# M1D4: Complete CometChip experiment and load cells for sub-nuclear foci assay

09/25/18

1. Quiz
2. Electrophoresis of CometChips
3. Seed cells for γH2AX assay

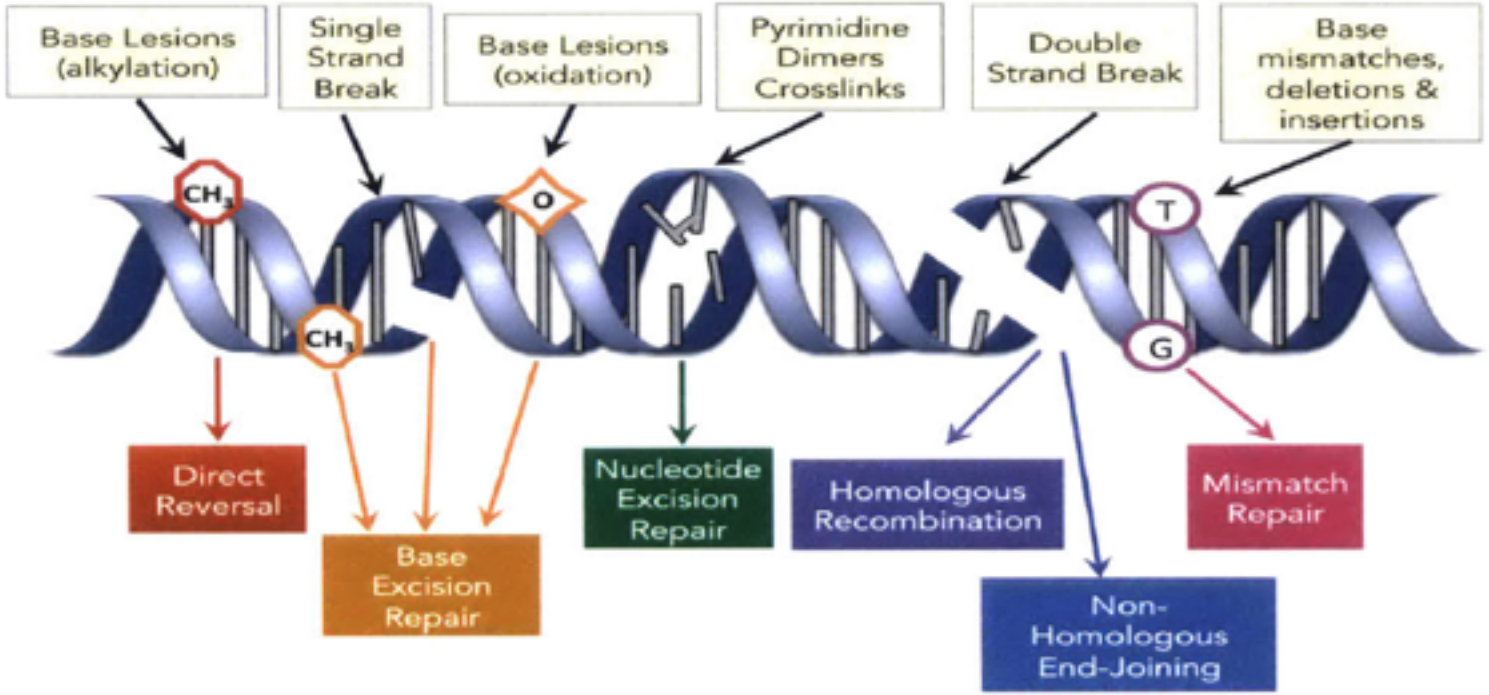
Reminders

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

*Remember to empty benchtop buckets at end of day

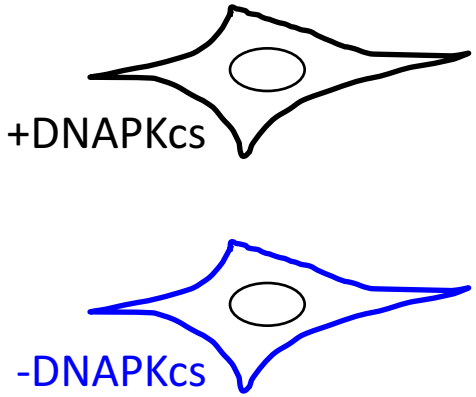
# There are many DNA repair pathways

$H_2O_2$- induced DNA damage is typically repaired by ___BER___

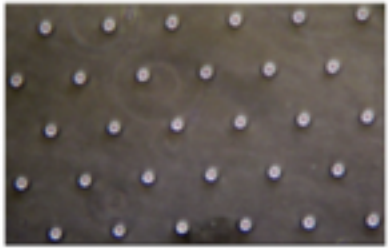


# Does DNAPKcs have a role in DNA repair in response to oxidative damage?

- BER produces single strand breaks (SSBs)
- Having many SSBs can produce double strand breaks (DSBs)
- DSBs can be repaired through NHEJ, a pathway that involves DNAPKcs

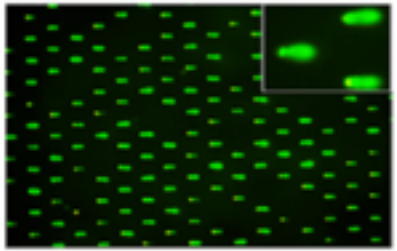


