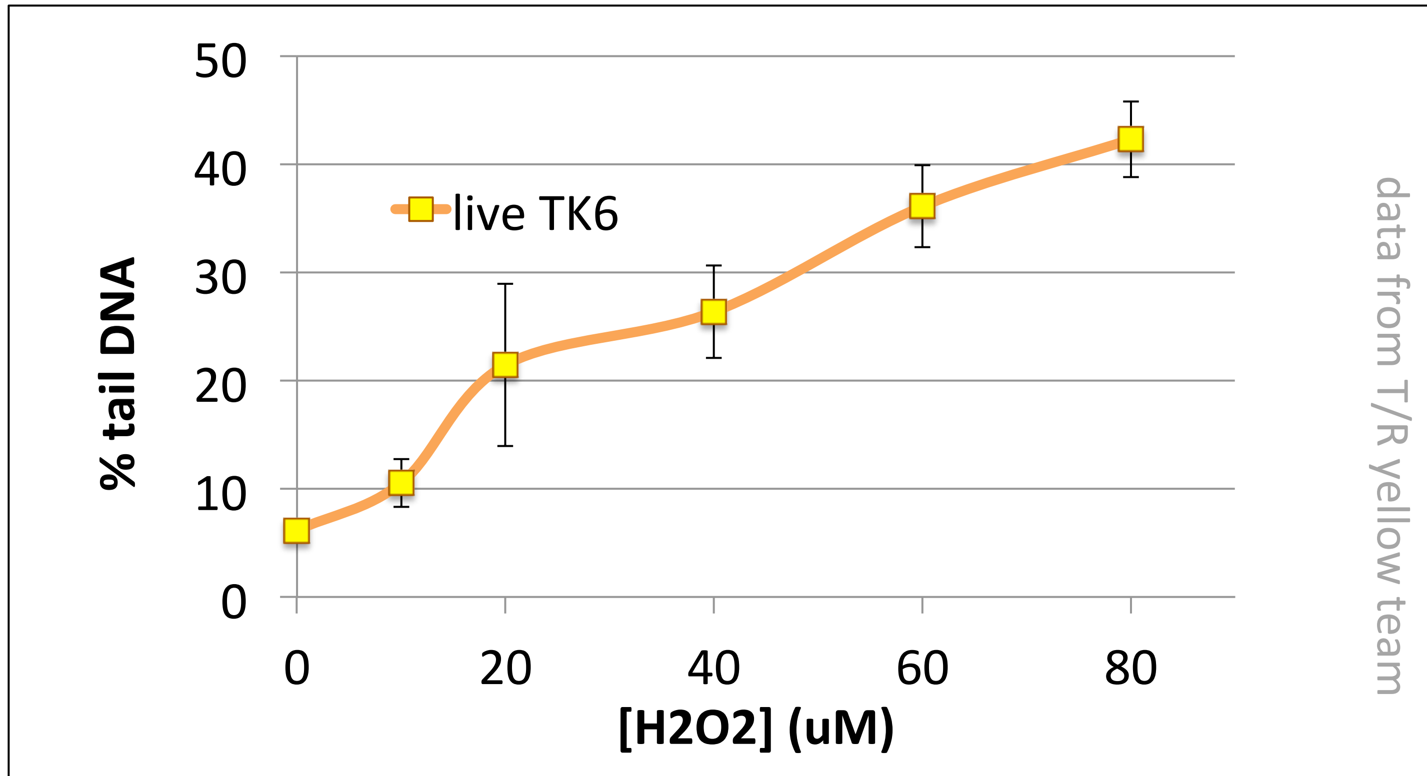
M1D4:
Assess DNA repair variability

09/28/2016

H_2O_2 dose response of live TK6 cells



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

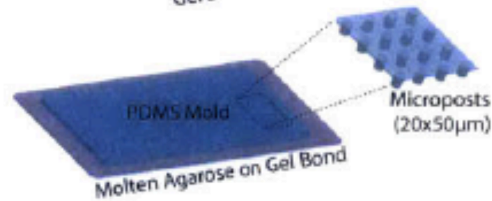
40 uM

Making a CometChip (with zoomed view)

