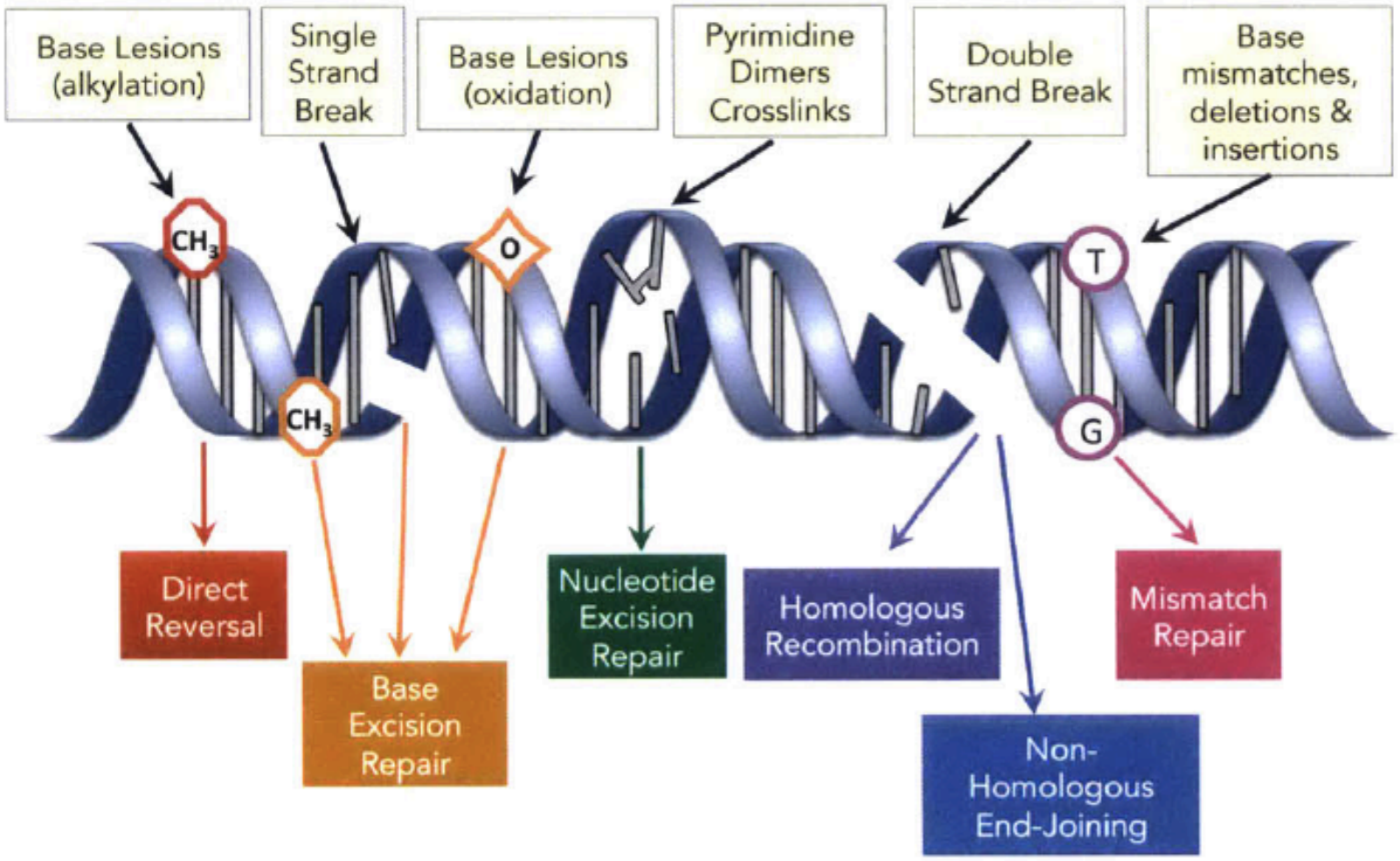
M1D4:
Test role of biochemical factors in genomic stability