# Overview of Module 1: Measuring Genomic Instability



**1. Optimize comet chip assay**

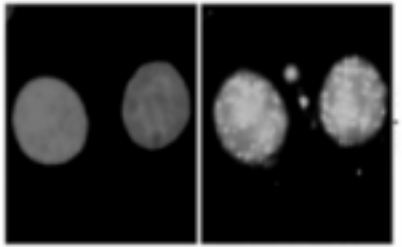
- Test loading variables



**2. Use comet chip assay to measure DNA damage**

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

Direct/indirect measure of DNA damage



**3. Use immuno-fluorescence assay to measure DNA damage**

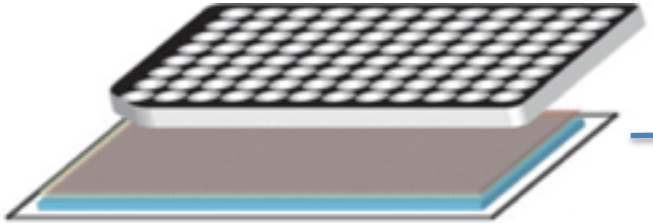
- Examine effect of $H_2O_2$ on γH2AX foci formation

Direct/indirect measure of DNA damage

Today

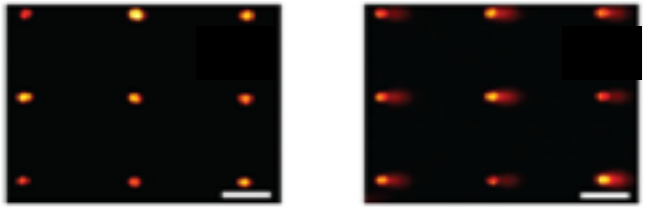
# Assess DNA damage in tumor cells with & without DNAPKcs

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

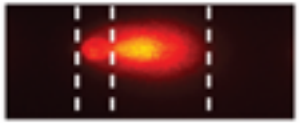


Lyse cells & unwind DNA (DNA still captured agarose in overlay)

Agarose Electrophoresis

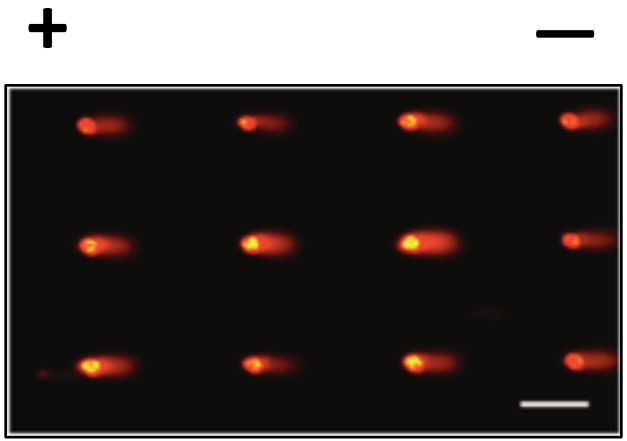
Stain DNA and image via fluorescence microscopy