A

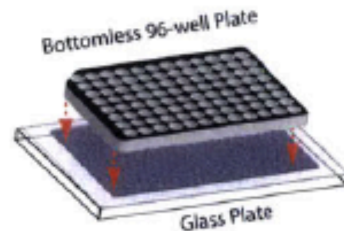
1) Pour Molten Agarose

2) Apply PDMS Mold
Allow Agarose to Gel

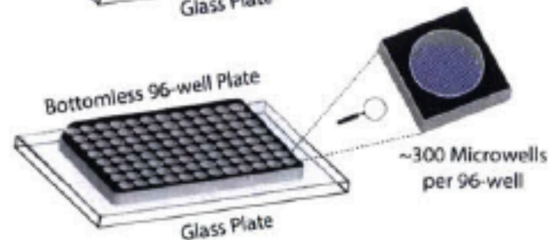


3) Peel Off PDMS Mold

4) Align 96-well Plate

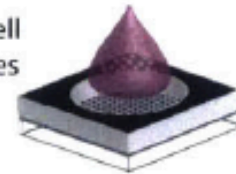


5) Clamp 96-well Plate

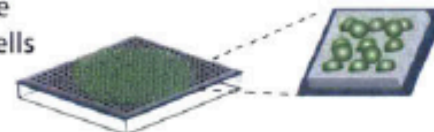


B

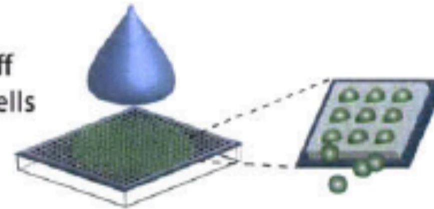
Load Cell
Samples



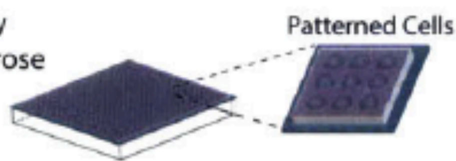
Aspirate
Excess Cells



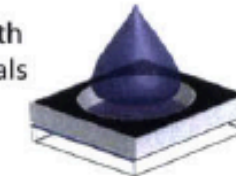
Wash off
Excess Cells



Overlay
with Agarose

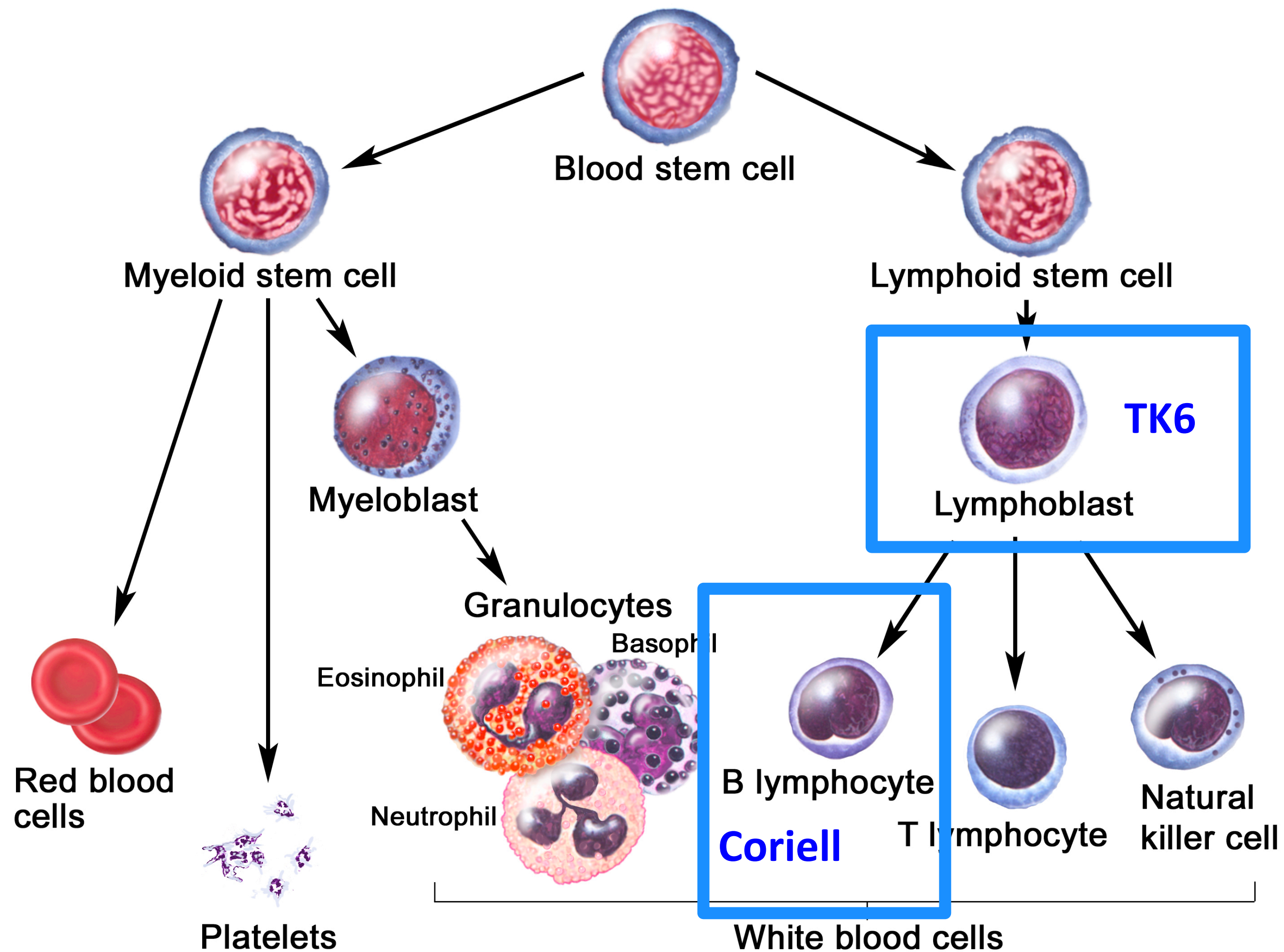


Dose with
Chemicals



Jing Ge's thesis, Engelward Lab, 2015

Coriell cells ~ differentiated TK6 cells

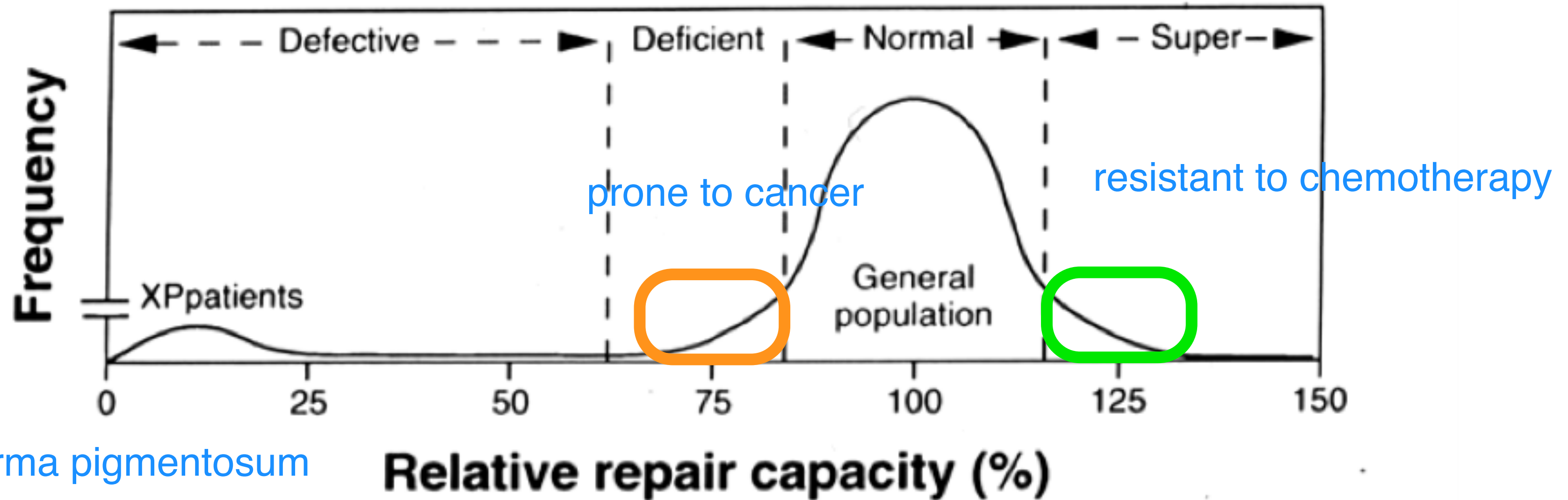


Coriell human B-lymphocyte cell lines

- from Coriell Institute for Medical Research
  - # 10: GM15221 doubling time 40 h
  - # 20: GM15242 20 h
  - # 24: GM15061 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?

Why do we care about DNA repair capacity?