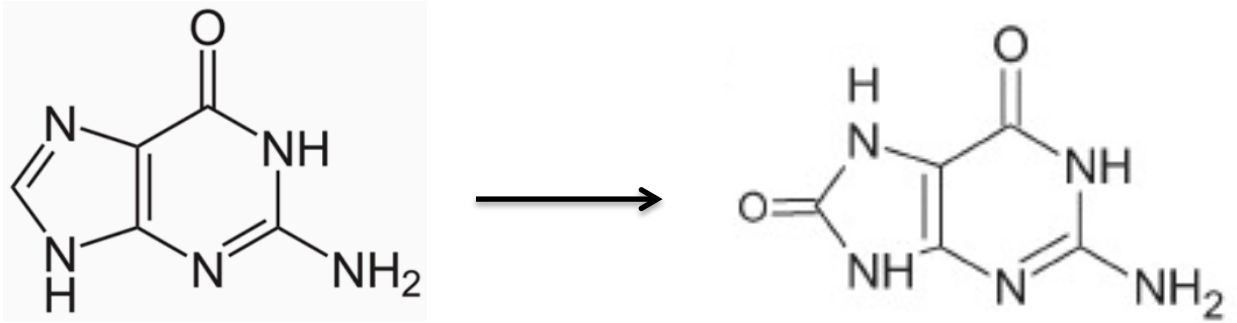
1. Prelab discussion
2. Benchwork
   - Load CometChips
   - Induce DNA damage
   - Lyse cells

H_2O_2- and MMS-induced damage is corrected by base excision repair (BER)



