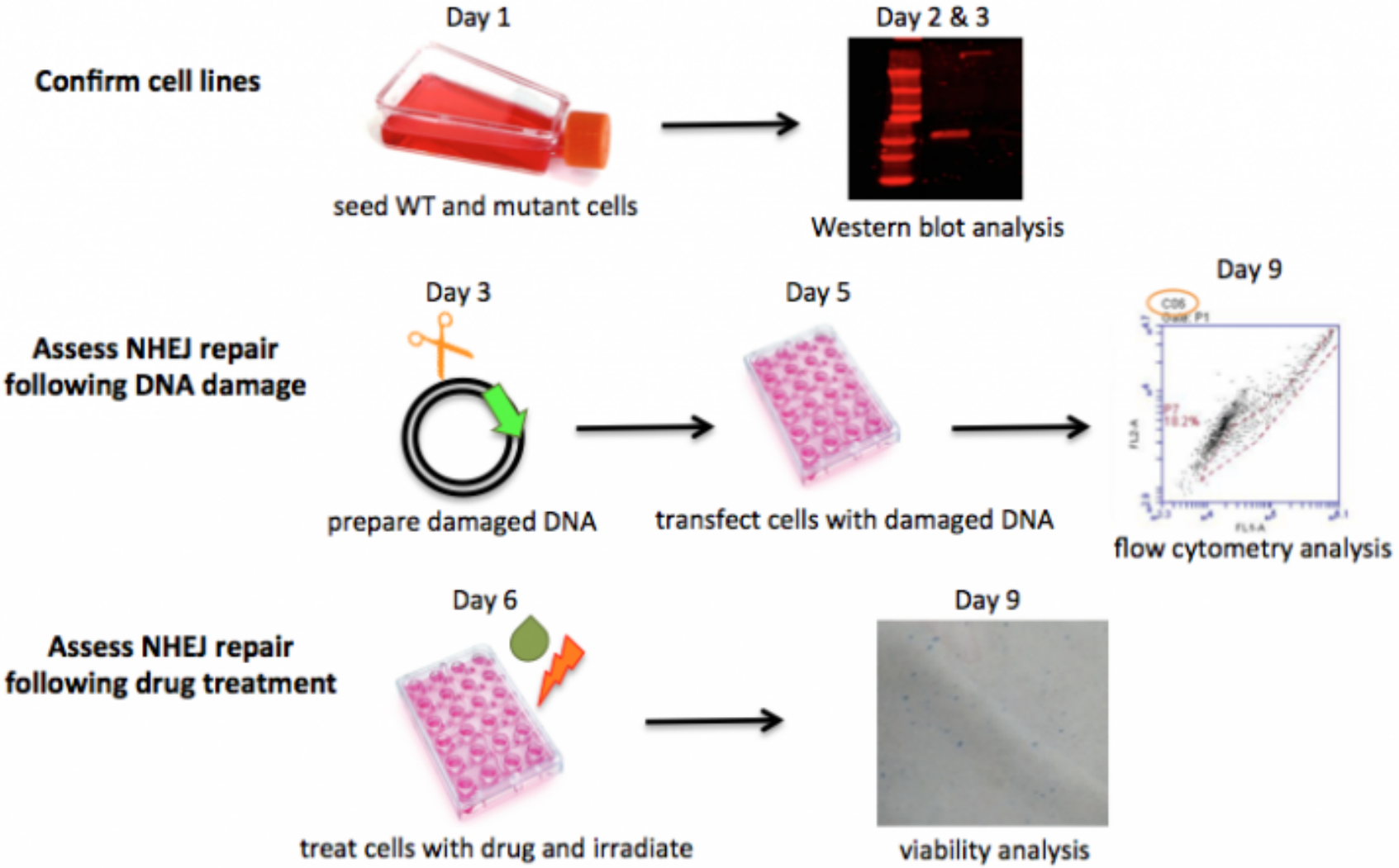
M2D6: DNA repair assay

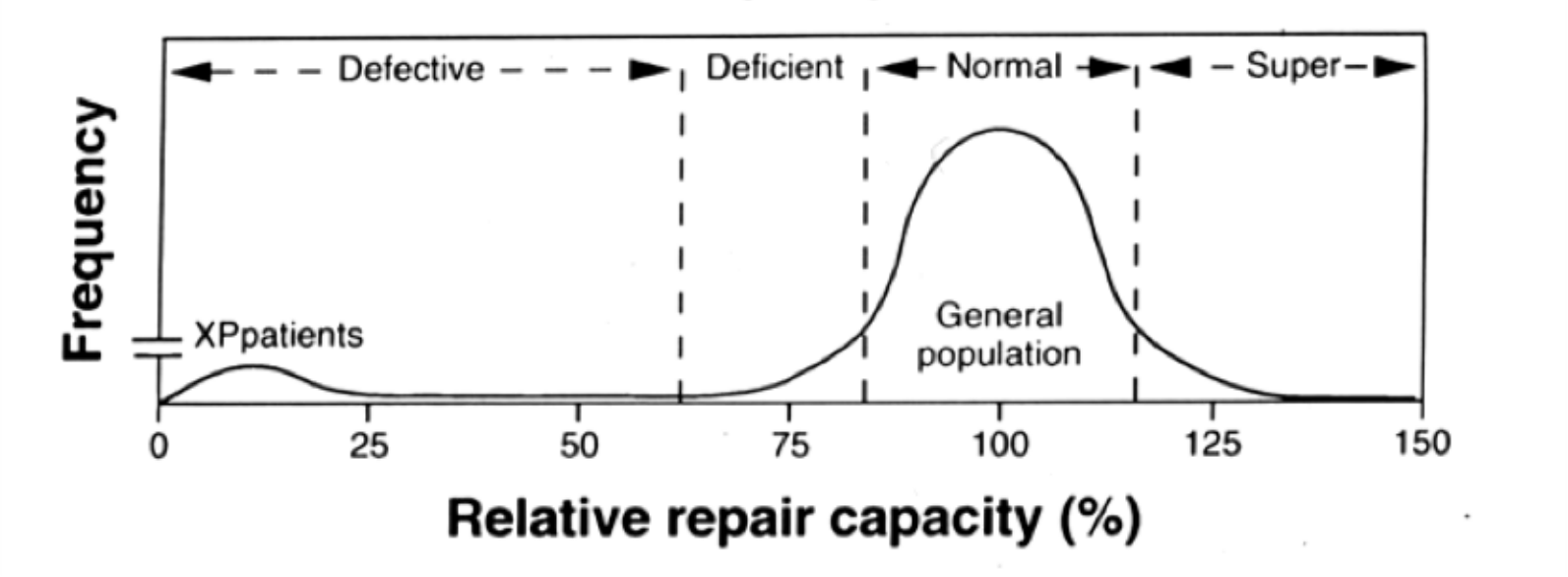
03/31/16

1. Pre-lab discussion
2. ½ group: Tissue Culture
3. ½ group: Start reading Dietlein *et al.*, "A functional Cancer Genomics Screen Identifies a Druggable Synthetic Lethal Interaction between *MSH3* and *PRKDC*"

Mod 2 experimental overview



Why do we care about DNA repair capacity?