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

DNA repair capacity varies among individuals

Knowing a particular individual's repair profile can inform decisions

Slide from M2D1 Lecture — Prof. Samson

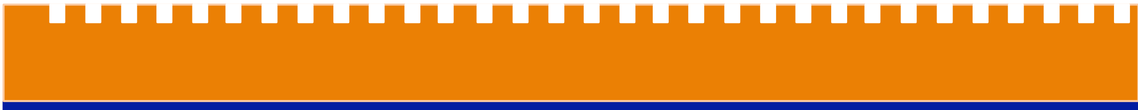
20 and # 24 are at 1,000,000 cells/mL
10 at 150,000 cells/mL

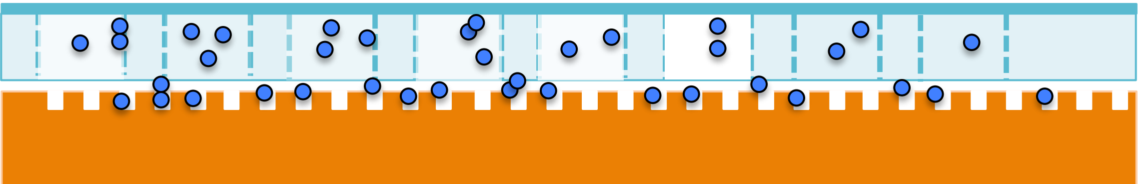
In lab today

	1	2	3	4	5	6	7	8	9	10	11	12	
A													Coriell #10
B													
C													Coriell #20
D													
E													Coriell #24
F													
G													
H		No drug		0 min		20 min		40 min		60 min			

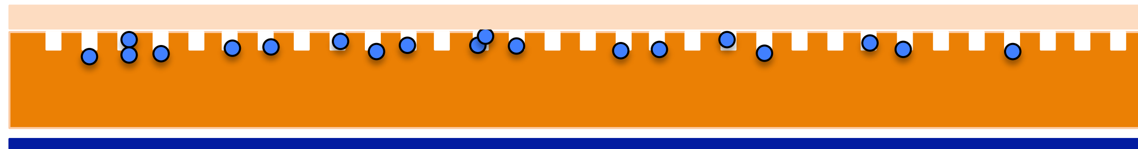
- Load Coriell cells:
  - 100 µL cells
  - for 30 min at 37 °C
- Trap with 1% LMP agarose
  - 3 min at room temperature + 3 min at 4 °C
- Treat 30 wells of CometChip with H_2O_2 drug
  - 40 µM
  - on ice
  - 20 min at 4 °C
- Allow cells to recover at 37 °C in medium
  - 0 min = directly in lysis buffer
  - or 20 min, 40 min, 60 min = cut band with scissors, then add it to lysis tray

