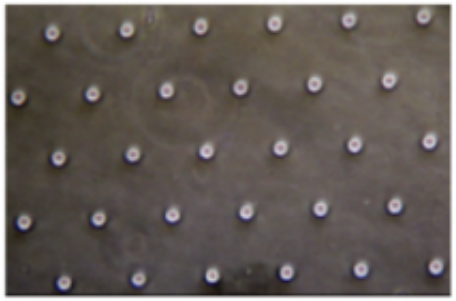
M1D5: Develop approach for sub-nuclear visualization of DNA damage

09/26/17

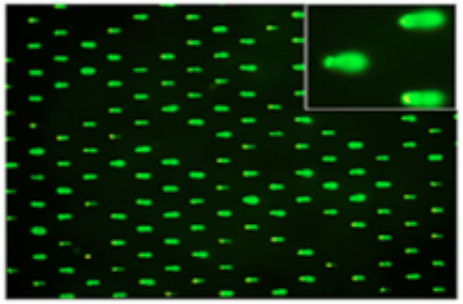
1. Comm lab workshop: Titles and Abstracts
2. M1 Quiz1
3. Pre-lab Discussion
4. Enzyme treatment of CometChips
5. Drug treat cells for H2AX assay
6. Electrophoresis of CometChips

Overview of
Module 1: Measuring Genomic Instability



1. Optimize comet chip assay

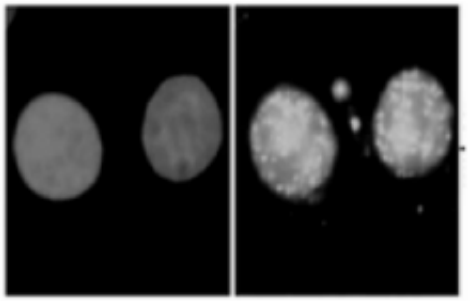
- Test loading variables



2. Use comet chip assay to measure DNA repair

- Measure effects of MMS and H_2O_2 on BER

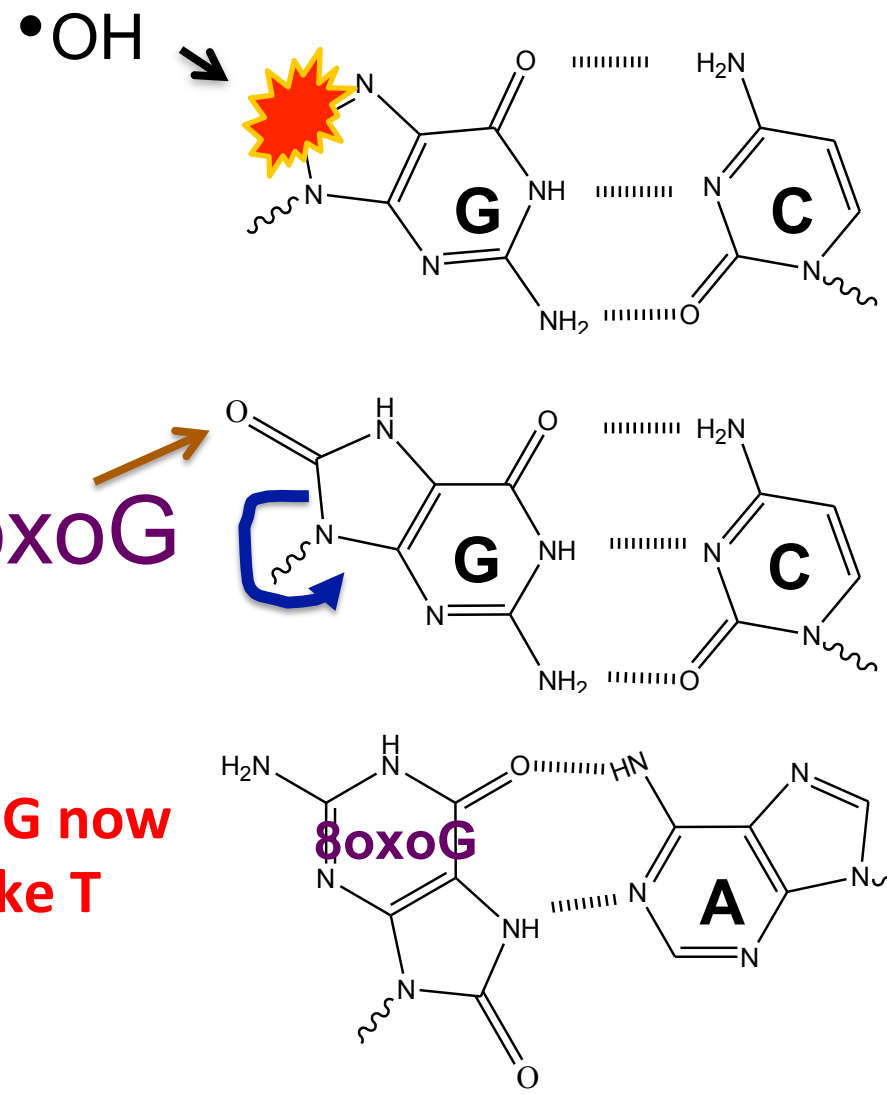
Measure (Quantify) effect of DNA repair activity in response to alkylation damage and oxidative damage.



