M1D3: Prepare and treat cells for CometChip Experiment

09/18/16

1. Communication workshop 56-614
2. Determine # cells to load
3. Load CometChips with +DNAPKcs and –DNAPKcs cells
4. Induce DNA damage with H_2O_2 and lyse cells

Announcements

*Remember to spray & wipe benches with 70% ethanol before and after work

*Remember to empty benchtop buckets at end of day

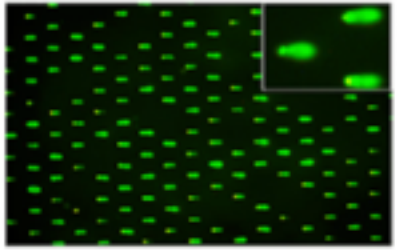
*No lab on Thursday!

Overview of
Module 1: Measuring Genomic Instability

1. Optimize comet chip assay

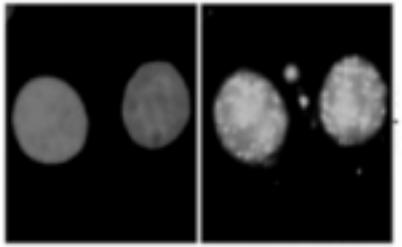
- Test loading variables

Today



2. Use comet chip assay to measure DNA damage

- Measure effects of H_2O_2 on +/- DNA-PK cell lines

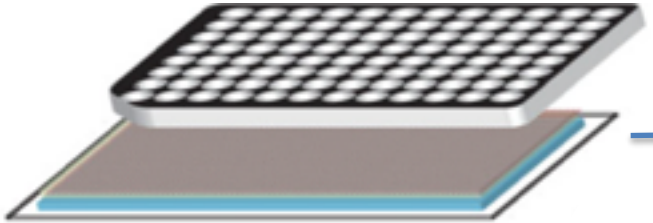


3. Use immuno-fluorescence assay to measure DNA damage

- Examine effect of H_2O_2 on γH2AX foci formation

Assess DNA damage in tumor cells with & without DNAPKcs

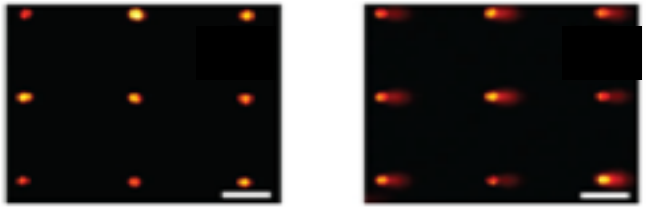
Treat captured cells in comet chip with H_2O_2 (oxidative damage)



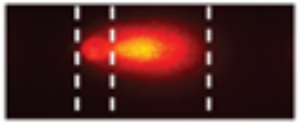
Lyse cells & unwind DNA (DNA still captured agarose in overlay)

