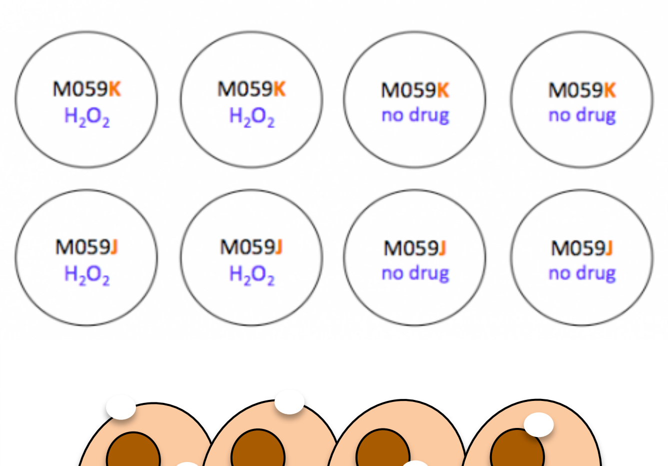
M1D6:
Complete immunofluorescence assay
Continue CometChip data analysis

10/05/2016

Start with Part 2 protocols…

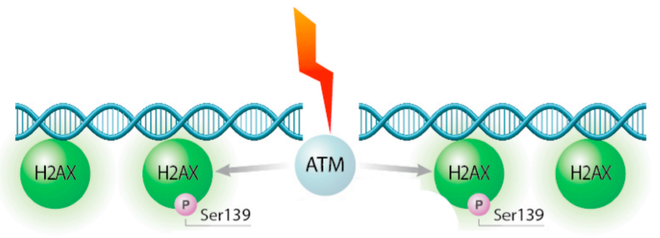
- Quiz
- Workshop on abstracts
- Permeabilization and primary γH2AX antibody



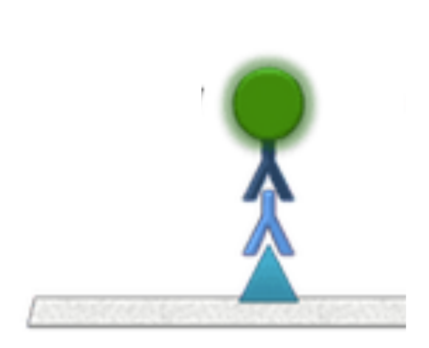
- fix(ed) cells with 4% formaldehyde
- permeabilize cells with 0.2% Triton X
- block (nonspecific binding) with 1% BSA
- pause: 1 h incubation
- add primary antibody

- Prelab during incubation in BSA blocking solution
- Statistics practice and data analysis continued

Using immunofluorescence:
γH2AX assay to detect double-strand DNA breaks



- histone H2AX phosphorylated at Ser139 if DSB
- use antibodies against γH2AX

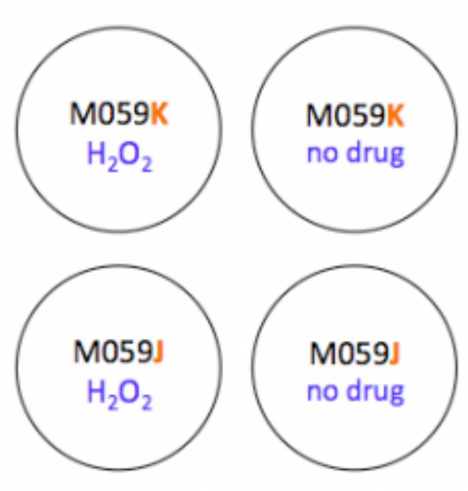


today	protein of interest	▲	γH2AX
today	primary antibody	⅄	mouse anti-human anti-γH2AX
Friday	secondary antibody	⅄	goat anti-mouse
Friday	fluorescent dye exc./ em. wavelengths	●	488 / 520 nm

… imaging on M1D7

… then 1-page "Results and Interpretation " homework due on Friday at 10pm

What do we hypothesize we'll observe?



All 4 coverslips will show blue (DAPI-stained) nuclei

With H2O2, more green yH2AX foci

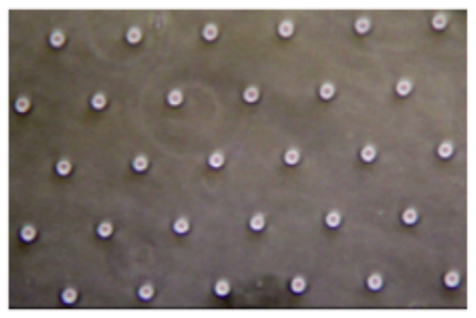
The (NHEJ) repair-deficient M059J cells may show even more green yH2AX foci than the wild-type M059K cells

What are our controls?

