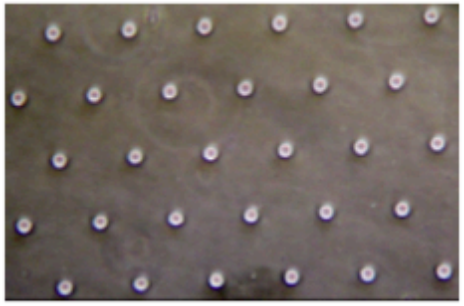
M1D4: Query inter-individual variability in exposure susceptibility

09/27/16

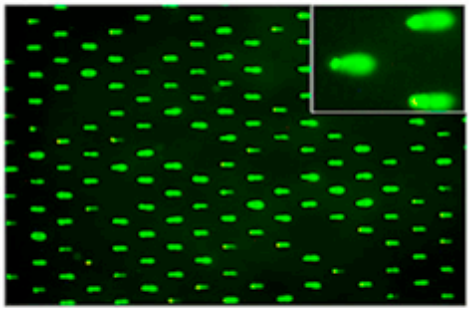
1. Quiz
2. Pre-lab Discussion
3. Load CometChip
4. Induce DNA damage, repair time course
5. CometChip lysis

Overview of
"M1: Measuring Genomic Instability"



1. Optimize comet chip assay

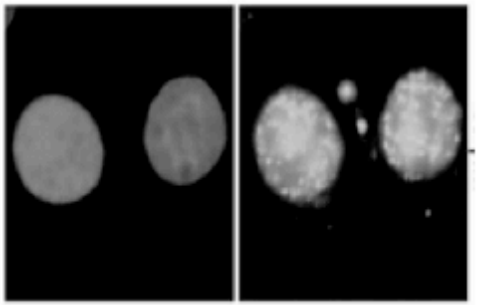
- Test loading variables



2. Use comet chip assay to measure DNA damage / repair

- Measure effects of MMS and H_2O_2 on BER
- Assess repair variability in healthy individuals

TODAY



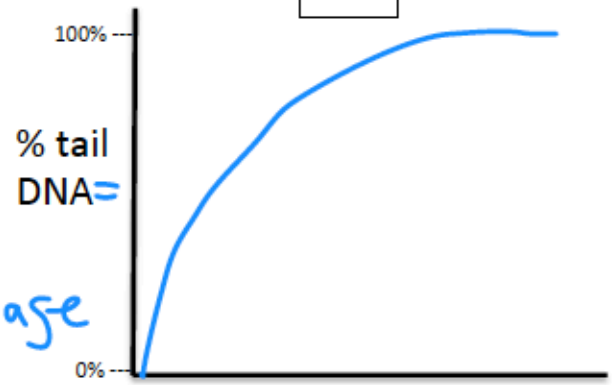
3. Use immuno-fluorescence assay to visualize DNA repair

- Examine effect of H_2O_2 on DSB abundance

Why analyze dead cells?

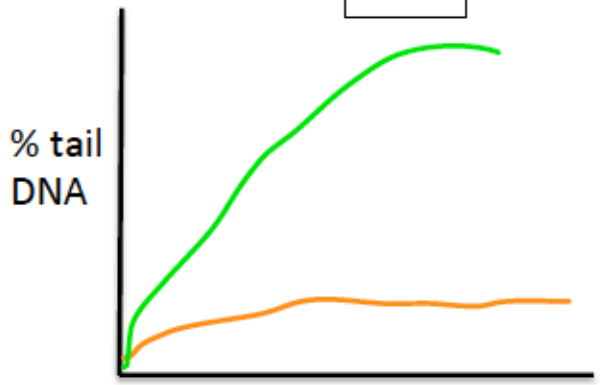
→ enzyme dead

live



DNA damage

dead



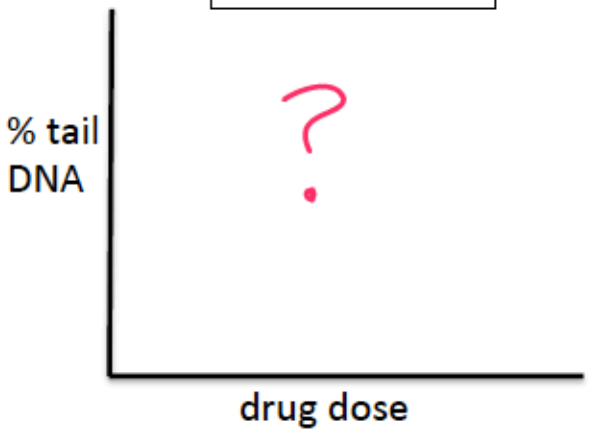
no tail=
no damage

tail=
DNA damage

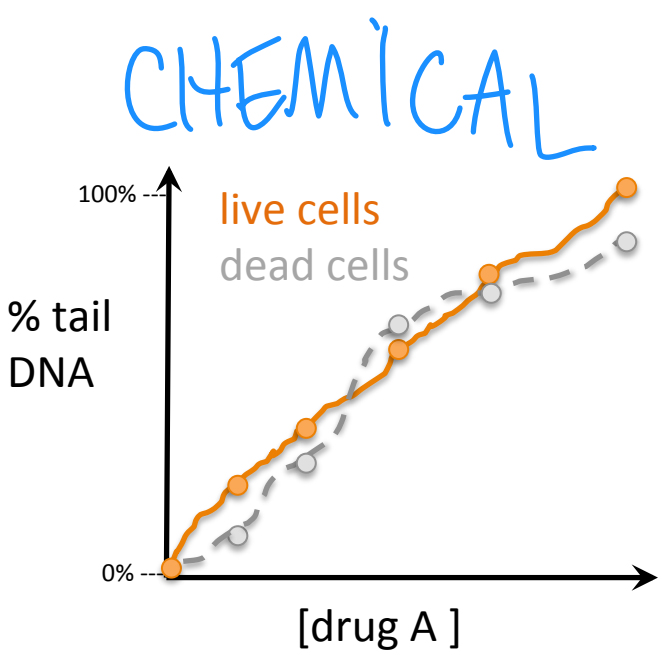
Note: W/F students will treat their TK6 cells with another drug, MMS.

