M1D3: Prepare and treat cells for CometChip Experiment

09/19/18

1. Communication workshop 16-220
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

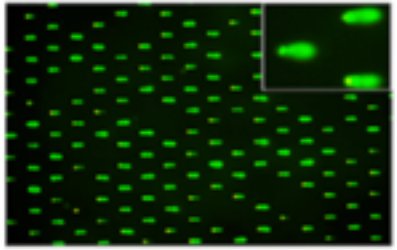
*Remember to empty benchtop buckets at end of day

*No lab on Friday!

Overview of
Module 1: Measuring Genomic Instability

1. Optimize comet chip assay ✓

- Test loading variables



2. Use comet chip assay to measure DNA damage

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

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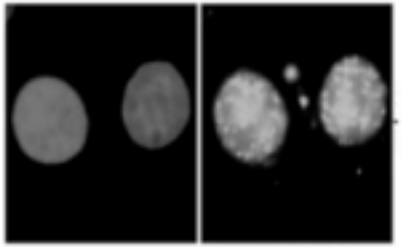


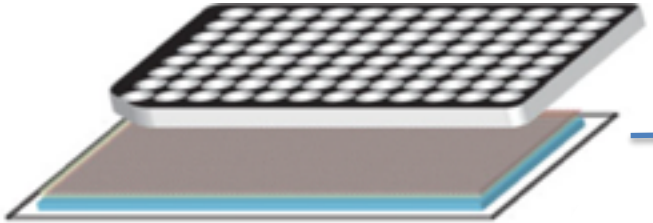
3. Use immuno-fluorescence assay to measure DNA damage

- Examine effect of H_2O_2 on γH2AX foci formation

Assess DNA damage in tumor cells with & without DNAPKcs

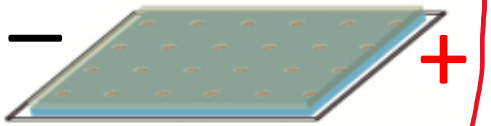
Next week

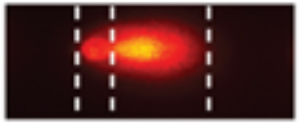
Treat captured cells in comet chip with H_2O_2 (oxidative damage)



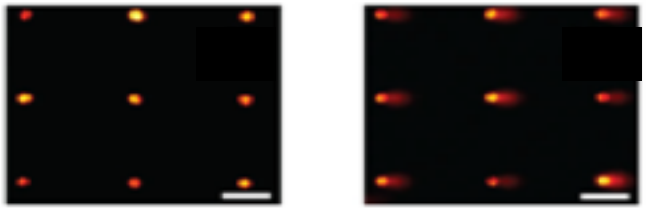
Lyse cells & unwind DNA (DNA still captured agarose in overlay)

Agarose Electrophoresis





Analysis via Matlab



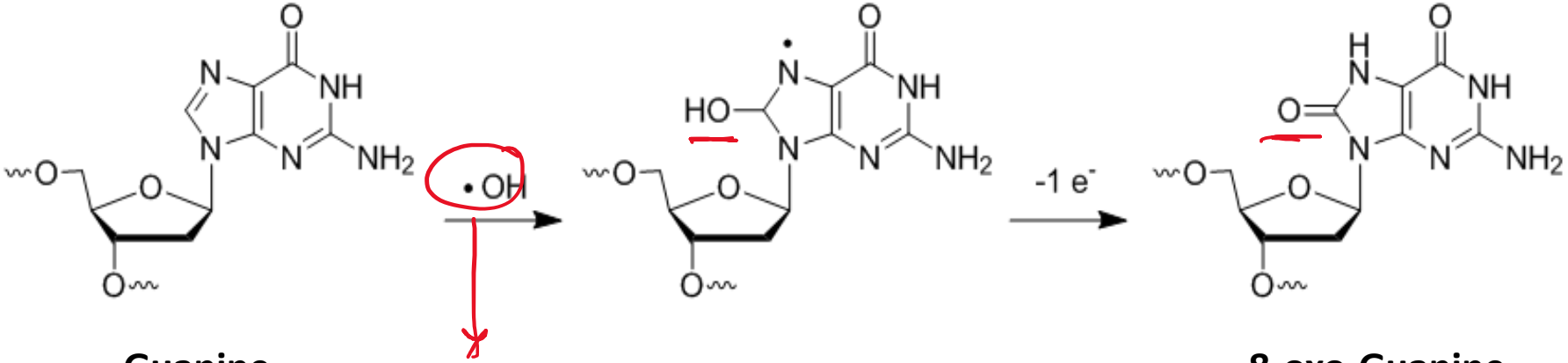
Stain DNA and image via fluorescence microscopy

How does H_2O_2 damage DNA?

