

M2D9: Complete cell viability assay

1. Quiz
2. Complete cell viability assay
3. Complete RNA-seq & TCGA analysis

Grading M2D4 lab notebook, complete by 10pm tomorrow (Sat. 4/14)

Extra (+ usual) Office Hours Next Week

- **Tuesday April 17th** 56-322 (lab):
 - 10:30am-1:30pm (Leslie)
 - 2:00pm-5:00pm (Noreen + Josephine's regular hour)
- **Wednesday April 18th** 56-322 (lab):
 - 10:00am-1:00pm (Josephine)
 - 2:00pm-5:00pm (Noreen + Leslie's regular hour)
- **Thursday April 19th** (56-341c), 10-11am (Josephine)
- **Friday April 20th** (56-341c), 4-5pm (Leslie)

Mod2 Research Report (20% of final grade)

Due Saturday 4/21 at 10pm

- Title, Abstract
- Introduction
- Methods
- *Results (Figures and captions)*
- Discussion
- References
- ***Use class data in at least one figure***

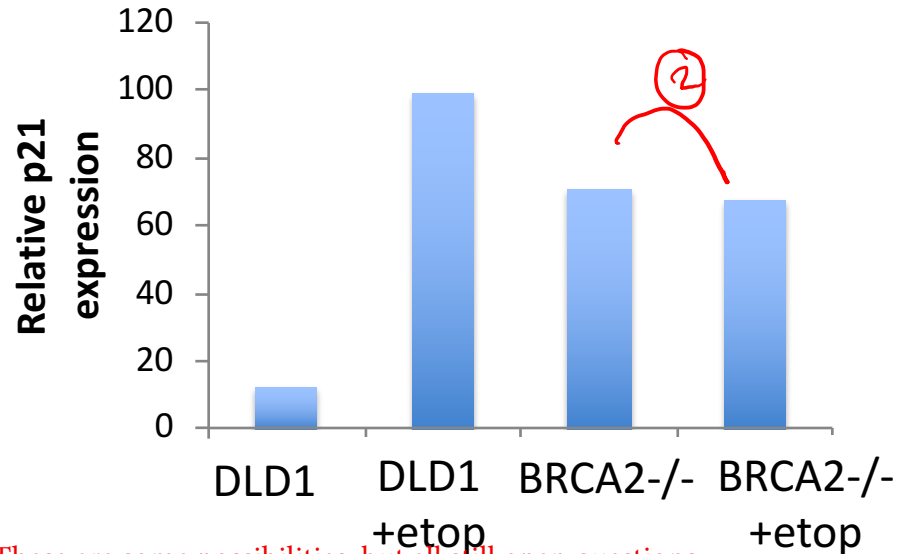
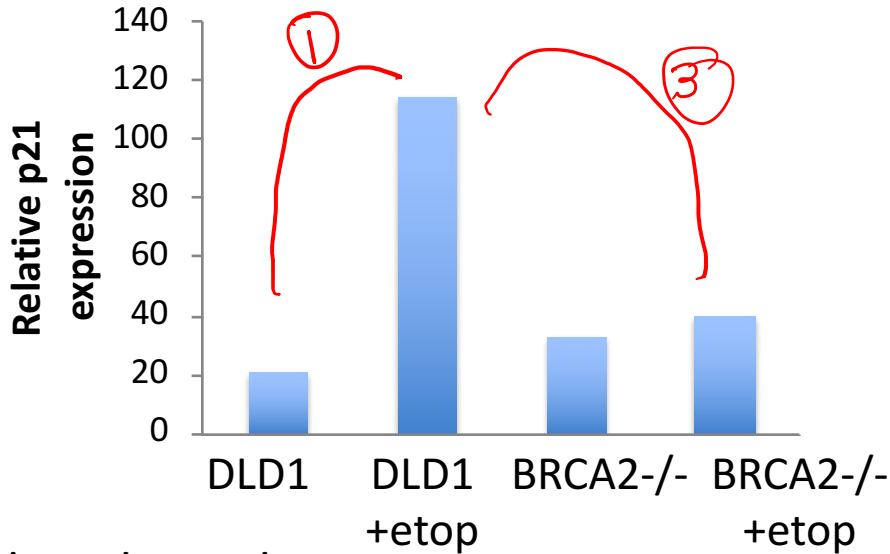
Individual assignment

