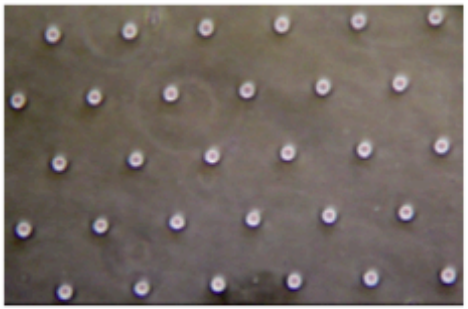
M1D6: Query DNA repair capacity in tumor cells

10/04/16

1. Quiz! (in 16-275)
2. Communications lab workshop (in 16-275)
3. Prelab during block incubation
4. Permeablization and primary H2AX antibody
5. Statistics practice and Data analysis continued

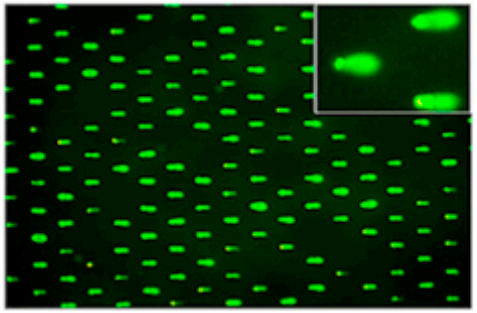
Overview of
"M1: Measuring Genomic Instability"



1. Optimize comet chip assay

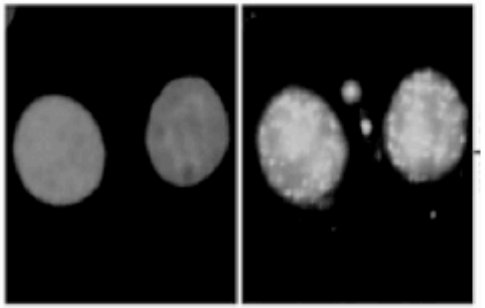
- Test loading variables

Data Summary



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

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

Extra office hours

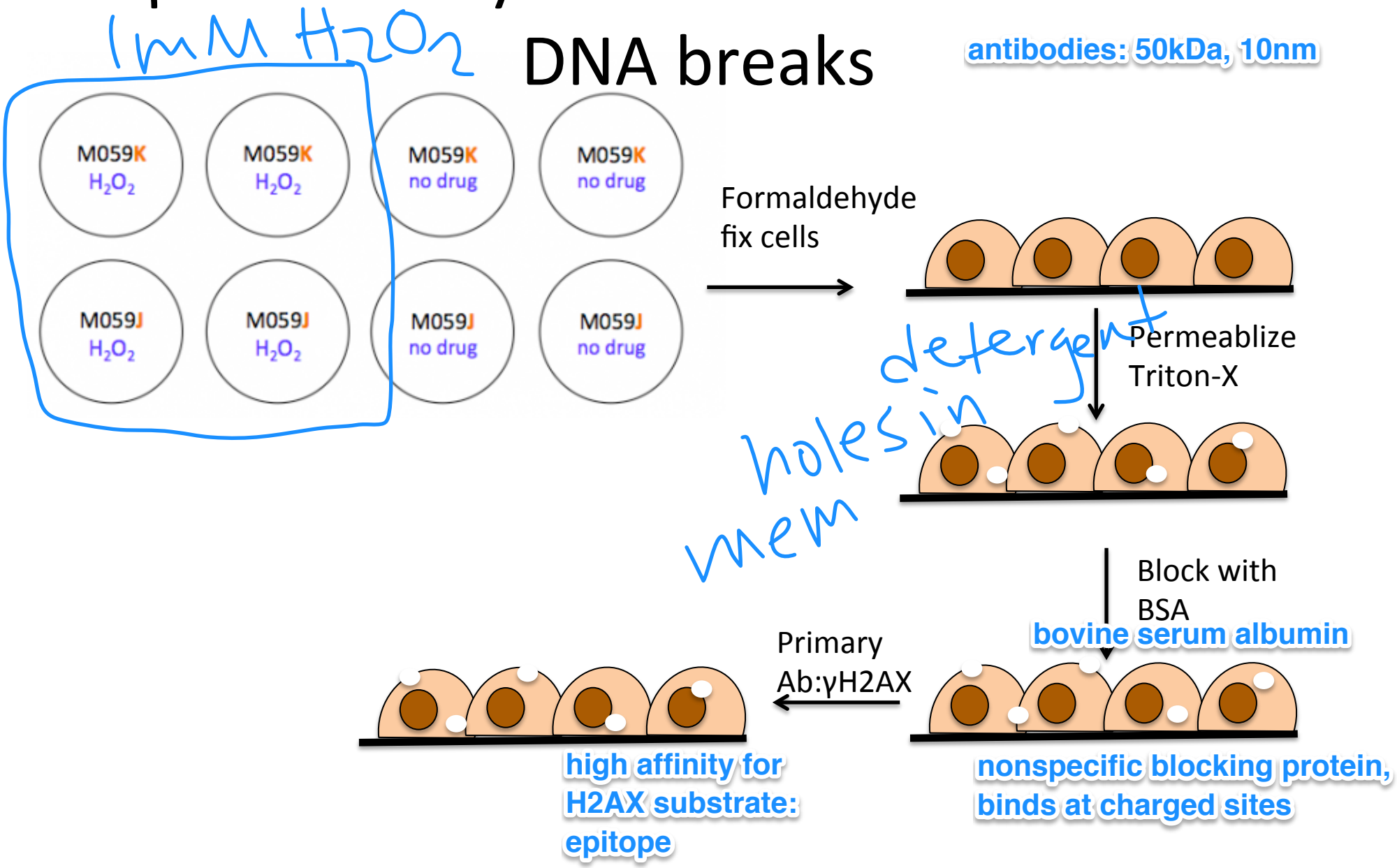
- Sunday, 10/9, 10am-12pm
- Monday, 10/10, 3pm-5pm
- Tuesday, 10/11, 1pm-4pm