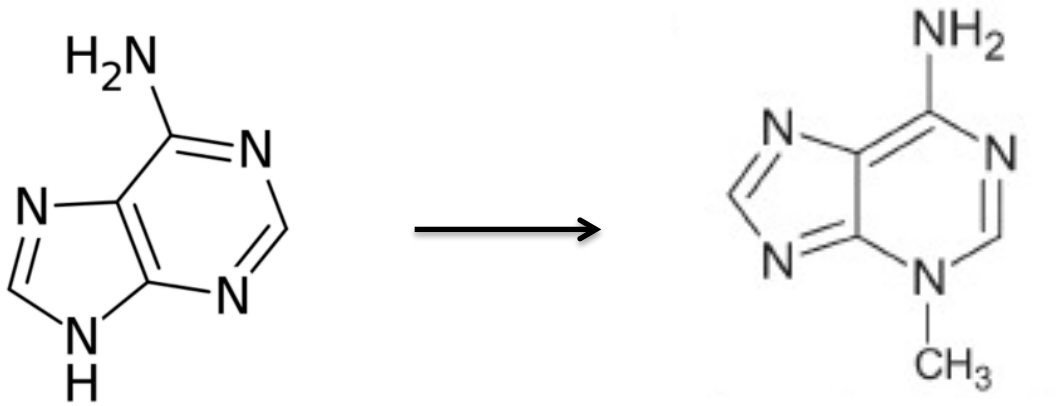
Damaging agents ‘decorate’ bases

Oxidative damage: H_2O_2



guanine 8oxoG

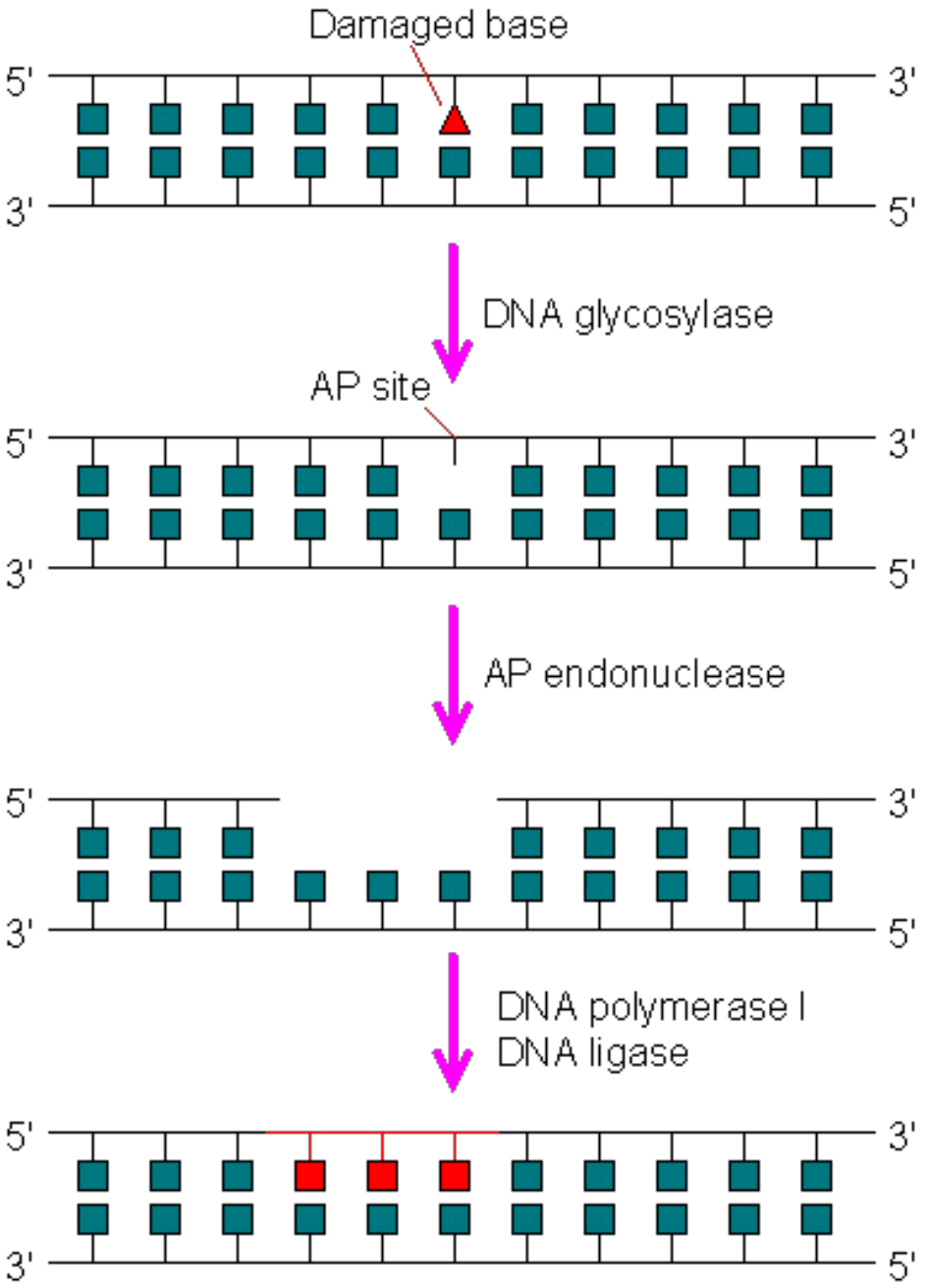
Alkylating damage: MMS



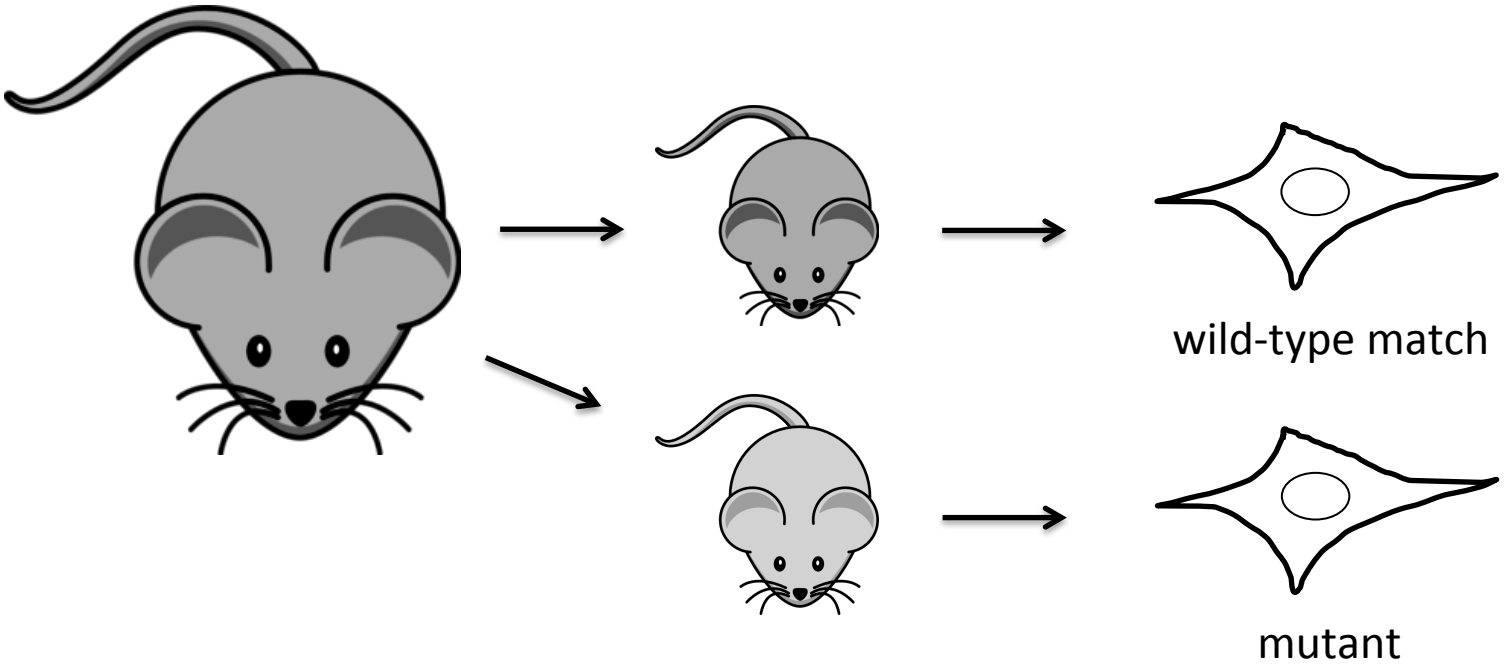
adenine 3meA