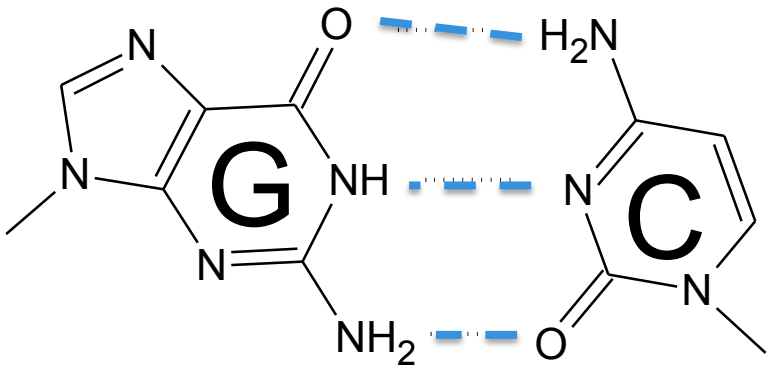
3. Use immuno-fluorescence assay to measure DNA repair

- Examine effect of MMS and H_2O_2 on DSB abundance

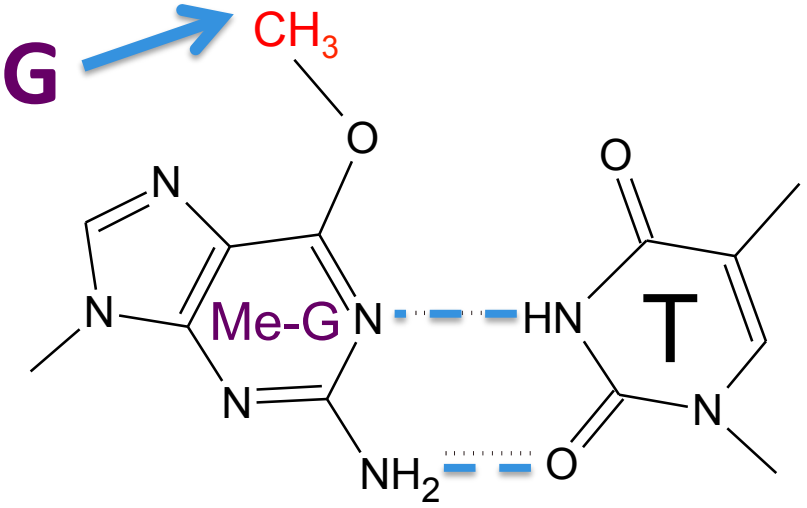
Reactive oxygen species: H2O2



Alkylating agent: MMS



7Me-G

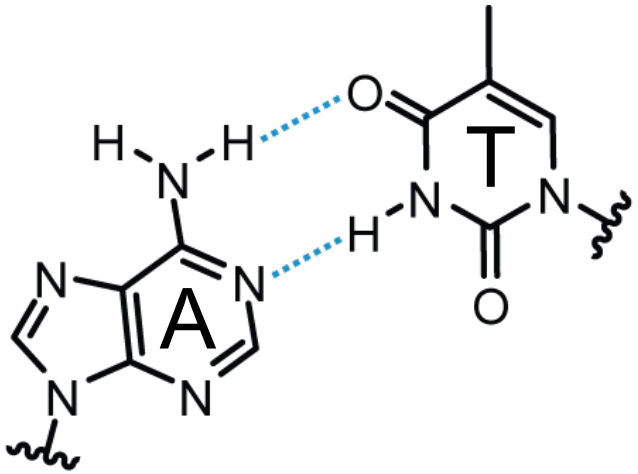


Damaged G now looks like A

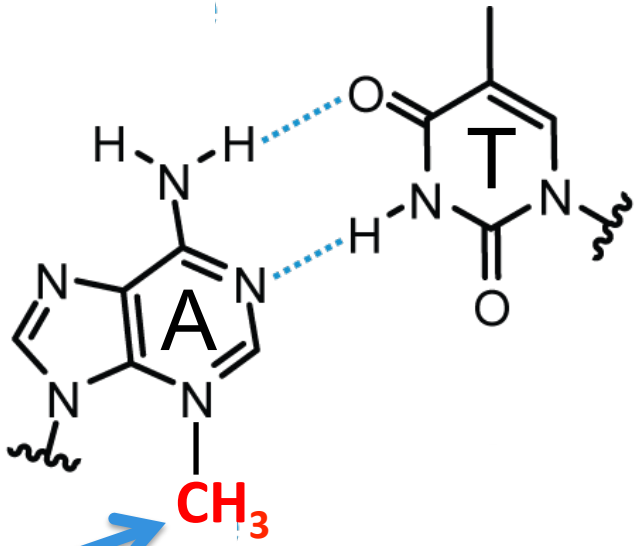
Mutation if replicated GC →AT

Alkylating agent: MMS

Primary damage type corrected by AAG glycosylase

