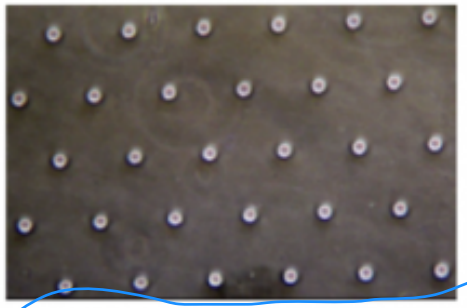
M1D4: Test role of biochemical factors in genomic stability

09/21/16

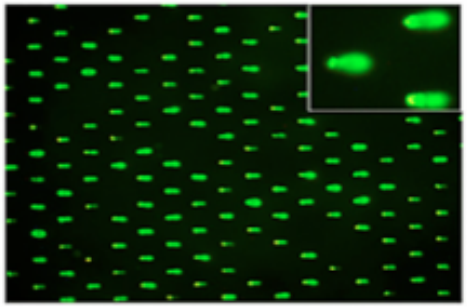
1. Pre-lab Discussion
2. Choose experimental parameters
3. Load CometChips with wild type and mutant MEFs
4. Induce DNA damage with mutagen and lyse

Overview of
Module 1: Measuring Genomic Instability



1. Optimize comet chip assay

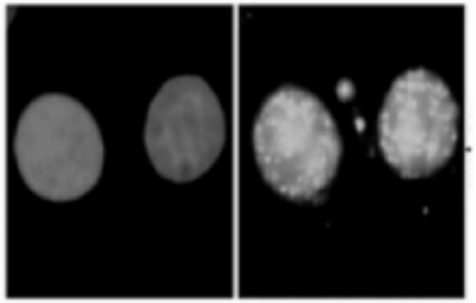
- Test loading variables



2. Use comet chip assay to measure DNA repair

- Measure effects of MMS and H_2O_2 on BER

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



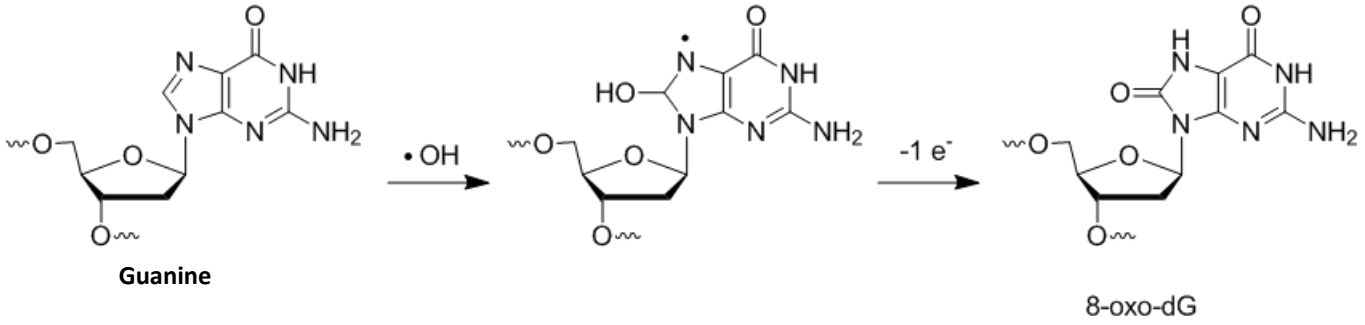
3. Use immuno-fluorescence assay to measure DNA repair

- Examine effect of MMS and H_2O_2 on DSB abundance

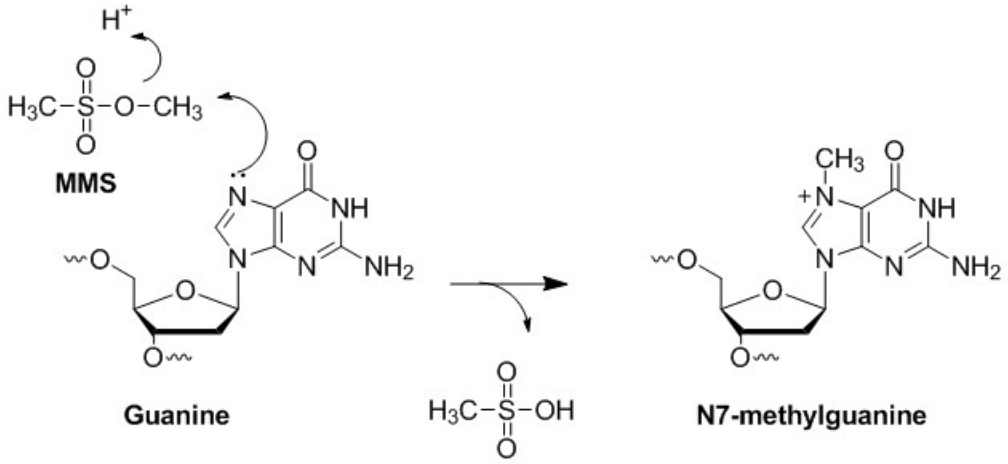
How do H_2O_2 and MMS damage DNA?