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

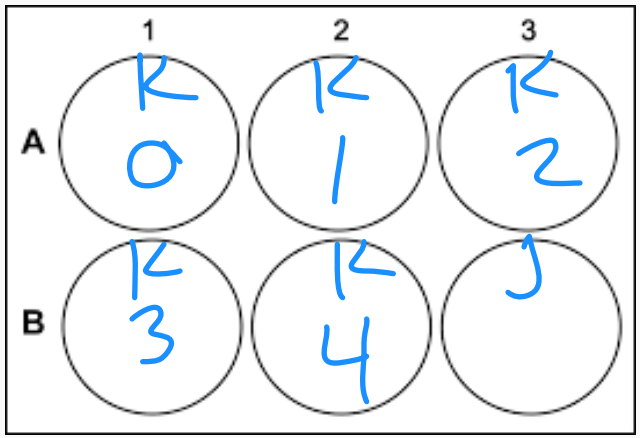
***DNA Repair is variable**

*** Quantifying DNA repair can inform risk assessment and disease treatment choices**

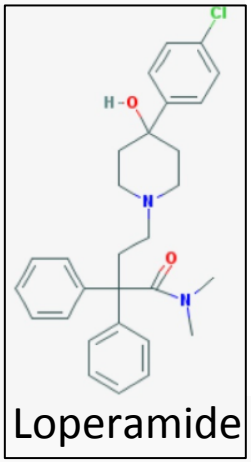
Slide from M2D I Lecture — Prof. Samson

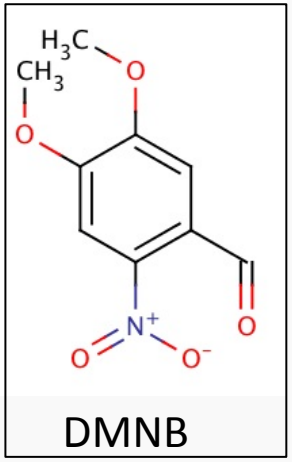
What evidence shows our inhibitor works in MO59 J/K cells?