Next time

Agarose Electrophoresis



Stain DNA and image via fluorescence microscopy



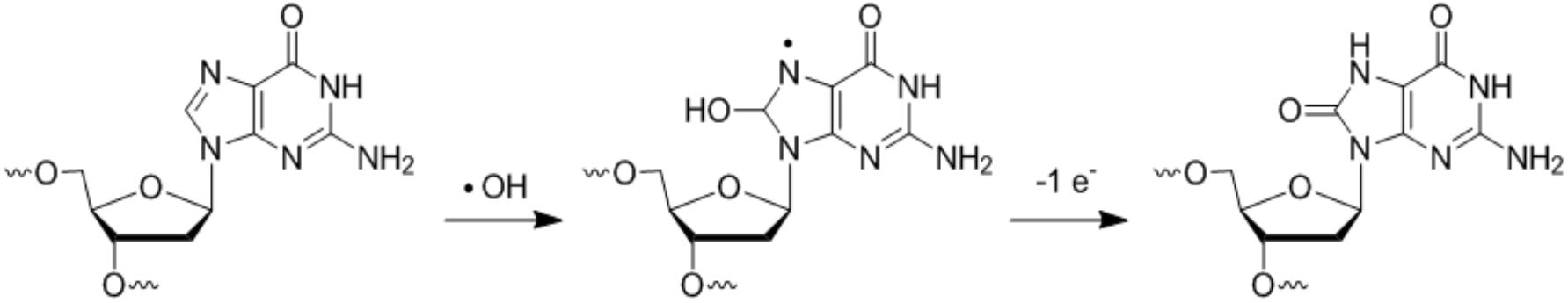
Analysis via Matlab

How does H_2O_2 damage DNA?

$H_2O_2 + e^- \rightarrow HO^- + \bullet OH$

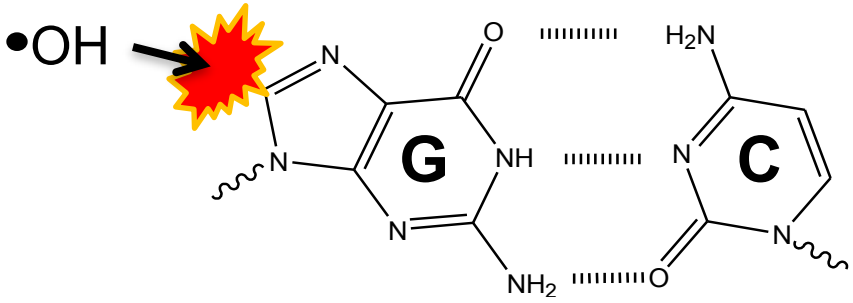
ROS = reactive oxygen species

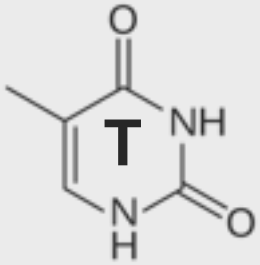
$2\ H^+ + 2\ e^- + H_2O_2 \rightarrow 2\ H_2O$



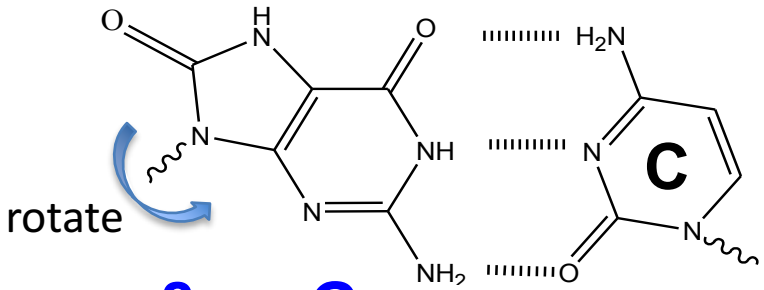
Guanine **8-oxo-Guanine**

How does H_2O_2 damage DNA?