$H_2O_2 + e^- \rightarrow HO^- + \bullet OH$ → hydroxyl radical

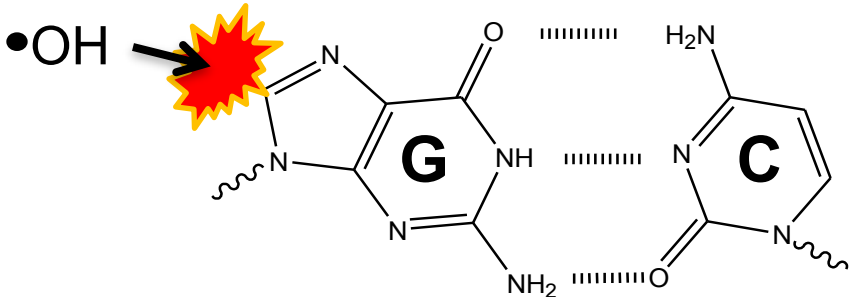
ROS = Reactive oxygen species

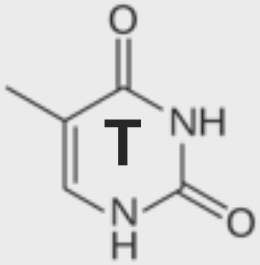
$2\ H^+ + 2\ e^- + H_2O_2 \rightarrow 2\ H_2O$



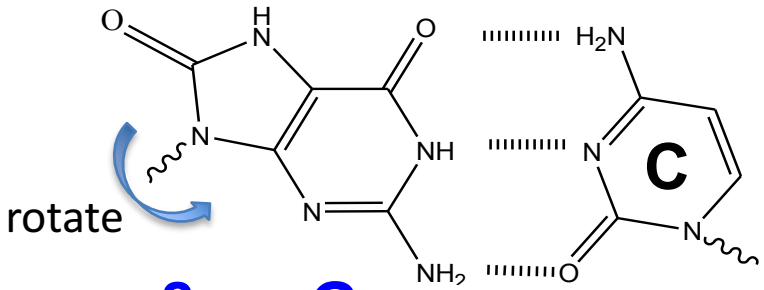
hydroxyl radical attacks bond + adds carbonyl group

How does H_2O_2 damage DNA?

