

M2D8:Cell viability; quantitative PCR; identification of regulatory motifs

04/10/2018

1. Treat cells with DNA damaging agents and inhibitors for cell viability
2. Analyze qPCR results
3. R: Identify regulatory motifs in RNA-seq data

Extra Office Hours

- Leslie: Tuesday April 17th 56-322 (lab),
10:30am-1:30pm
- Josephine: Wednesday April 18th 56-322,
10:00am-1:00pm
- Noreen: Tuesday and Wednesday 56-322,
2:00-4:00pm

Mod2 Research Report (20% of final grade)

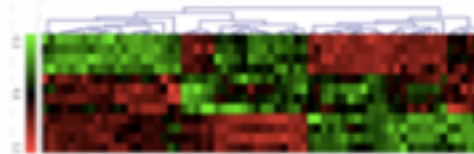
Due Saturday 4/21 at 10pm

- Title, Abstract
- Introduction
- Methods
- Results (Figures and captions)
- Discussion
- References

Last week of Mod2!

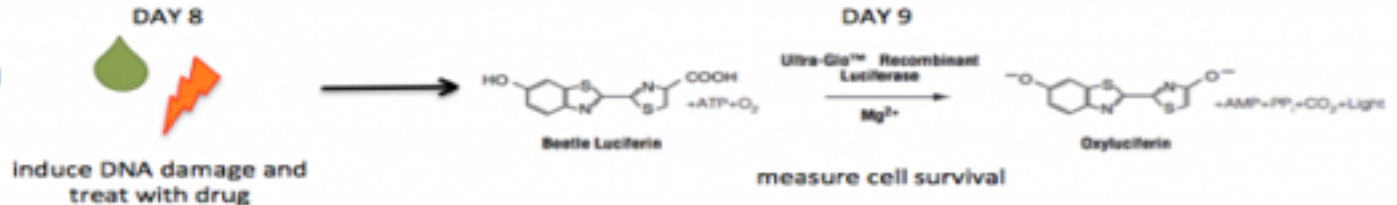


Analyze RNA-seq results



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

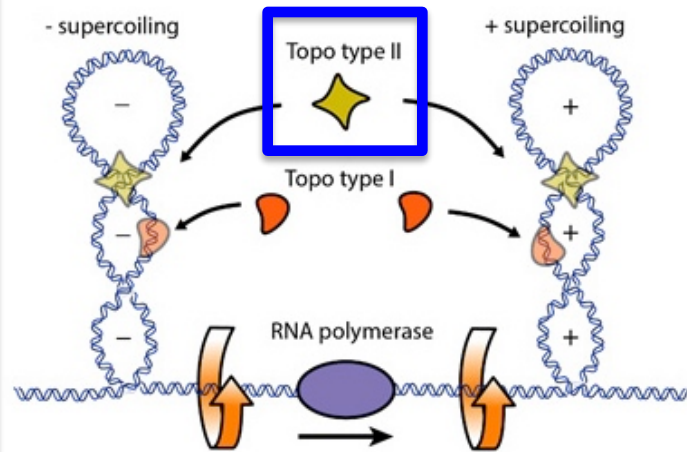
Examine effect of drug treatment on cell survival



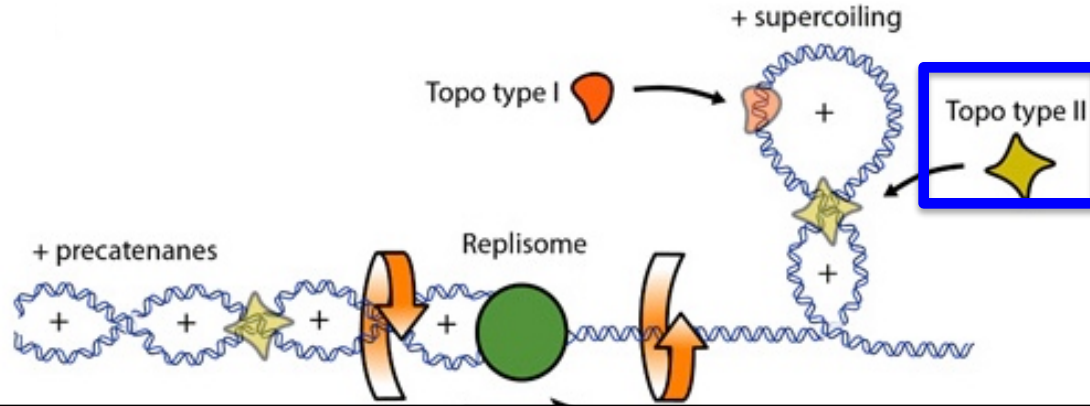
Etoposide is a drug/chemotherapy that causes DNA double strand breaks