Day 1: Seed MO59J and K cells at low density



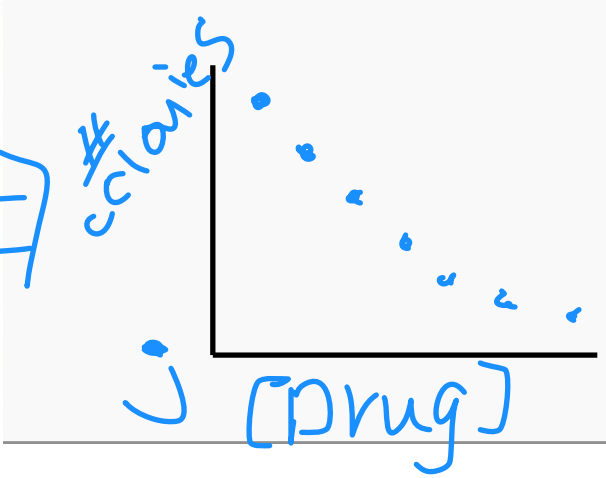
Day 3: Dose response of NHEJ inhibitor around IC50 and expose to plate to ionizing radiation



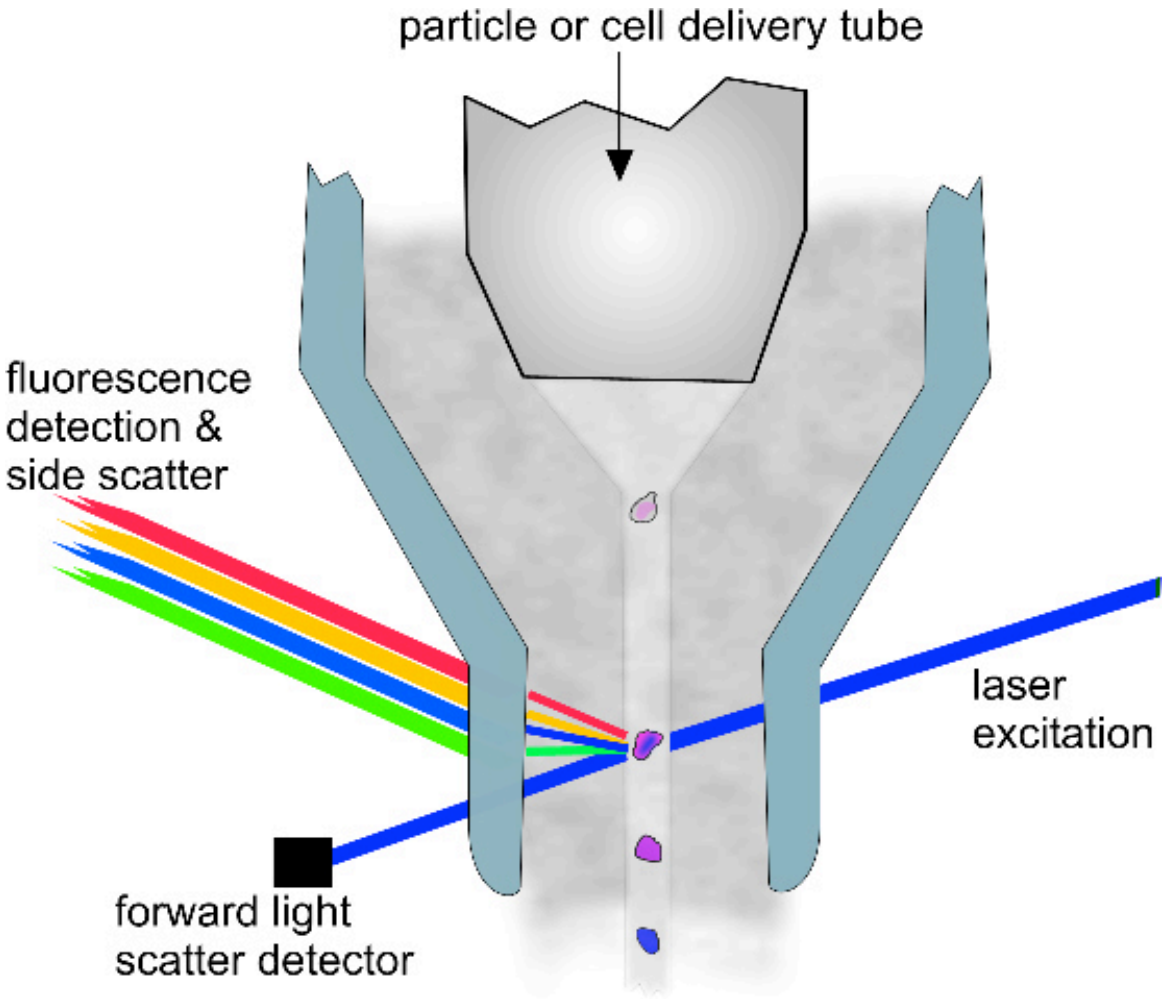


- -don't go higher than 3X IC50