Example list—not exhaustive or required

- Schematics (intro / results)
- PCA
- heatmap / dendrogram
- GO table
- Comparison to TCGA (\geq figure), help generalize / contextualize data
- qPCR (spot check RNA-seq), include primer comparison, compare to RNA-seq data
- cell viability data (compare class data)

Representing qPCR results—

Remember to include C.I. and statistical significance



These are some possibilities, but all still open questions:

Discussion topics:

① Why p21↑?
p21 functions in cell cycle arrest,
apoptosis

② etop. fails to induce additional arrest
BRCA2- already upregulates DNA repair machinery

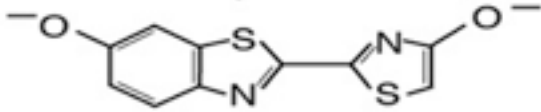
③ BRCA2-/- respond via other pathways
resistance, survival mechanism not to
Stall cell cycle

Note: clarification of “old primer” vs “new primers”—don’t write this on your report

+ AMP
+ PP_i
+ CO₂



Oxyluciferin

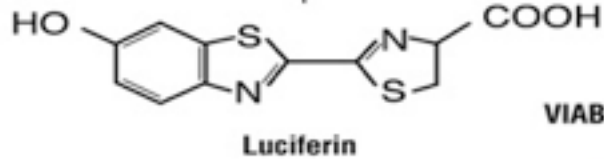


Ultra-Glo™
Luciferase

O₂

ATP

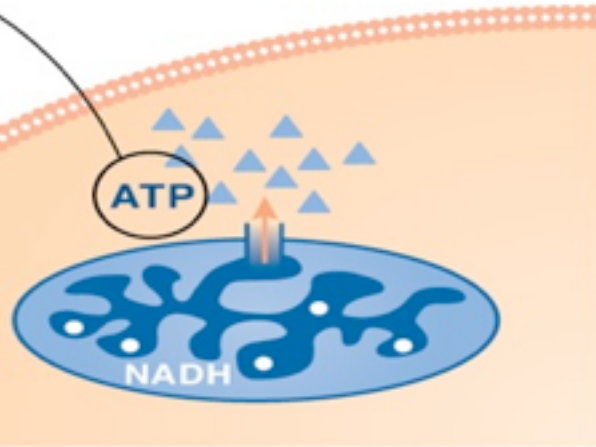
Mg²⁺



VIABLE

CellTiter-Glo® 2.0
Assay is lytic.