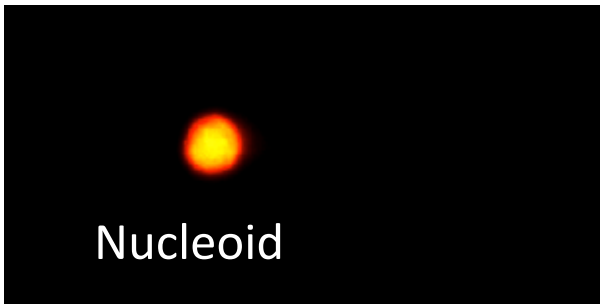


Damaged A blocks replication fork



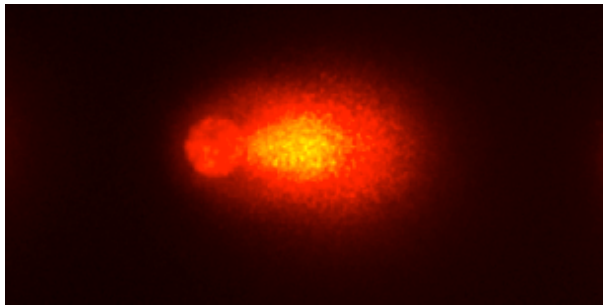
3-MeA

Output of CometChip Assay



No Damage

- Supercoiled nucleoid
- Little or no migration

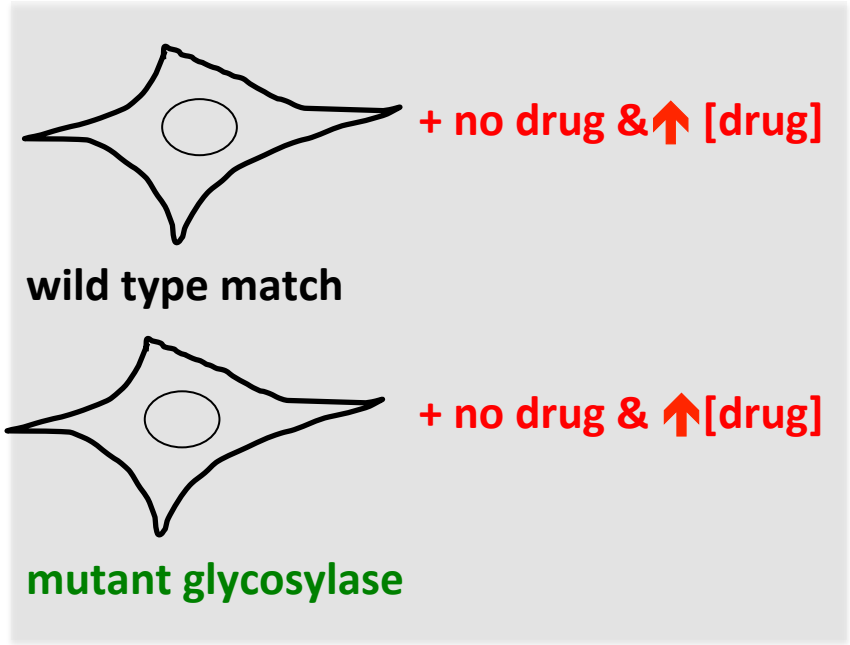


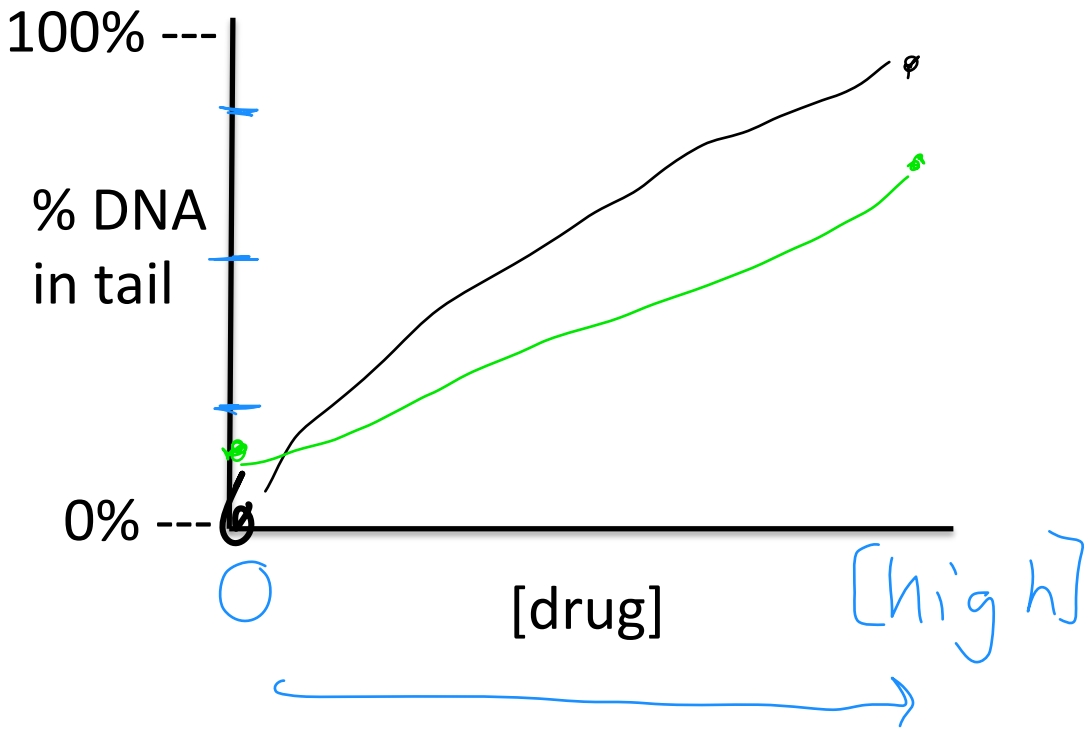
High Damage

- SSBs, DSBs, abasic sites, alkali labile sites
- forms a "Comet tail"

Genomic damage (instability) from direct strand breaks from MMS/H2O2 and repair intermediates of BER