- -keep DMSO volume same in all wells

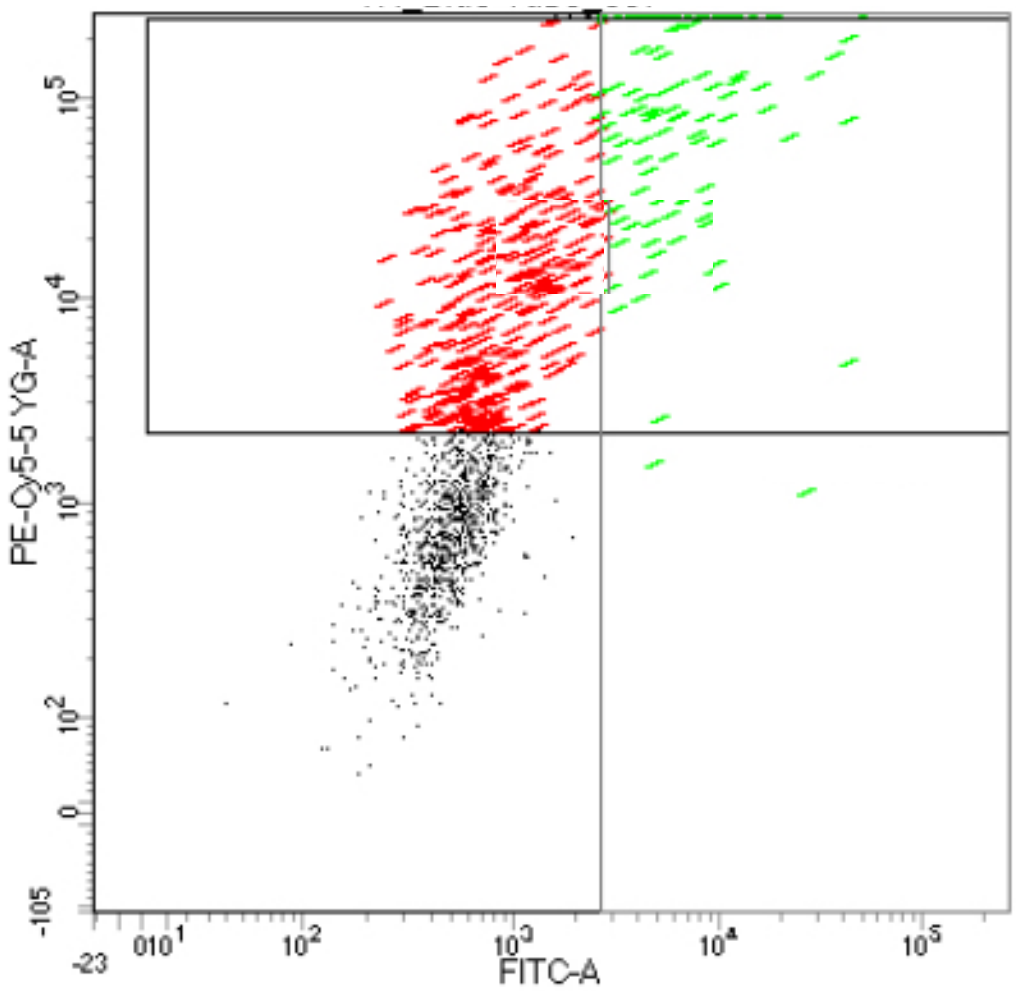
Day 8: Count surviving cells via colony formation assay



Flow cytometer



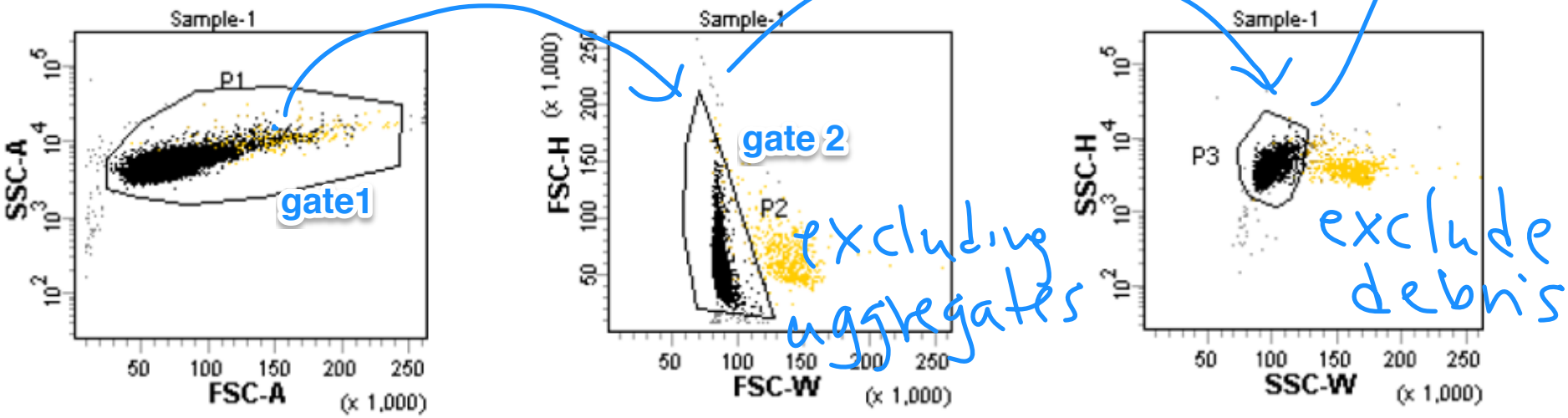
Analysis of Flow Cytometry Data: There are a lot of steps before you get this plot!