reaction chemistry

H_2O_2

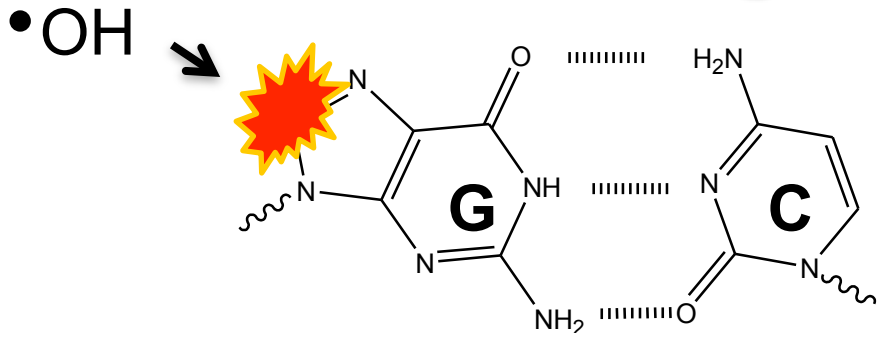


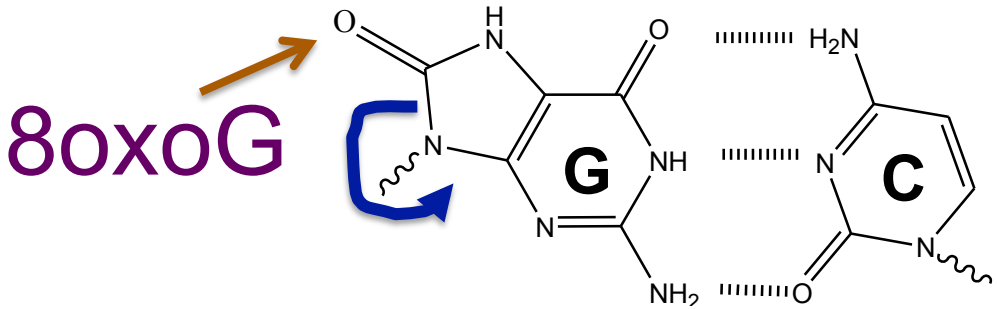
MMS



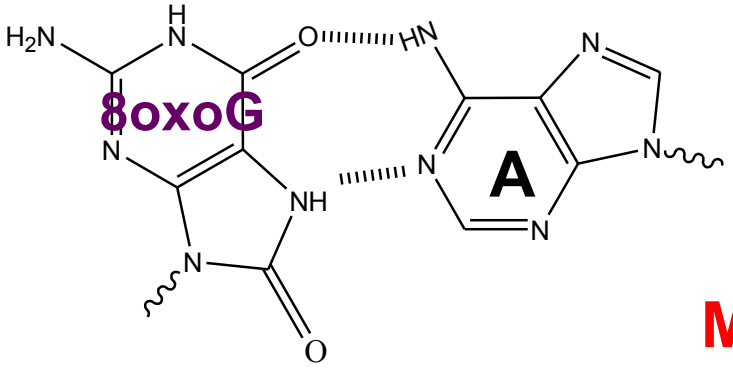
Reactive oxygen species: H2O2

effect of damage





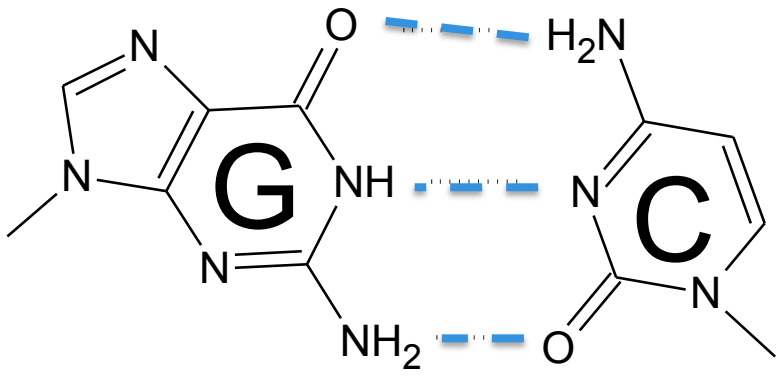
Damaged G now looks like T



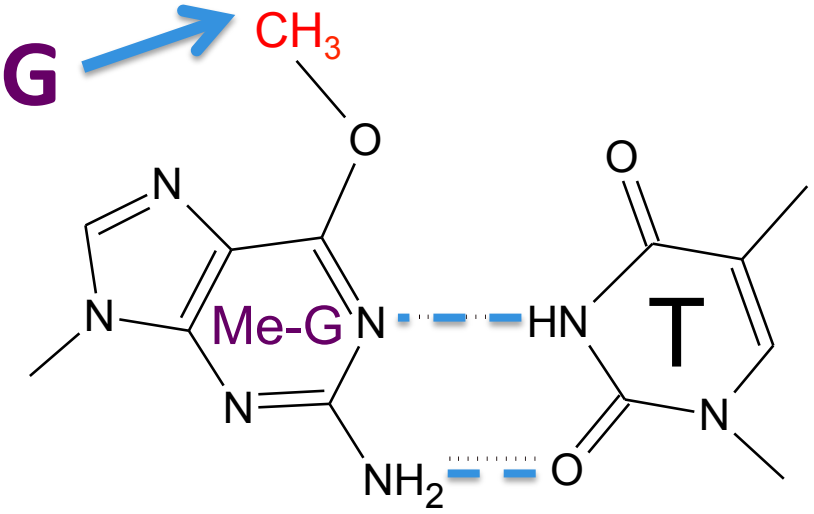
Mutation if replicated
GC →AT

effect of damage

Alkylating agent: MMS



