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

09/26/18

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

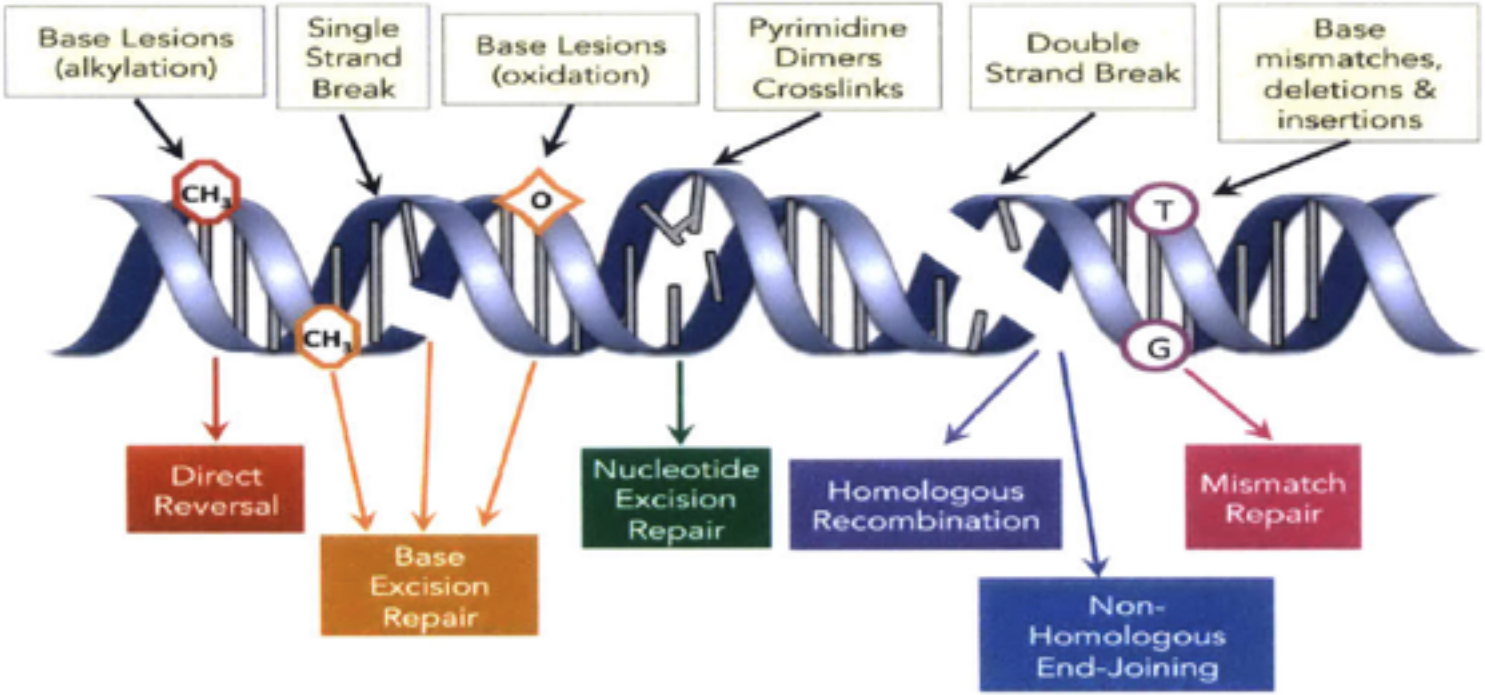
<u>Reminders</u>

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

*Remember to empty benchtop buckets at end of day

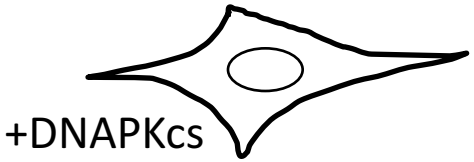
There are many DNA repair pathways

H_2O_2- induced DNA damage is typically repaired by BER

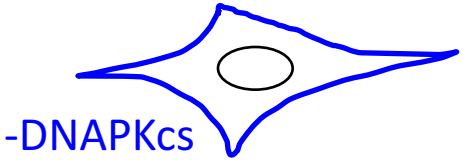


Does DNAPKcs have a role in DNA repair in response to oxidative damage?