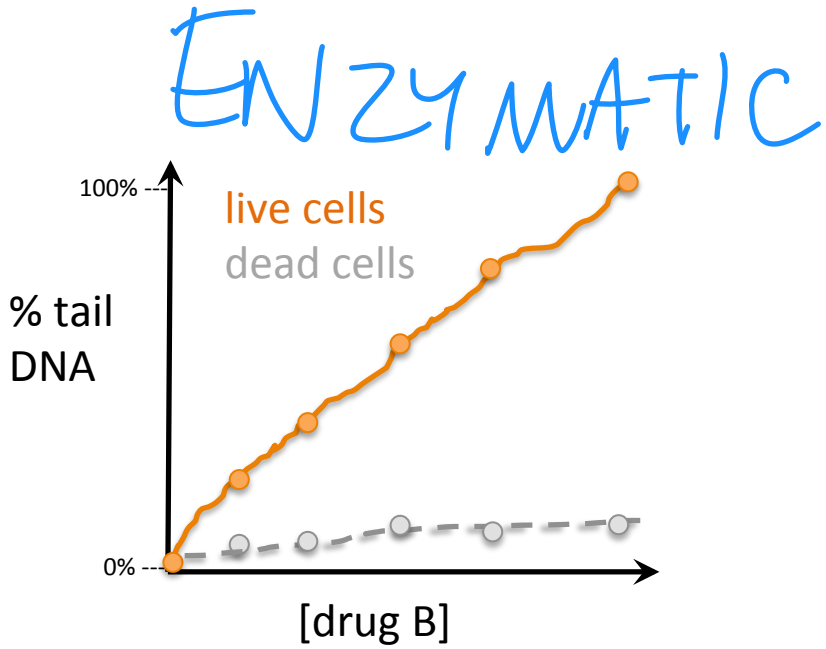
- Will the two chemicals have the same DNA damaging effect?

H2O2 vs MMS



What about chemical damage vs. enzymatic damage?

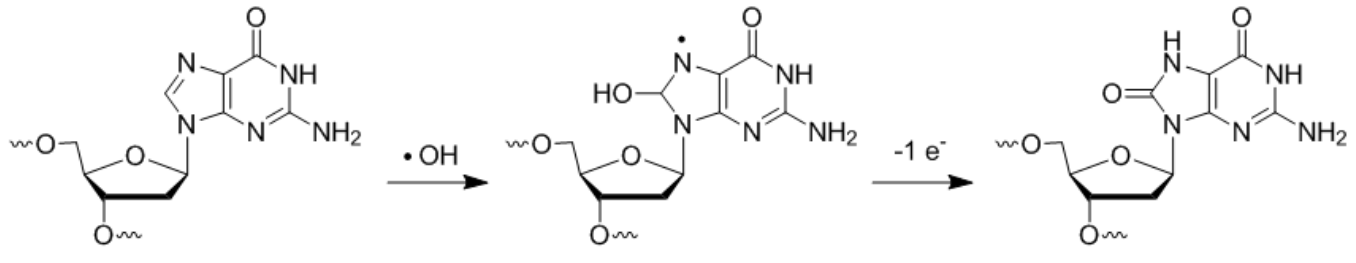




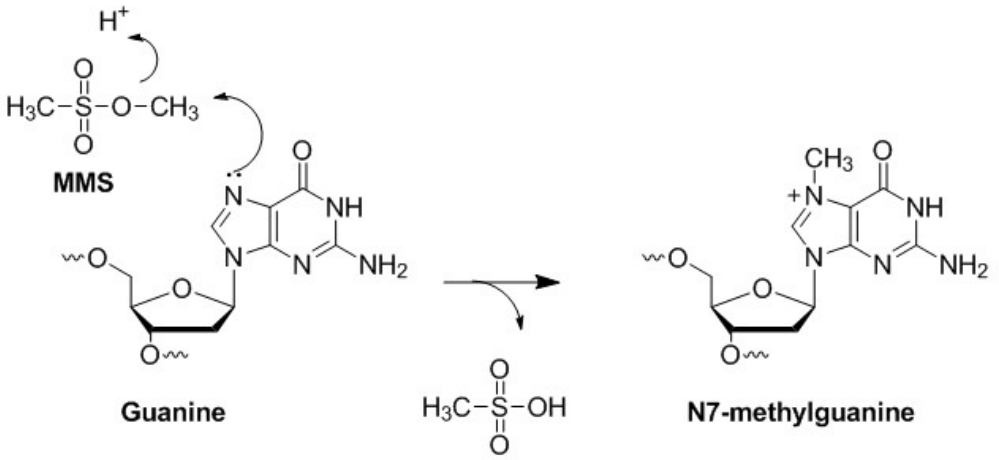
How do H_2O_2 and MMS damage DNA?