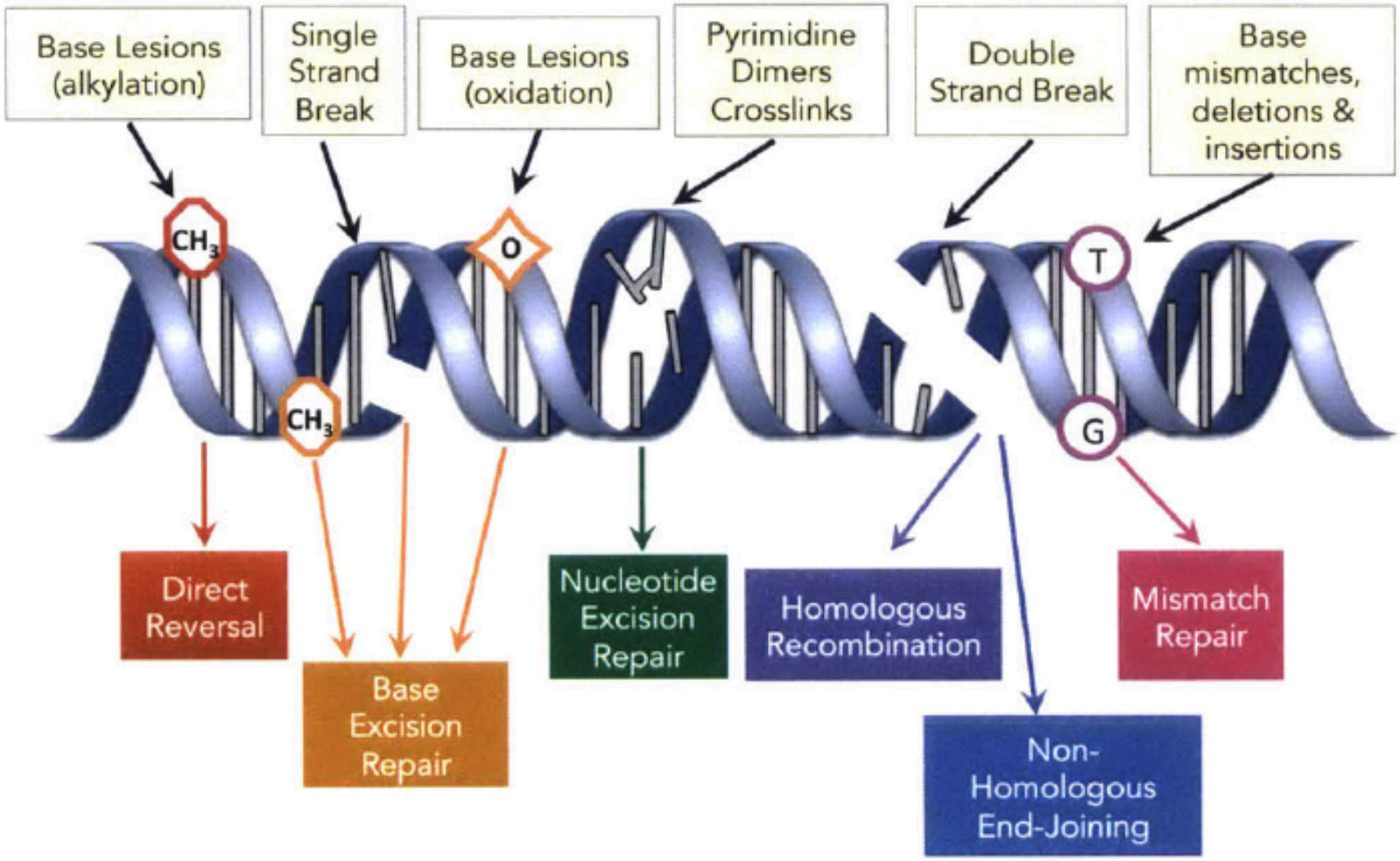
Me-G



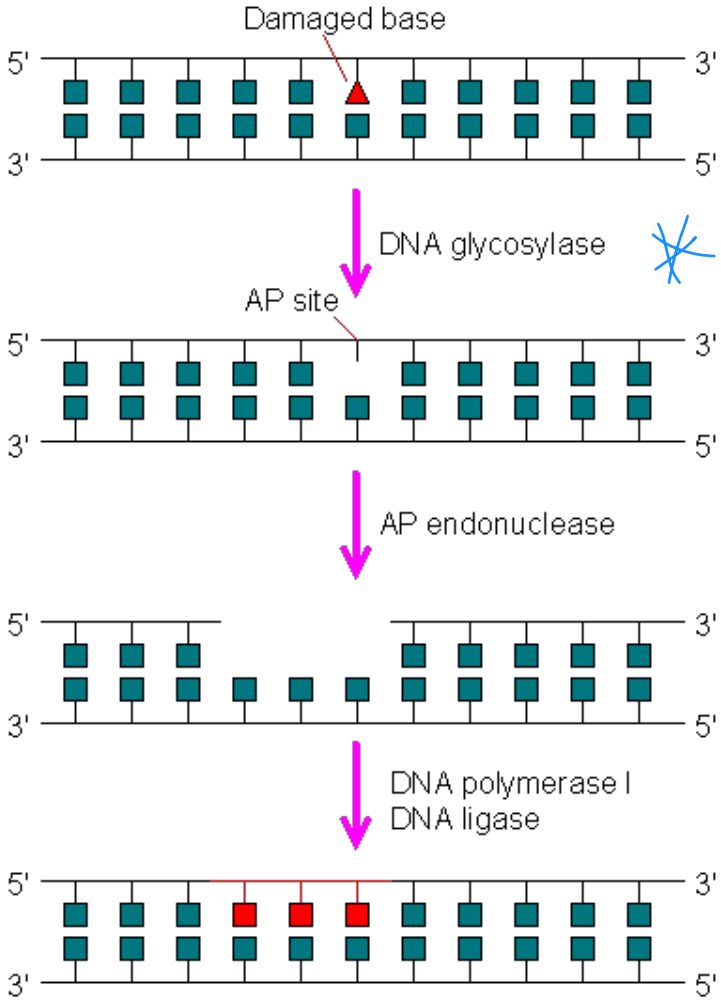
Damaged G now looks like A

Mutation if replicated GC →AT

H_2O_2- and MMS-caused DNA damages are repaired by base excision repair (BER)



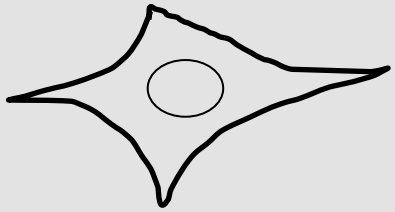
How do our cells respond?
Base Excision Repair (BER) Pathway



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

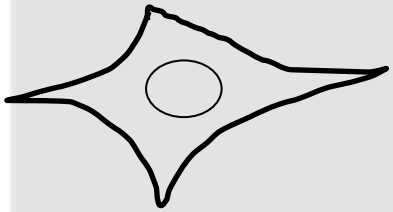
How do we study the response of the BER pathway to MMS and H2O2 damage?

study the extremes



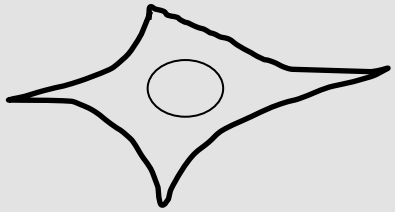
+ no mutagen & [MMS]

wild type match



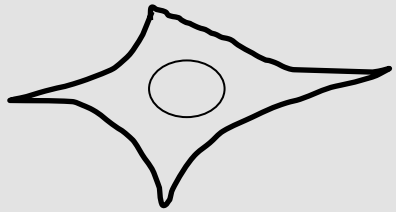
+ no mutagen & [MMS]

deletion of gene encoding
3-alkyladenine DNA glycosylase
AAG -/-



+ no mutagen & [H2O2]