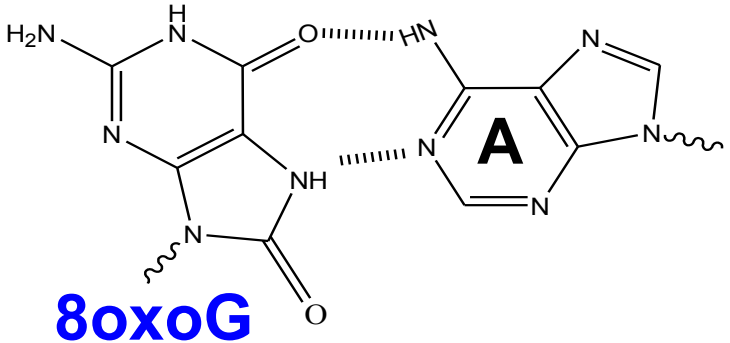




Damaged G now looks like T



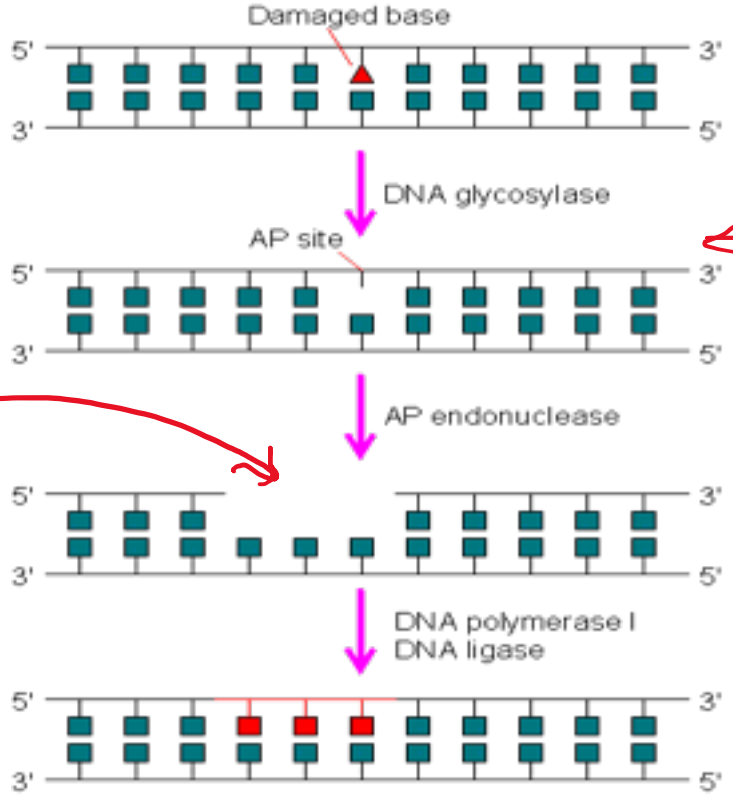
8oxoG



8oxoG

Mutation if replicated GC → AT

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



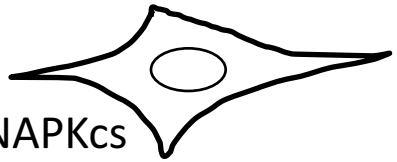
AP site = abasic site (apurinic apyrimidinic)

Single strand breaks (SSB)

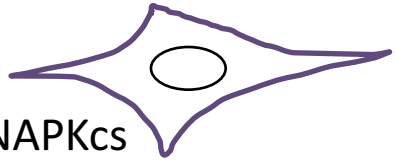
http://www.web-books.com/MoBio/Free/Ch7G.htm

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 NHEJ (nonhomologous end joining)
- DNAPKcs = DNA-dependent protein kinase, catalytic subunit
- DNAPKcs involved in NHEJ



+DNAPKcs



-DNAPKcs

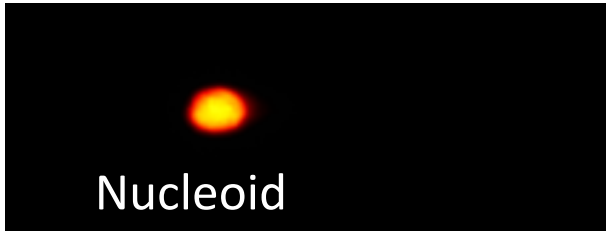
Control:

+ H_2O_2 or PBS alone

+ H_2O_2 or PBS alone

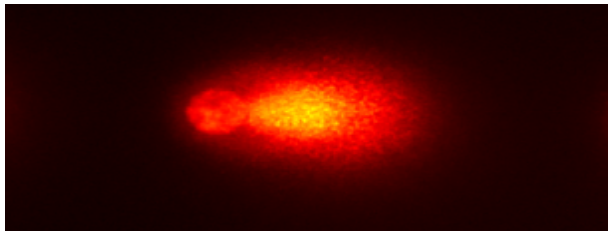
Measure "damaged" DNA via CometChip

Output of Alkaline CometChip Assay



No Damage

- Supercoiled nucleoid
- Little or no migration



High Damage

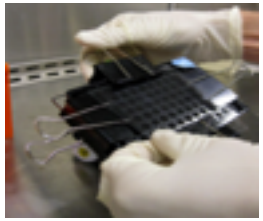
- SSBs, abasic sites, alkali labile sites
- forms a "Comet tail"