The no-drug (untreated) coverslips

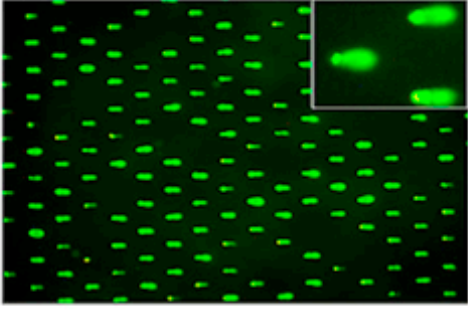
"K" WT cells

Only report on M1D1-M1D4 in your Data Summary



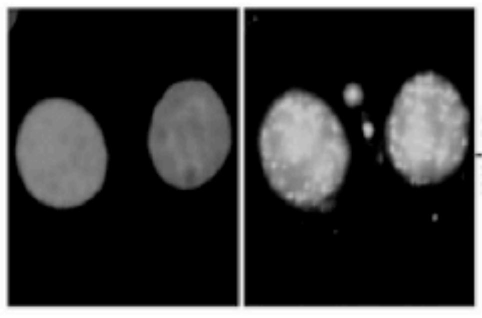
1. Optimize comet chip assay

- Test loading variables



2. Use comet chip assay to measure DNA damage / repair

- Measure effects of MMS and H_2O_2 on BER
- Assess repair variability in healthy individuals



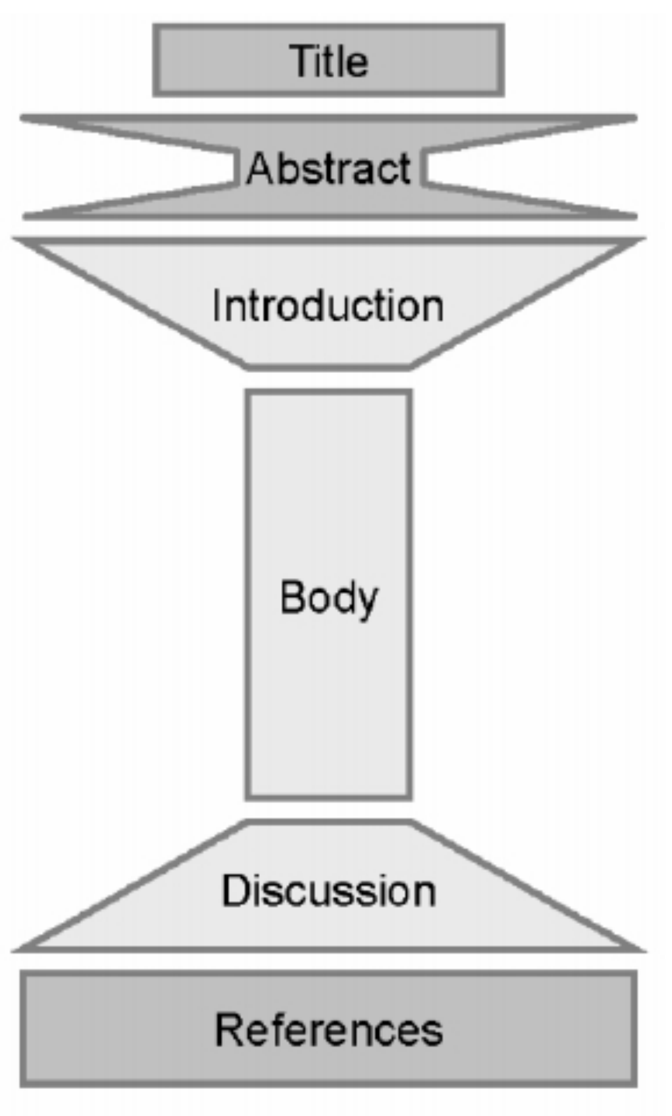
3. Use immuno-fluorescence assay to visualize DNA repair

- Examine effect of H_2O_2 on DSB abundance

Extra office hours

- M1 Data Summary draft
  - due 5pm on Wednesday, October 12
- Office hours:
  - Friday, October 7, 9am-10am: Maxine in 16-336
  - Sunday, October 9, 10am-12pm: Maxine in 56-302
  - Monday, October 10, 3pm-5pm: Leslie in 56-302
  - (regular Monday office hours cancelled)
  - Tuesday, October 11, 1pm-4pm: Noreen in 56-302
  - Wednesday, October 12, 10am-12pm: Leslie in 16-429b
- Come early, even with nothing ;-)
  - ask questions, get feedback on the fly

Your M1 Data Summary



Title: take-home message

Abstract: the only page *not* in bullet points

In bullet points:
Introduction: background and motivation

Results and
interpretation / discussion
bundled together

Both MMS and H_2O_2 dose response curves
Your data elsewhere (you may use others' too)

transitions from one page to the next

Implications and future work
References (*not* in bullet points)

see Communication tab for format

What goes into an introduction?

Specific background

Knowledge gap
Statement of problem

Here we show…

- Your research is anchored in a general topic that your audience cares about.
  - focus on outsiders
  - include references
- All information connects your project with the general topic.
  - minimum essential information
  - accurately represents the field
  - correctly referenced, give credit
- The question you address is clearly articulated, connected to the background, and appears meaningful.
  - give evidence of incompleteness of current understanding, of value of investigation
  - CLEARLY state your hypothesis
- A preview of your findings and their implications fills the demonstrated gap.
  - light on Methods

The meat of your paper

- Figures and captions
  - Decide on these first
  - Use subpanels
  - Text: limited on figure, explicit in caption
  - reasonable size
  - descriptive title
  - caption purely descriptive of image
  - intro sentence in caption
- Results
  - goal / intent / purpose of experiment = intro topic sentence
  - What you did: experiments and expectations, including controls
  - What you found:
  - transition

What goes into the Discussion / Interpretation?