wild type match

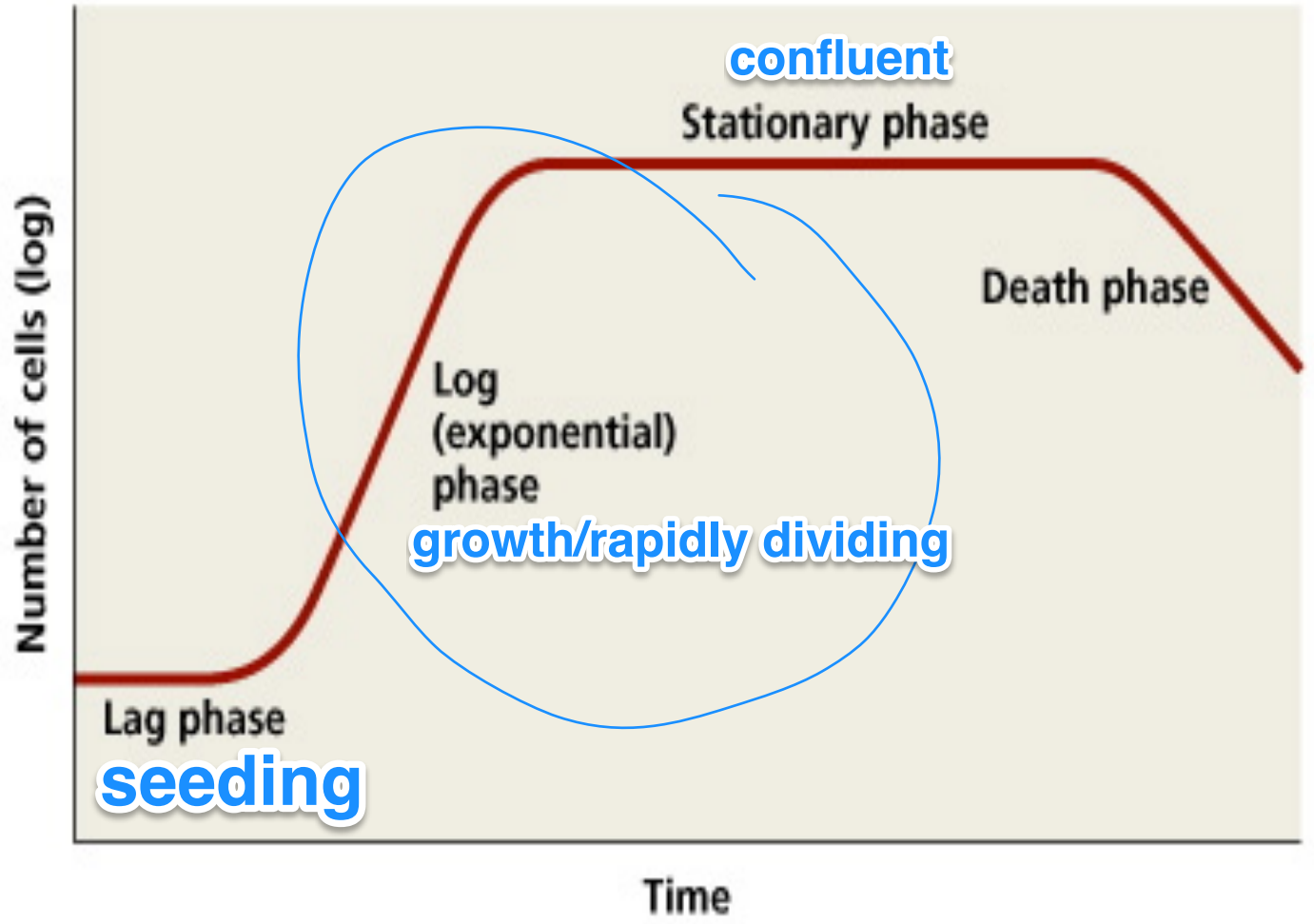


+ no mutagen & [H2O2]

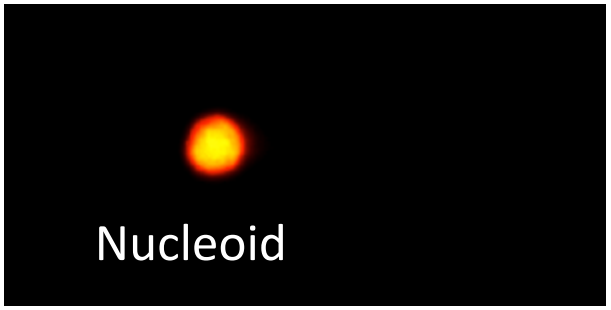
deletion of gene encoding
8-Oxoguanine glycosylase
OGG1 -/-