- mechanism of action: forms a ternary complex with DNA and topoisomerase II enzyme and prevents re-ligation of the DNA strands = dsDNA strand break
- cancer cells (quickly dividing cells) rely on topoisomerase II more than normal cells

transcription



replication



Measuring synthetic lethality in our parental and BRCA2-/- cell line

What is synthetic lethality? *Combination of deficiencies (knockout, LOF, drug treatment) in 2 or more genes leads to cell death*

What parallel pathways are we perturbing in this experiment?

*double strand breaks → HR (BRCA2-/-)
→ NHEJ (drug - top. or mib.)*

What is the output of this assay?

*living cells
cell titer glo: measures ATP → light signal*

Six compounds identified that target NHEJ and / or HR

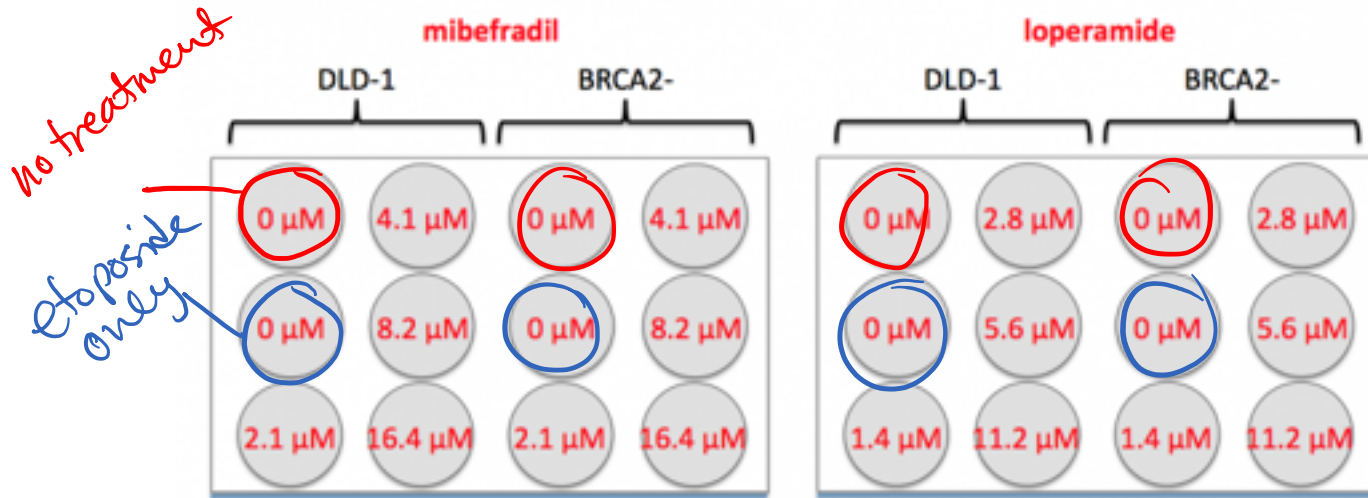
1 = DMSO, no effect
0 = total loss of activity

Drug name	Repair activity	
	NHEJ	HR
Pimozide	0.28	0.55
→ Loperamide	0.20	0.57
→ Mibefradil	0.28	0.57
Etoposide	0.65	0.08
SR 59230A	0.27	0.58
AMN082	0.19	0.92

- Loperamide = slows contractions of intestines, treatment for gastrointestinal ailments
- Mibefradil = blocks calcium channels, treatment for heart conditions