- 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



+ H_2O_2



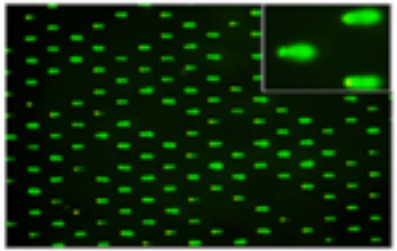
+ H_2O_2

Measure "damaged" DNA via CometChip and γH2AX assays

Overview of Module 1: Measuring Genomic Instability

1. Optimize comet chip assay

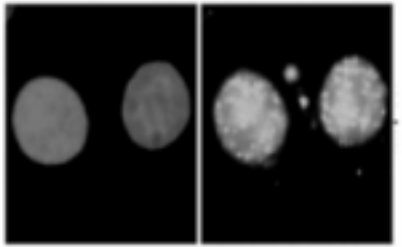
- Test loading variables



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



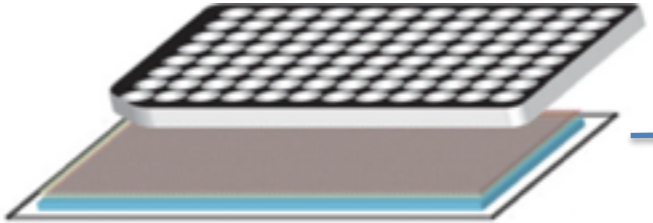
3. Use immuno-fluorescence assay to measure DNA damage

- Examine effect of H_2O_2 on γH2AX foci formation

Direct/indirect measure of DNA damage

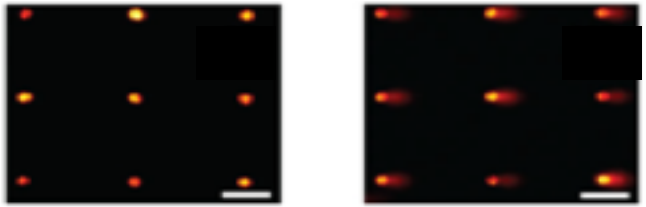
Assess DNA damage in tumor cells with & without DNAPKcs

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

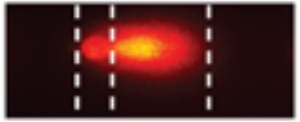


Lyse cells & unwind DNA (DNA still captured agarose in overlay)

Agarose Electrophoresis

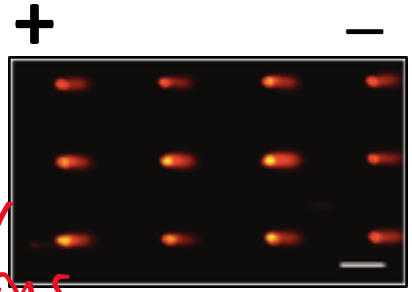


Stain DNA and image via fluorescence microscopy



Analysis via Matlab

Lysis & staining in CometChips



- Alkaline lysis solution (pH 10) alkaline
  - 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris
  - Triton X-100 detergent

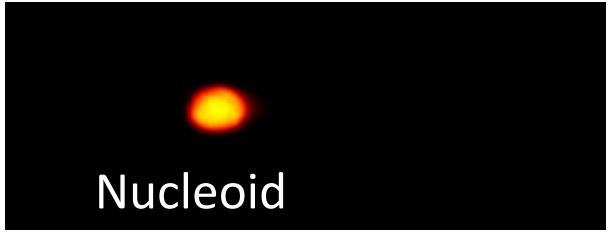
  breakdown of cell membrane/ denature proteins
- Unwinding/ electrophoresis buffer (pH 13.5)
  - 0.3M NaOH, 1mM Na_2EDTA
- Neutralize dye (pH 7.5)
  - 0.4M Tris

  need neutral pH for SYBR gold staining
- Florescent stain for DNA
  - SYBR Gold in PBS

  ↑1000X signal when bound to DNA
  DNA intercalator
- What are impt. considerations for visualizing DNA?