BER pathway corrects damaged bases

- Glycosylases are specific to type of damage
  - Oxoguanine DNA glycosylase (Ogg)
  - Alkyladenine DNA glycosylase (Aag)

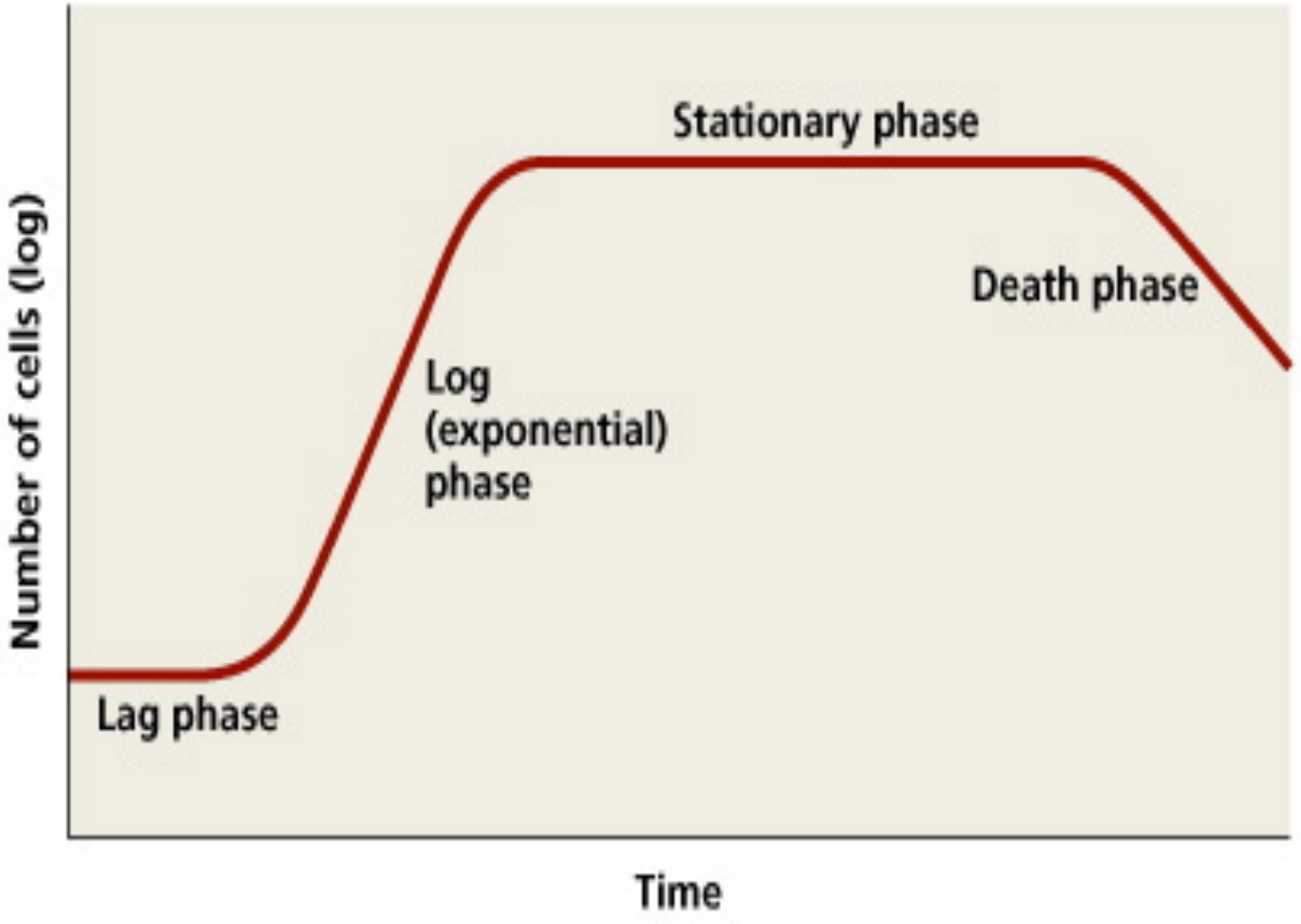


How will we study the effects of H_2O_2 and MMS?