Analysis via Matlab

# Lysis & staining in CometChips

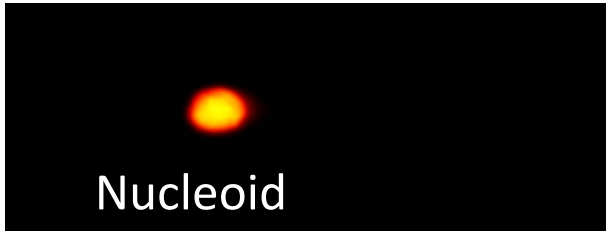
- Alkaline lysis solution — breakdown cell membrane / denature proteins
  - 2.5 M NaCl, 100 mM $Na_2EDTA$, 10 mM Tris
  - pH 10 alkaline
  - Triton X-100 detergent




- Unwinding / electrophoresis Buffer — pH 13.5
- Neutralize ~~& dye~~ — optimize SYBR gold stain binding to DNA
  - 0.4M Tris
  - pH 7.5
- stain — SYBR Gold DNA stain (in PBS) — DNA intercalator, increases 1000x signal when bound to DNA

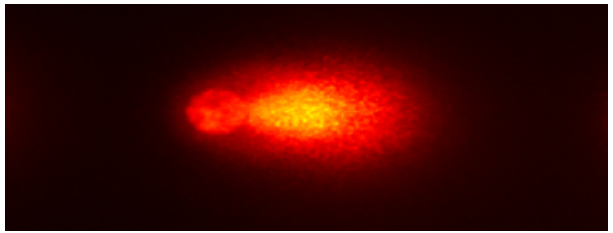
- What are impt. considerations for visualizing DNA? ssDNA, dsDNA, visualize (UV), sensitivity

# 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**

# Crafting a hypothesis

## Motivation for your study…

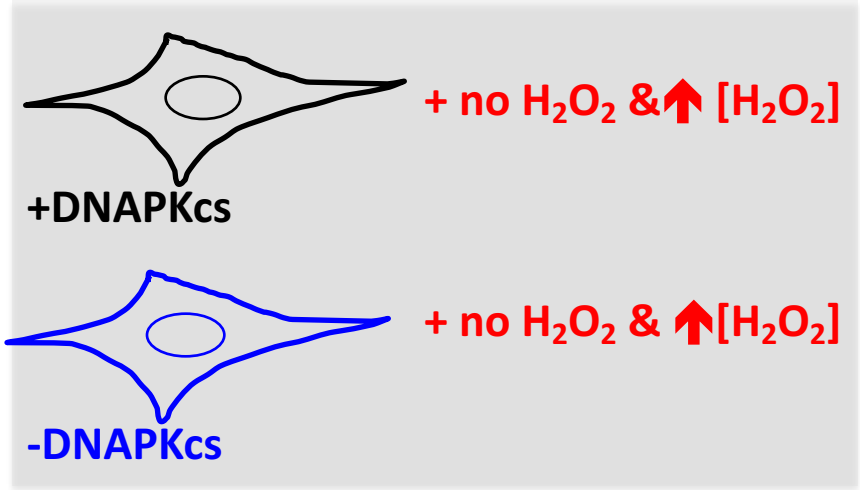
- Study DNA damage in response to environmental factors, i.e. oxidative stress
- Understanding role of DNAPKcs in oxidative DNA damage/repair

