC_T for a gene

- The analysis output of a qPCR reaction is a C_T value
 - You can not directly compare C_T values of samples due to variation between qPCR reactions and experiments
 - In the excel sheet there are three C_T values for each gene that represent three triplicate wells in the experiment
 - The three tabs in the excel represent 3 experiments carried out on different days
- You must normalize your C_T to a gene you know should not change between your samples and in response to treatment
 - We chose the abundant housekeeping gene TBP, TATA binding protein
 - You need to use the TBP C_T values in the experiment you chose to calculate (TBP on the same tab of the excel)
- Finally exponentially transform each normalized value to the ΔCT expression

10



11



12

Calculating Confidence interval in excel

= CONFIDENCE.t(confidence level, standard dev., size)

↑
0.05

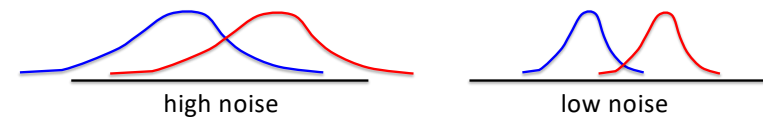
↑
Need to calculate
standard deviation
in separate cell
=STDEV

↑
n

13

Student's *t*-test used to determine if populations are significantly different

- Assume data follows *t*-distribution
- At $p < 0.05$, there is less than a 5% chance that populations are the same (95% chance that populations are different)
- Examines signal (means):noise (variance) ratio



14

Calculating Student's *t* in excel

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

Use the fewest assumptions: two-tailed
unequal variance

Can only compare two data sets at a time
*Make sure it is clear on your plots/writing which conditions are being compared

15

M2D7 "Lab" Checklist

1. Watch qPCR video at the top of the Protocol section and read through Part 1.
 2. Calculate the ΔC_T values for the 4 genes you would like to investigate further
 3. Practice calculating confidence intervals and p-values with the Student's *t*-test
 - We suggest practicing using the ΔC_T values you will use for your research article
- Homework due M2D8: Peer review methods
 - We will email you another students methods today
 - You should comment on the methods similarly to instructor's feedback – add a number to the place you'd like to comment and submit a separate document with comments
 - There are overview questions in the homework prompt you should address at the bottom of your specific comments

16