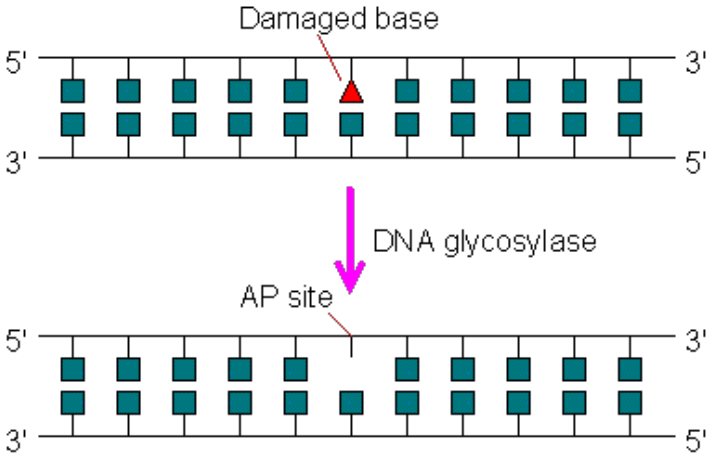
What result do we expect from our assay so far…





adding back glycosylases to REVEAL damage not seen in the initial experiment

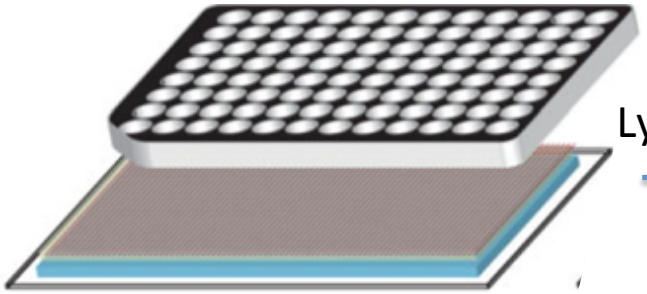
Purified glycosylases can release damaged bases in lysed cells



	Oxidative damage	Alkylating damage
Chemical treatment:	H_2O_2	MMS
Mutant cell line:	Ogg1-/-	Aag-/-
Enzyme:	Fpg (*E. coli* Ogg1)	hAAG (human AAG)

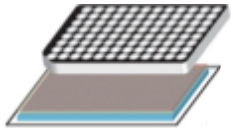
Complete experiment to test role of biochemical factors (MMS or H2O2) in genomic stability (DNA damage)

Load and capture cells in 2 CometChips, then treat with MMS or H2O2

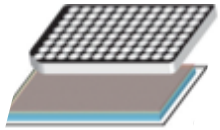


Lyse cells

Chip 1: No enzyme treatment (buffer control)

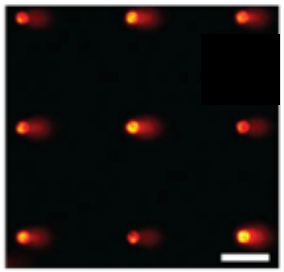


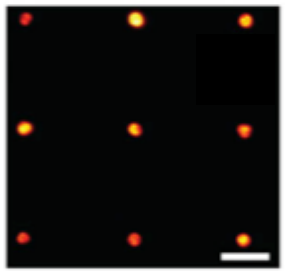
Chip 2: Treat with FPG (*E. coli* Ogg1) OR human AAG

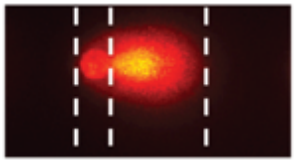


Alkaline unwinding

Electrophoresis



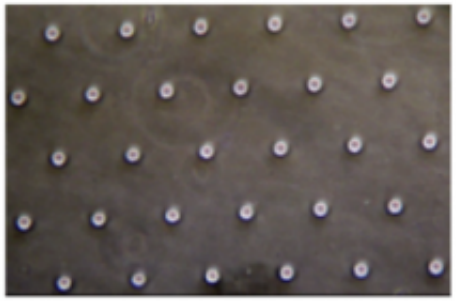




stain DNA and image via fluorescence microscopy

analysis via Matlab

Overview of
Module 1: Measuring Genomic Instability



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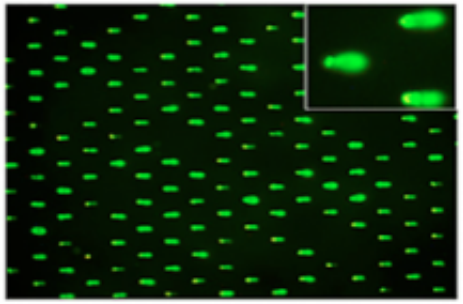