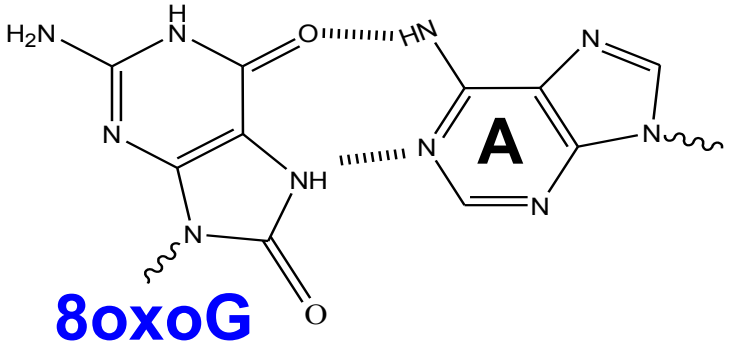




Damaged G now looks like T



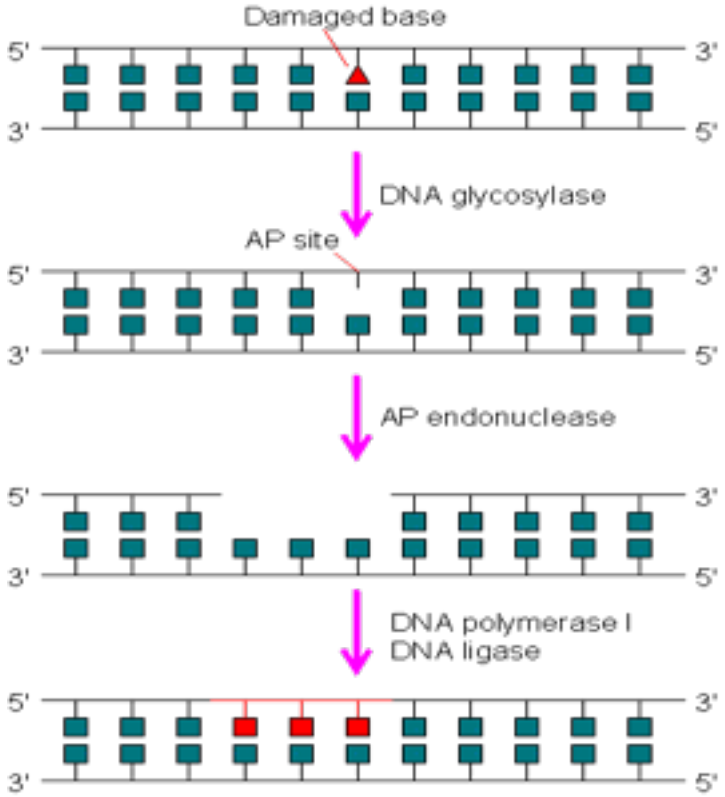
8oxoG



8oxoG

Mutation if replicated GC → AT

How do our cells respond?
Typically, Base Excision Repair (BER) Pathway



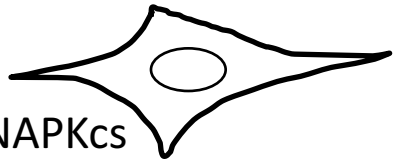
AP site = abasic site

single strand break

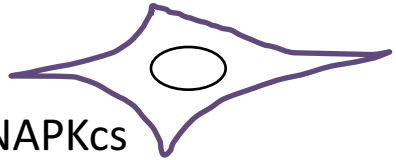
http://www.web-books.com/MoBio/Free/Ch7G.htm

Does DNAPKcs have a role in DNA repair in response to oxidative damage?

- BER can lead to Double strand breaks, which can be repaired by non-homologous end joining (NHEJ)
- DNAPKcs = DNA-dependent protein kinase, catalytic subunit
- DNAPKcs involved in NHEJ



+DNAPKcs



-DNAPKcs

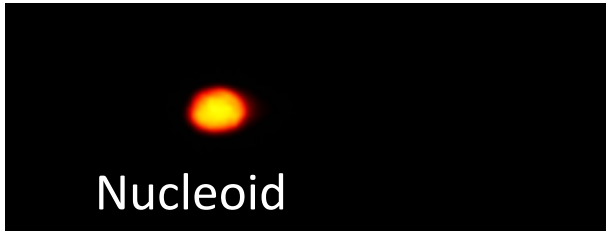
Control:

+ H_2O_2 or PBS only

+ H_2O_2 or PBS only

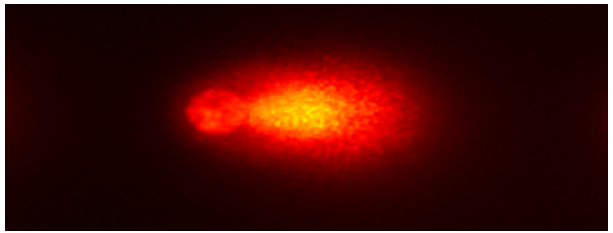
Measure "damaged" DNA via CometChip

Output of Alkaline CometChip Assay



No Damage

- Supercoiled nucleoid
- Little or no migration



High Damage

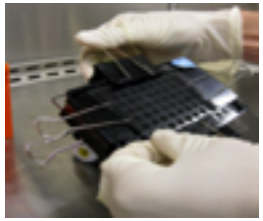
- SSBs, abasic sites, alkali labile sites
- forms a “Comet tail”

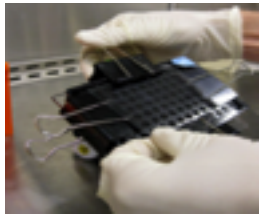
Genomic damage from direct strand breaks

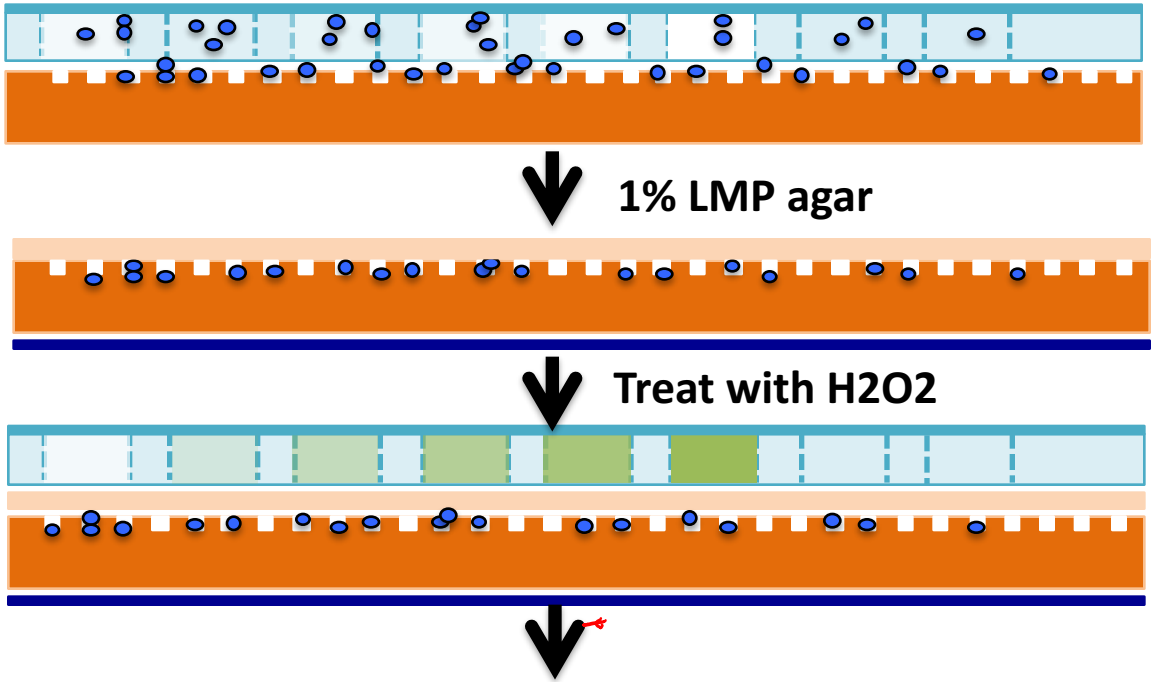
AND REPAIR INTERMEDIATES

Logistics of today's experiment

Be sure to mix your cells right before loading!!!