Or

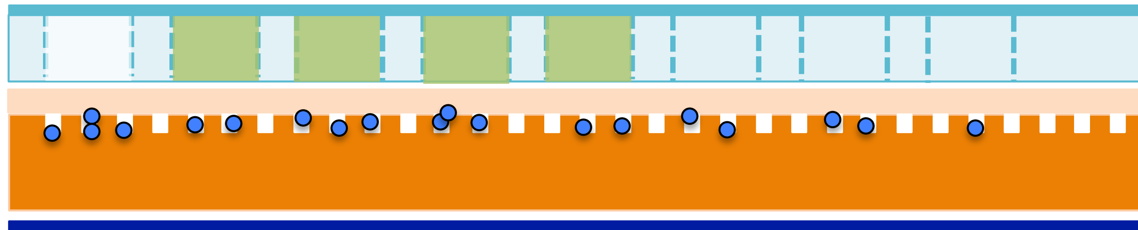




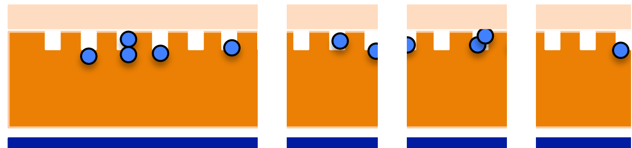
load Coriell cells



1% LMP agar



add H_2O_2
<u>except</u> in
“no drug” column



let recover
lyse

<u>no</u> 0 20 40 60 min

… and this evening, tomorrow and Friday morning

3) Exposure to DNA damaging agents

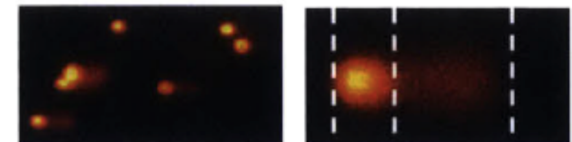
4) Cell Lysis

5) Alkaline Unwinding (alkaline comet assay)

6) Gel Electrophoresis

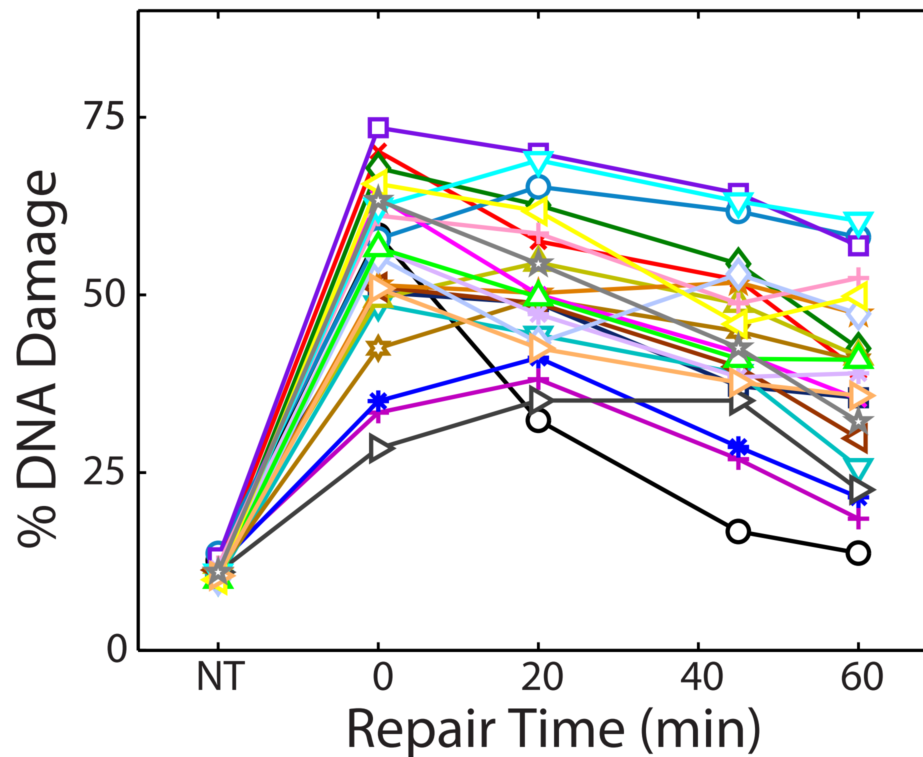
7) Neutralization & Staining

8) Imaging & Comet Scoring



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

- Load CometChip with 3 Coriell cell lines

Don't mix them during wash!

- Treat with H_2O_2

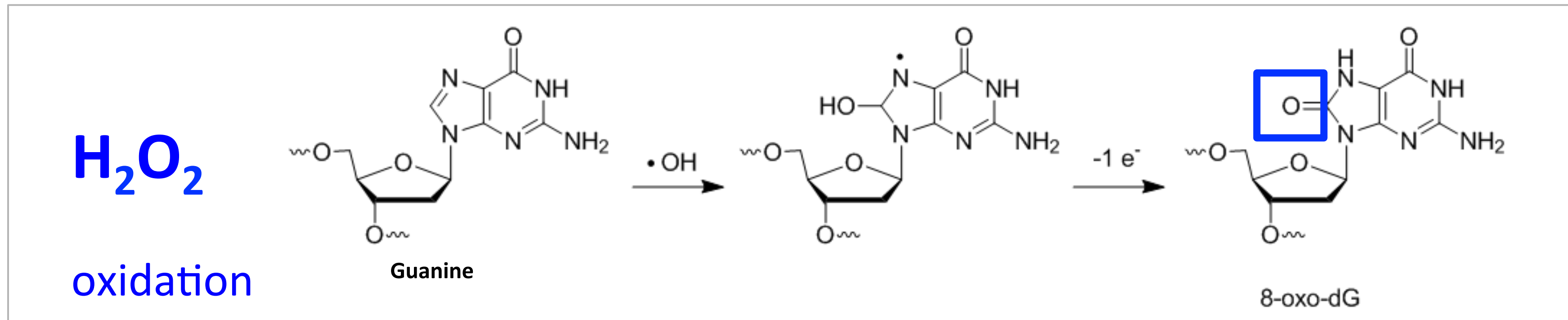
✧ Immediately lyse "no drug" and "0 min recovery" strips