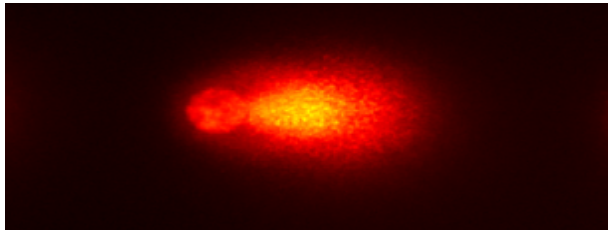
  ssDNA, dsDNA, visualize via UV, sensitivity

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 <u>REPAIR INTERMEDIATES</u>

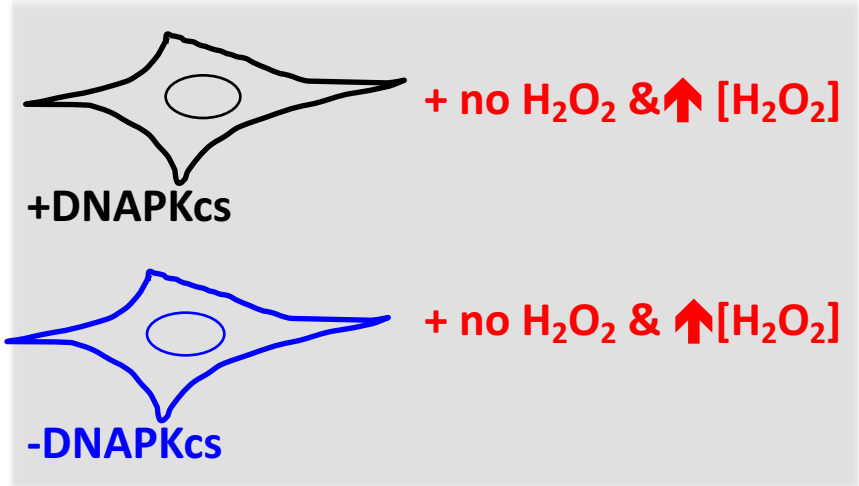