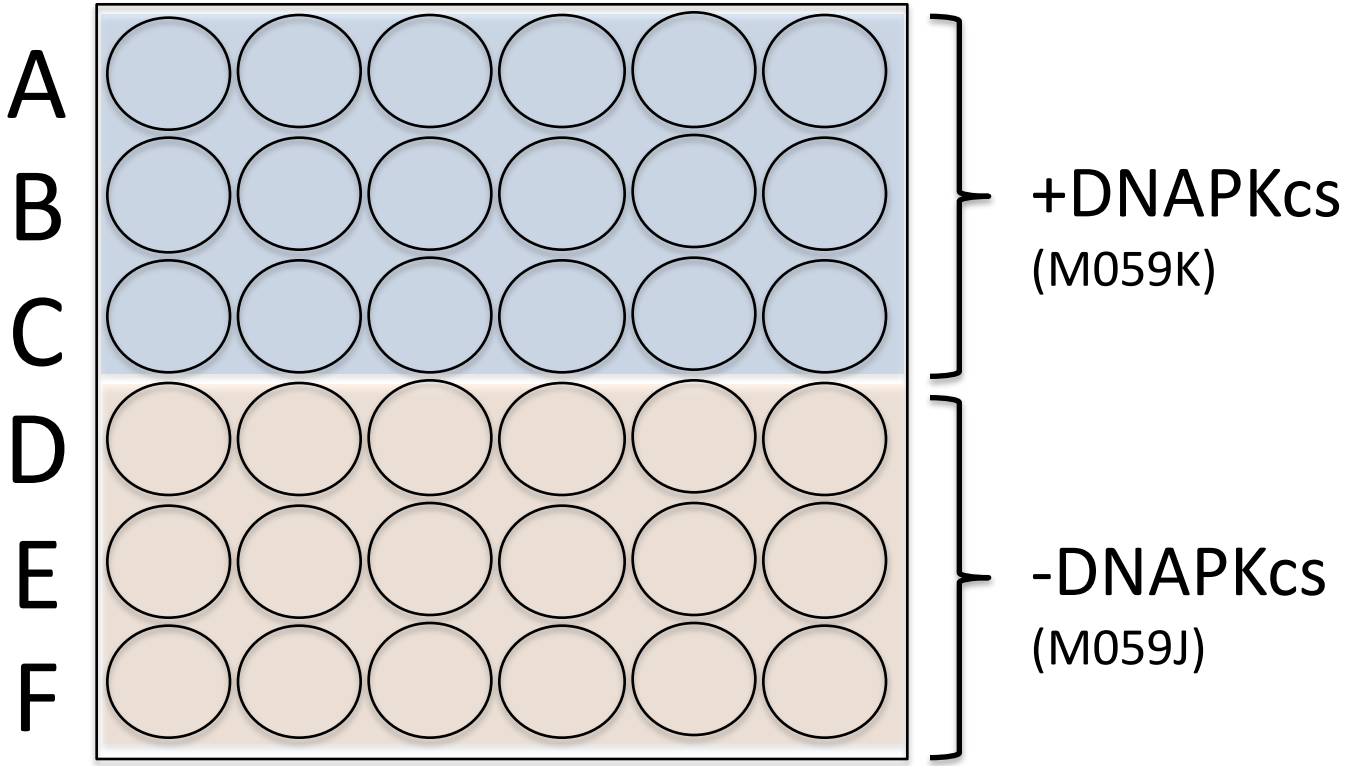




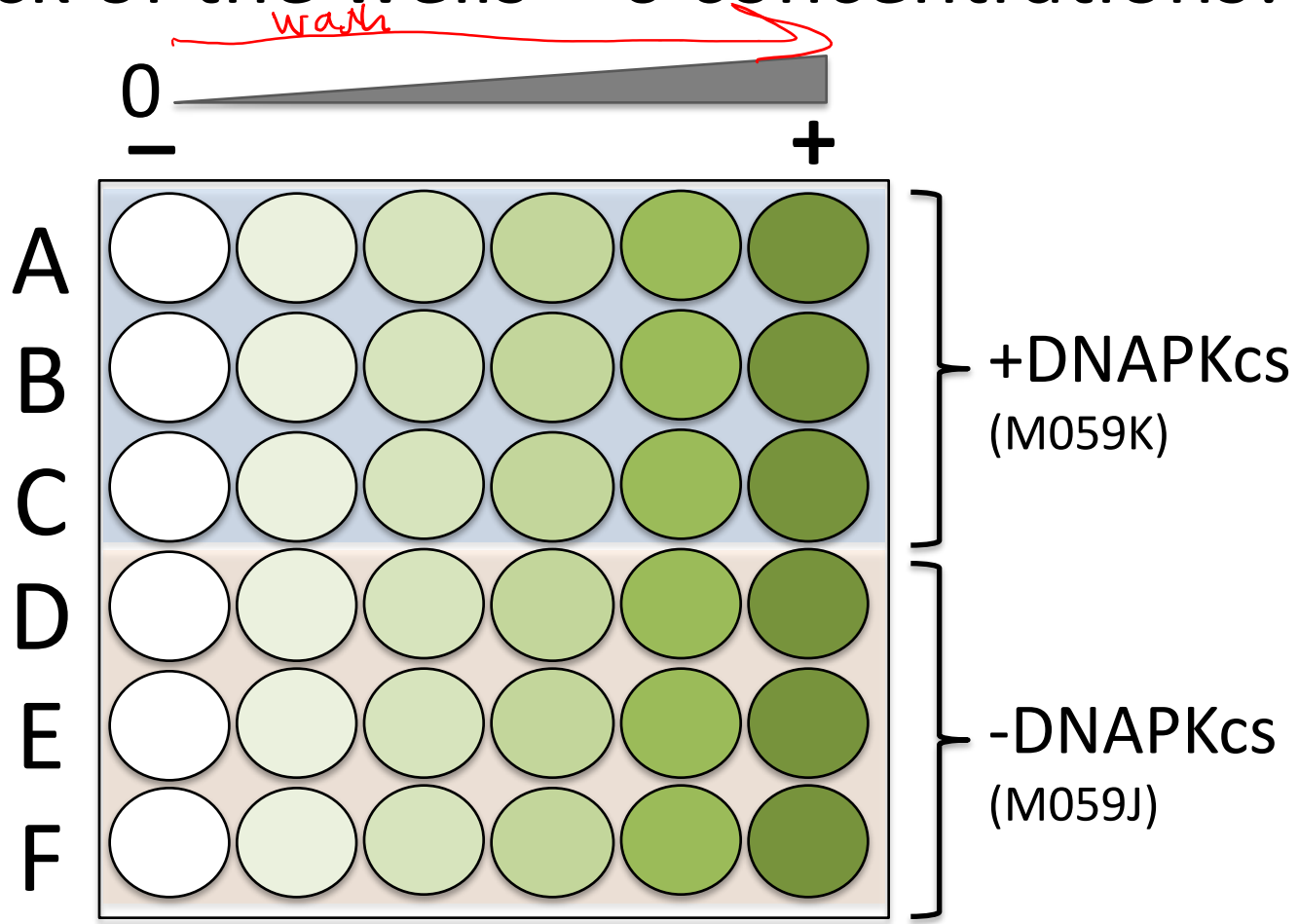


Wash off mutagen (carefully!) and leave CometChip in lysis buffer

Keep track of the wells – 2 cell lines!



Keep track of the wells – 6 concentrations!



Preparing H_2O_2 dilution series:

Handling tips:
- Concentrated H_2O_2 (10M) should be left at front bench
- Keep H_2O_2 **on ice** at all times
- Minimize waste!