Step 1: Determine the relevant cell population

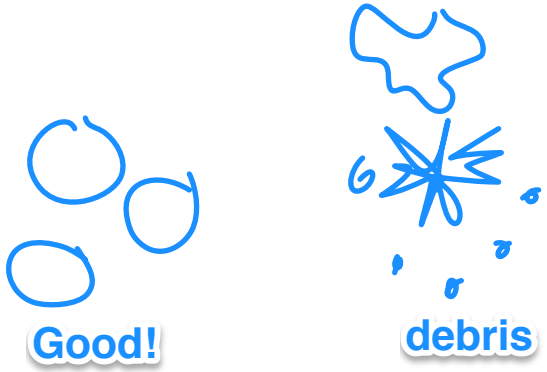
MOCK transfected MO59K (no DNA=no fluorescent molecules)

relevant



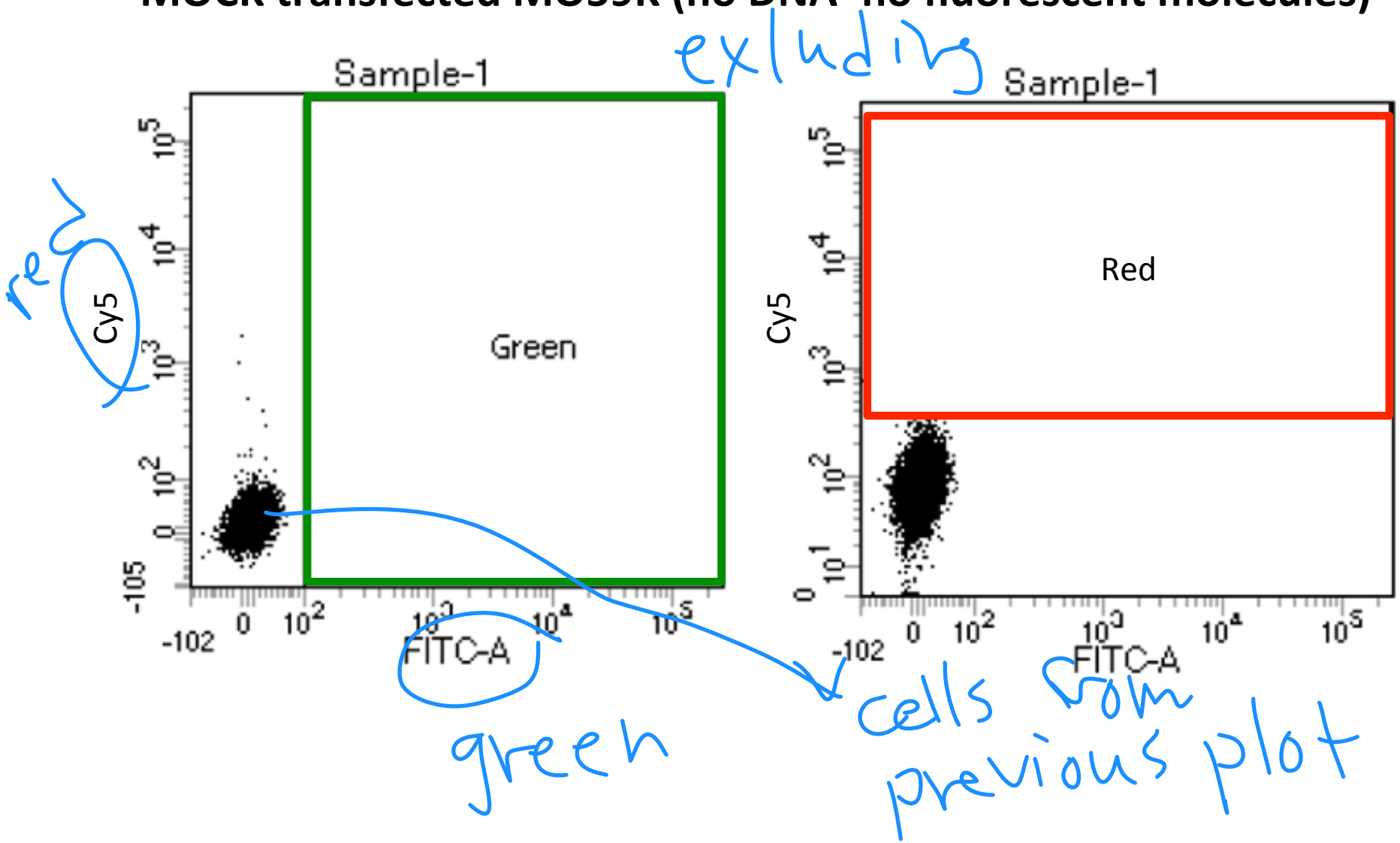
SSC, Side Scatter: Shape

FSC, Forward Scatter: Size



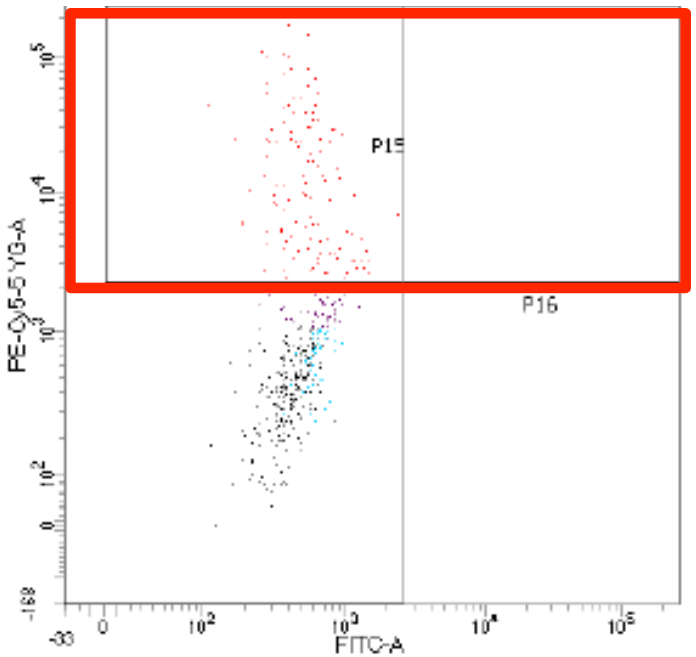
Step 2: Set negative gates

MOCK transfected MO59K (no DNA=no fluorescent molecules)