Genomic damage from direct strand breaks

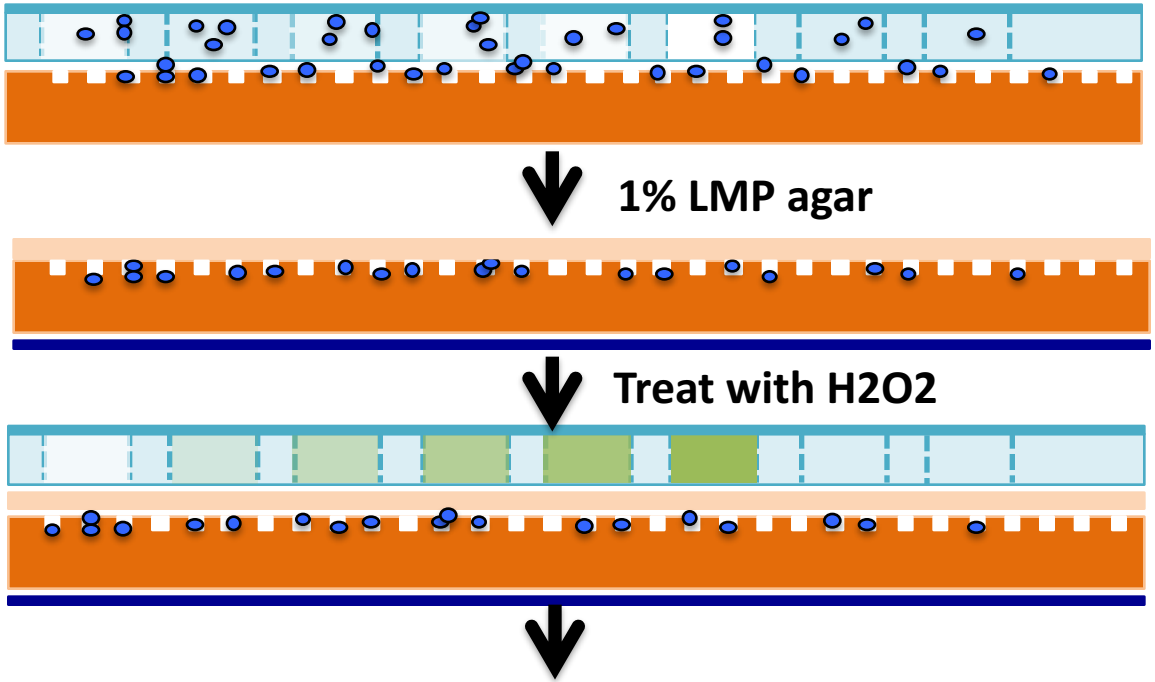
AND Repair Intermediates

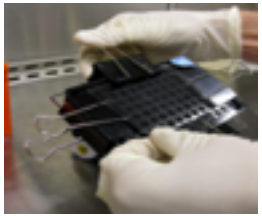
Logistics of today's experiment

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



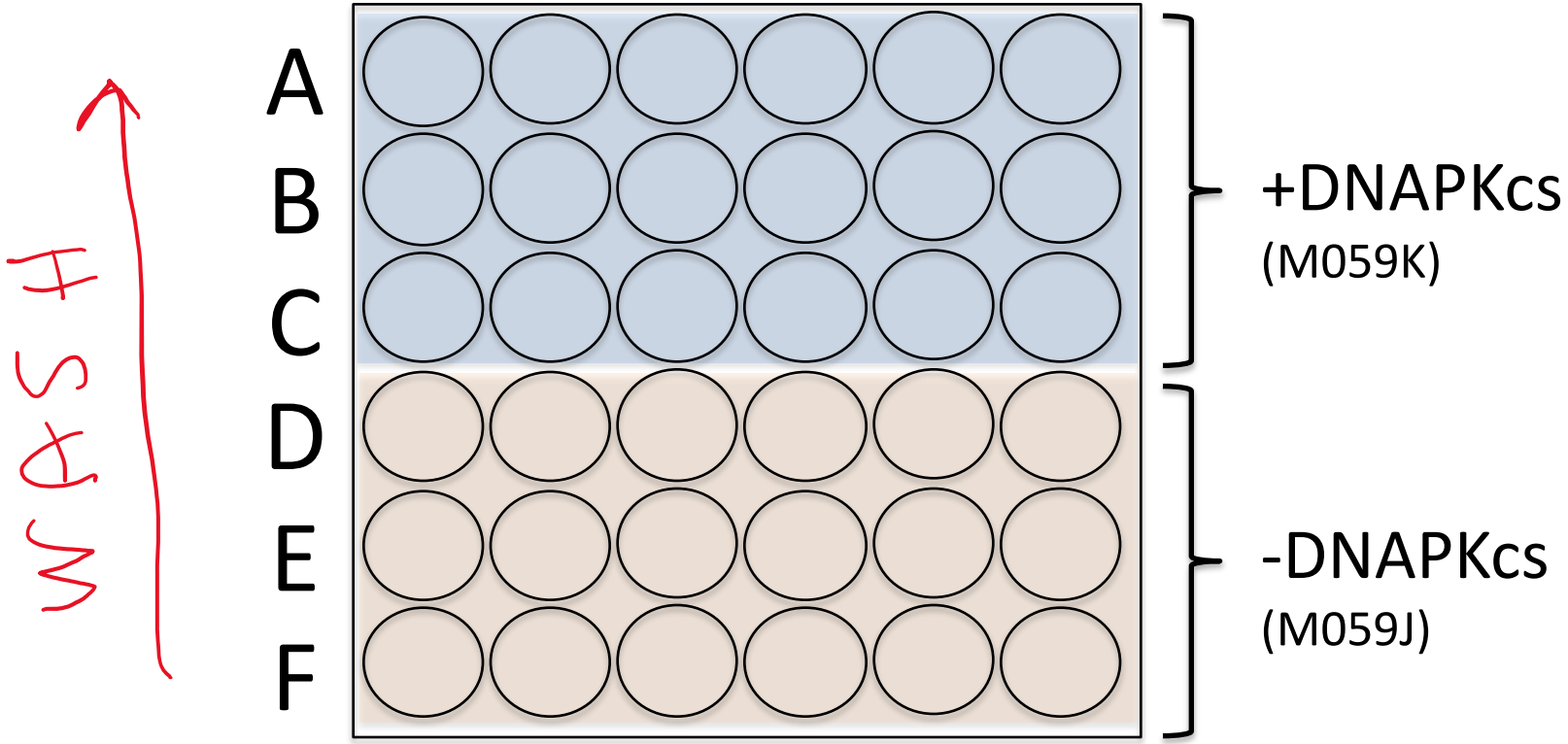