- Damage ≠ strand break
- Comet assay only detects strand breaks

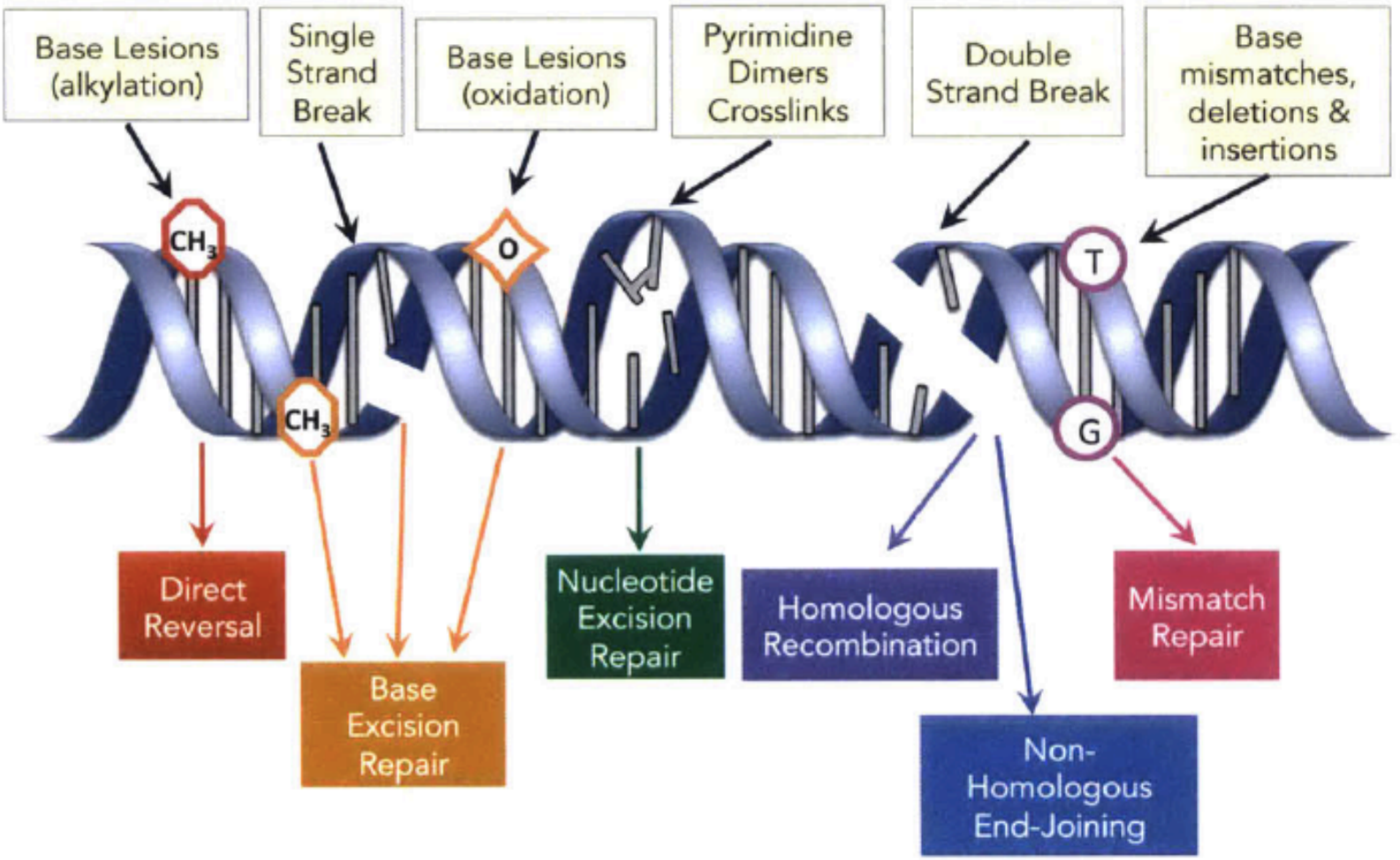
H_2O_2



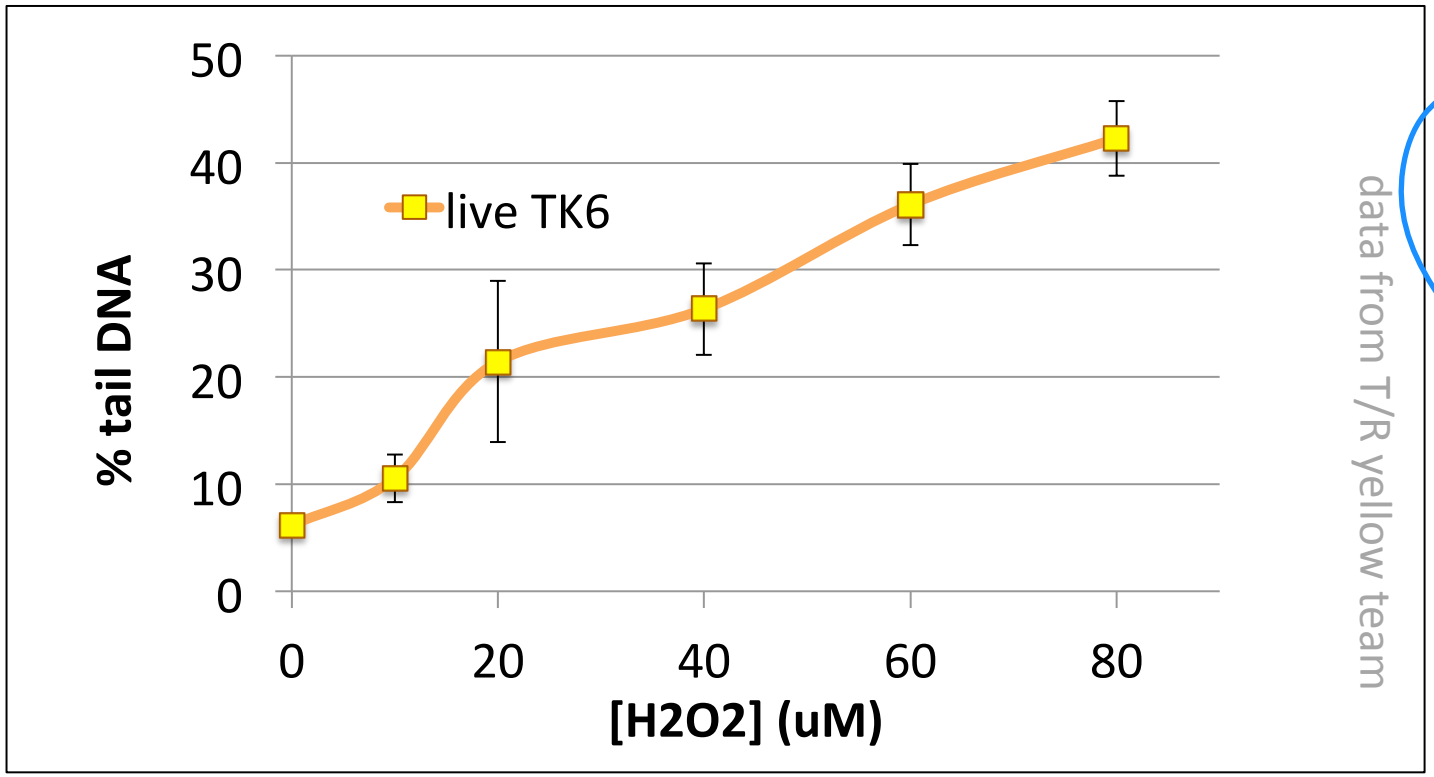
MMS



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



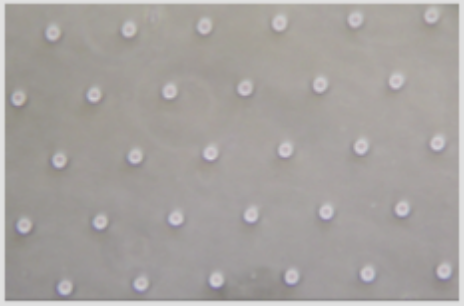
H_2O_2 dose response of live TK6 cells



40uM

- Which concentration of hydrogen peroxide are we choosing for today's drug treatment?

Overview of
“M1: Measuring Genomic Instability”



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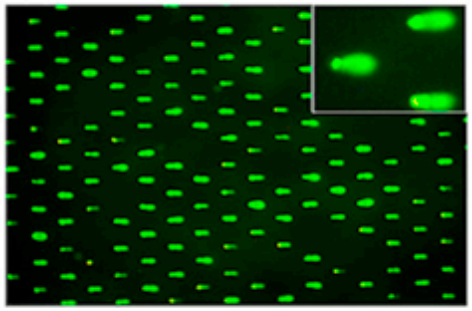

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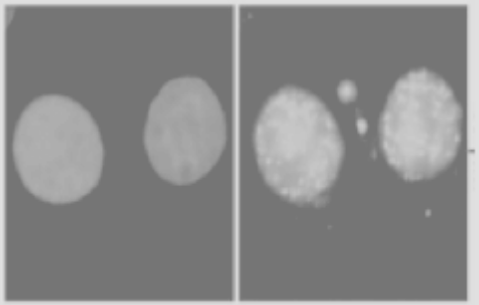
- Measure effects of MMS and H_2O_2 on BER
- Assess repair variability in healthy individuals