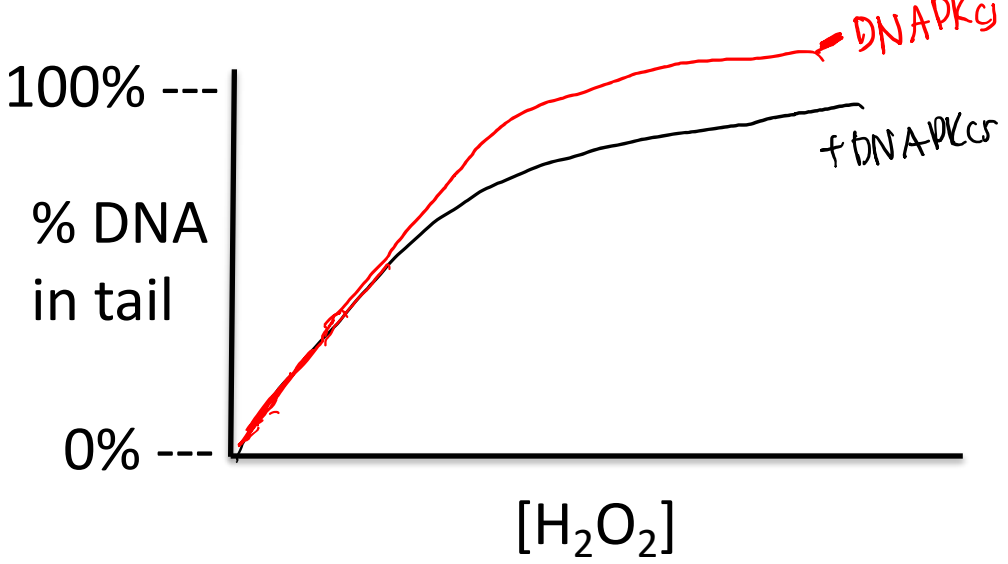
## What is your research question?

1) what's difference between +DNAPKcs & -DNAPKcs in response to $H_2O_2$
   - if little damage, maybe no difference in BER
   - if high damage, ↑ stress in -DNAPKcs cells
   - ?

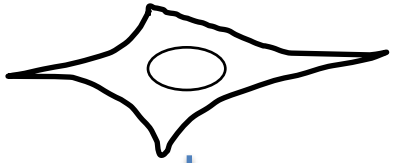
2) ↑ $[H_2O_2]$ → ↑ damage?

# What result do we expect from the CometChip?

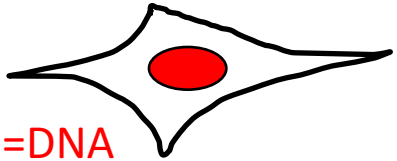


# The DNA damage response



exposure to DNA damaging agent



red=DNA sustained damage

Stall the cell cycle and **repair** the damage before replication

~ minutes (~30min)

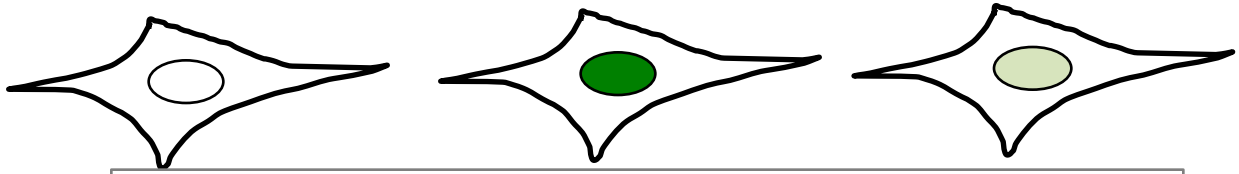
Damage goes unrepaired and **mutations** arise after next round of replication

cell cycle (~24 hours)