2. Use comet chip assay to measure DNA repair

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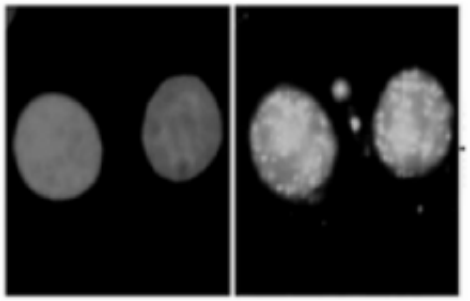
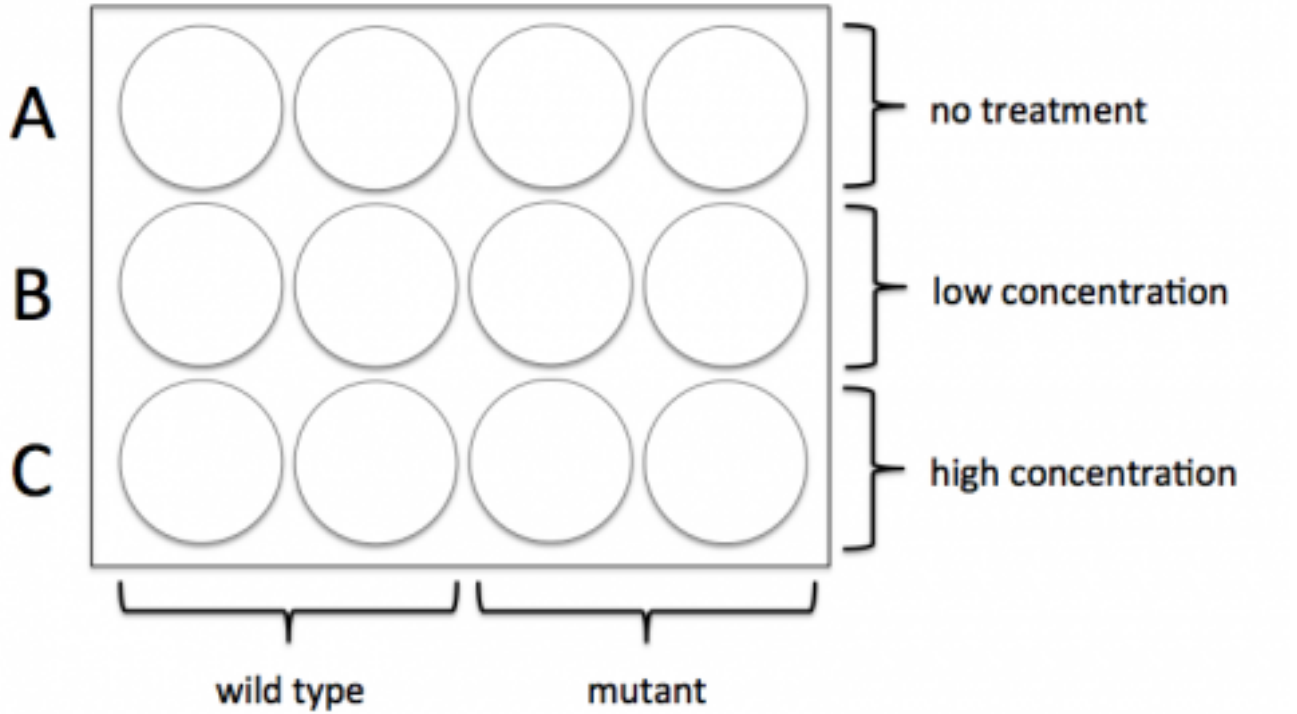


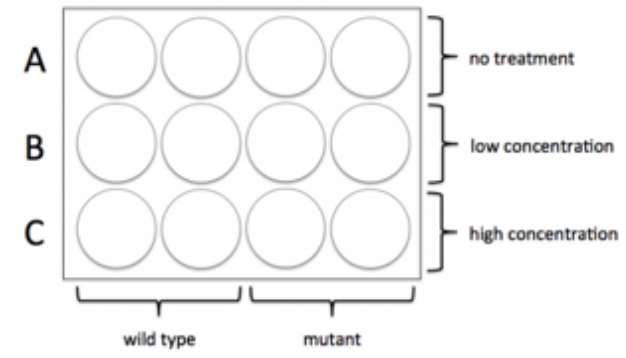
3. Use immuno-fluorescence assay to measure DNA repair

- Examine effect of MMS and H_2O_2 on DSB abundance

Quantify effect of DNA damage response (DNA repair and recovery) in response to alkylation damage and oxidative damage induced DSBs.

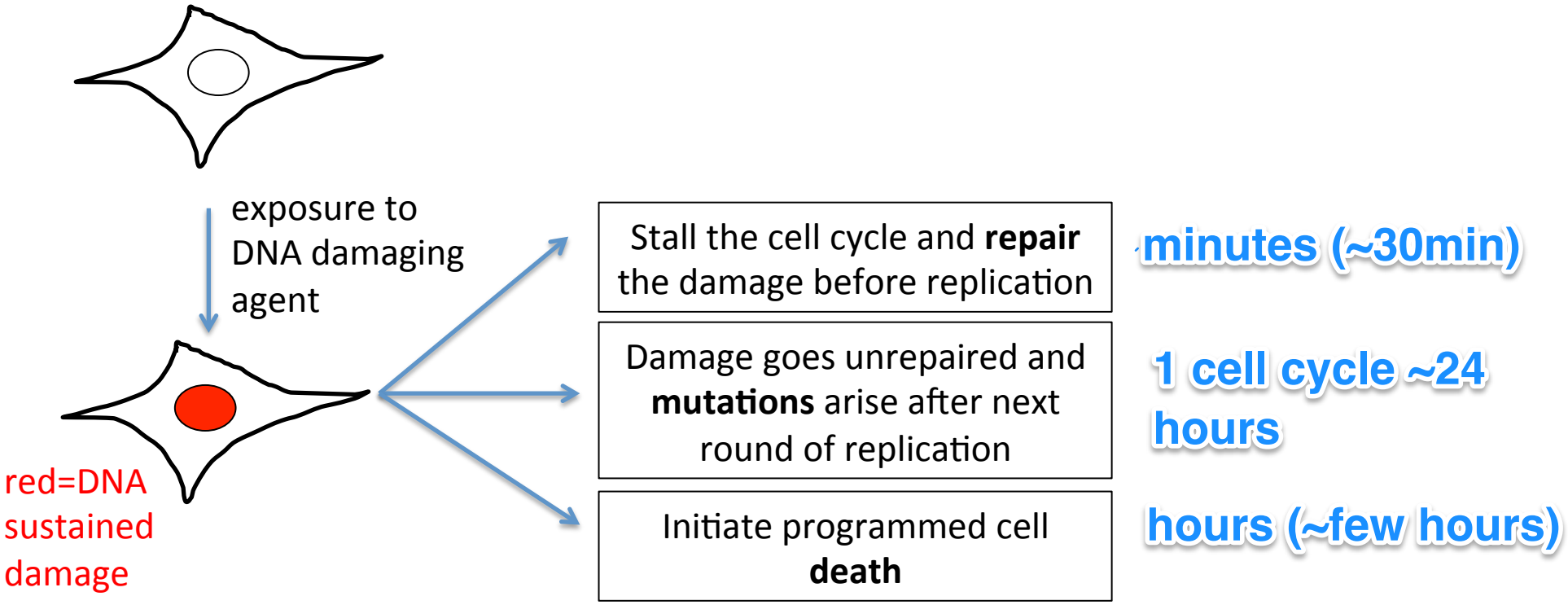
Design your experiment!