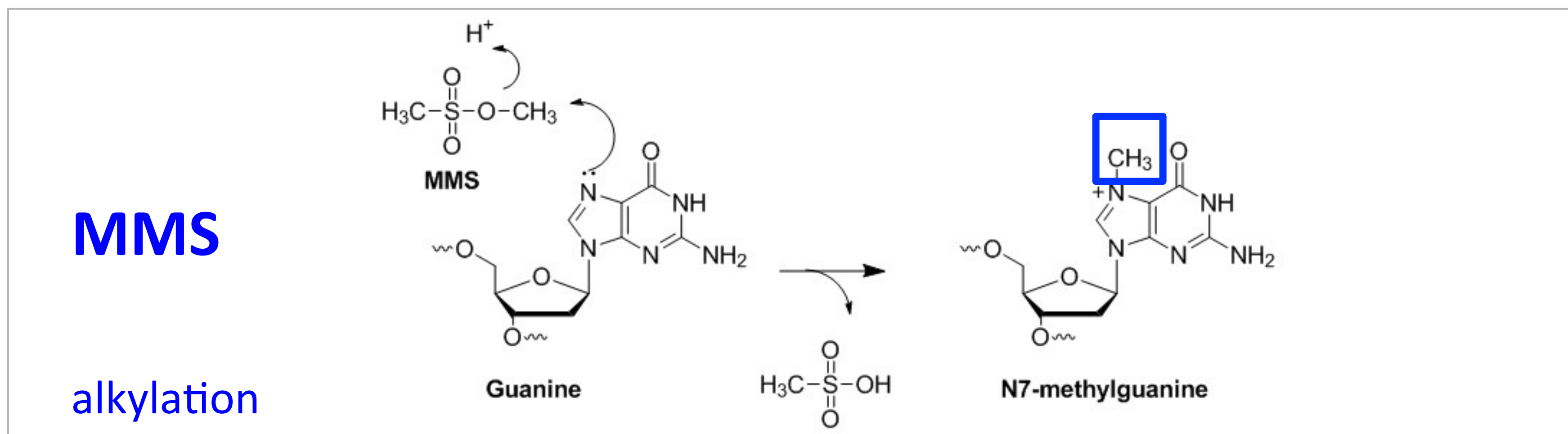
✧ Allow other time points to recover before lysis

- During pauses / incubation times
  - Review H_2O_2 vs. MMS dose responses
  - Introduction of a paper (homework due M1D5 = Friday!)