Initiate cell **death**

~ hours

# Measuring double strand breaks via γH2AX Assay



no treatment, $H_2O_2$ treatment, recovery condition

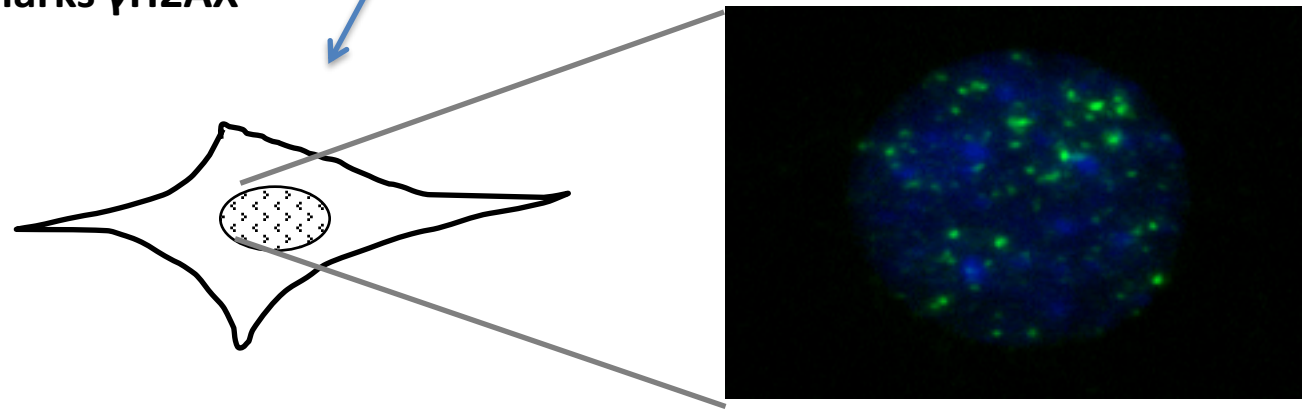
30 min or 1 hr

**γH2AX =**

phosphorylated H2AX (histone)

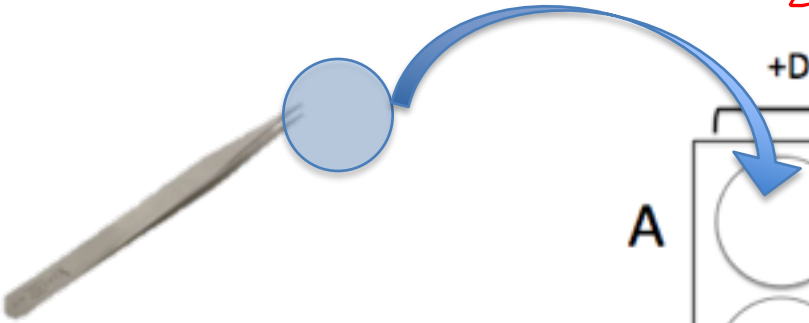
↳ mostly correspond to DSBs (but maybe other DNA damage as well?)

**Fix cells and stain with antibody that marks γH2AX**

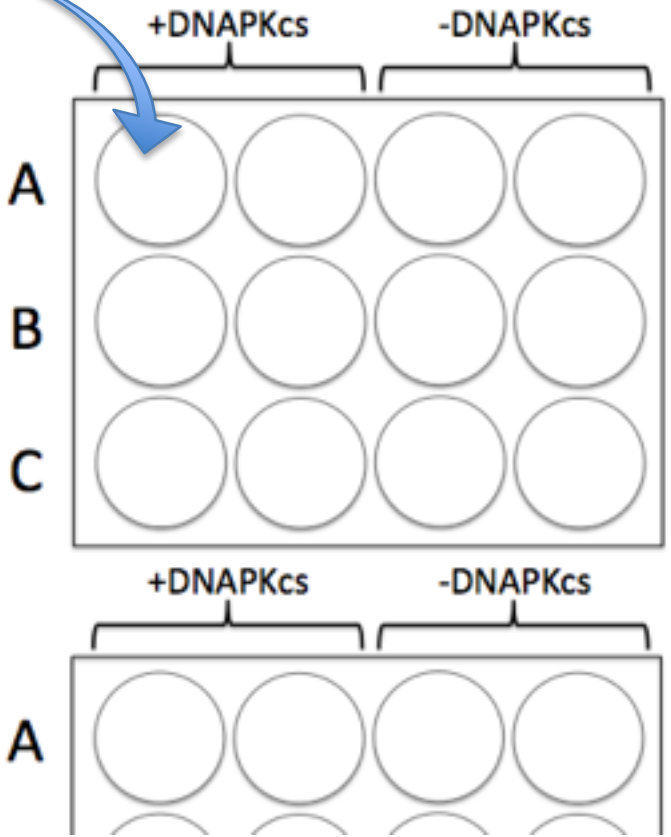


Blue: DNA
Green: γH2AX staining

# Prepare plates for γH2AX experiment



25k cells/well




- First put coverslips in each well
- Then, put cells in the wells (cells will grow on coverslips)
- We will remove the coverslips for immunofluorescence staining later.