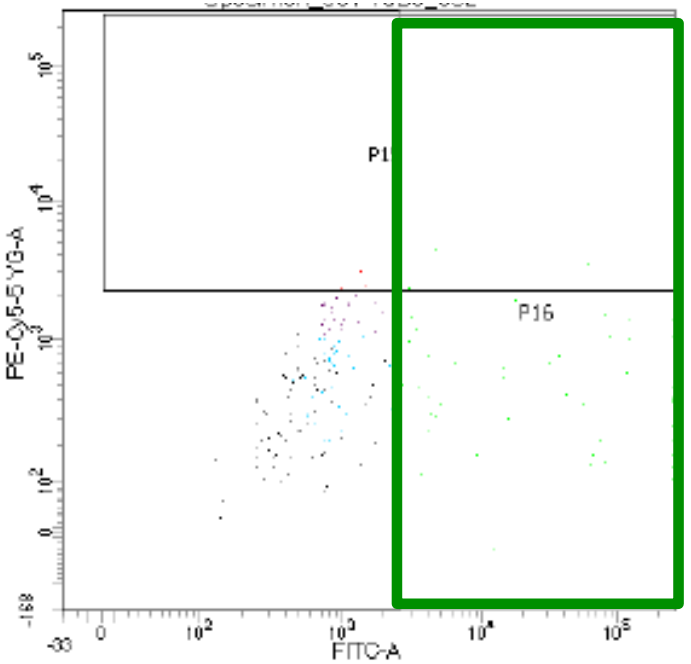
Step 3: Set positive gates

M059K transfected with pMAX-mCherry only



including red cells; refine gate

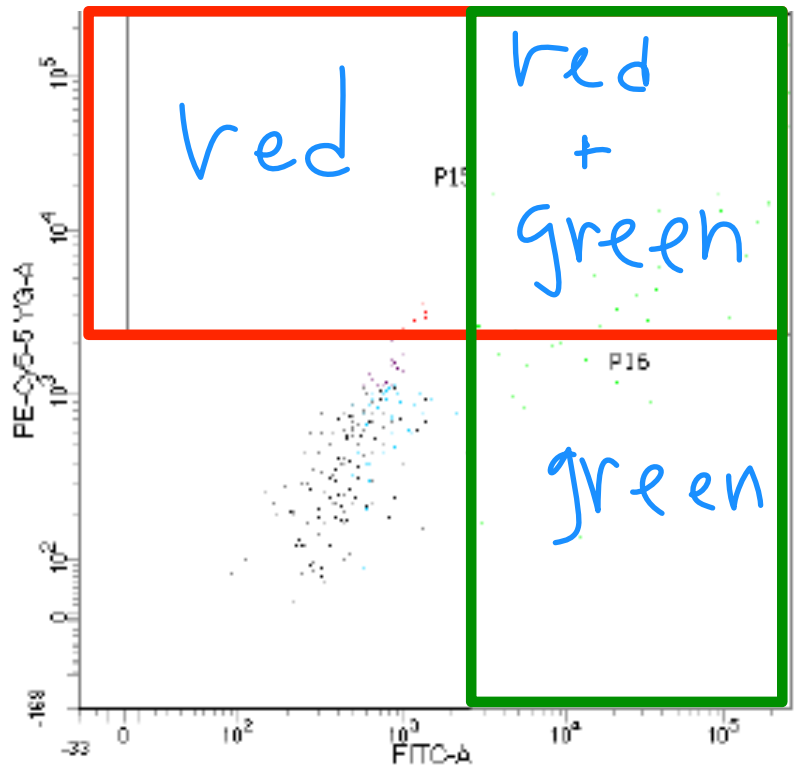
M059K transfected with pMAX-EGFP only



including green refine gate

