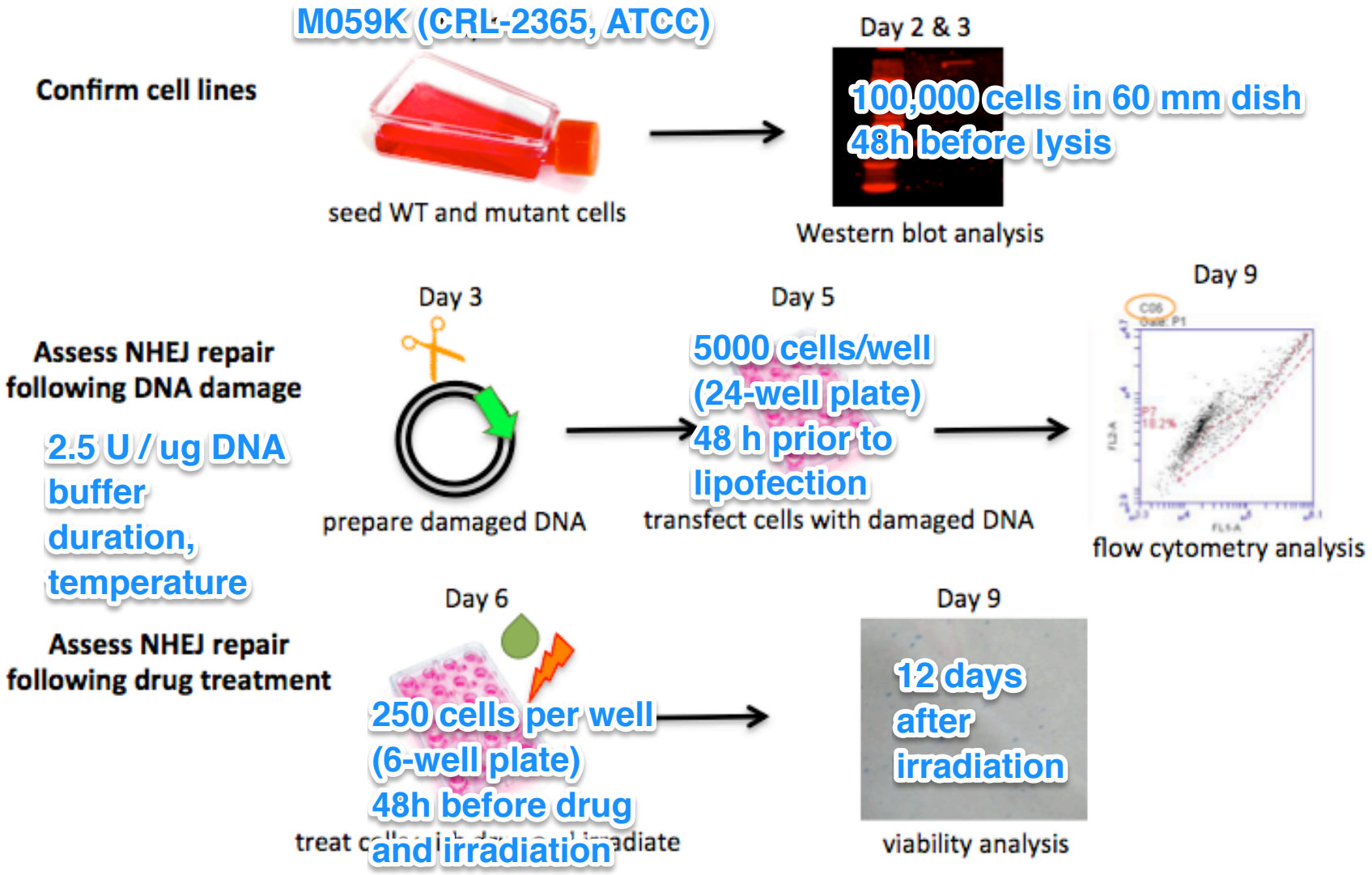
M2D7:
Flow cytometry data analysis

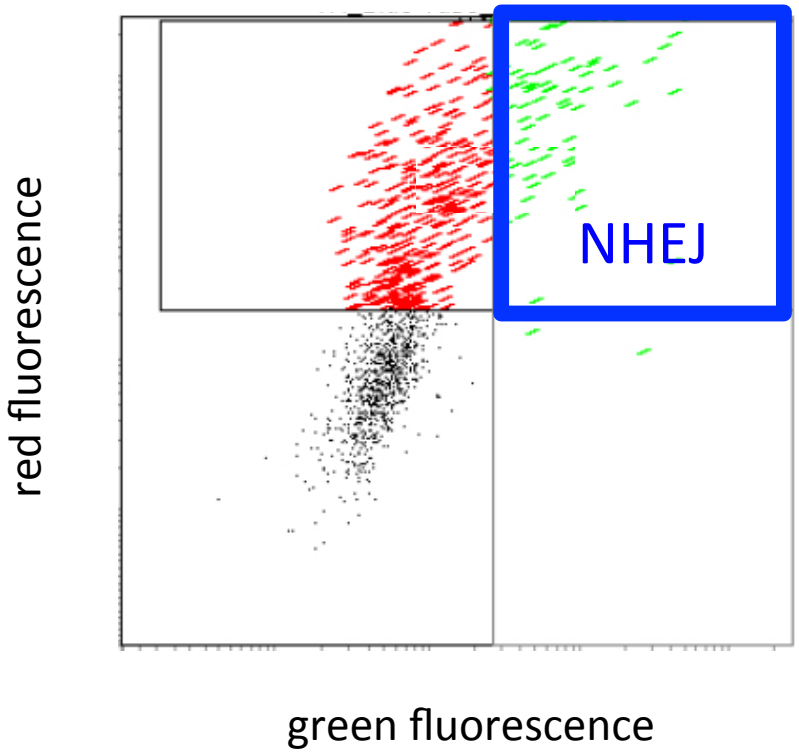
04/06/2016

M2: Experimental overview



Analysis of flow cytometry data

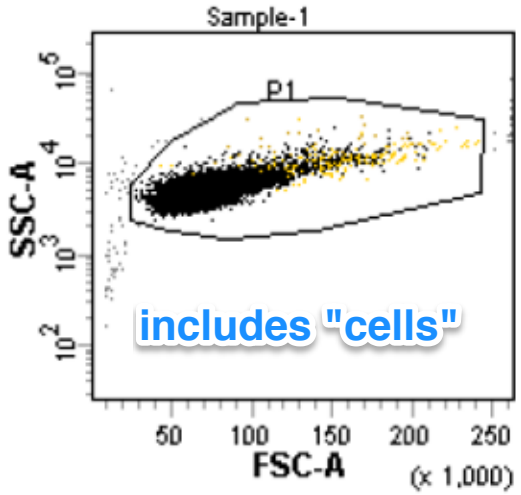
- Show in your M2 research article
  - gating plots (using controls)
  - representative experimental plots