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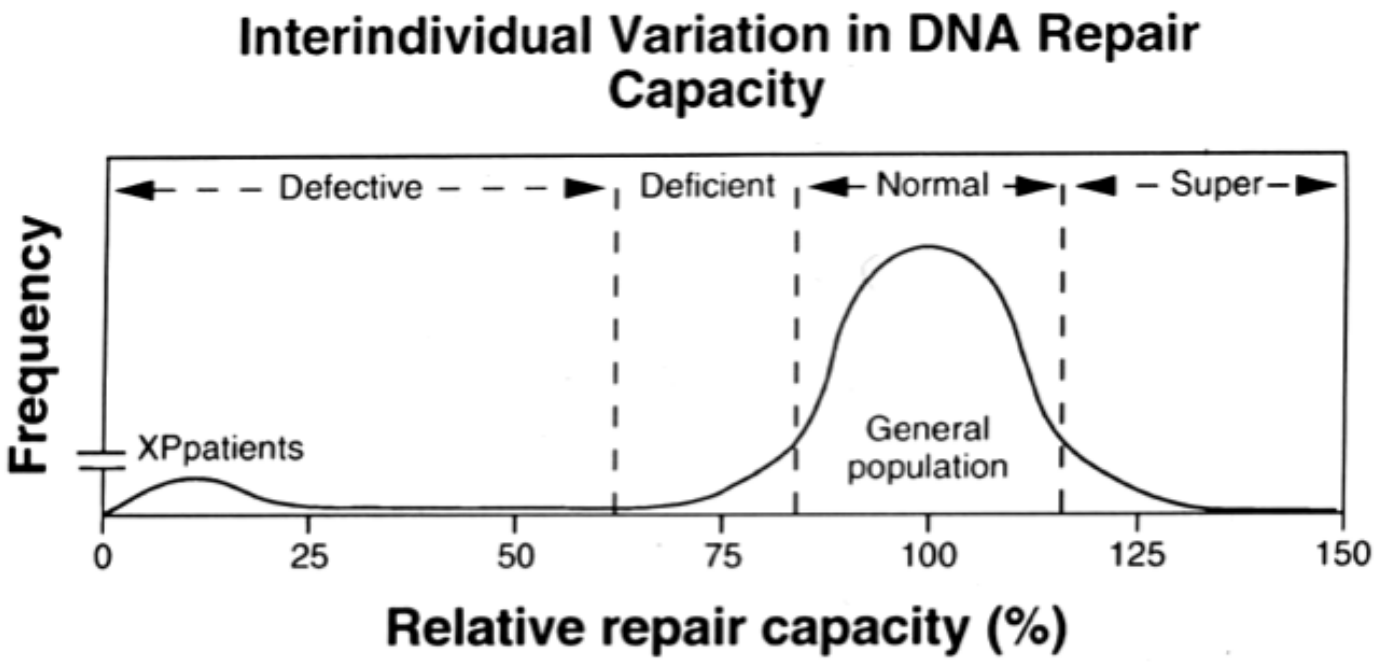
- Examine effect of H_2O_2 on DSB abundance



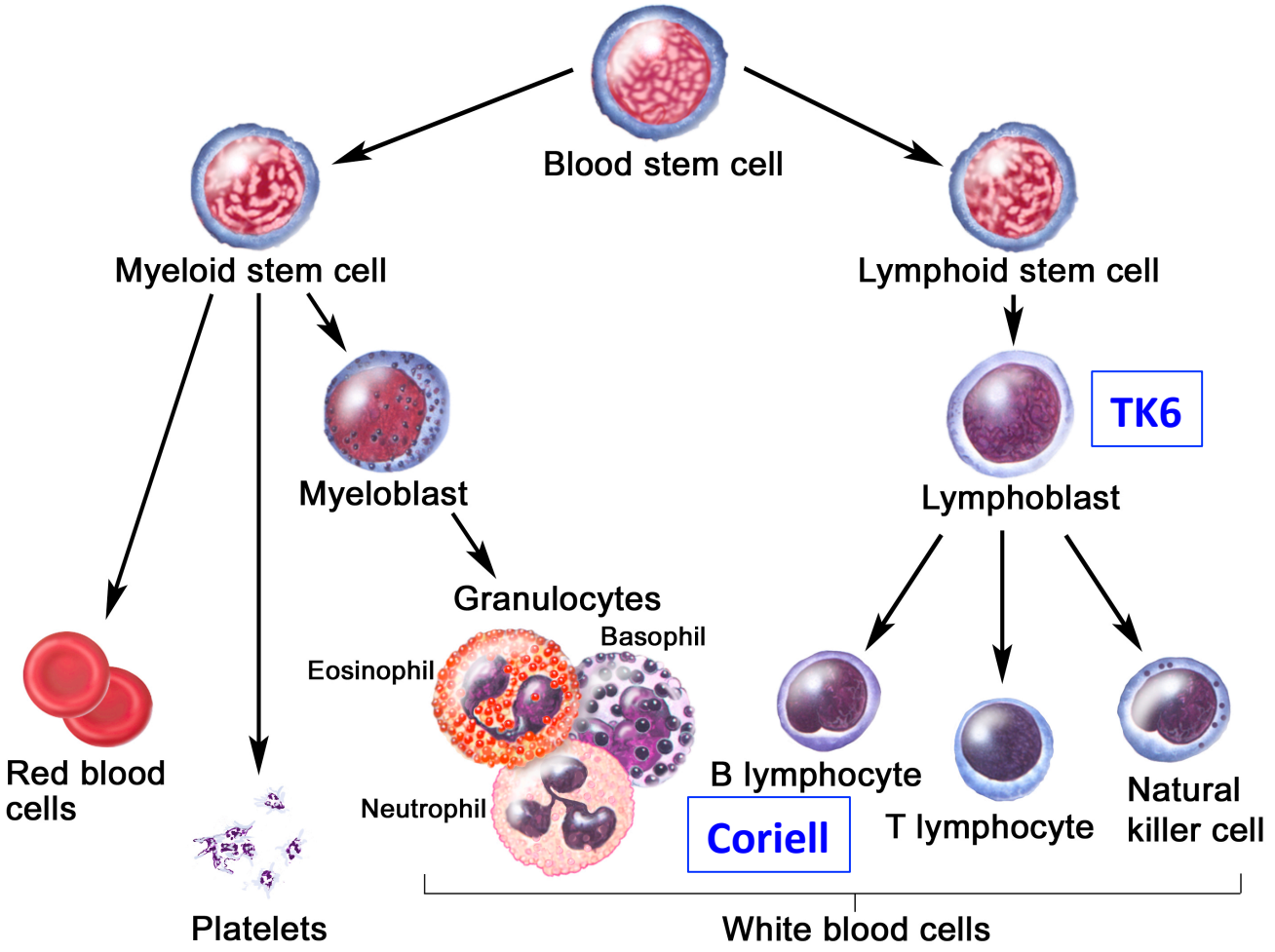
Adapted from **GROSSMAN and Wei (1995)** Clinical Chem 41: 1854-1863

XP frequency = ~1:250,000 giving a theoretical maximum of ~28,000 cases worldwide with 2,000-fold increased risk

Even if just 1% of the population is relatively repair deficient, could have tens of millions with several-fold increased risk