	Oxoguanine DNA glycosylase	**Alkyladenine DNA glycosylase**
wild-type match	WT (Ogg1-/-)	WT (Aag-/-)
mutant	Ogg1-/-	Aag-/-

Cell culture growth is not constant



In what phase do we want the cells for our experiment?

Keep track of the players!

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

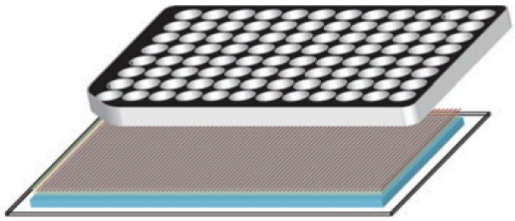
M1D4

Enzyme:	Fpg	hAAG

M1D5

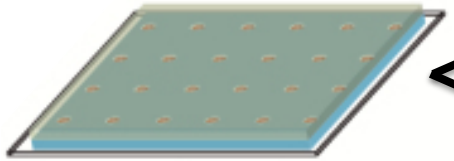
Exp2: Biochemical testing using CometChip

M1D4:



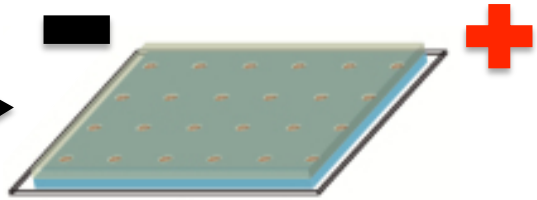
Load → Treat with DNA damaging agent → Lyse

M1D5:

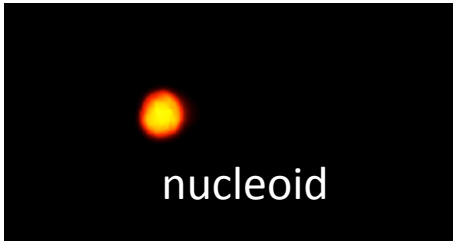


Chip 1: no enzyme control

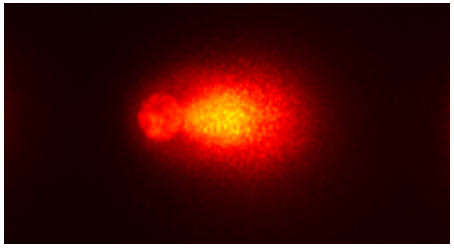
Chip 2: purified enzyme treatment



M1D6 and D7:

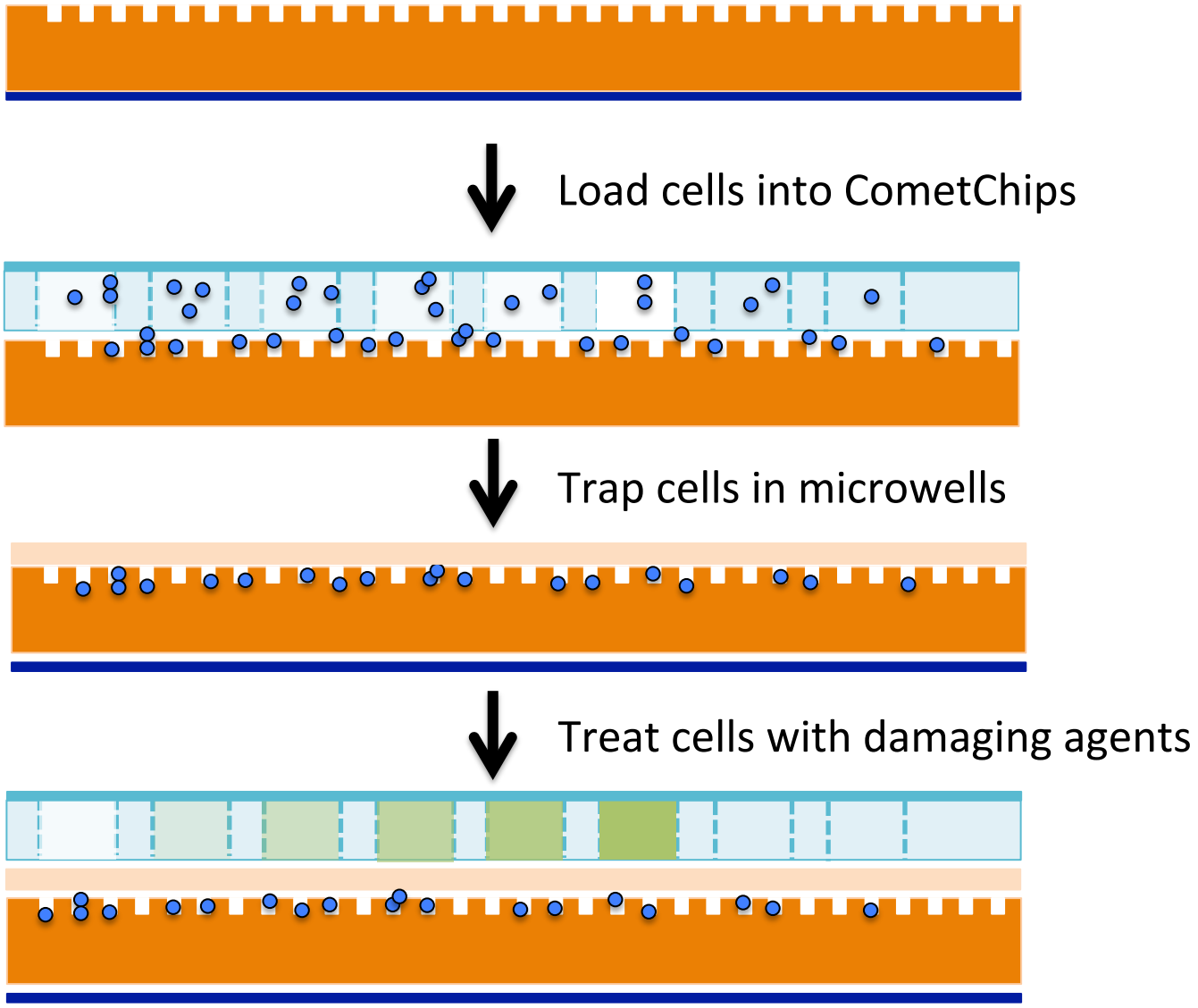


No damage: supercoiled

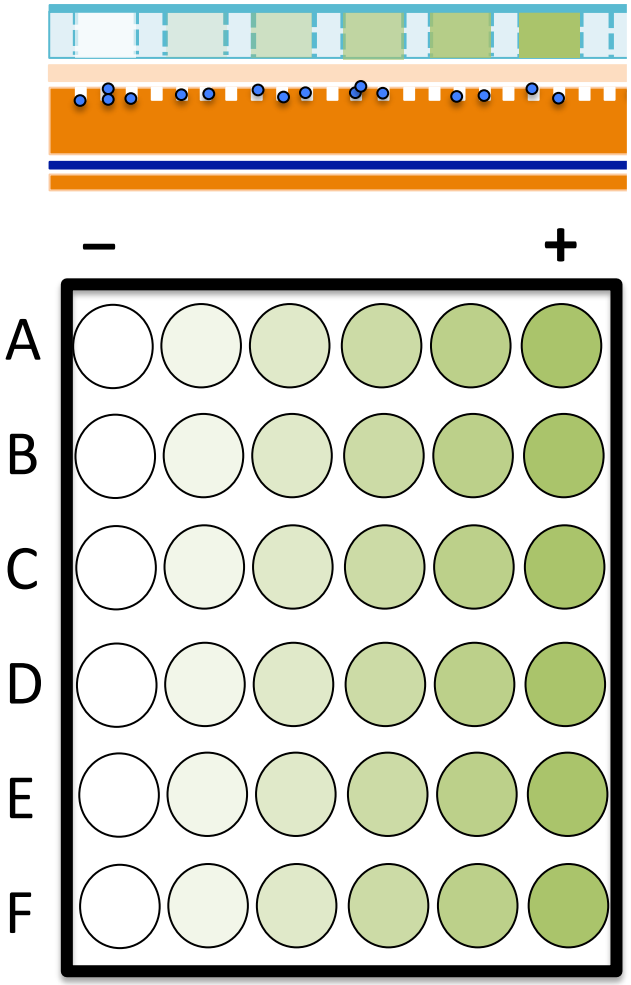


Damage: SSBs, DSBs, abasic sites, alkali labile sites

Let’s take a closer look



Be careful with DNA damaging agents!