Treat with: 0, 0.01, 0.1, 0.25, 0.5, 1 µM H_2O_2
- 4°C for 20min
- Add **100µl** of drug dose to each macrowell
- **Triplicate**: each concentration will have three macrowells for each cell line
  - Make 1 mL of each concentration

Stock 1: 10 M
Stock 2 Provided: 1 mM
Stock 3: 1 uM

1:1000

$C_1V_1 = C_2V_2$

$(1mM)\,V_1 = (1\mu M)(2mL)$

$V_1 = 2\mu L$

2µL of 1mM H_2O_2 + 1998 µL of PBS ⇒ 2mL of 1µM H_2O_2

Note: 2 uL minimum volume on your pipettes

	0	0.01	0.1	0.25	0.5	1 uM
Stock 3						1 mL
PBS						0 mL

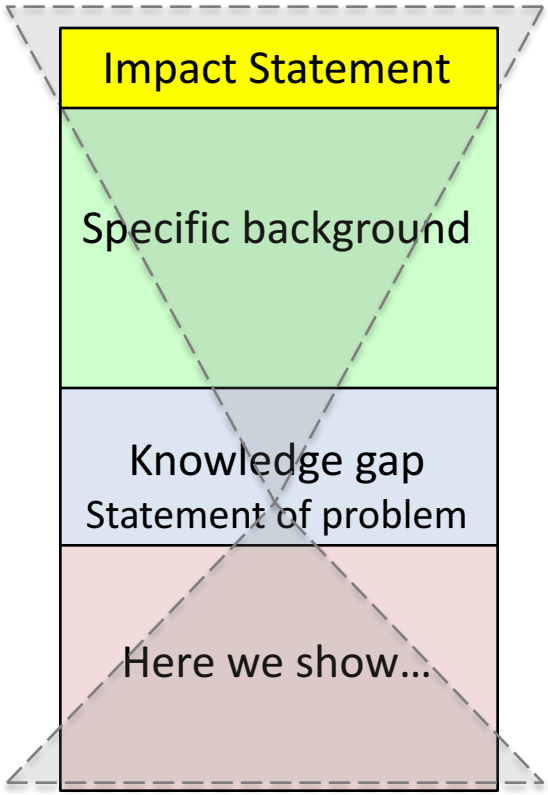
In lab today

1. Calculate volume of cells to load, obtain cells from instructors and load cells onto CometChip
2. Calculate volumes necessary for mutagen dilutions and check with instructors.
3. Treat CometChip with mutagen and carefully wash.
4. Leave CometChips in lysis buffer.

For next time

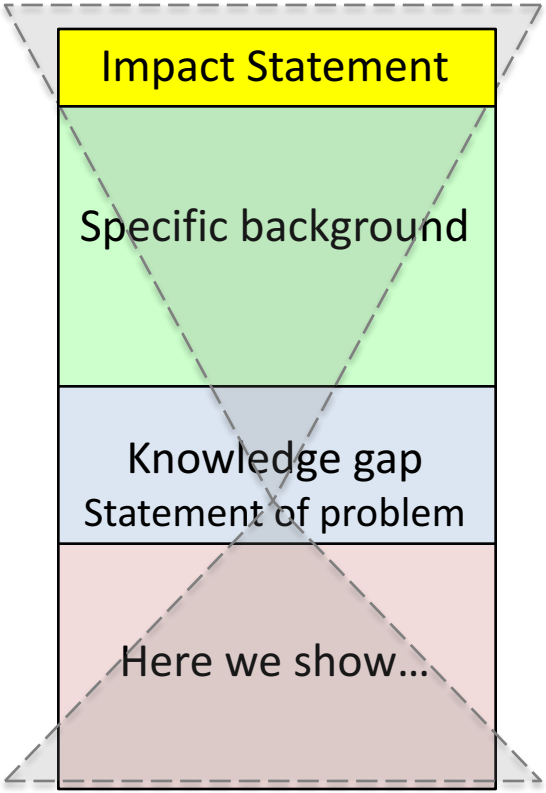
1. Write topic sentences (1st sentence) for each paragraph that would be in your Data Summary's Background and Motivation section
   - Remember to include references with summary & why you chose it
2. Visit Comm Lab before M1D5.