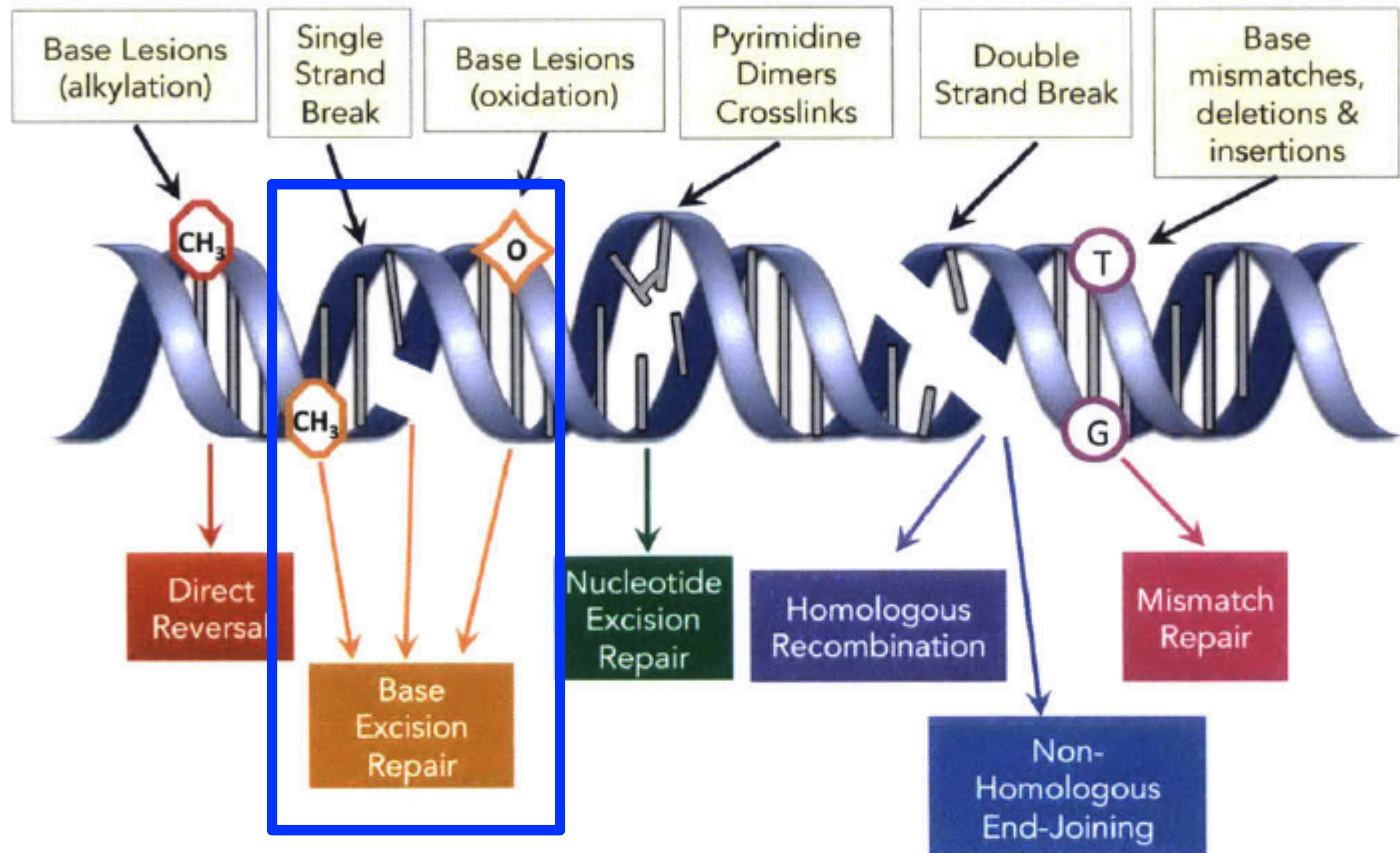
DNA damage induced by H_2O_2 *vs.* MMS

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



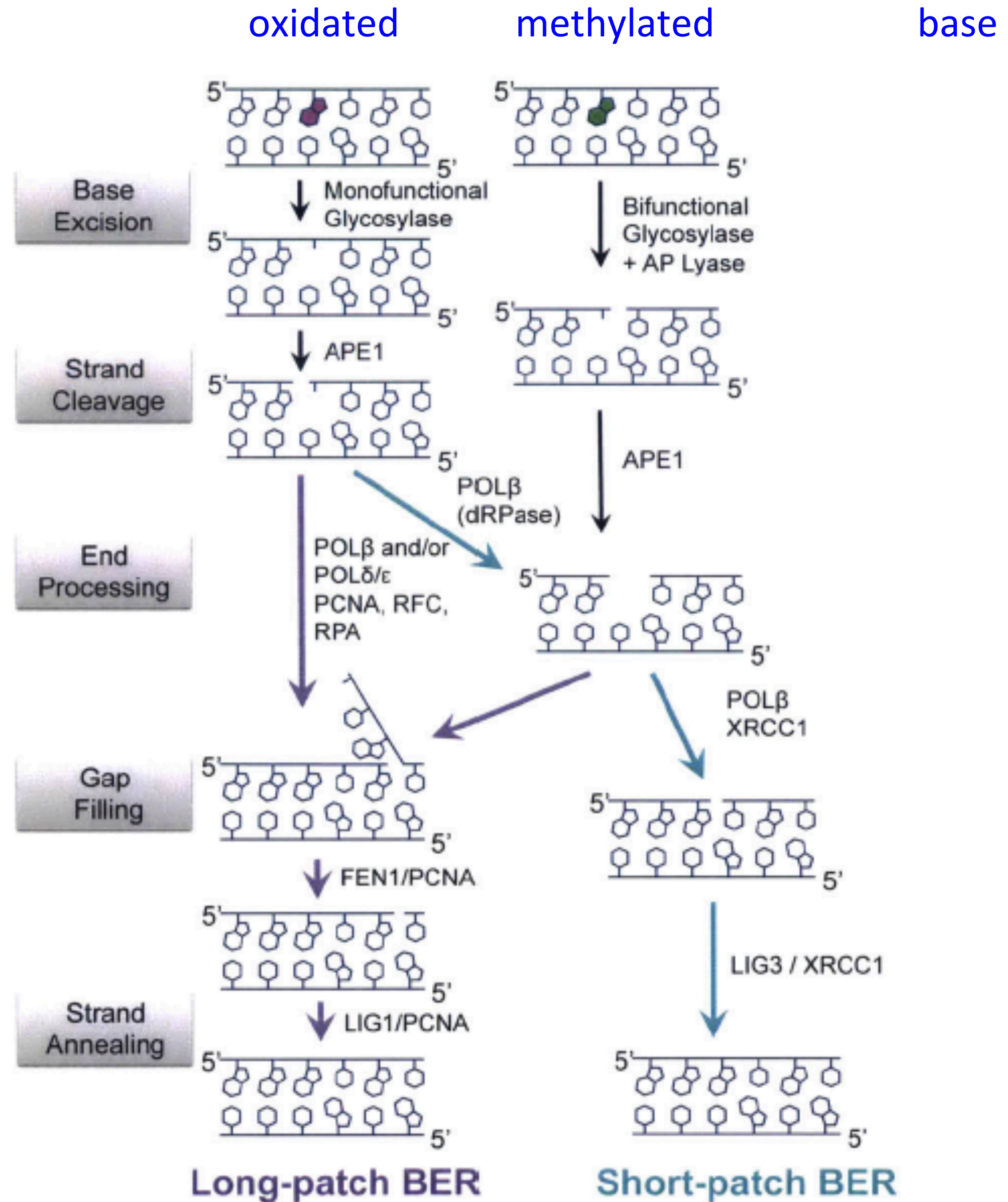


Both H_2O_2- and MMS-caused DNA damages are repaired by base excision repair (BER)