56-302

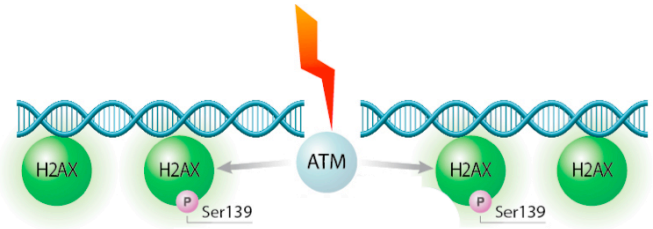
Regular office hours

- Next week Monday OH canceled
- Leslie OH, Wed. 10am-12pm, 16-429b

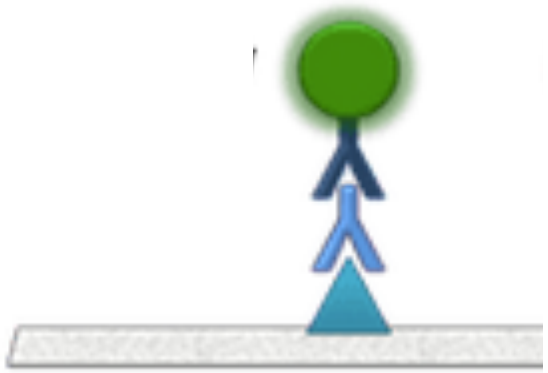
γH2AX assay to detect double-strand DNA breaks



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



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



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

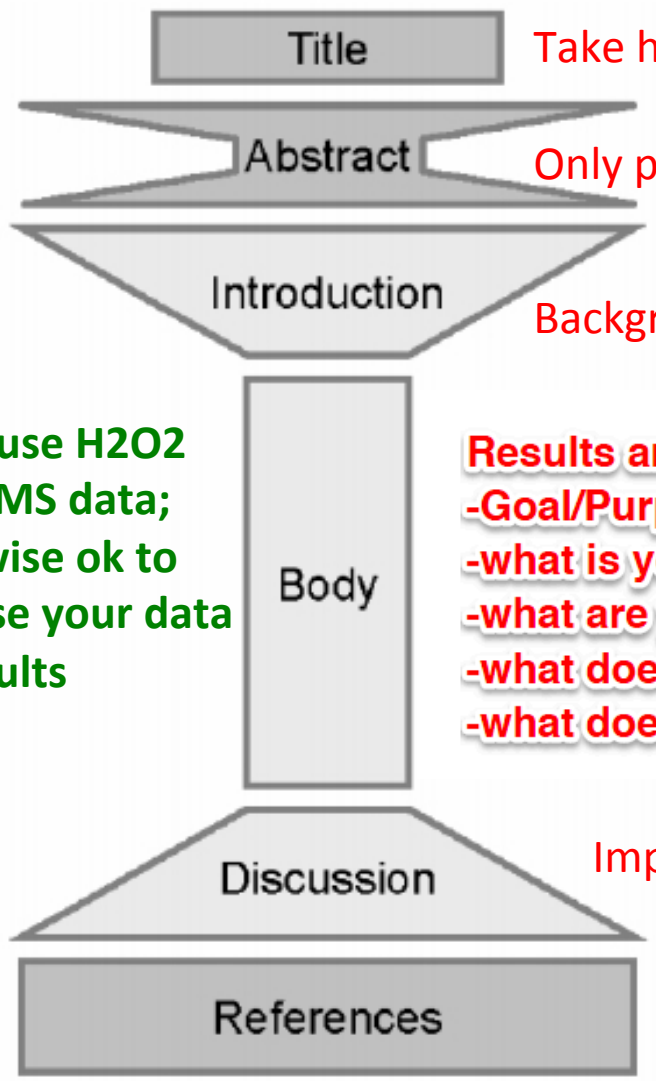
What do we hypothesize we will see?