measure damaged DNA via CometChip

Cell culture growth phases

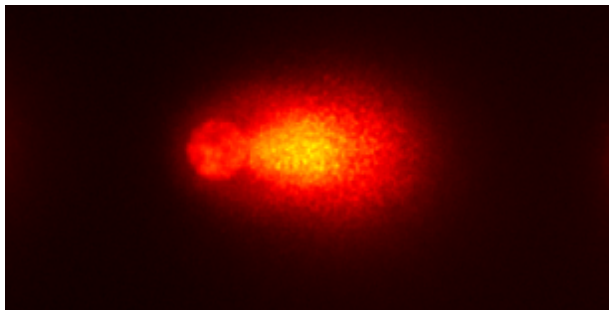


Output of CometChip Assay



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

Crafting a hypothesis

Motivation for your study…

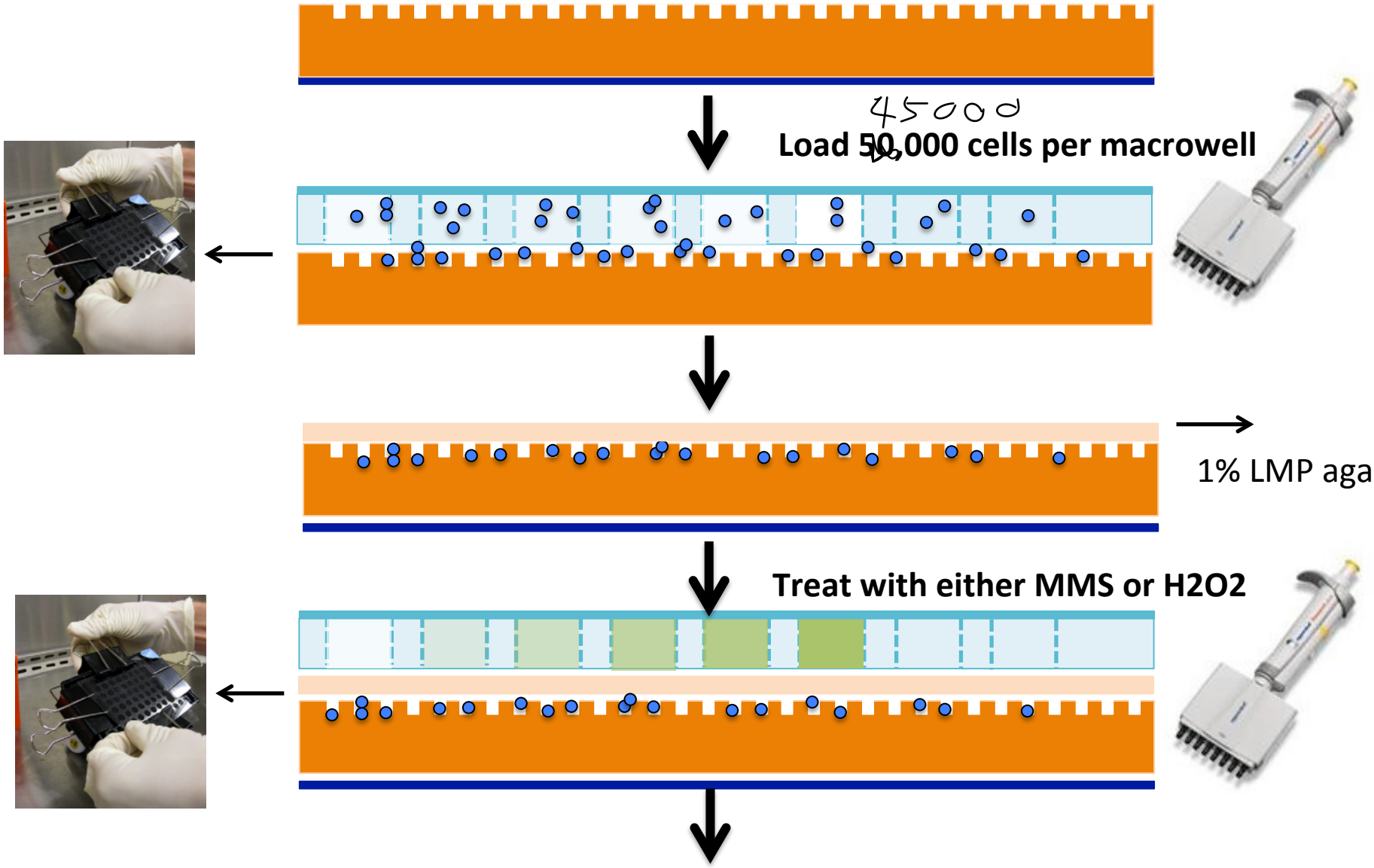
CometChip measures differences in DNA damage in response to external factors.

What is your research question?

I hypothesize we will be able to measure effect of BER activity in Wt vs. AAG-/- response to MMS.

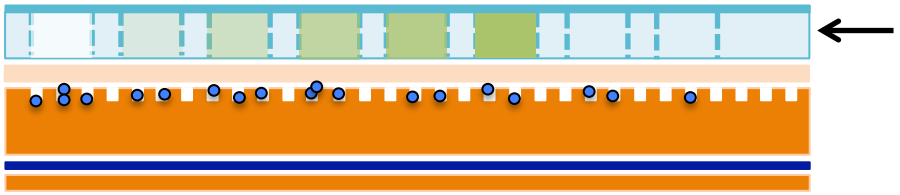
wt vs. mut
direct strand breaks vs. repair intermediates

Logistics of today's experiment

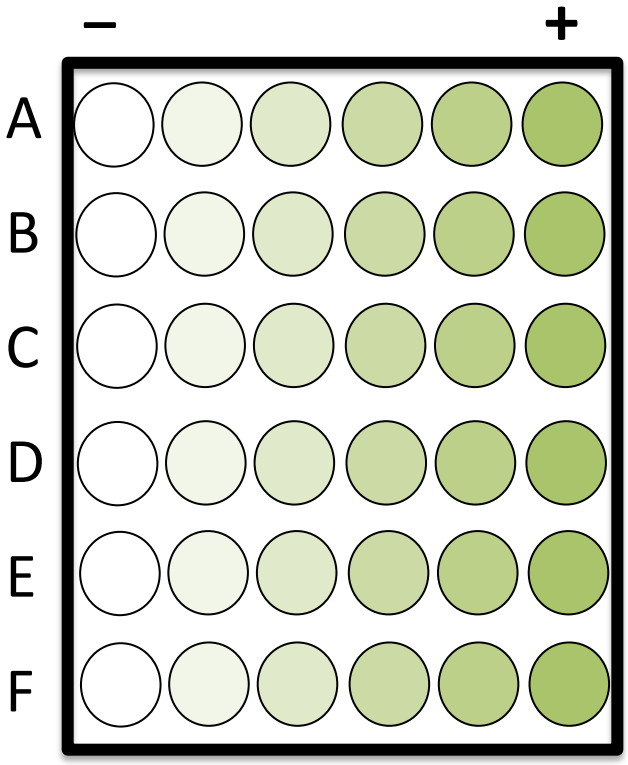


Wash off mutagen (carefully!) and leave CometChip in lysis buffer

Treat cells with 6 different doses of Mutagen: MMS or H2O2



drug treatment
(in macrowells of 96-well plate)