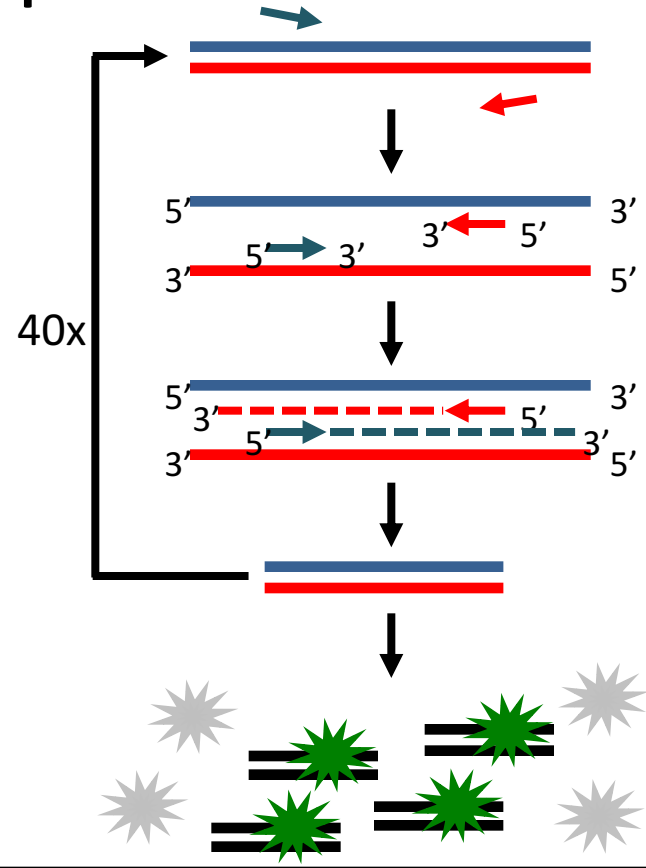
Synthetic lethality part 1: experiment overview

1. Choose miberfradil or loperamide, sign up at front bench
2. Induce double strand breaks (etoposide 37°C for 60min)
3. Remove etoposide media and incubate with appropriate concentration of miberfradil or loperamide till M2D9

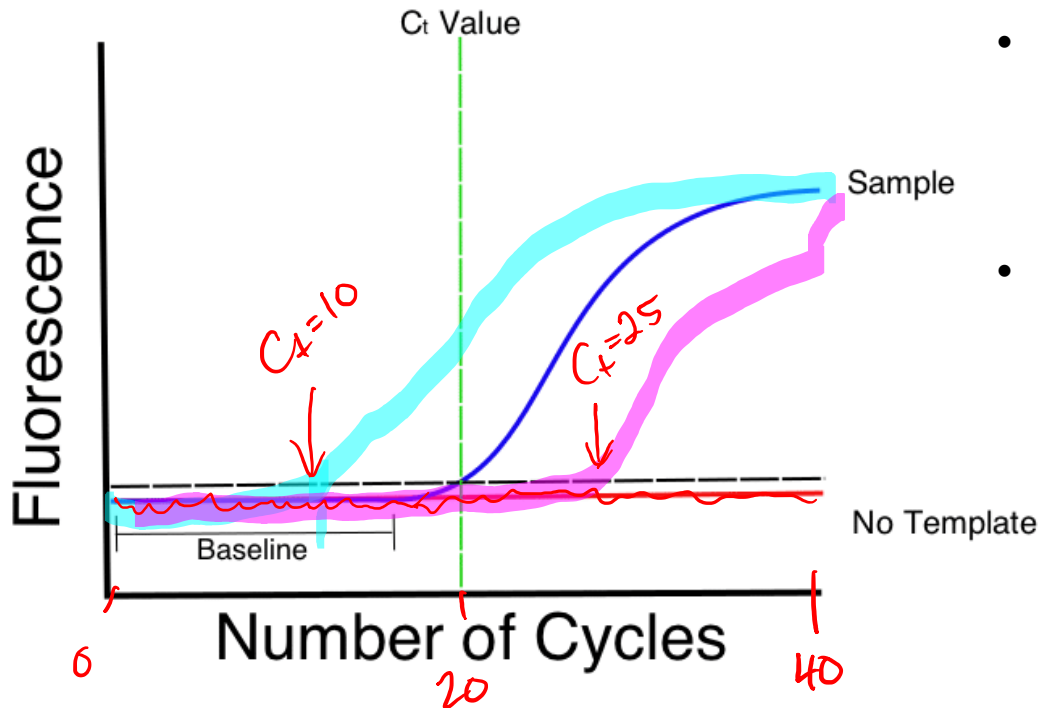


qPCR (quantitative PCR) is used to detect and quantitate gene expression

- Fluorescence is a function of dsDNA concentration via SYBR green dye
- dsDNA concentration is proportional to RNA purified from cells and used to make complementary DNA (cDNA)
- We can compare expression of a particular gene in different conditions by measuring the abundance of the gene-specific transcript
- Expression of the gene of interest is normalized to a housekeeping gene, GAPDH
- Plot the exponential transformation (\log_2) of the change in the threshold values (ΔC_T) for each condition