What goes into a background/motivation section?

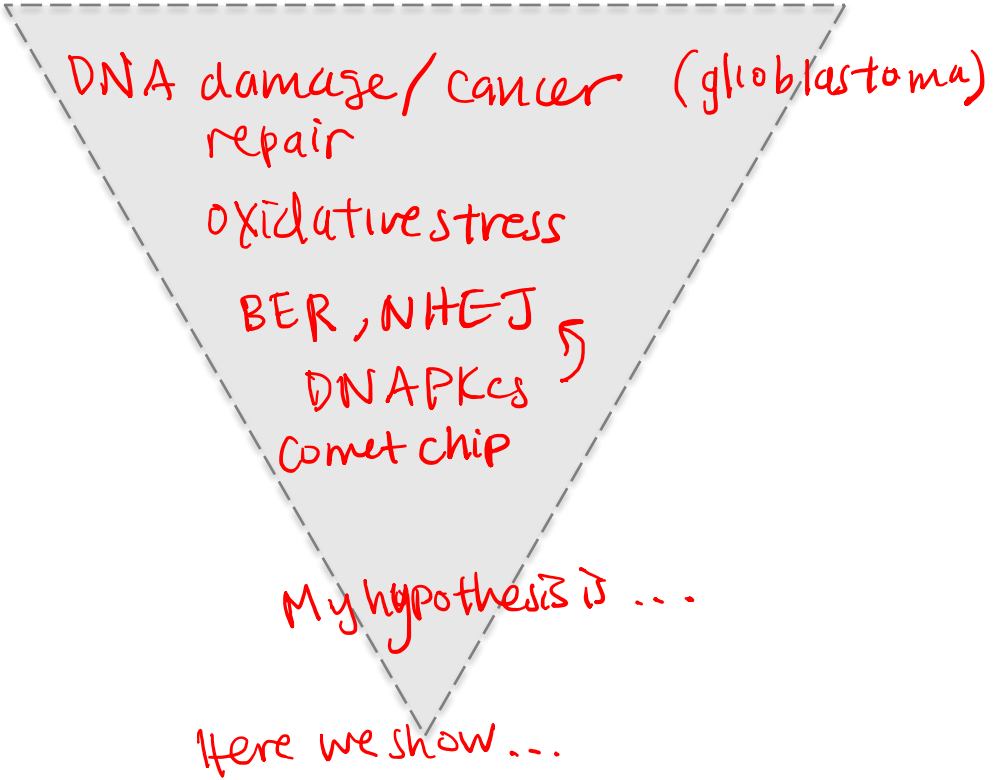


- Your research is anchored in a general topic that your audience cares about or could be interested in.
  - Focus on describing previous work in the field
- Specific background connects your project with the general background.
  - Minimum essential information
  - References current work in the field
  - Introduce specific technologies necessary for understanding the project
- The question you address is clearly articulated, connected to the background, and has appropriate scope for the project
  - Give evidence of incompleteness of current understanding, therefore motivating the investigation
  - Include a space holder for your hypothesis (or come up with one)
- A preview of your findings and their implications
  - Light on Methods

What goes into your introduction?



Choose one narrative



Let's combine our data from last time:
How many cells should we load per microwell?

50k / microwell

	M059J (-DNAPKcs)		M059K (+DNAPKcs)	
Team	Row B cell # loaded (% filled)	Row C cell # loaded (% filled)	Row B cell # loaded (% filled)	Row C cell # loaded (% filled)
Red	19k (9%)	375k (19%)	19k (13.5%)	375k (46%)
Orange	9k (10%)	128k (19%)	9k (50%)	128k (58%)
Green	16k (54%)	50k (54%)	16k (76%)	44k (76%)
Blue	25k (33%)	65k (30%)	25k (47%)	65k (48%)
Purple	35k (27%)	150k (24%)	35k (50%)	150k (56%)