- When using MMS wear **flock-lined gloves** (and lab coats) in addition to standard gloves
- Special waste stream for ***everything*** in contact with MMS in fume hood
- Concentrated H2O2 should left at the front bench

$C_1V_1 = C_2V_2$

Preparing mutagen dilution series:

Dilutions should minimize waste!

- **MMS** stock is at 12M **1:1000=12mM**
- Add 100 µL of drug dose to each macrowell
- 2x: prepare 2 chips in parallel 1.5mL
- Prepare dilutions in DMEM (no FBS, P/S)
- Treat with : 0, 0.1, 0.2, 0.5, 6, and 8 **mM**
- 37°C for 30min

0.2mM

media = 1475 µl

stock = 25µl

- **H_2O_2** stock is 10M 2 dil = 50mM
- Add 100µl of drug dose to each macrowell
- 2x: prepare 2 chips in parallel 1.5mL
- Prepare dilutions in cold PBS
- Treat with : 0, 5, 10, 15, 20, 25 **µM**
- 4°C for 20min

10mM

PBS = 1200µl

stock = 300µl

Keep track of the players!

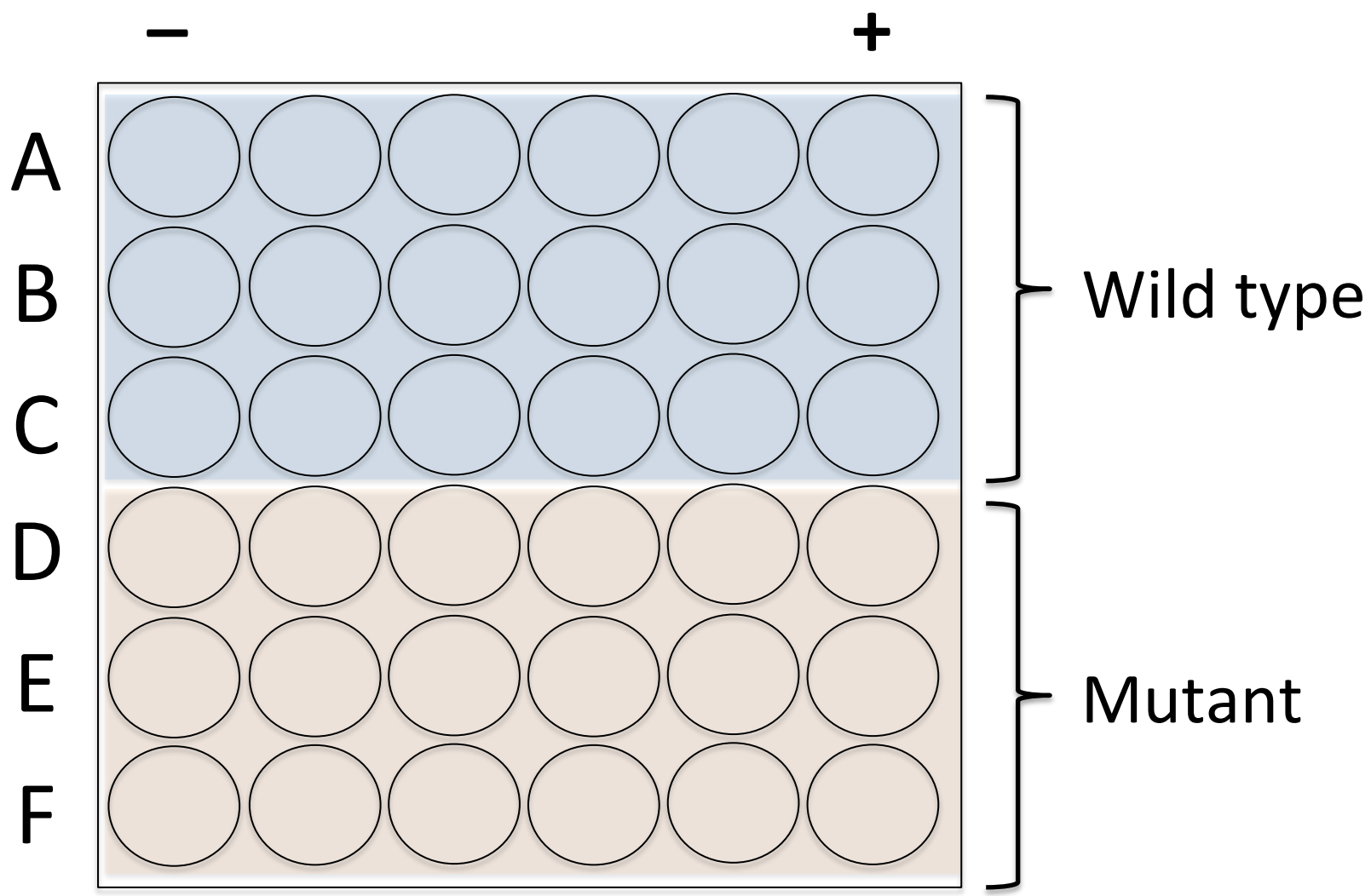
	Oxidative damage	Alkylating damage
Chemical treatment:	H_2O_2	MMS
Mutant cell line:	Ogg1-/-	Aag-/-