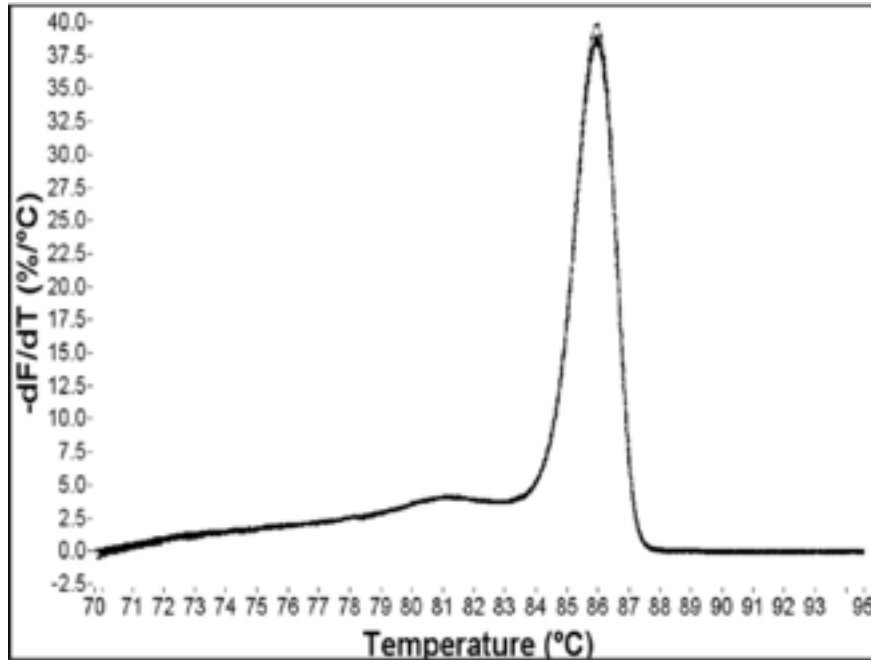
Threshold cycle (C_T) is calculated from qPCR after all cycles complete



- What would be a C_T value for a gene expressed lower than the transcript in blue? *pink, $C_T=25$*
- Higher? *light blue, $C_T=10$*

qPCR melt curve indicates the number of dsDNA products in rxn

negative derivative of fluorescence
vs. temperature



What would cause multiple peaks?

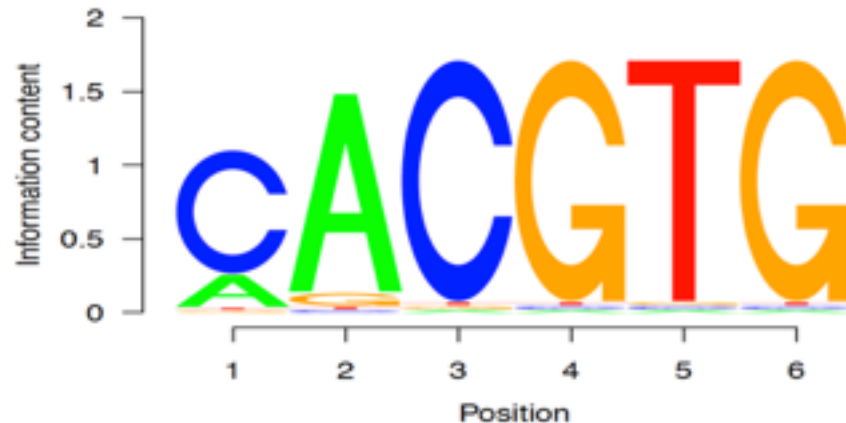
multiple dsDNA products

↳ primers created off target product, seq. similarity

↳ splice variants

Computational exercise—transcription factor binding site motifs

- Calculate position weight matrices
- Search public database of transcription factor binding
- Scan sequences to look for matching motifs
- Practice expectation-maximization algorithm for de novo motif discovery



Today in lab

1. Drug treat cells in tissue culture:
 - 1st: Red, Orange, Yellow, Green
 - 2nd: Blue, Pink, Purple, White, Grey
2. Analyze qPCR data
3. Complete “Transcription Factor Motifs” R exercise
 - HW due M2D9:
 - Create figure with qPCR analysis/RNA seq analysis (including figure title and caption)
 - associated results section
 - associated discussion section