Slide from M2D1 Lecture — Prof. Samson

Coriell cells: differentiated TK6 cells

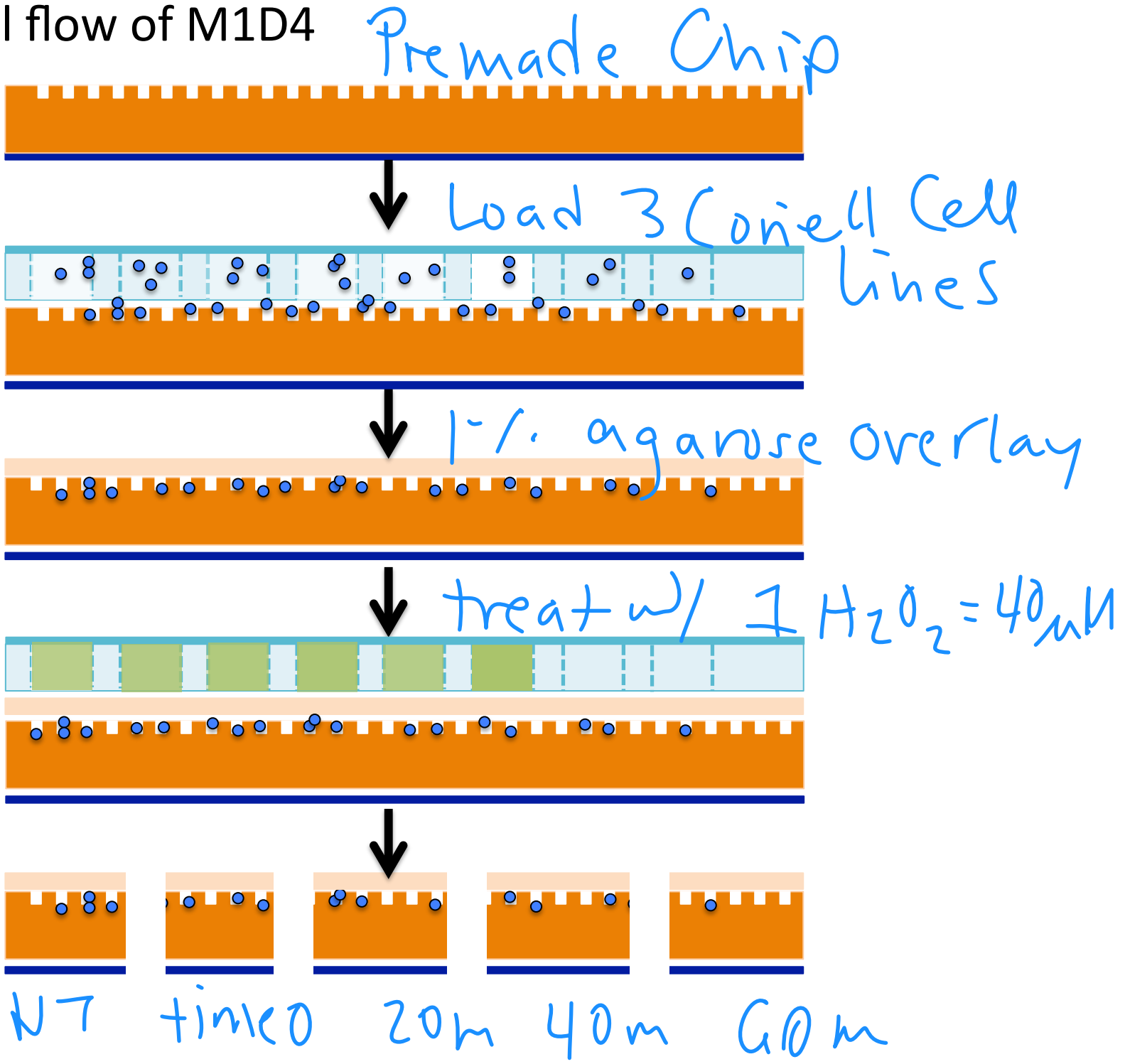


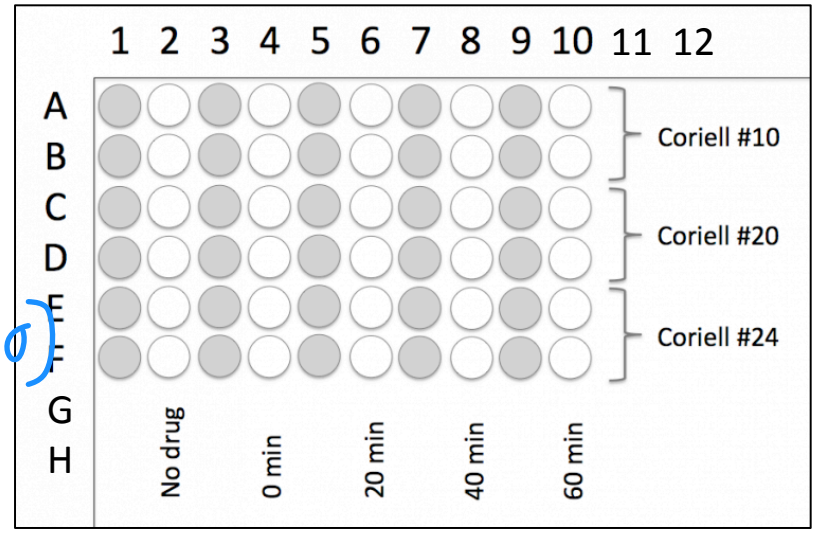
Coriell human B-lymphocyte cell lines

- from Coriell Institute for Medical Research
  - # 10: GM15221 doubling time 40 h
  - # 20: GM15242 doubling time 20 h
  - # 24: GM15061 doubling time 21 h
- healthy individuals with no known DNA repair deficiencies
- derived from ethnically diverse populations
  - ideal for inter-individual variation studies

➢ Do they have the same DNA repair kinetics profile?