Crafting a hypothesis

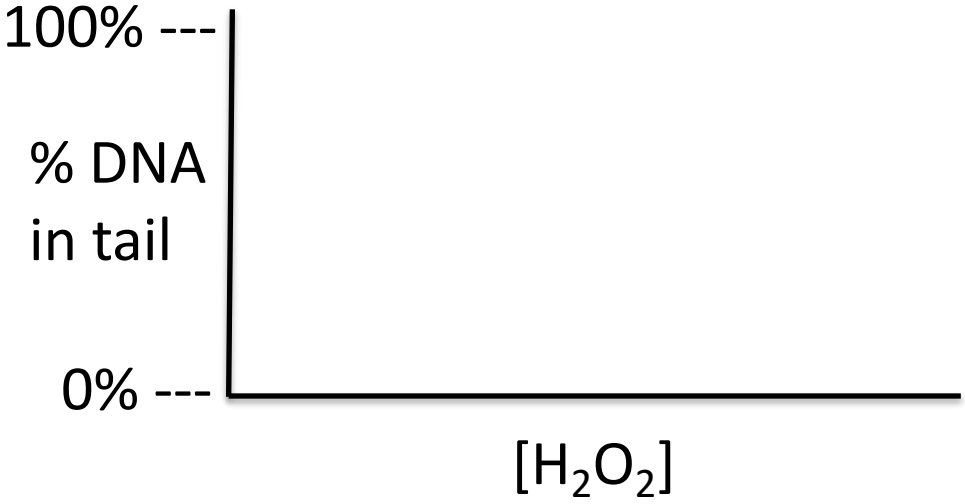
Motivation for your study…

Using comet chip

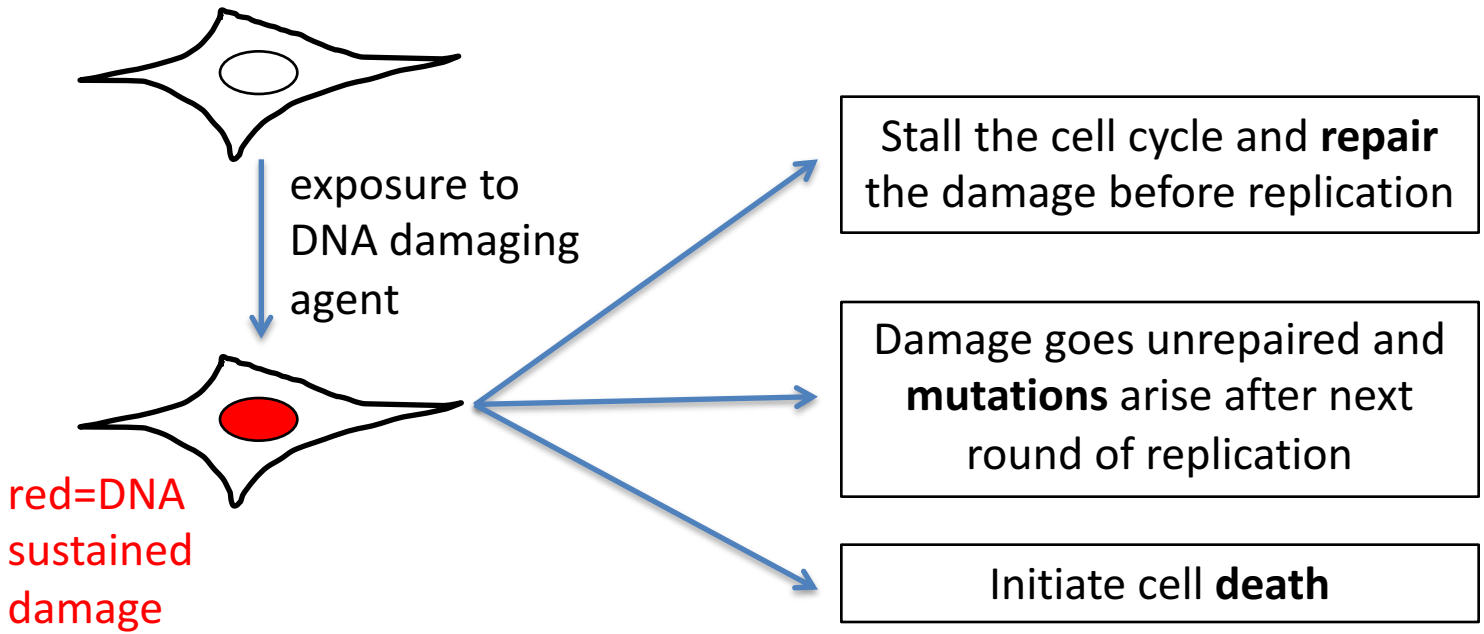
What is your research question?

What result do we expect from the CometChip?