Load __TBD___cells per macrowell



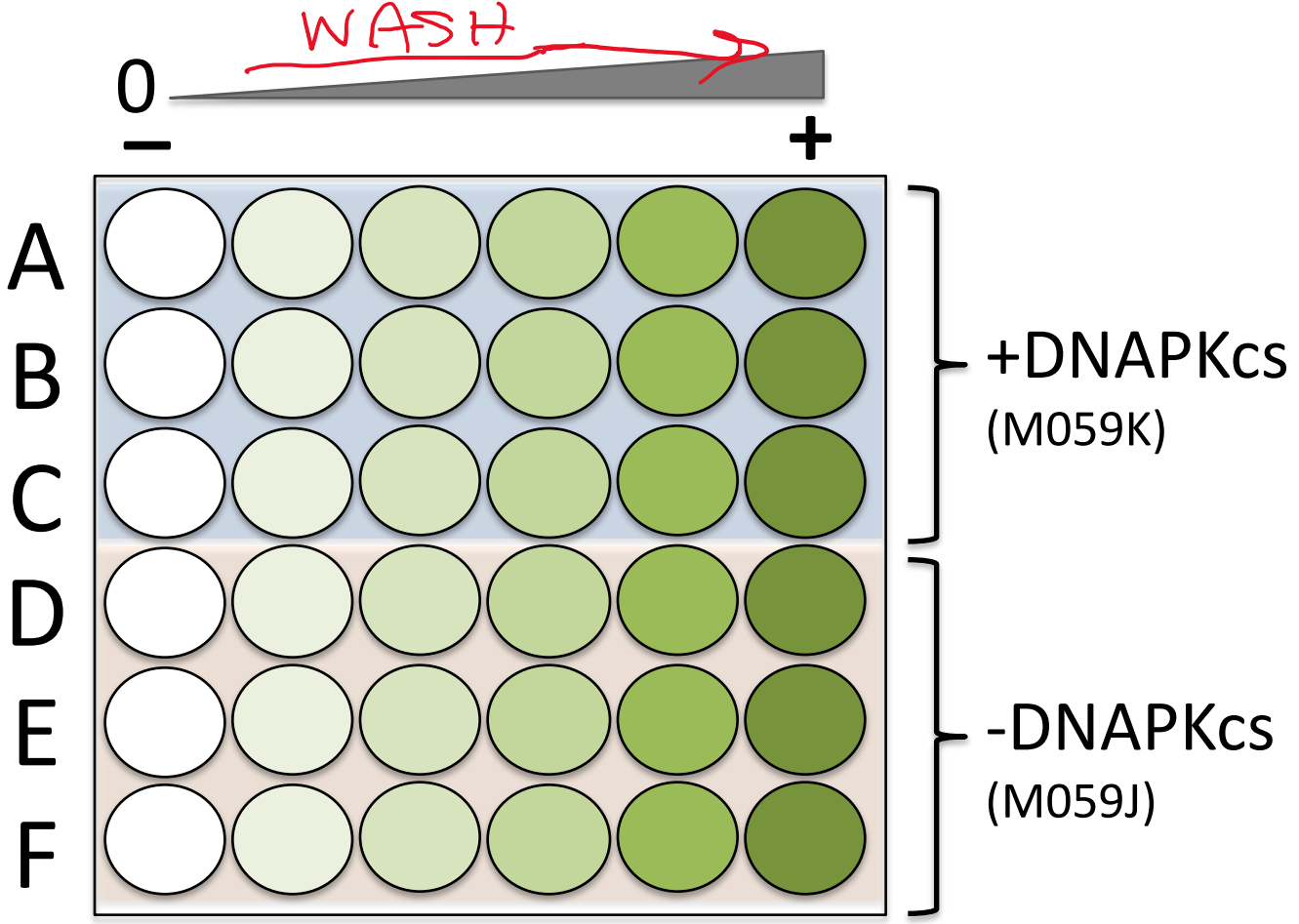


Wash off mutagen (carefully!) and leave CometChip in lysis buffer

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

$C_1V_1 = C_2V_2$

$(1\mu M)(X) = (0.5\mu M)(1000\mu l)$

↳ 500ul of Stock 3