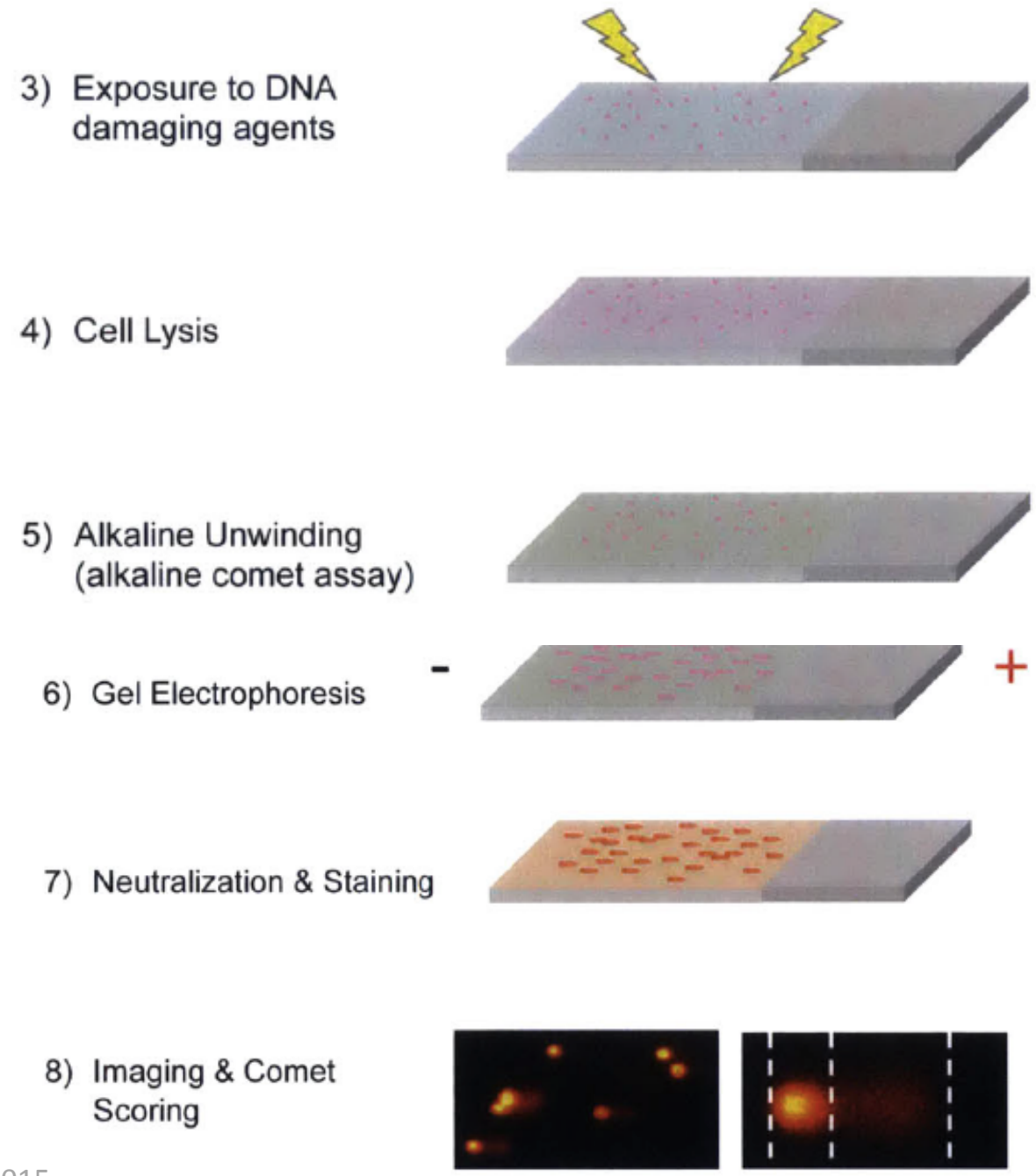
Experimental flow of M1D4





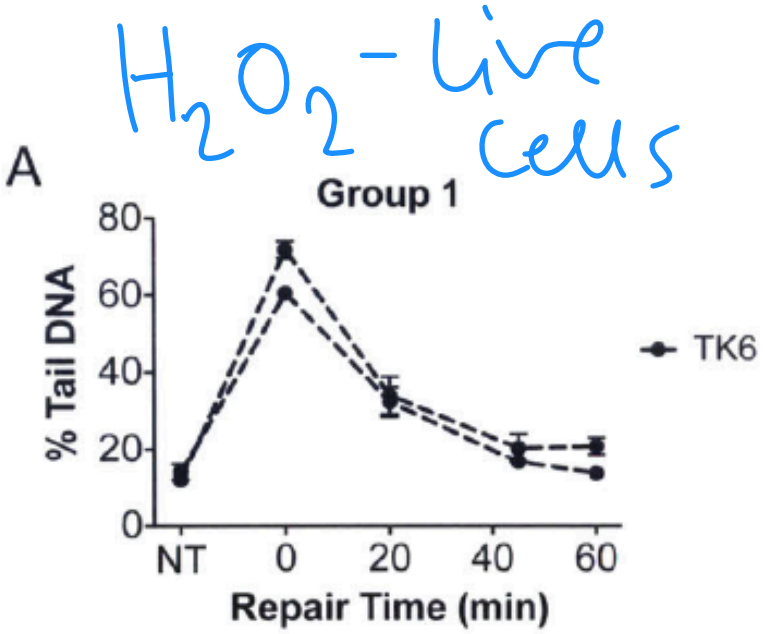
- Load Coriell cells:
  - 25,000 cells/well (#10 ~ 10,000)
  - for 30 min at 37 °C
- Trap with 1% LMP agarose
  - 3 min at room temperature + 3 min at 4 °C
- Treat 30 wells of CometChip with H_2O_2 drug
  - 40 µM
  - on ice
  - 20 min at 4 °C
- Allow cells to recover at 37°C in TC media
  - No drug and 0 min = directly in lysis buffer
  - or 20 min, 40 min, 60 min = cut band with scissors, then add it to lysis tray

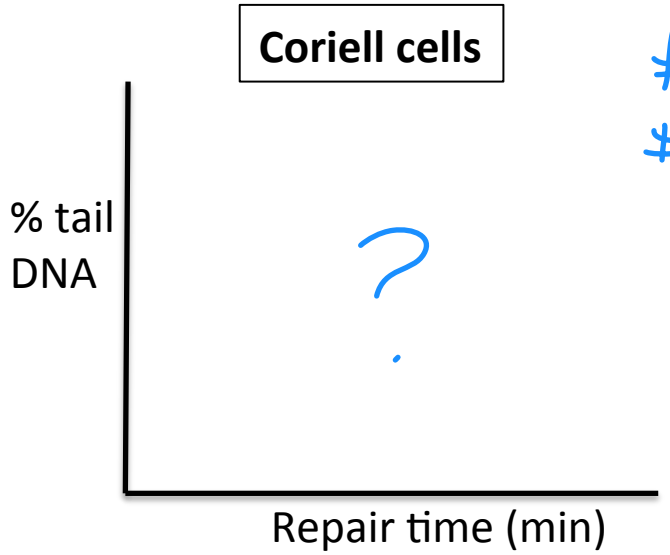
… and this evening, tomorrow and Thursday morning



Jing Ge's thesis, Engelward Lab, 2015

Cell lines vary in susceptibility to DNA damage and in the kinetics of repair





Jing Ge's thesis, Engelward Lab, 2015

In lab today…

1. Load chip with 3 Coriell cell lines
2. Treat cells with H_2O_2
3. Immediately lyse no drug treatment and time=0
4. Allow 3 other time points to recover and add to lysis buffer when appropriate
5. During incubation time we will have a background/ motivation discussion (HW due M1D5, Thursday!) and a preview of our results