	# of H2AX foci (DSB) hypothesis
M059J (deficient for repair) +H2O2	many
M059J (deficient for repair)	many
M059K +H2O2	some
M059K	few

What are the experimental controls?

primary alone, nothing; no fluor. marker
secondary alone, nothing; no epitope

Manuscript architecture *vs.* M1 Data Summary



Take home message

Only part of report in paragraph form

Background and Motivation (bullets)

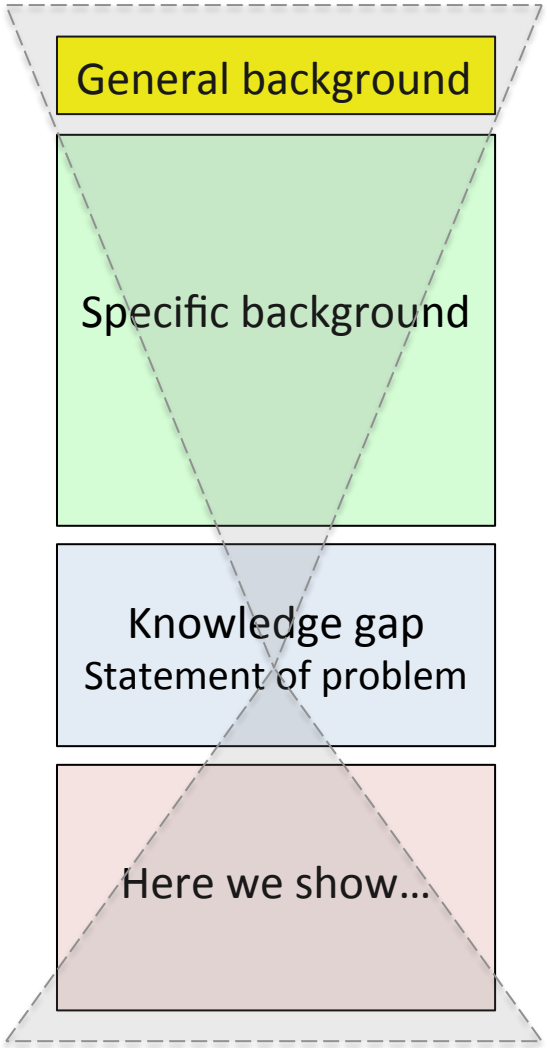
MUST use H2O2 and MMS data; otherwise ok to only use your data for results

Results and Interpretation (bullets)
-Goal/Purpose
-what is your expected result?
-what are your actual results?
-what does this suggest/indicate?
-what does this motivate to do next?

Implications and Future Work (bullets)

References from Intro (see wiki for format)

What goes into an introduction?



- 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

Example Results slide (from Wiki)

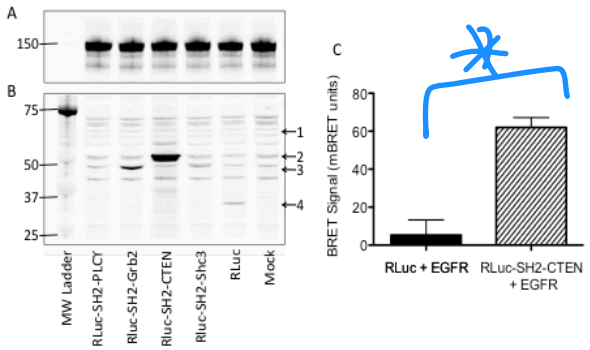


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. 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.

Make strides on your statistical analysis!

- On **M1D6**, you'll continue creating Results figures:
  - Plot your data with 95% confidence intervals

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

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

  - 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.