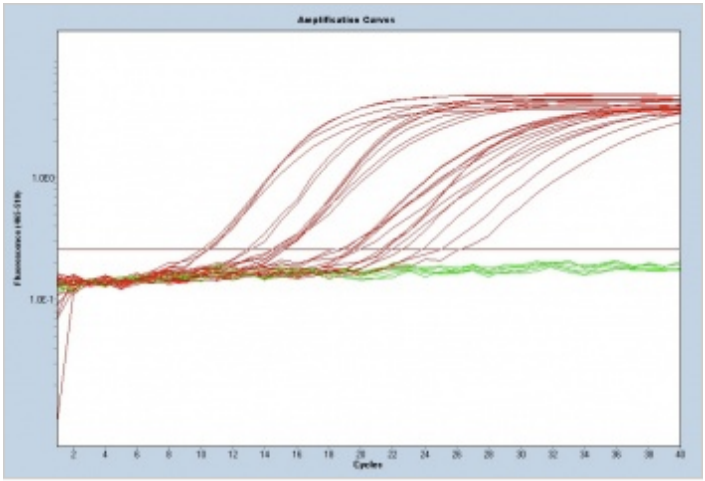
M2D9:
Complete data analysis

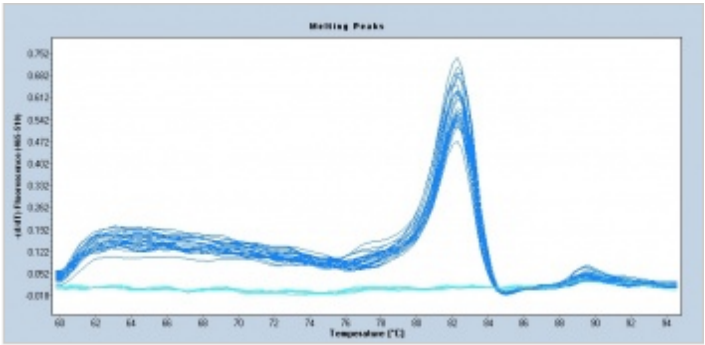
QUIZ

1. Prelab discussion
2. Analyze qPCR data
3. Measure cell viability

qPCR measures gene expression

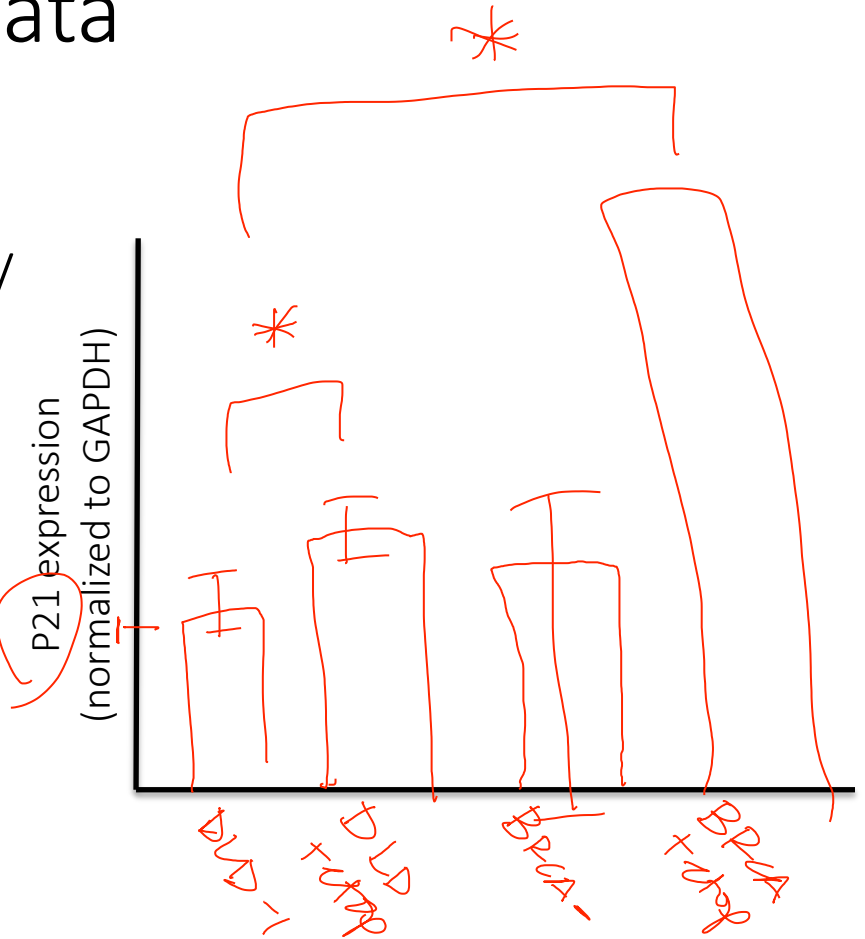
- Amplification curve
  - C_T value corresponds to amount of transcript in sample
  - What is the C_T value?
  - What does the C_T value measure?
- Melt curve
  - Confirms single amplification product