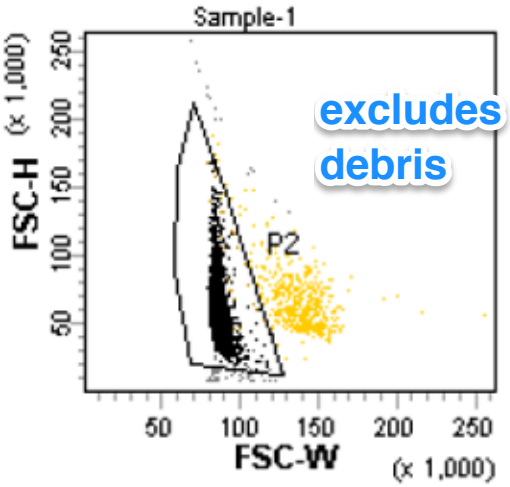


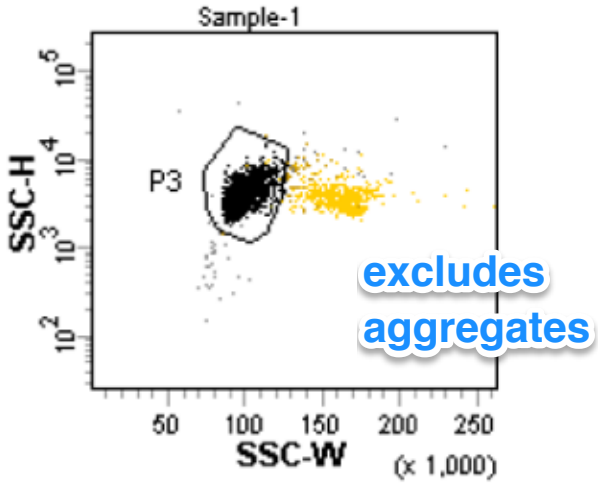
1. Determine the relevant cell population