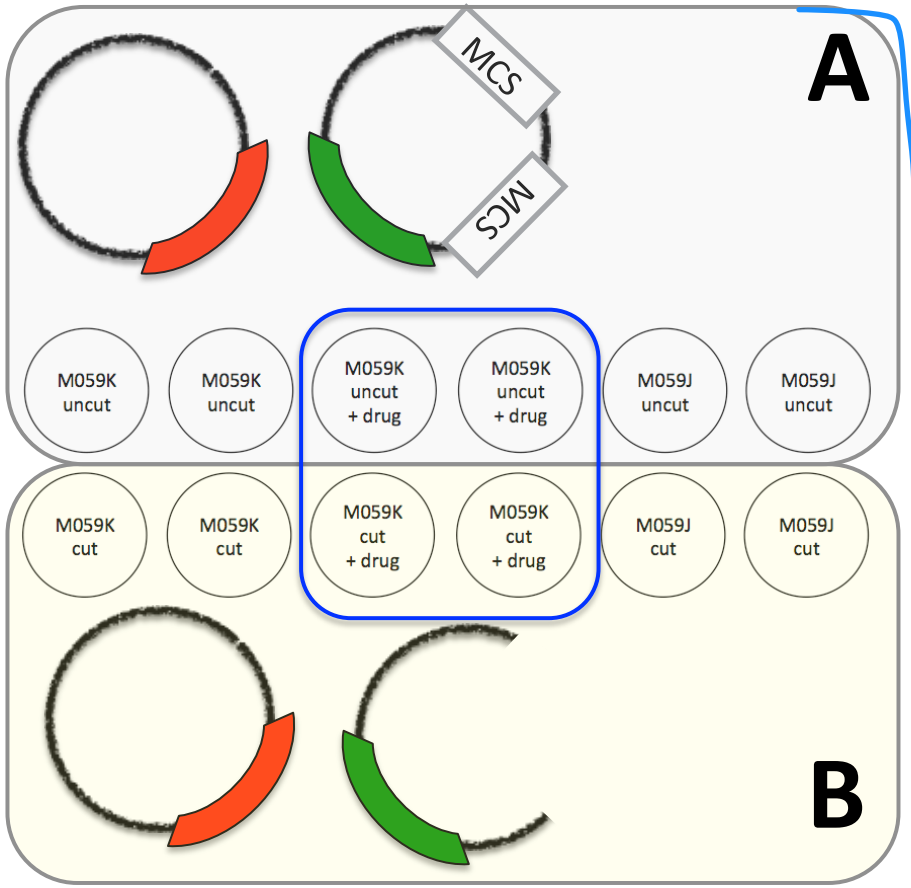
Step 4: Quantify experimental conditions

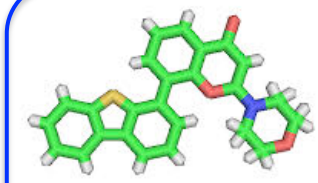
M059K transfected with pMAX-mCherry and pMAX-EGFP



% cells red + green

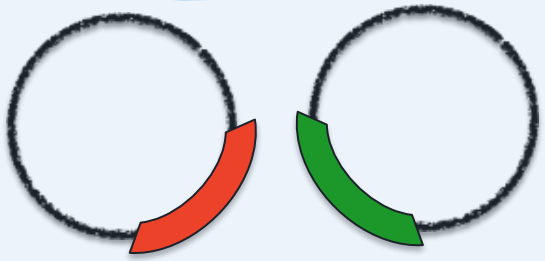
What experiments did we carry out?