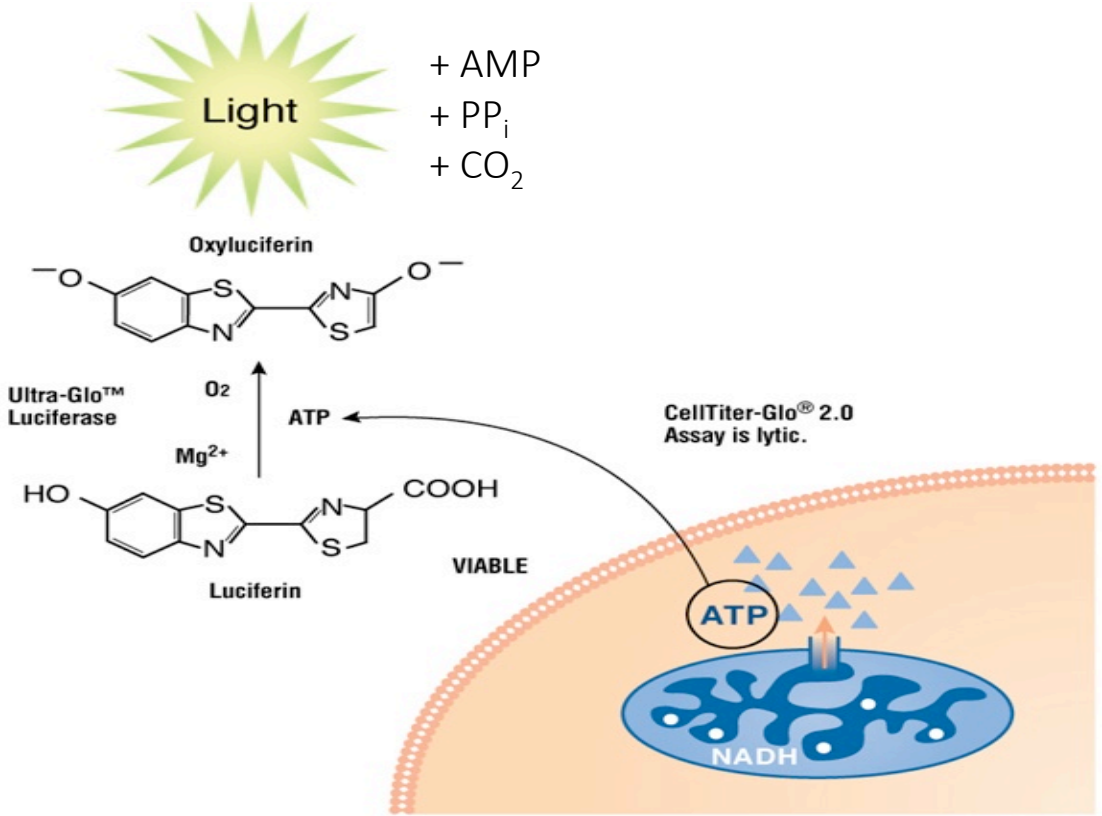
Analyzing your qPCR data

1. Subtract GAPDH C_T value
   - Normalizes to cell number / total transcript
2. Transform to ΔCT expression
3. Average replicates
4. Calculate 95% CI and p-value(s)



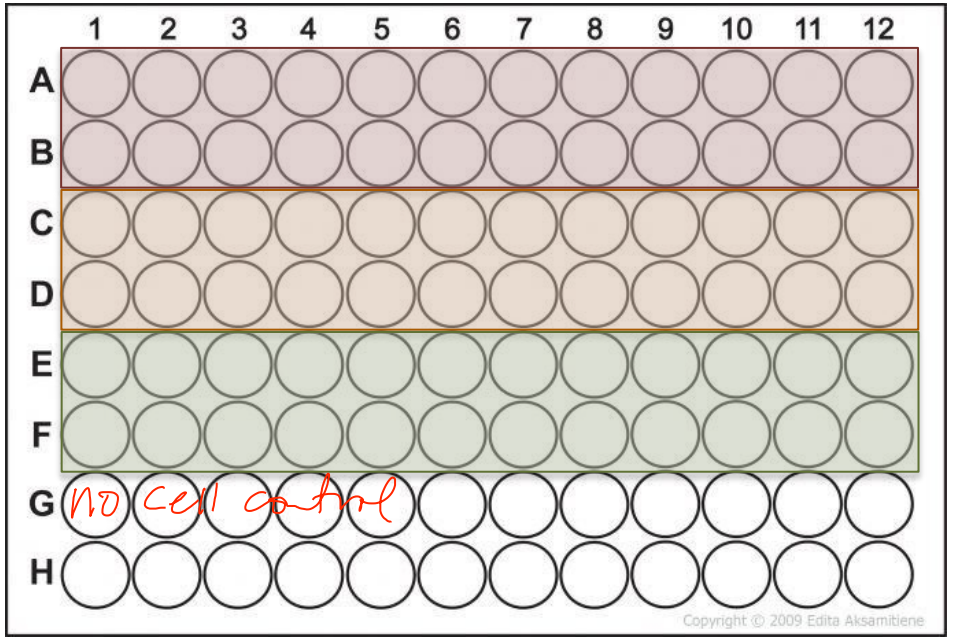
CellTiter Glo assay measures cell viability