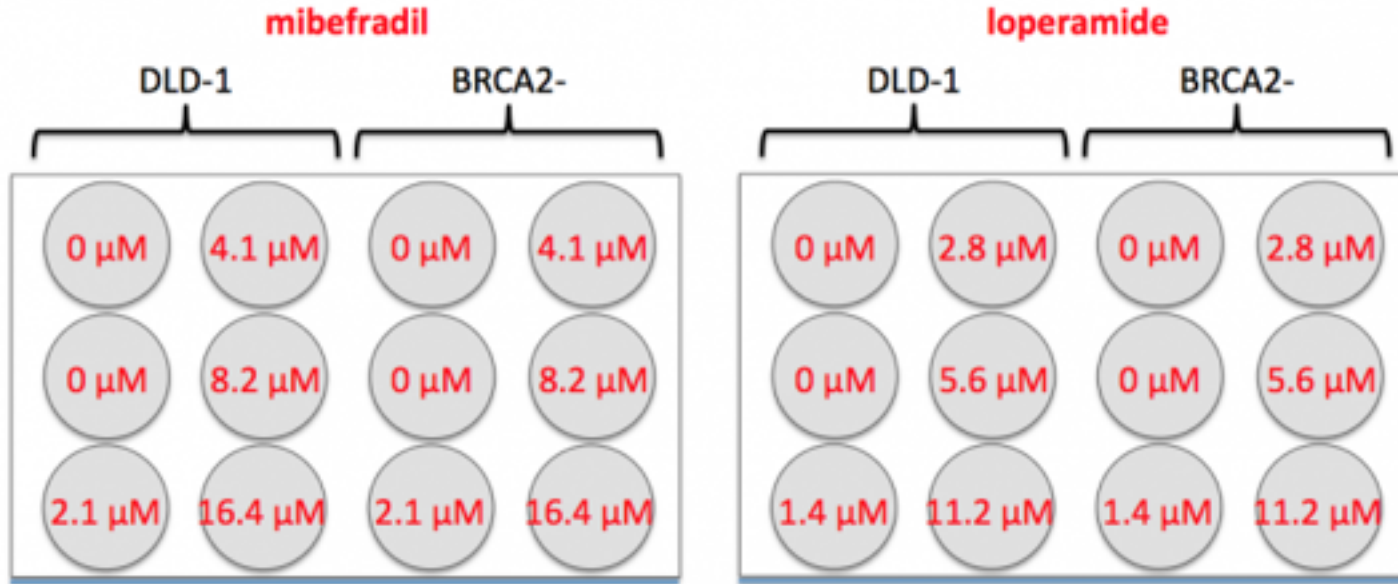
ATP



CellTiter Glo luminescent cell viability assay

- Number of live (metabolically active) cells proportional to number of ATP molecules
- Mix well to make sure cells lyse!

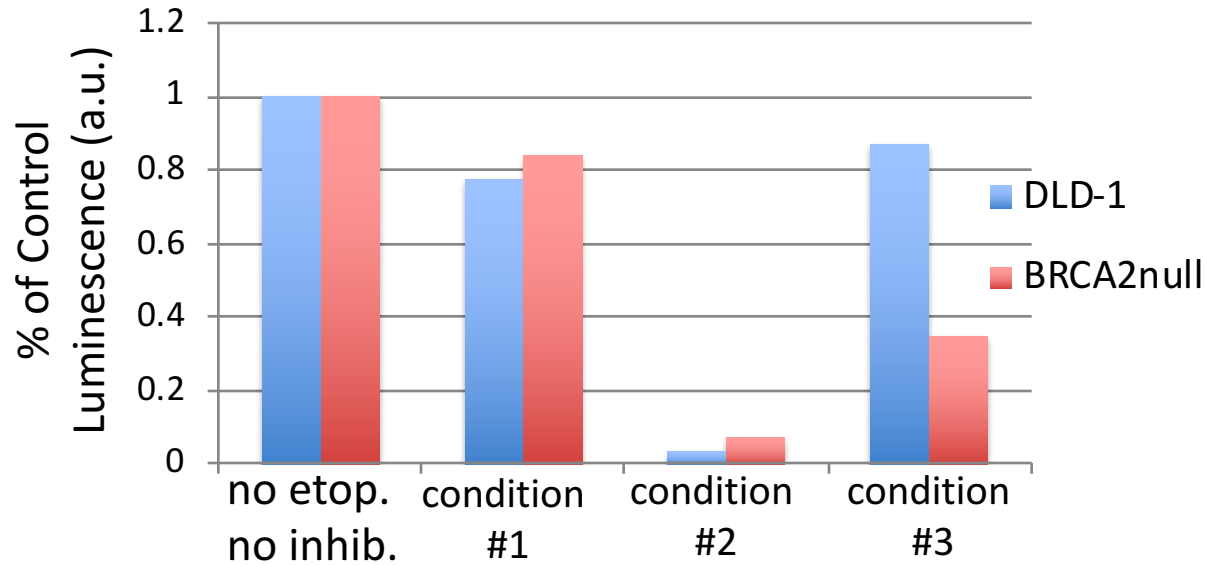
What do we hypothesize?



Highest viability: A1, A3

Lowest viability: C4

Analyze cell viability data, with error bars (C.I.) and find the statistical significance (Student's t-test)



1. Subtract values from media only wells (media + CellTiter-Glo, no cells, completed by instructors)
2. Divide by control wells with cells, but no etoposide, and no drug

Today in lab

1. Retrieve cells from TC
 - Make a note of their confluency and morphology
 - Start CellTiter-Glo assay
2. Complete any additional analysis necessary for your report!