- MMS
  - Must wear flock-lined gloves over nitrile gloves
  - All waste must be collected in dedicated waste stream
- H_2O_2
  - Must wear nitrile gloves
  - Concentrated stock must be collected in dedicated waste stream

Minimize waste when preparing damaging agents

MMS:

- 12M stock 12 mM
- 100 uL added to well
  make 1.5 mL per dilution
- 6 wells / chip (x2)
- Dilute in pure DMEM
- Concentrations:

  0, 0.1, 0.2, 0.5, 6, 8 mM
- Incubate 30 min at 37°C

H_2O_2:

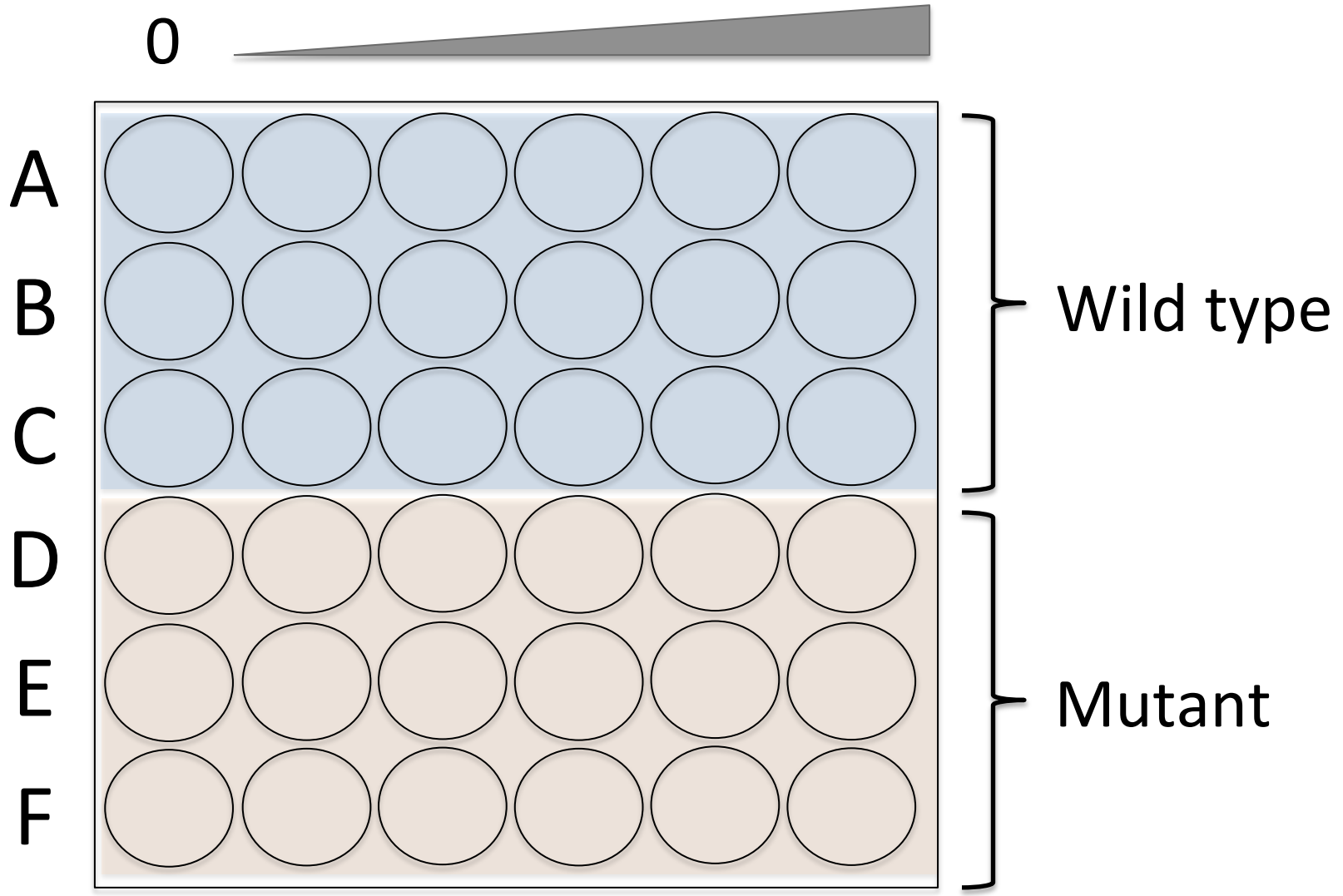
- 10M stock 50 uM
- 100 uL added to well
- 6 wells / chip (x2)
- Dilute in cold PBS
- Concentrations:

  0, 5, 10, 15, 20, 25 uM
- Incubate 20 min at 4°C

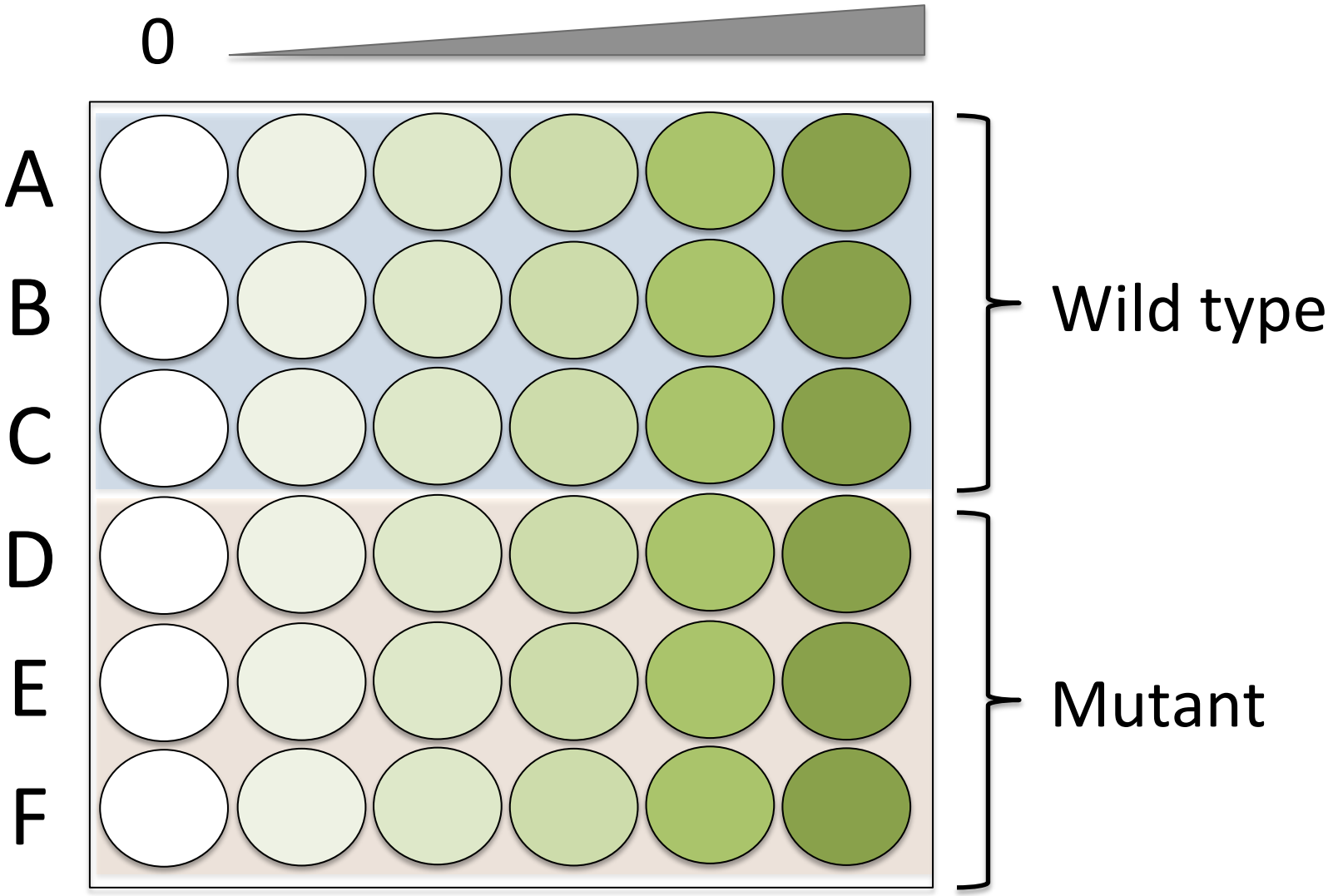
  on ice at all times!!

C1 V1 = C2 V2

Keep track of the wells – 2 cell lines!



Keep track of the wells – 6 concentrations!



Crafting a hypothesis

What is the motivation for your research?

the effect of increasing concentration of drug treatments using two cell lines -- mutant and wild type

What is your research question?

higher concentration of drug, the more DNA damage

mutant will have more DNA damage than WT

How/why do we communicate our protocol?

- Include information needed to replicate the experiment
  - list manufacturers name and location (City, ST)
  - Organize steps in logical order
- Use subsections with descriptive titles
  - Separate by experimental protocol, NOT chronologically
  - 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

Let's practice!

Tissue Culture:

human lymphoblast (TK6)..... (gift of Engelward Lab, MIT, Cambridge MA)

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.

For example,

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 and cell numbers 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% CO_2 and 95% relative humidity.

Today in lab…

- Select DNA damaging agent for experiments
- Calculate volume of cell suspension needed for cell loading (stock = 500k / mL)
- Calculate DNA damaging agent dilutions
- Load / treat cells in CometChips
- Store CometChips in lysis buffer