500ul of PBS

Stock 1: 10 M

Stock 2 Provided: 1 mM

Stock 3: 1 uM ← 1:1000

Need 2 mL total

Note: 2 uL minimum volume on your pipettes

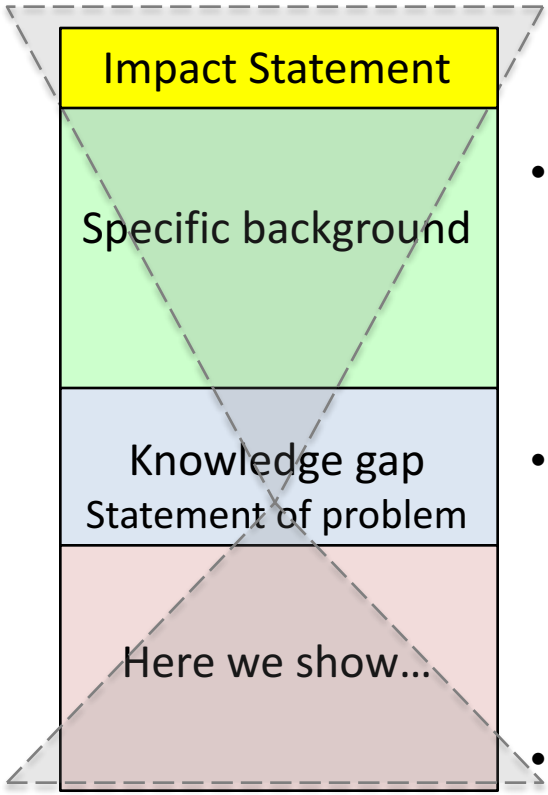
	0	0.01	0.1	0.25	0.5	1 uM
Stock 3					500ul	1000µl
PBS					500ul	0

Let's combine our data from last time:

How many cells should we load per microwell? 25,000 cells per macrowell

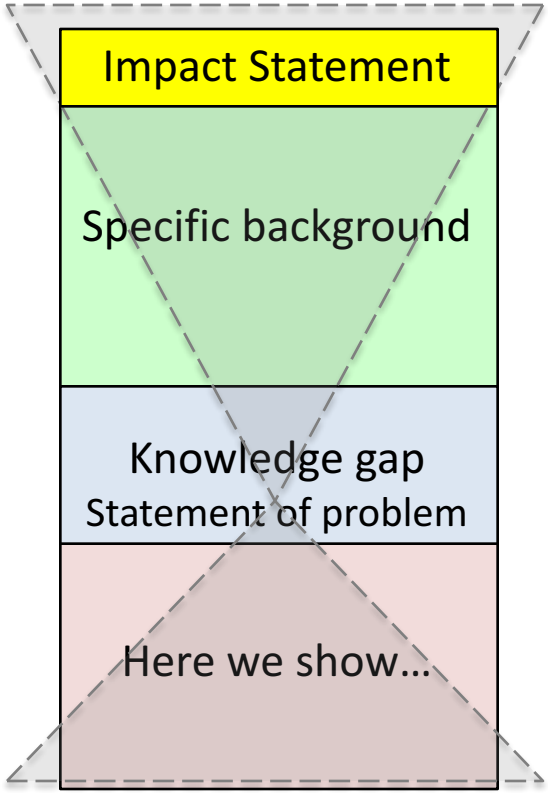
Team	M059J (-DNAPKcs)		M059K (+DNAPKcs)	
	Row B cell # loaded (% filled)	Row C cell # loaded (% filled)	Row B cell # loaded (% filled)	Row C cell # loaded (% filled)
Yellow	50K (32%)	250K (25%)	50K (25%)	250K (35%)
Pink	38K (48%)	100K (40%)	38K (36%)	100K (47%)
Green	25K (13%)	150K (28%)	25K (67%)	150K (62%)
Blue	5K (48%)	50K (40%)	5K (36%)	50K (47%)

What goes into a background/motivation section?

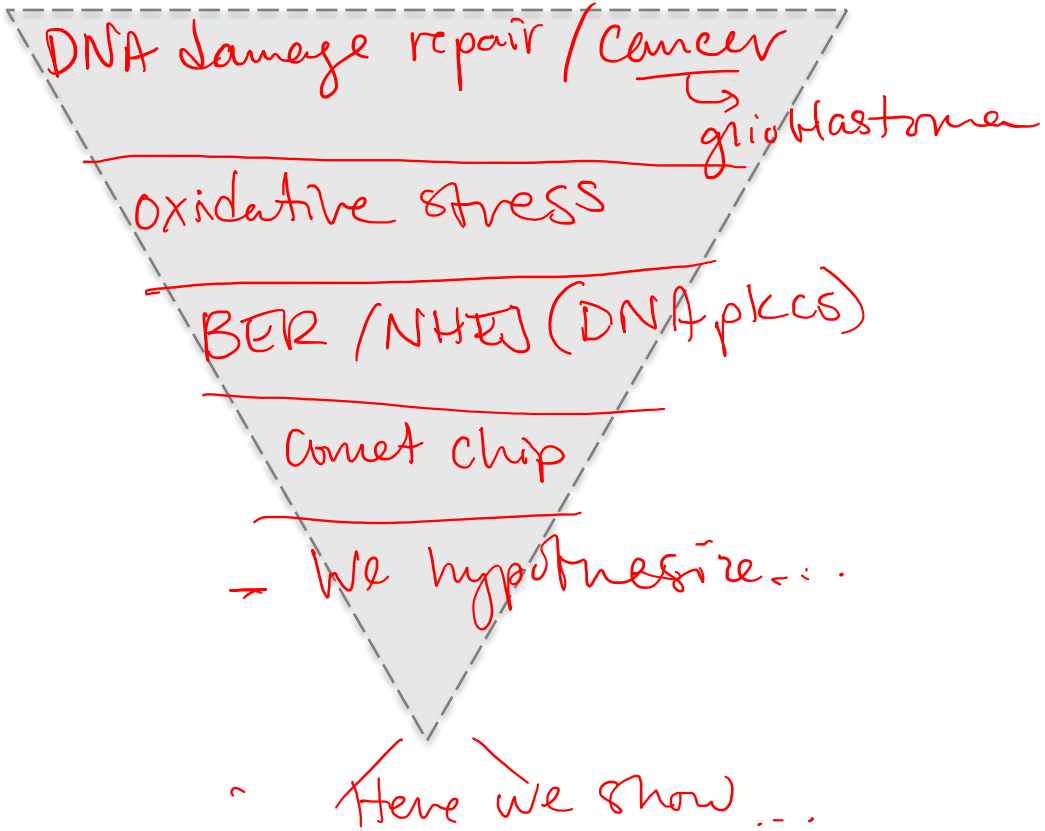


- Your research is anchored in a general topic that your audience cares about or could be interested in.
  - Focus on describing previous work in the field
- Specific background connects your project with the general background.
  - Minimum essential information
  - References current work in the field
  - 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



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.

M1D4HW (9/26/18)

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

- Reminder: Visit Comm Lab before M1D5
- First Mod1 Quiz at 1:05pm M1D4