NHEJ inhibitor

Additional experiment by instructors: Maximum Repair



if all "13" was repaired by NHEJ

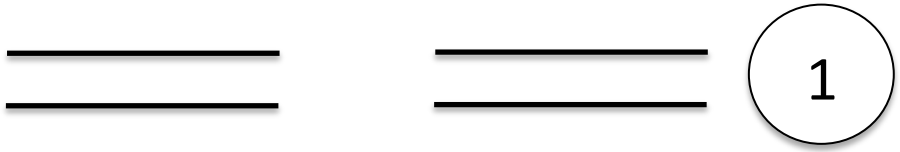
vary depending on experiment

What questions can we ask with our data?

Hypothesis for NHEJ Repair capacity:

From class discussion 3/10

blunt ends



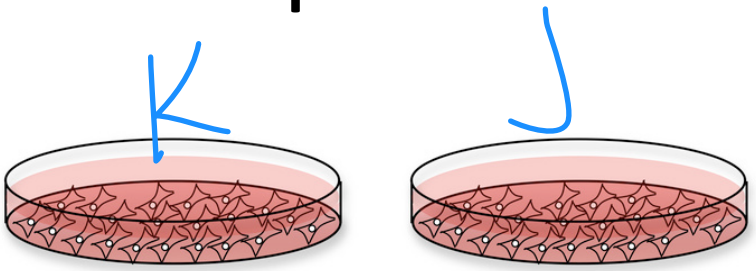
compatible overhangs

incompatible overhangs

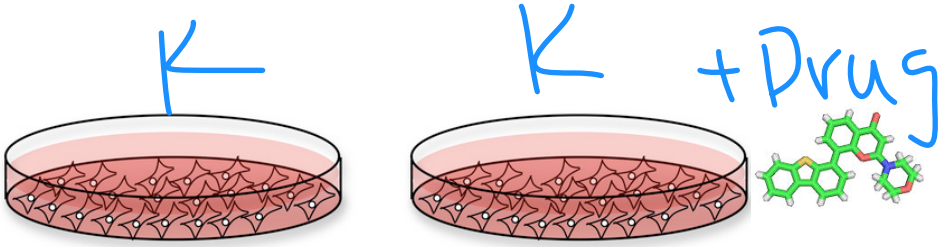
From class discussion 3/29,30

Team Color	DNA damage type	NHEJ inhibitor
T/R Red	compatible overhangs	DMNB
T/R Orange	blunt	DNMB
T/R Yellow	incompatible overhangs	DNMB
T/R Green	blunt	Loperamide
T/R Blue	compatible overhangs	Loperamide
T/R Pink	compatible overhangs	DMNB
T/R Purple	blunt	DNMB
W/F Red	incompatible overhangs	loperamide
W/F Orange	compatible overhangs	loperamide
W/F Blue	blunt	loperamide
W/F Pink	incompatible overhangs	loperamide
W/F Purple	incompatible overhangs	DMNB

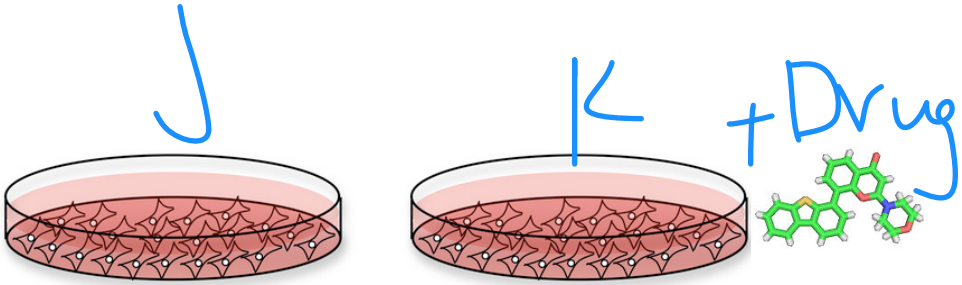
What questions can we ask with our data?



Does loss of DNApk change NHEJ in our assay?



Does drug affect NHEJ 1.0A.?



Is there difference between loss of DNApk and drug inhibition of NHEJ 1.0A?

Craft your story carefully!

Big Picture:

- Cancer
- Immunology
- HIV

Data Analysis:

- Statisical Analysis
- How many variable to compare
- all experimental conditions

Today in lab

- Tissue Culture (TC)
  - Make sure instructors check your drug dilutions before entering tissue culture
  - Group order to TC:
    - 1st: Red, Orange Pink
    - 2nd: Yellow, Green, Blue, Purple

- Being reading "A functional Cancer Genomics Screen Identifies a Druggable Synthetic Lethal Interaction between *MSH3* and *PRKDC*" for class discussion next week.

Let us help you

commit a random act of kindness.

The MIT Dept of Biological Engineering will be handing one Tech Cash card, loaded with a $5 value, to every BE / Course 20 student (undergraduate & graduate). All we ask is that you use the card on someone who could use a little break, a little diversion, a little humor, a little kindness.

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Thusday, March 31, 2016
Anytime between 10:30am and 4:30pm
Room 16-267, The Course 20 / BE Academic Office

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