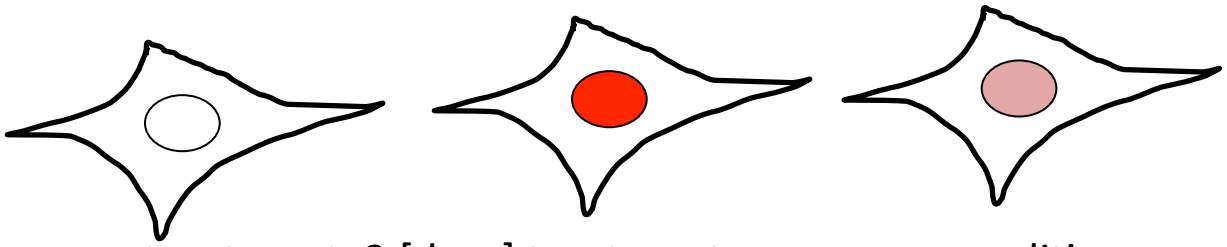


Recovery plate: same concentration of MMS or H2O2, choose a time point to assess your cell's ability to recover from damage

The DNA damage response

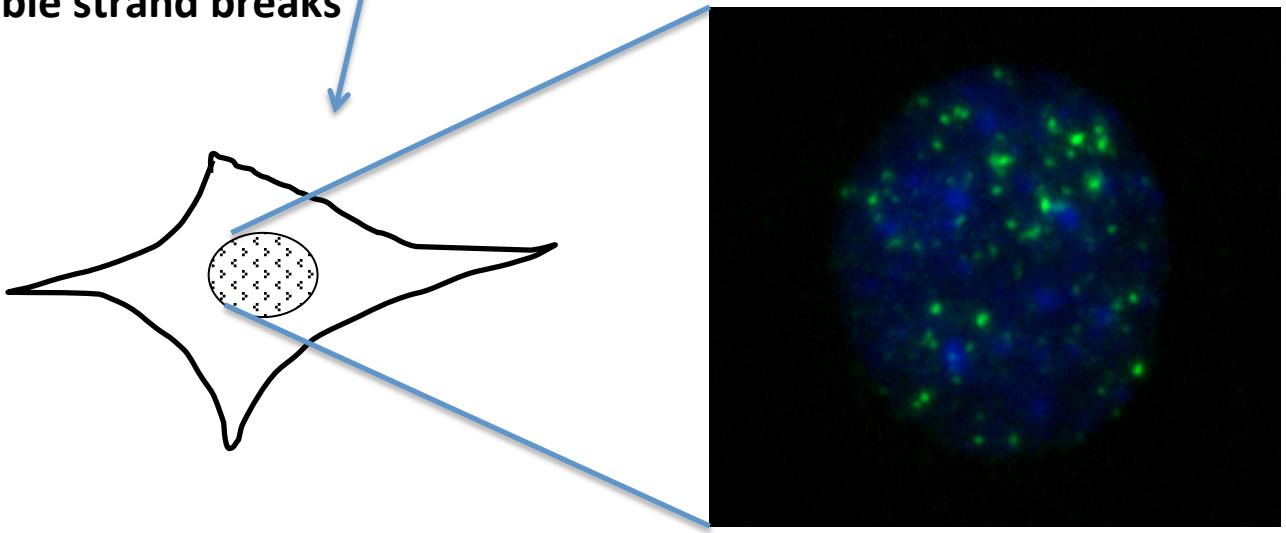


Output of H2AX Assay



no treatment, 2 [drug] treatments, recovery conditions

Fix cells and stain with antibody that marks double strand breaks

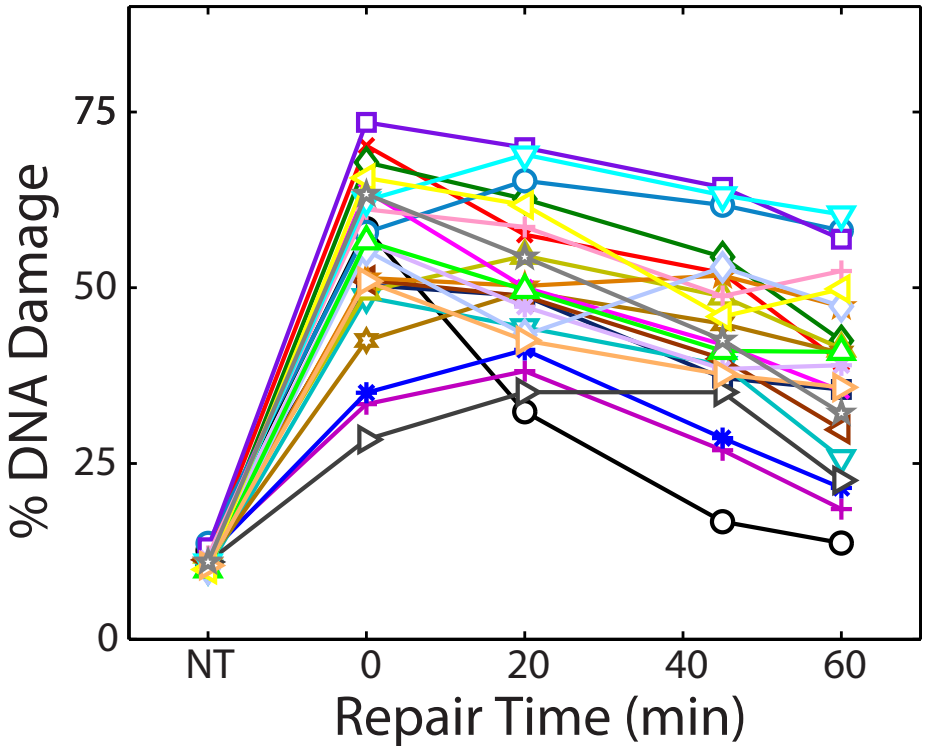


Blue: DNA
Green: H2AX staining= double strand breaks