Assignments for M1

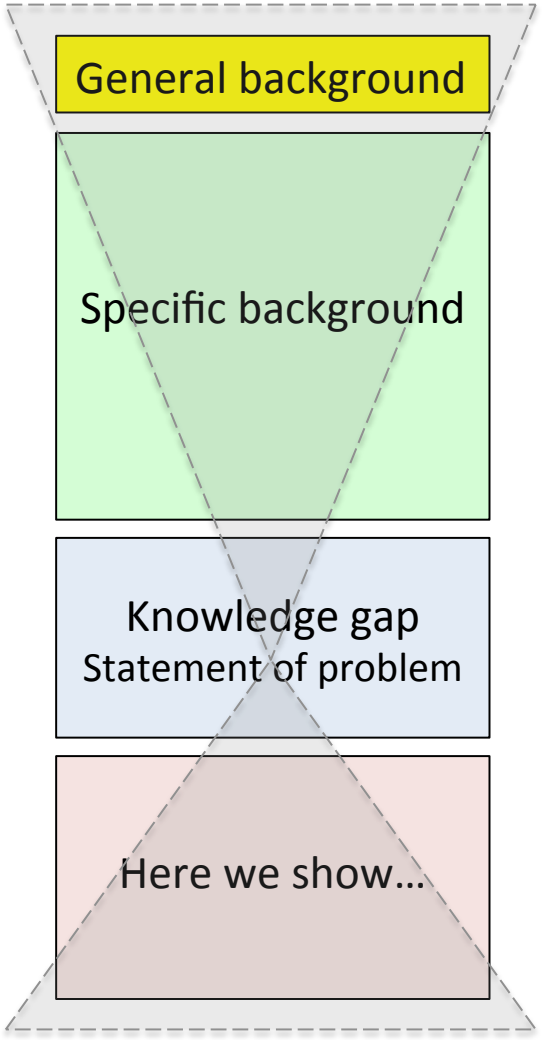
- Data summary draft
  - due by 5pm on Wed., October 12
  - revision due by 5pm on Mon., October 24

Summary content

1. Title
2. Abstract
3. Background, Motivation
4. Figures, Results & Discussion, Interpretation
5. Implications, Future Work

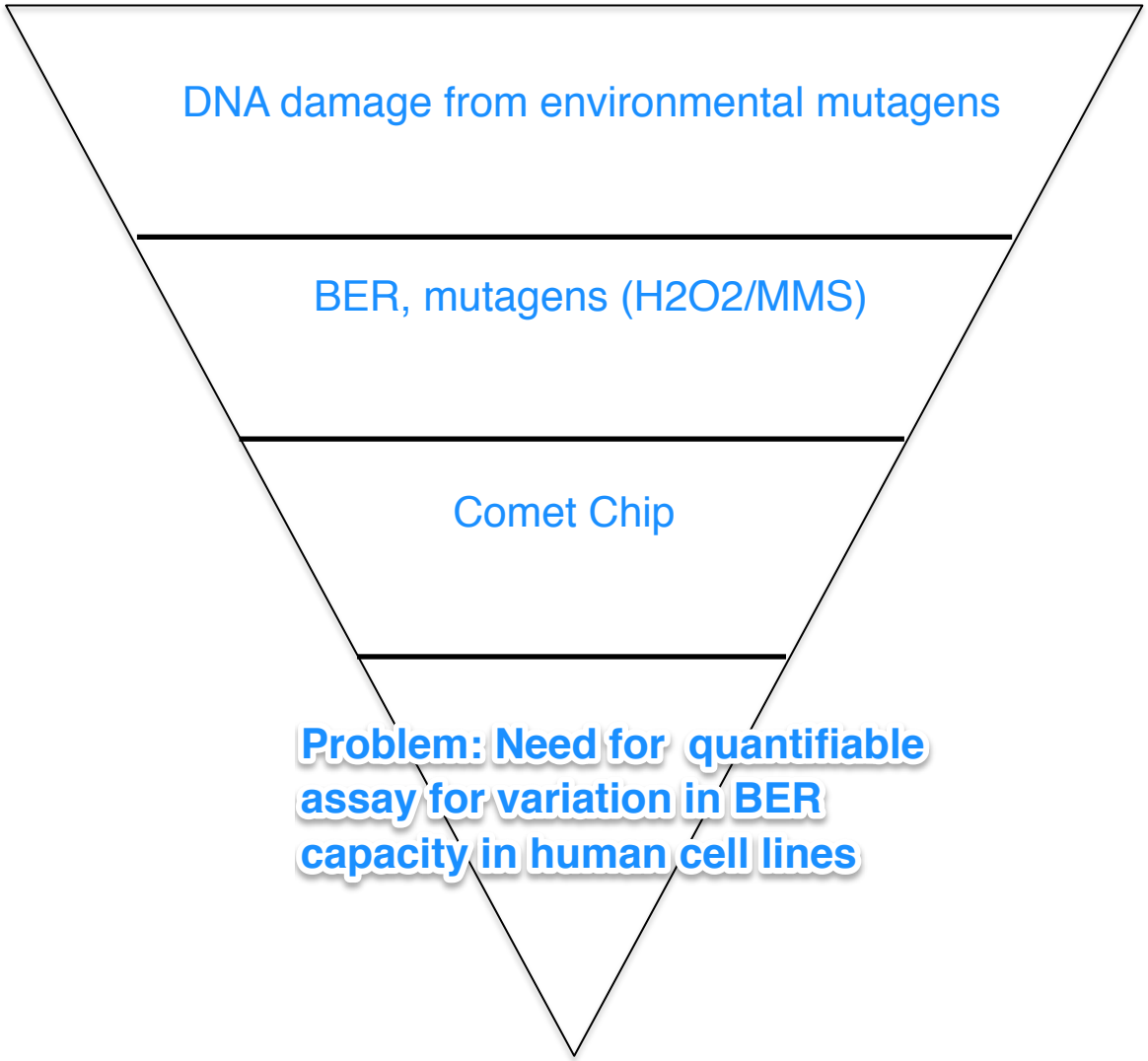
- Mini presentation due by 10pm on Sat., October 15
- Blog post for M1 due by 5pm on Tue., October 25

What goes into an introduction?