- Use **M059K wild-type cells, "mock" transfected**

  no DNA plasmid intake, no fluorescence





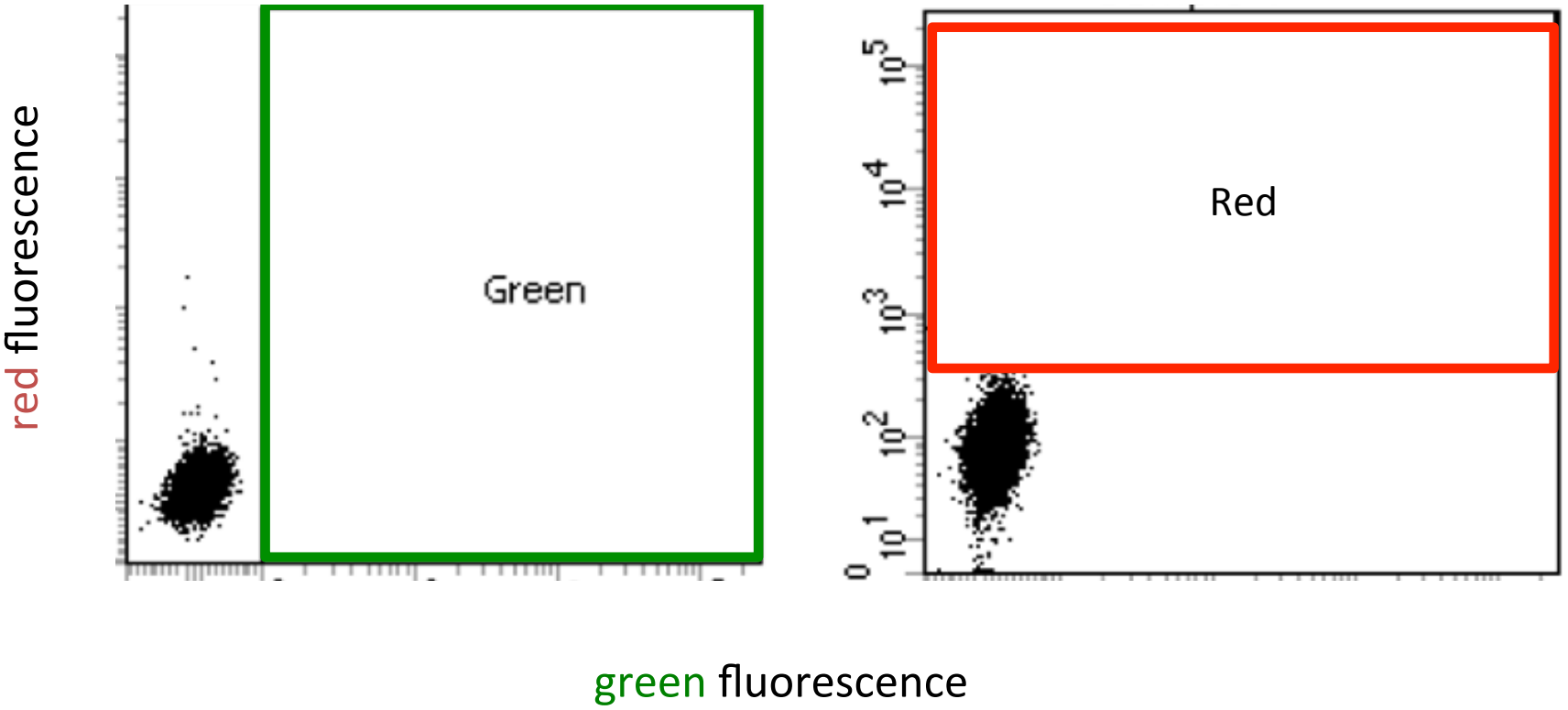


- FSC: **size**
- SSC: **shape**

2. Set negative gates

don't show these

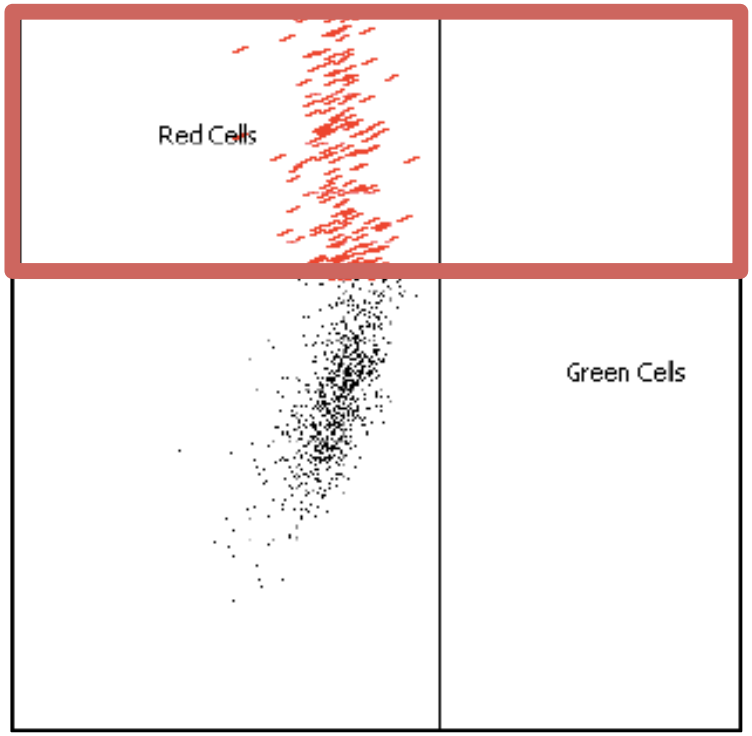
- Use “mock” transfected M059K
  - no plasmid DNA in lipofection = no fluorescence