- Interpret
- Put in context: how does this fit with other studies?
- Highlight significance: how might this impact this/other field?
- Discuss controversial or surprising results
- transition

What goes into Implications & Future Work?

- Describes caveats and suggest remedy
- Conjecture (one layer only!) implications
- Propose future work, identify new questions that arise

- Follow same order as in Figures/Results
- Make sure you come back to big picture introduced in intro
- Don’t overreach / overpromise!

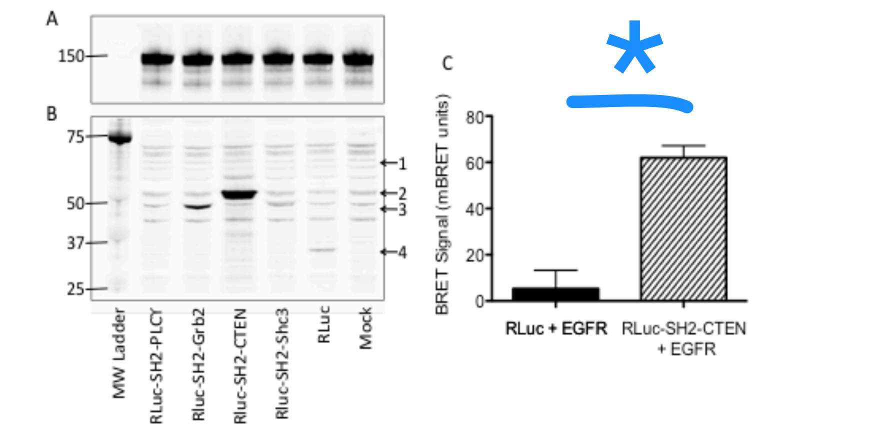


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. Arrowheads indicate the expected molecular weight of the RLuc-tagged proteins; (1) RLuc-SH2-PLCg, (2) RLuc-SH2-CTEN, (3) RLuc-SH2-Grb2 and RLuc-SH2-Shc3, and (4) RLuc alone. Mock indicates no cDNA was utilized during transfection. (C) For CTEN only, BRET signal was quantified using a luminometer after stimulation of CHO-K1 with 100 ng/mL EGF for 15 min.

BRET system effectively measures EGFR activation:

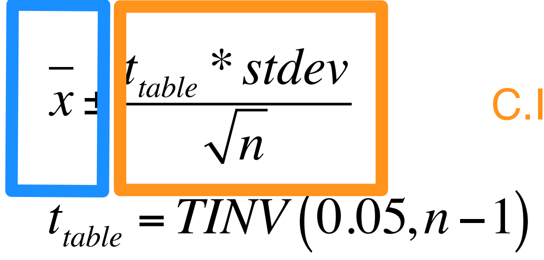
- To determine if the BRET system could be used to monitor EGFR activation, CHO-K1 cells were transfected with fluorescent EGFR and luciferase-tagged SH2 domains and a BRET assay was performed after growth factor stimulation.
- CHO-K1 were transfected with Citrine-EGFR in all conditions as indicated by correct molecular weight band at 150 kDa (Figure 1A).
- Several protein bands are present in Mock transfection lane suggesting off-target binding of the RLuc antibody (Figure 1B).
- RLuc alone, RLuc-SH2-Grb2, and RLuc-SH2-CTEN were successfully transfected as indicated by correct molecular weight bands (Figure 1B).
- RLuc-SH2-PLCg and RLuc-SH2-Shc3 did not appear by Western blot analysis -- bands different from those in the Mock lane are not identifiable. This outcome could be due to protein expression levels below the detection limit by Western blot or to unsuccessful transfection of cDNA.
- BRET signal increased in cells transfected with Citrine-EGFR and RLuc-SH2-CTEN versus Citrine-EGFR and RLuc alone after EGF stimulation. This difference suggests that the BRET signal is specific for an SH2-EGFR interaction versus randomly localized RLuc.
- In sum, these data suggest that the RLuc-SH2 constructs can be utilized to monitor EGFR phosphorylation, as SH2 domain-EGFR association occurs only at sites of EGFR tyrosine phosphorylation. Next, we determined the dynamic range of the BRET assay.

Example M1 “Results & Interpretation” slide

* denotates p-value of 0.05 for Student's t-test

Continue your statistical analysis!

- Create some Results figures:
  - Plot your data with 95% confidence intervals



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

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

  - 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.

Assignments for M1

- Data summary draft
  - due by 5pm on Wed., October 12
  - revision due by 5pm on Mon., October 24

Summary content

1. Title
2. Abstract
3. Background, Motivation
4. Figures, Results & Discussion, Interpretation
5. Implications, Future Work

- Mini presentation due by 10pm on Sat., October 15
- Blog post for M1 due by 5pm on Tue., October 25