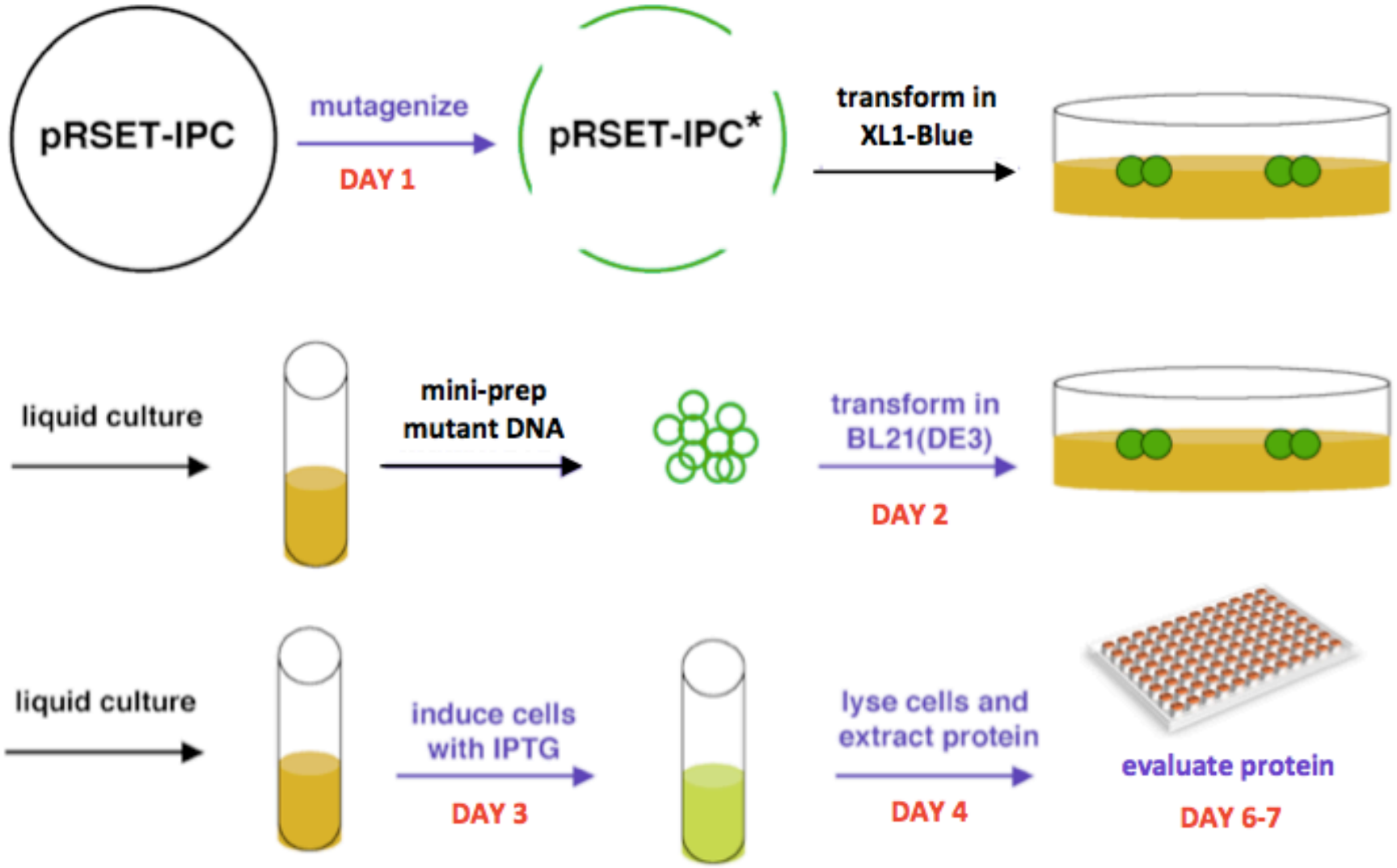
- Your research is anchored in a general topic that your audience cares about.
  - focus on outsiders
- All information connects your project with the general topic.
  - minimum essential information
  - accurately represents the field
  - correctly referenced, give credit
- The question you address is clearly articulated, connected to the background, and appears meaningful.
  - give evidence of incompleteness of current understanding, of value of investigation
  - include your hypothesis
- A preview of your findings and their implications fills the demonstrated gap.
  - light on Methods

HW M1D5: Background and Motivation



Effective schematics

-purpose of schematic in your summary
-explanatory
-little text as possible
-not too big
-all details have meaning



All data are consolidated on the wiki

http://engineerbiology.org/wiki/Talk:20.109(F16):Module_1

Module 1 Data

[edit]

M1D2: cell loading parameters [edit]

All teams' choices are recorded here.
The images from the doubling time experiments, CometChip t = 0 (see below), are illustrative of how many cells were loaded per microwell under each condition.

M1D2: doubling time, pictures and fluorescence signal [edit]

The 'data' below refer to Excel spreadsheets

- listing the fluorescence signal (a.u., with 1 cell corresponding to ~ 1600 a.u.) detected in each microwell in the field of view (rows 8 and above),
- together with the average signal in representative macrowells of quadrants A1, A2, B1, and B2 (row 5; see loading conditions above),
- and with the corresponding calculated doubling times (row 6).

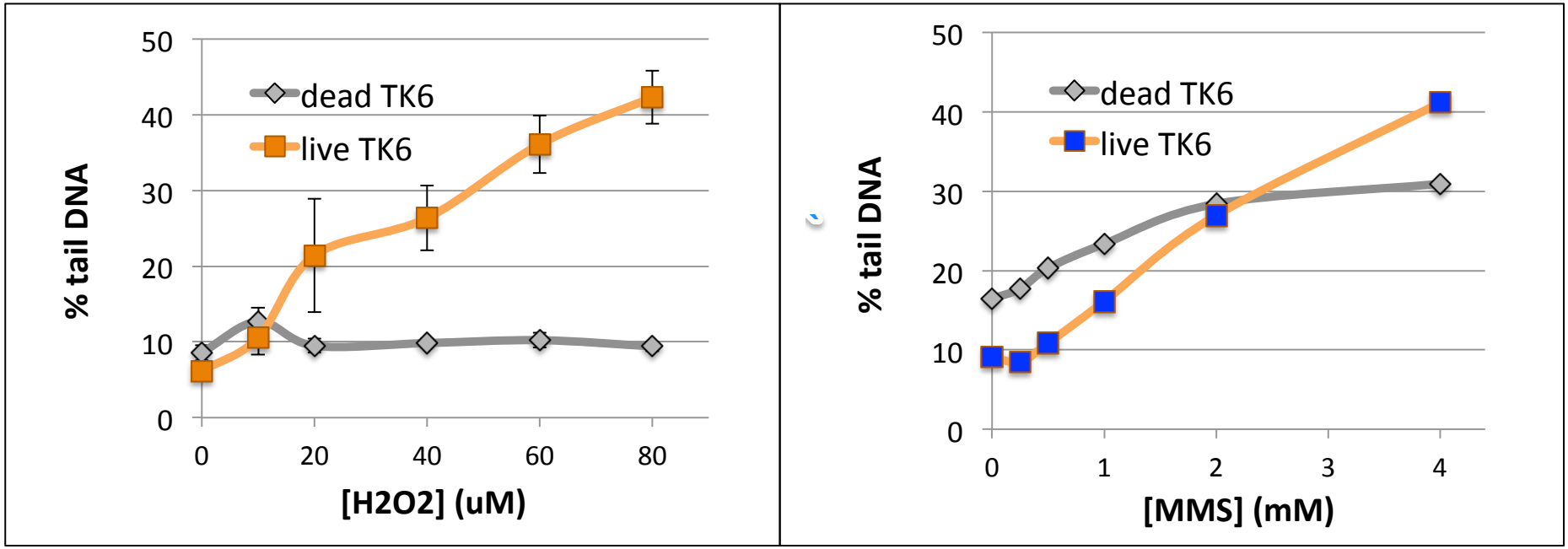
One image per quadrant was recorded. **Open images in ImageJ** for all encoded information to be displayed.

team	data summary	t=0 and t=2.5d images
T/R yellow	data	images
T/R green	data	images
T/R blue	data	images
T/R pink	data	images
T/R purple	data	images
W/F red	data	images
W/F green	data	images
W/F blue	data	images
W/F purple	data	images

M1D3: H_2O_2 and MMS dose response pictures and comet analysis [edit]

Your data: DNA damage by H2O2 *vs*. MMS

- Did the two chemicals have the same DNA damaging effect?



vs.

induced DNA damage