3. Set positive gates

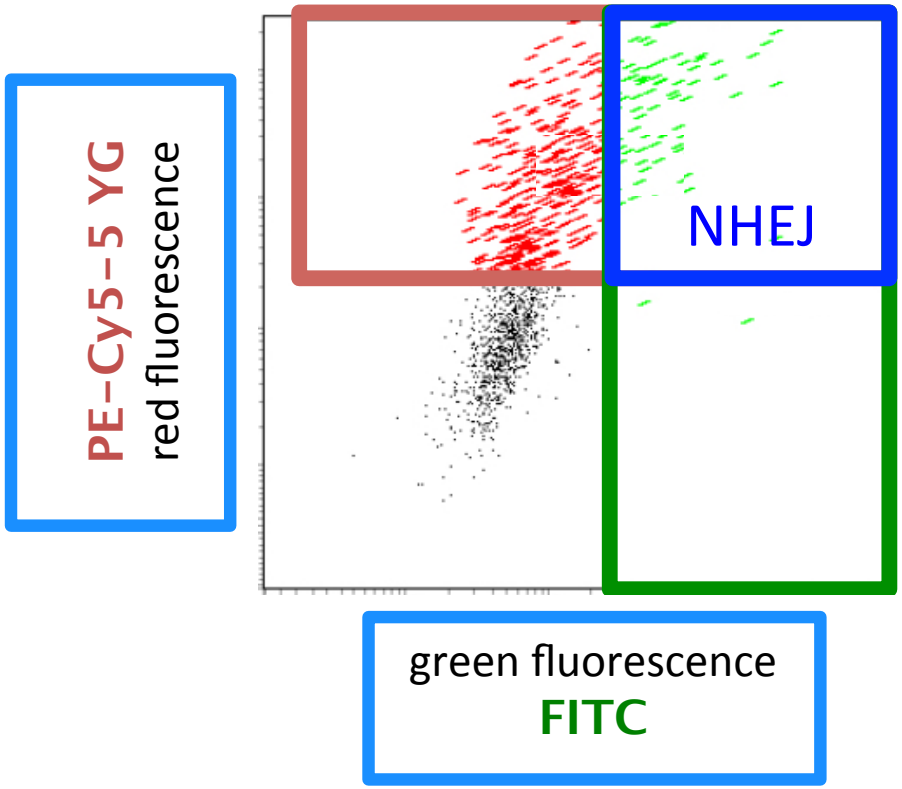
- Use M059K transfected with pMAX_EGFP only