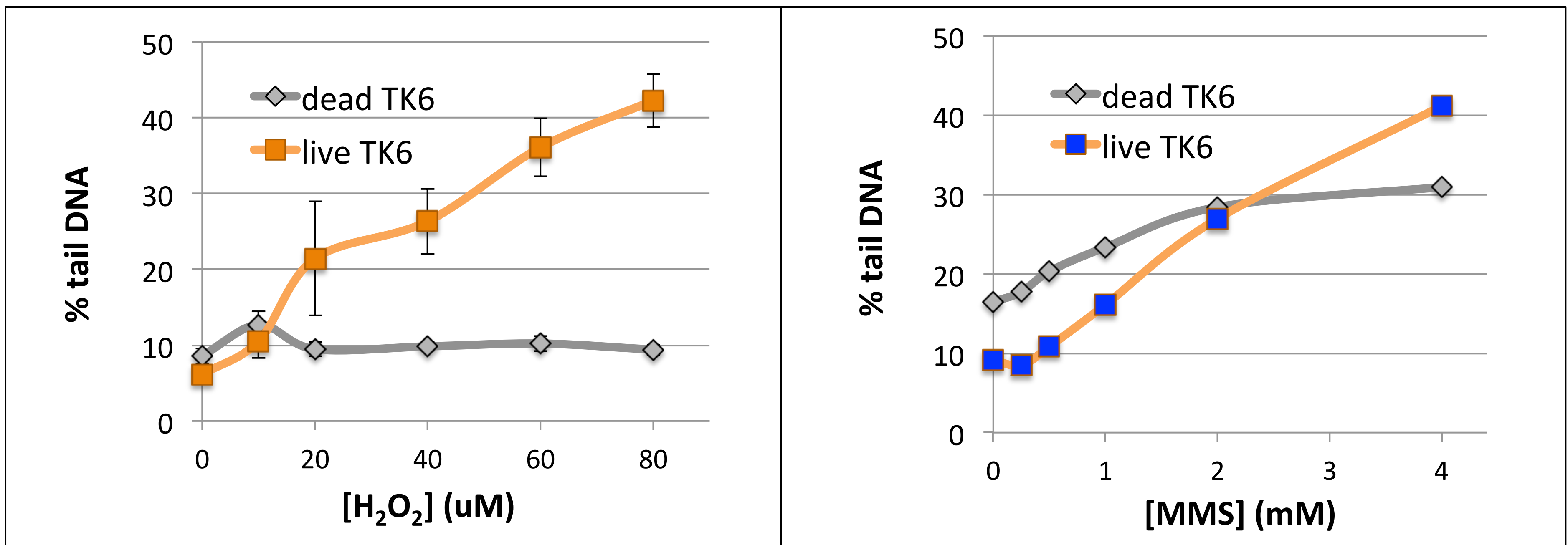
BER

oxidated methylated base



Your data: DNA damage by H_2O_2 *vs.* MMS

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



vs.

enzymatically induced DNA damage chemically

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]

Assignments for M1

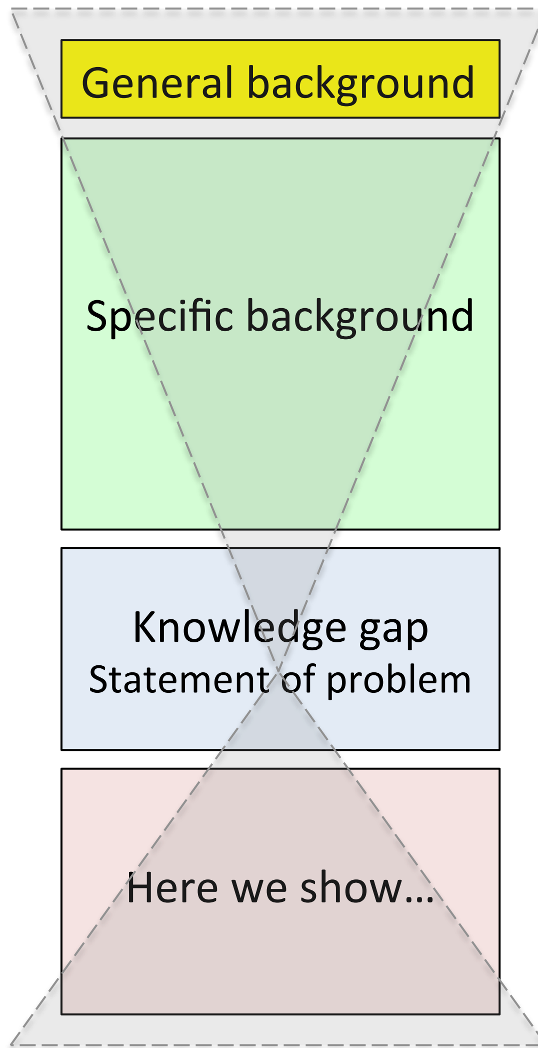
- Data summary draft
  - due by 5pm on Wed., October 12
  - revision due by 5pm on Mon., October 24

Summary content

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

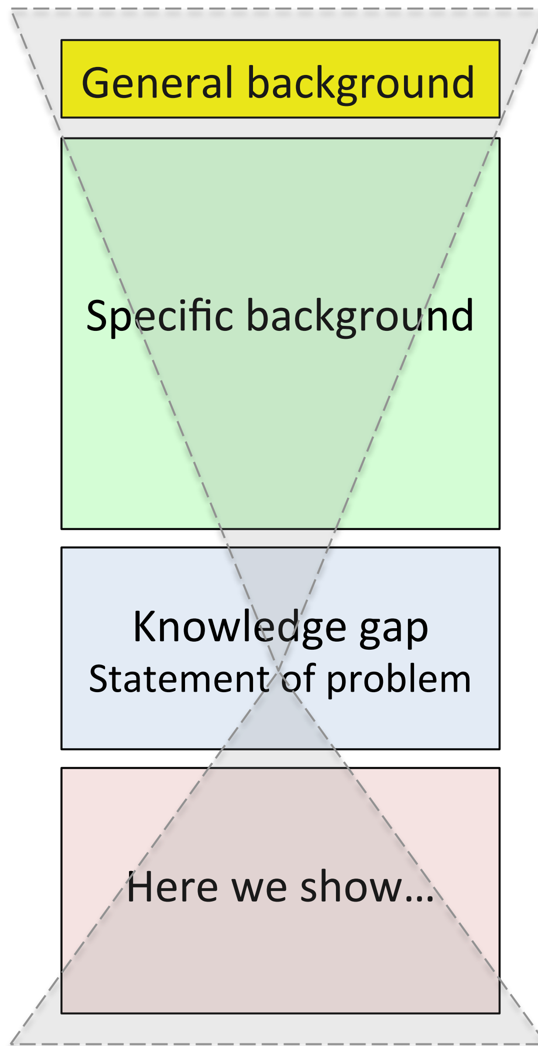
- Mini presentation due by 10pm on Sat., October 15
- Blog post for M1 due by 5pm on Tue., October 25

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

What goes into your introduction?