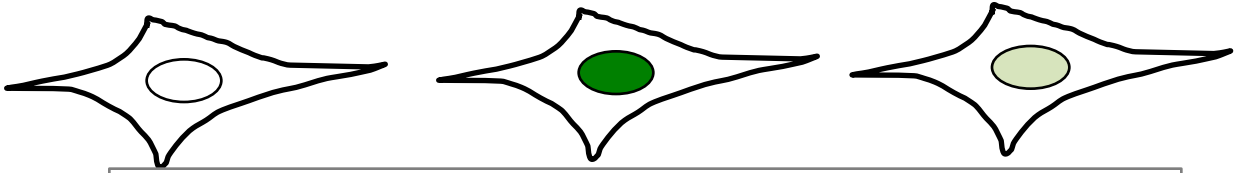




The DNA damage response



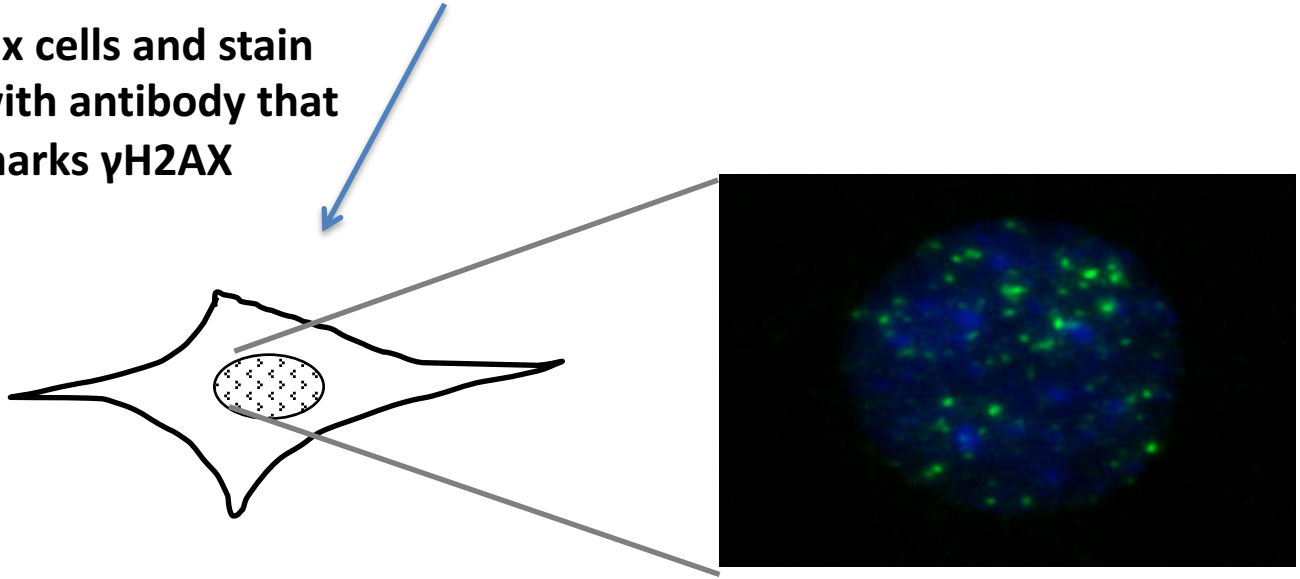
Measuring double strand breaks via γH2AX Assay



γH2AX =

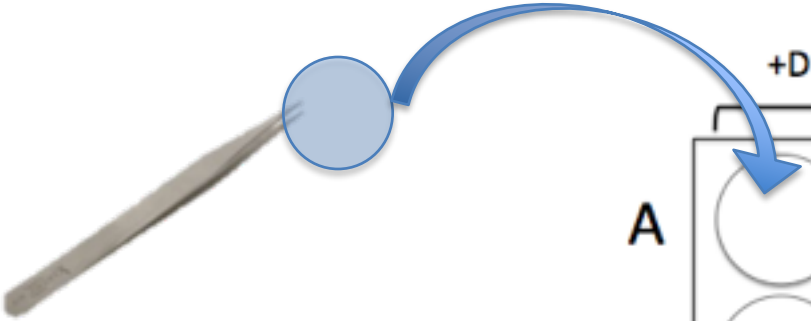
no treatment, H_2O_2 treatment, recovery condition