Luminescence is a proxy for metabolically active cells; based on amount of ATP



Workflow for cell viability assay

Samples transferred to **white plate**, then luminescence measured using plate reader



Team #1 | Team #2 | Team #3

Analyzing your cell viability data

1. Average ‘no cell’ control wells
2. Subtract average from each luminescence value
   - Normalizes to background
3. Divide by ‘no DNA damage’ control
   - Reports data as % of control
4. Calculate 95% CI and p-value(s)

For today…

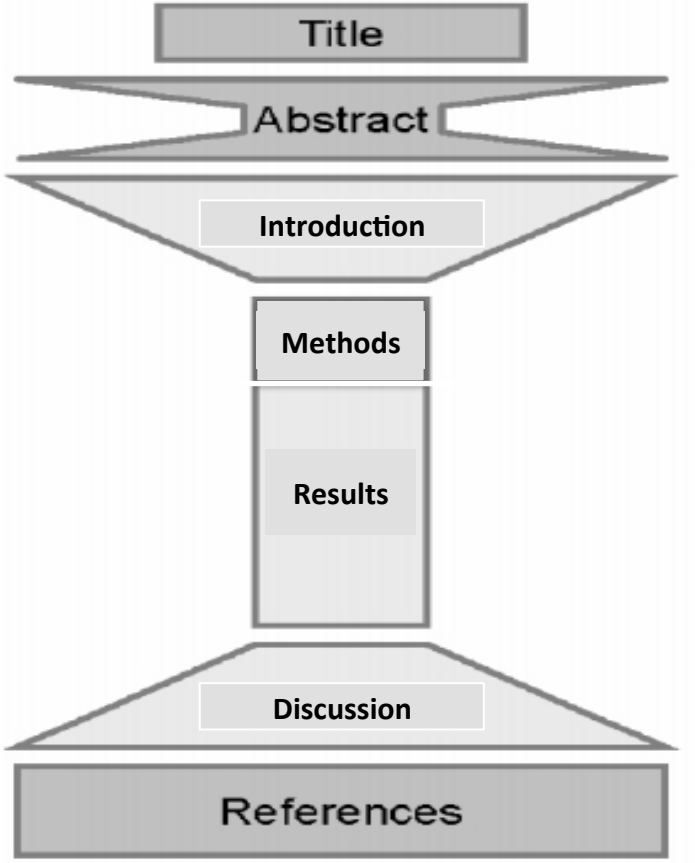
- Use downtime to work on your Research article!!
  - Due Saturday, April 20 by 10p to Stellar
  - (Extra) Office hours next week
    - Noreen: Tuesday 4-5p, Wednesday 5-7p, Thursday 4-7p
    - Leslie: Wednesday 2-5p, Friday 3-4p
- Notebook entry for M2D5 due tomorrow by 10p
  - Email .pdf to Michaela (mgold02@mit.edu)

For M3D1…

- Read Mod 3 overview and M3D1 introduction

How will you construct your Research Article?

- Structure is more formal
  - Title and Abstract
  - Introduction
  - Methods
  - Results
  - Discussion
- Complete sentences and paragraphs
- References



Notes on Research Article…

- What about the qPCR experiment?
- What about the RNA-seq data?
- What about the cell viability experiment?

On which experimental data should you focus??

How to present your RNA-seq data

1. DLD-1 and BRCA2-/-
   - Use heatmap with ALL of the genes to make broad comparisons about genotypes (what is more similar / different?)
   - Relate this to GO terms
2. A549
   - Integrate into / expand upon DLD-1 and BRCA2-/- data (how similar / dissimilar from DLD-1 and BRCA2-/- genotypes?)
   - Show something conceptual; comparisons in Venn diagrams or tables with a purpose / meaning

How to present your RNA-seq data

Bonus: CCLE

- How do these results relate to etoposide treated DLD-1 and BRCA2-/-??

At the very least, you should incorporate an experiment / question using the CCLE as a future work (be specific!)

Also, consider the methods / approaches discussed in lecture this week when considering future work

How to include RNA-seq analysis in your methods…

“RNA-seq was performed using Illumina HiSeq 2000 sequencing at the Massachusetts Institute of Technology, BioMedical Center. Data analysis was performed according to a workflow developed by Amanda Kedaigle and Ernest Fraenkel at the Massachusetts Institute of Technology using Rstudio.cloud. Transcriptomic data for A549 cell line was obtained from the Gene Expression Omnibus (Wang 2017).”