Choose one narrative

cancer, DNA damage, DNA repair

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explain DNA repair pathway(s):
6 existing, focus on BER

traditional comet assay and its limitations

inter-individual variability

---

how can we optimally use (load) the CometChip?

do chemicals creating DNA damage repaired by BER damage DNA in the same way?

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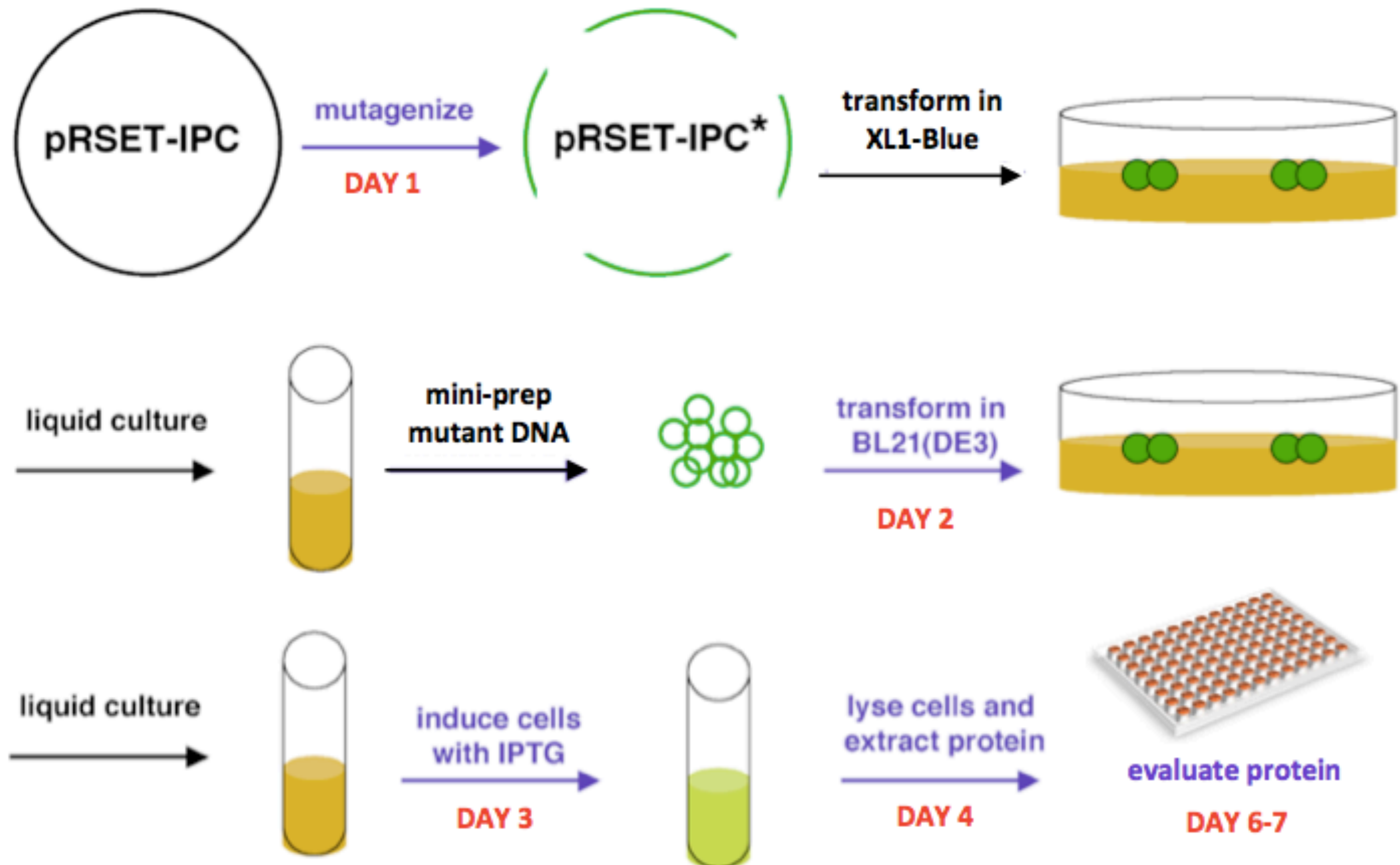
preview of results AND IMPLICATIONS

Judge this schematic as if published *figure*



Effective schematics

- Establish the purpose of the schematic, then think about its components and layout
  - no need to detail all Methods
- Schematics can have expository (rather than conclusive) titles
  - overview schematic often designed to support the first/overview paragraph of the Results section
- Very little text on schematic itself
  - A-B labels instead, and details in caption
- Only as large as needs to be
  - size icons intelligently, use white space sparingly
- All details must have a meaning
  - and the meaning must be spelled out in caption
  - otherwise they are distracting / confusing
  - different things should look different, same things should look the same

microwells are 40 microns in diameter and 40 microns in depth
separated by 240 microns