Fix cells and stain with antibody that marks γH2AX



Blue: DNA
Green: γH2AX staining

Prepare plates for γH2AX experiment



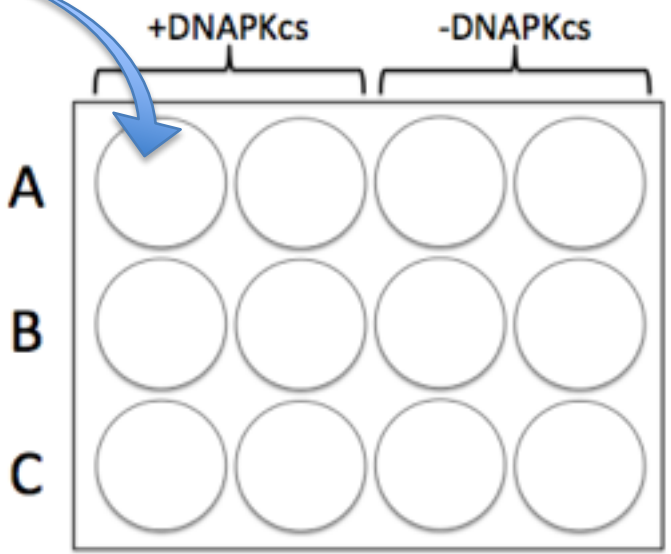


Plate #1

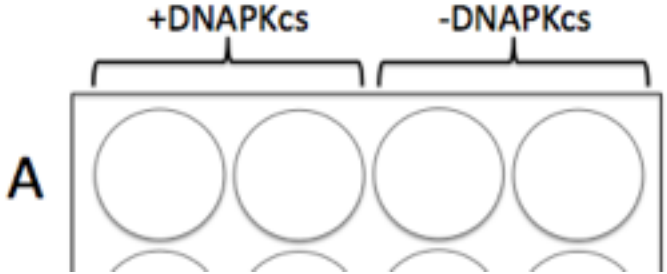
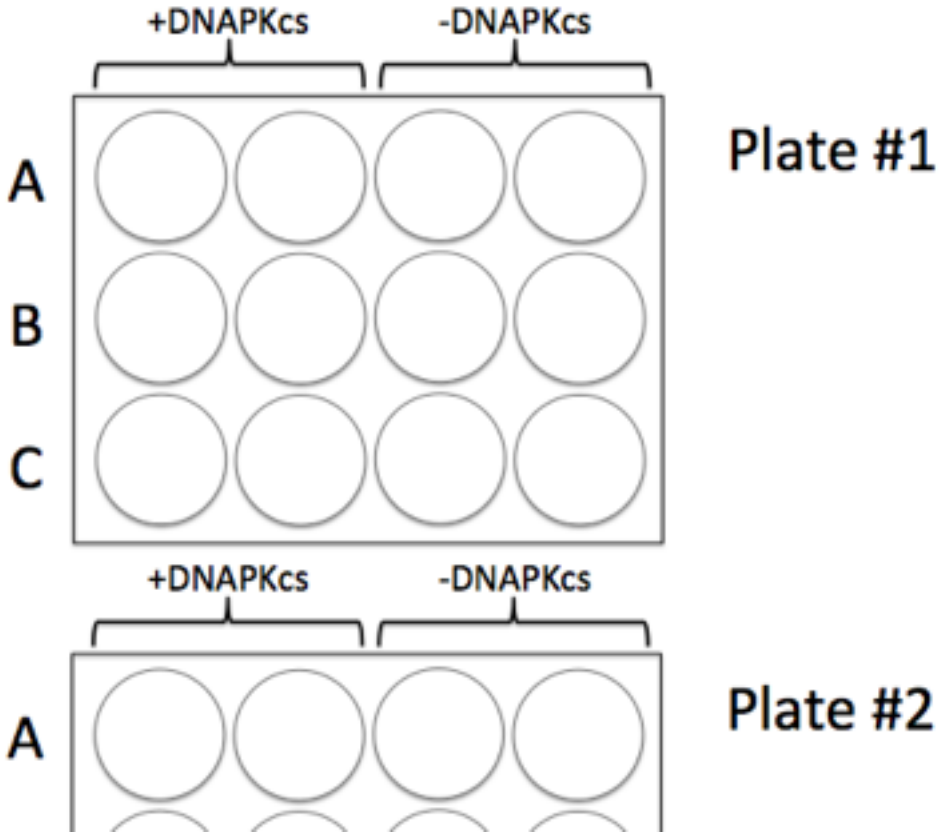


Plate #2

- 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



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)

- 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:

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

Improving a Methods paragraph

<u>Maintaining lymphoblastoid cell line(s):</u>

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. Start electrophoresis (30min) and once complete put CometChips in SYBRgold stain (overnight).
2. Move to TC to seed cells for γH2AX experiment.
3. During downtime work with your lab partner on methods homework.