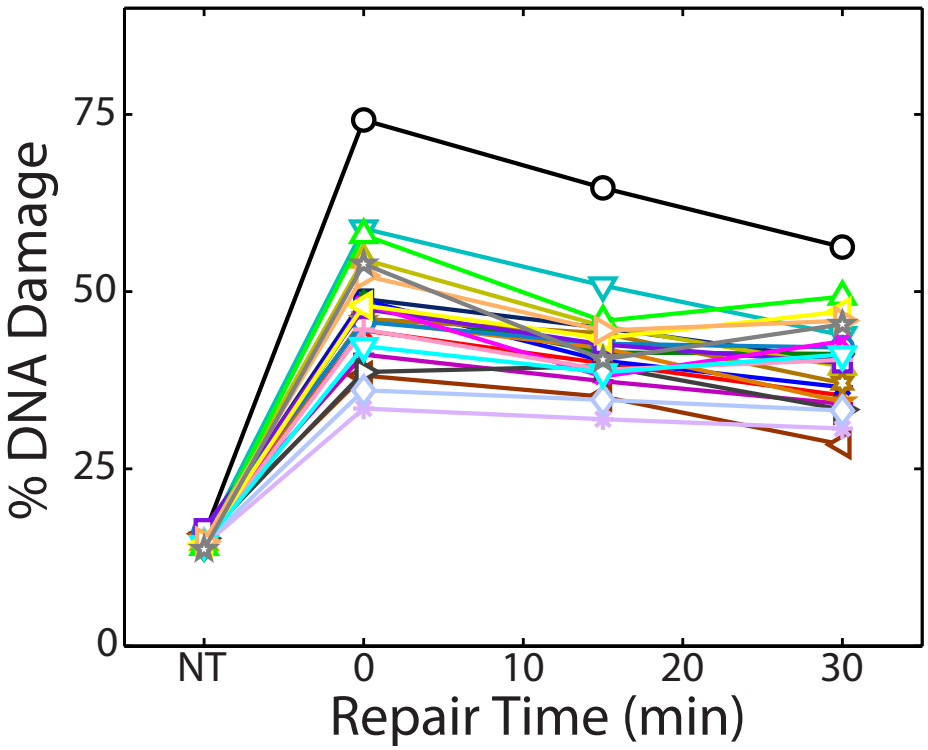
Detecting Repair over time in Human Cells

➔**Each color represents a different human cell line's response to mutagen after an initial exposure followed by recovery time**

Oxidative Damage



Alkylation Damage



from Prof. Engelward's lecture slides

Major assignments for Mod1

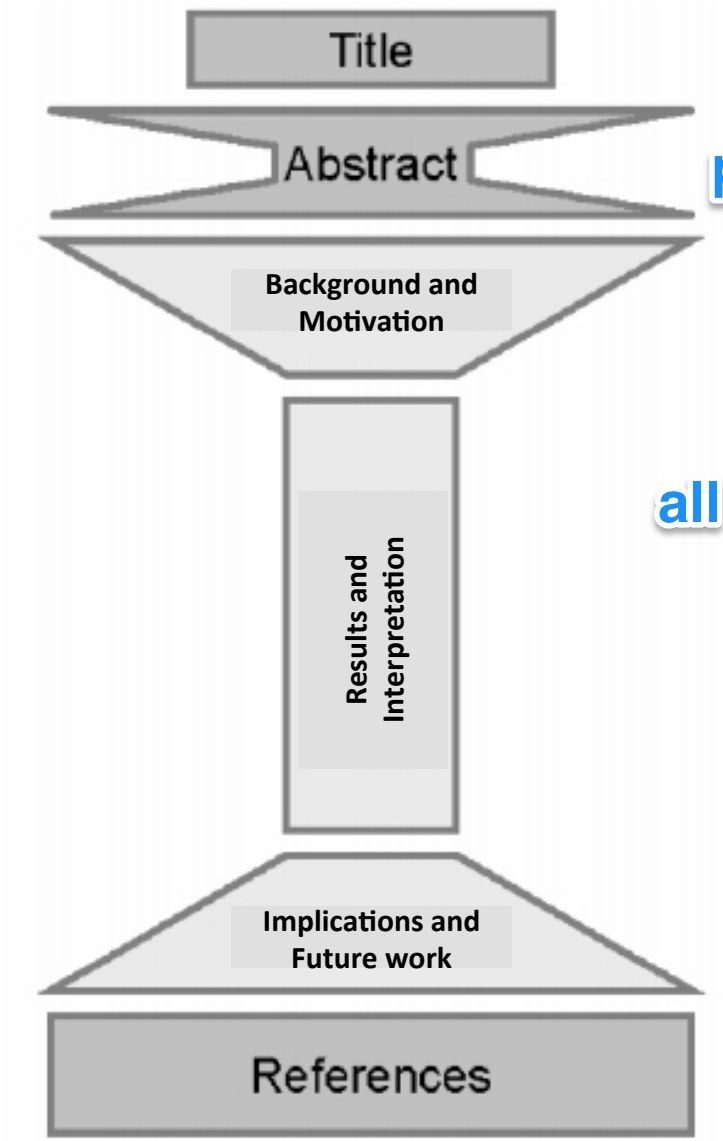
- Data summary draft
  - due by 10pm on Wed., October 11
  - revision due by 10pm on Sun., October 22