M1D4

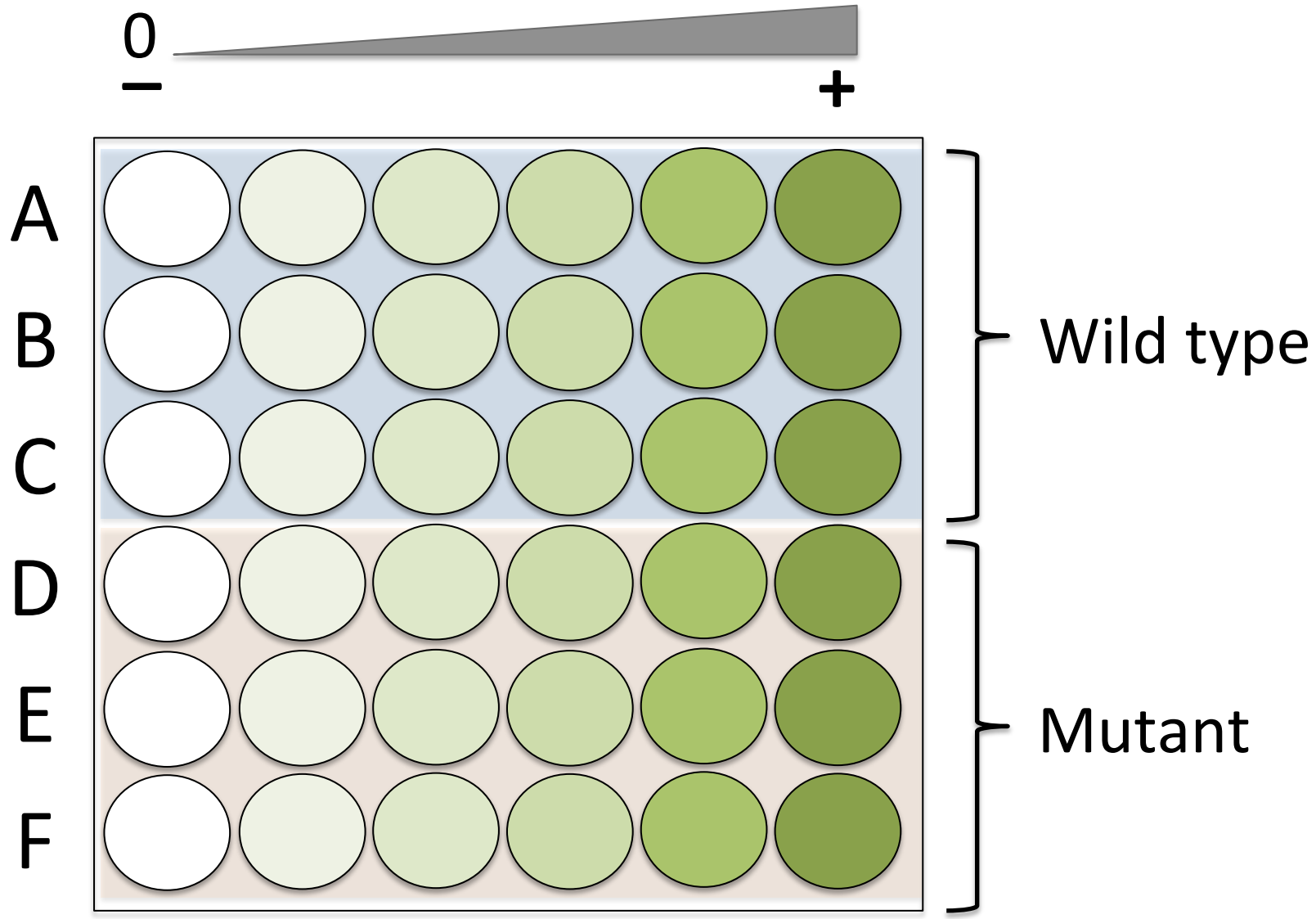
Enzyme:	Fpg	hAAG

M1D5

Keep track of the wells – 2 cell lines!



Keep track of the wells – 6 concentrations!



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)
  - Be **concise and clear** in your description
- 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

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

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

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. Choose your experimental parameters and check with instructors before you start.
2. Prepare your workspace for cell loading, obtain cells in suspension from instructors and load/capture wild-type and mutant MEFS onto 2 chips.
3. Calculate volumes necessary for mutagen dilutions and check with instructors.
4. Treat 2 CometChips with mutagen and carefully wash.
5. Leave both CometChips in lysis buffer.