# Seed cells for the γH2AX experiment

1°, 2° Ab controls →

media control →

1mM $H_2O_2$ →

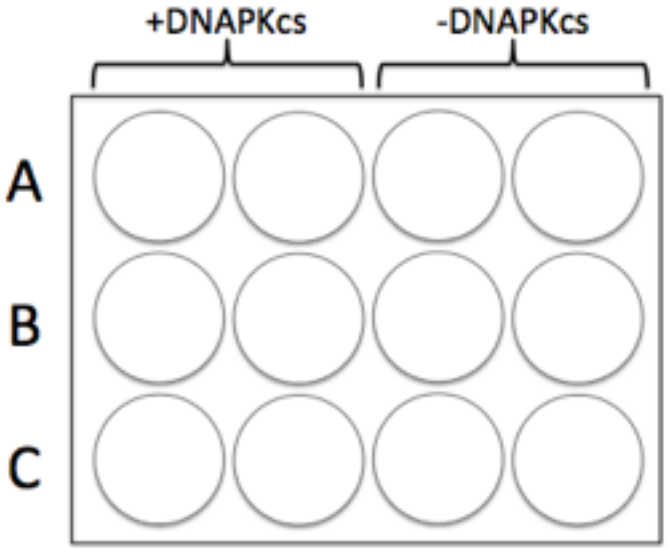



Plate #1

1mM $H_2O_2$ + recovery →

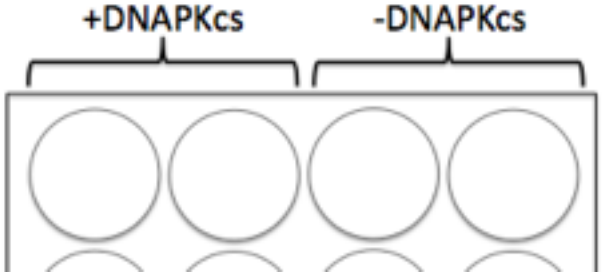



Plate #2

# Major assignments for Mod1

- Data summary draft
  - due by 10pm on Mon., October 8
  - revision due by 10pm on Sat., October 20

**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 13
- Blog post for M1 due by 10pm on Tues., October 9

# How do we communicate our protocol?

## Tips to write Methods (due M1D5)

with lab partners

- Include enough information to replicate the experiment
  - list manufacturers name and location (City, ST)
- Use subsections with descriptive titles
  - Put in logical order
  - Begin with topic sentence to introduce purpose
- Use **clear and concise** full sentences
  - NO tables and lists
  - Passive voice expected
- Use the most flexible units
  - Write concentrations (when known) rather than volumes
- Eliminate 20.109 specific details
  - Example “labeled Row A, Row B…”
  - Do not include details about tubes and water!
  - Assume reader has some biology experience

Example methods section

## Tissue Culture:

human lymphoblast cell line (gift of Engelward lab, MIT)

TK6 cells were grown in a flask with 12ml RPMI supplemented with 10% FBS. The cells were kept in an incubator at 37°C. A stain was used to assess if the cells were alive or dead.

Trypan blue

# Improving a Methods paragraph

Maintaining lymphoblastoid cell line(s):

TK6 human lymphoblastoids (gift of the Engelward Lab, MIT, Cambridge MA) were cultured at 1-9 x $10^5$ cells/mL, cell number calculated via hemocytometer and trypan blue stain. Cells were grown in RPMI medium 1640 (Invitrogen, Carlsbad CA) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Atlanta, GA) and 100 units/mL penicillin-streptomycin (Invitrogen). Culture conditions were maintained at 37°C, 5% CO2 and 95% relative humidity.

# In lab today

1. Prepare chips for electrophoresis. Two teams share one gel box (cut down the gelbond).
2. During unwinding step, start TC work.
3. Finish electrophoresis and put CometChips in SYBRgold stain.