Summary content

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

- Mini presentation due by 10pm on Sat., October 14
- Blog post for M1 due by 10pm on Mon., October 23

M1 Data summary Architecture



paragraph

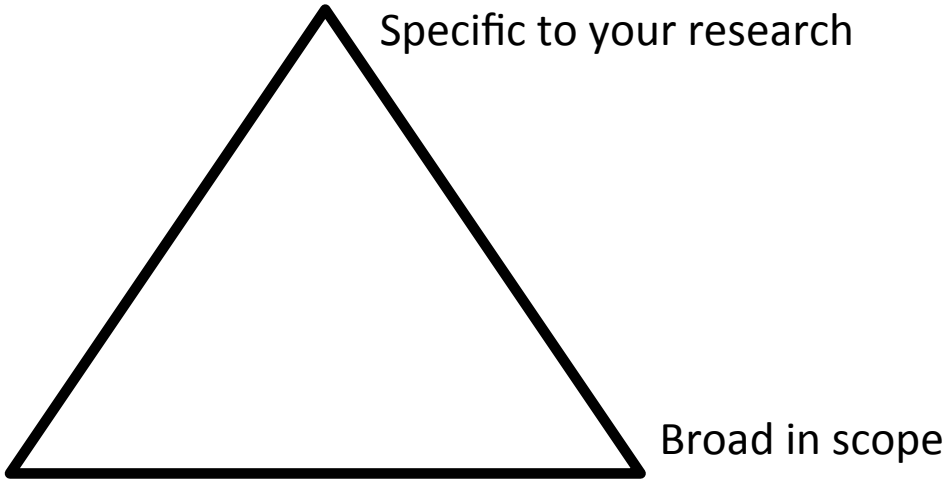
all the rest in bullets

HW M1D6: Implications and Future Works Draft

use loading expt. for draft

Implications and Future Work: potential topics [edit]

- ***Topic***: Did your results match your expectations?
  - If no, provide a putative explanation. If yes, how can you further test if your hypothesis is correct?
- ***Topic***: Based on the results, whether they matched your expectations or not, what experiments might you recommend next?
  - Follow-up experiments could distinguish between competing explanations of a given outcome or broaden the sample set for a question you already asked, to give just two examples.
- ***Topic***: How might this assay be improved?
- ***Topic***: How might this assay be used as a research tool? in the clinic? in industry?



In your Data summary tie together (and mirror) your background and motivation, and implications and future work

HW M1D6: Mini Presentation Outline

- Follow time and content guidelines
- Introduce yourself and your research
- Clearly state your hypothesis to identify main question
- Be quantitative when stating your findings (NOT "This was more/less than…"
- For this HW assignment put placeholder statements for key findings

Category	Approximate worth	Elements of a strong presentation
Content	50%	• Did you introduce your research? • Did you include the key findings (and the techniques used to gather these results, if necessary)? • Was the importance of your project clear?
Organization	25%	• Is the presentation logical and easy-to-follow? • Are the main points emphasized? • Did you include transition statements such that the presentation 'flows' and is easily followed/understood?
Delivery	25%	• Do you show confidence and enthusiasm? • Did you use appropriate language (technical or informal, as appropriate)? • Is your speech clear?

HW M1D6: Prepare for in-class paper discussion

- Consider discussion guidelines on wiki while reading the paper
- Contributing to the discussion is impt. for your participation score

Cytometry
PART A
Journal of the International Society for Advancement of Cytometry

Standard Fluorescent Imaging of Live Cells is Highly Genotoxic

Jing Ge,[1] David K. Wood,[2] David M. Weingeist,[1] Somsak Prasongtanakij,[3] Panida Navasumrit,[3] Mathuros Ruchirawat,[3] Bevin P. Engelward[1]*

In lab today

1. Teams start enzyme treatment in main lab and during this time determine your Part 4 experimental variables, check with instructors.
2. At ~3:30pm at least 1 person from each H2O2 team should start Part 4 in the tissue culture room.
3. At ~4:00pm at least 1 person from each MMS team should start Part 4 in the tissue culture room.
4. Complete Comet Chip electrophoresis and paraformaldehyde fix H2AX assay.