- Use M059K transfected with pMAX_mCherry only

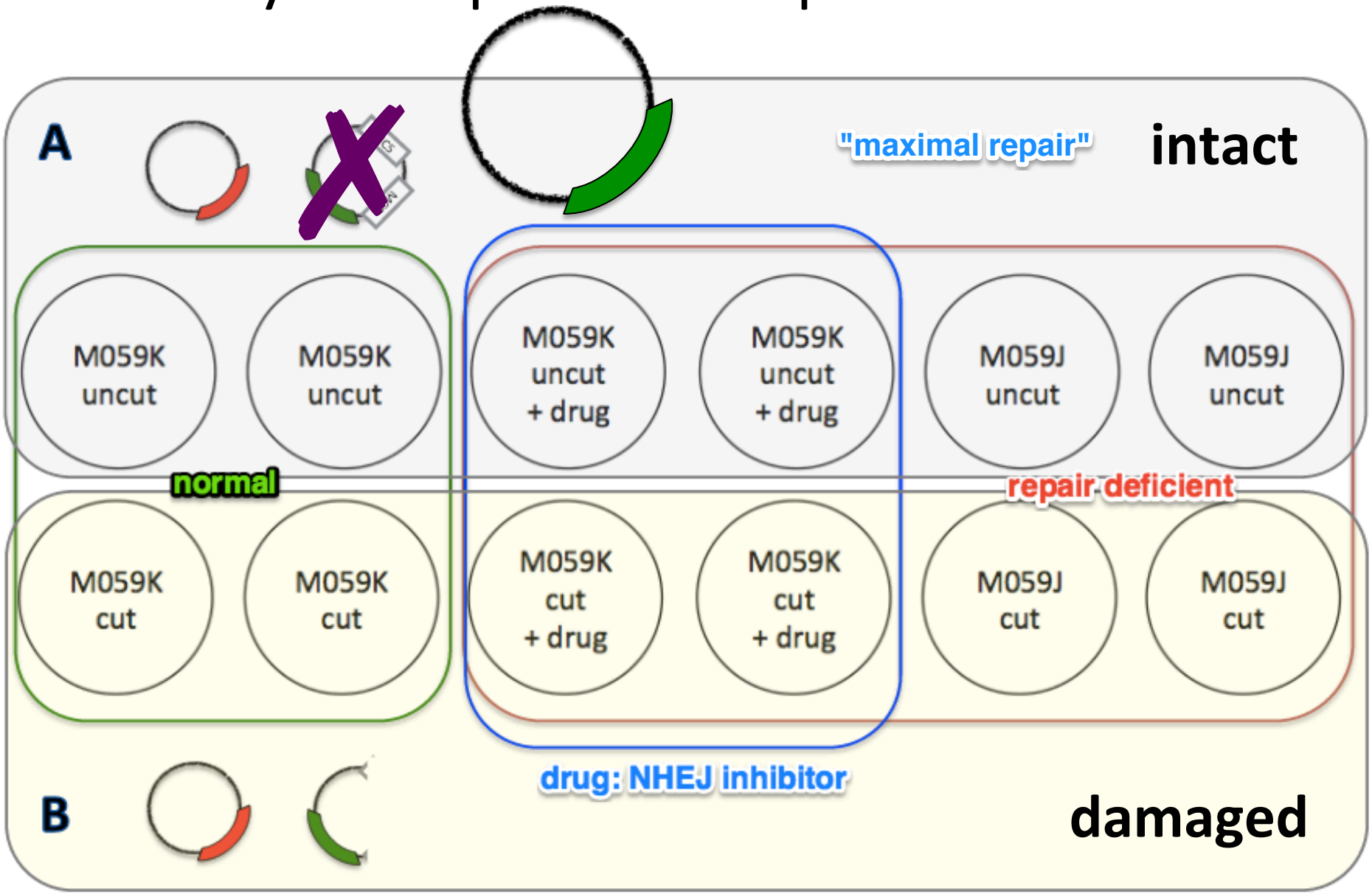


4. Quantify experimental conditions

- M059K with both pMAX_mCherry and pMAX_EGFP
  - "maximum repair"
- Cells with pMAX_mCherry and pMAX_EGFP_MCS repaired!

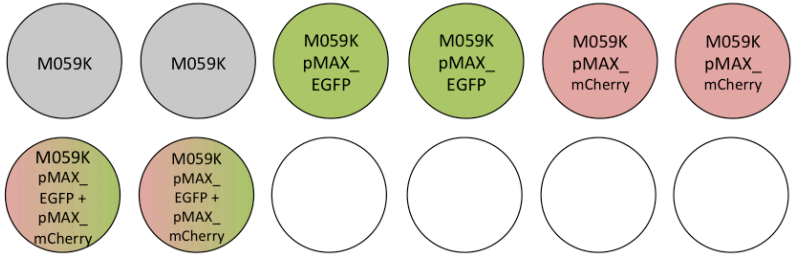


Recall your experimental plate from M2D5

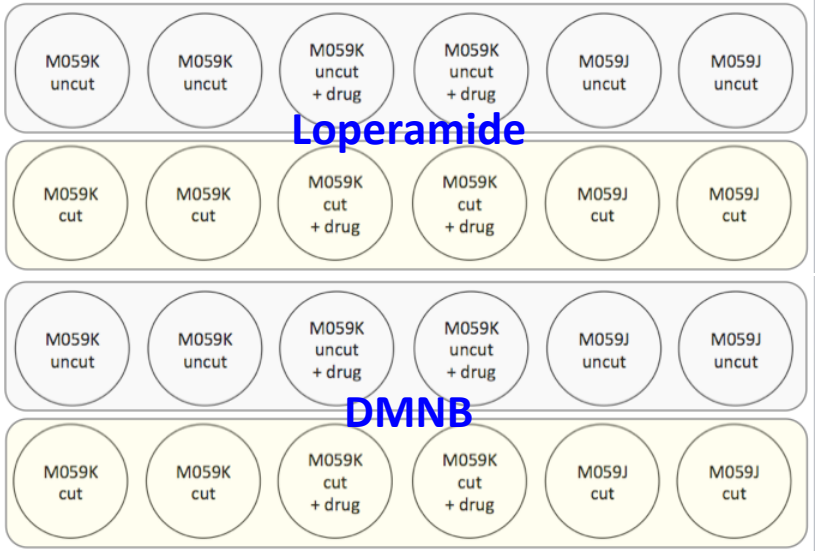


Instructor flow cytometry data

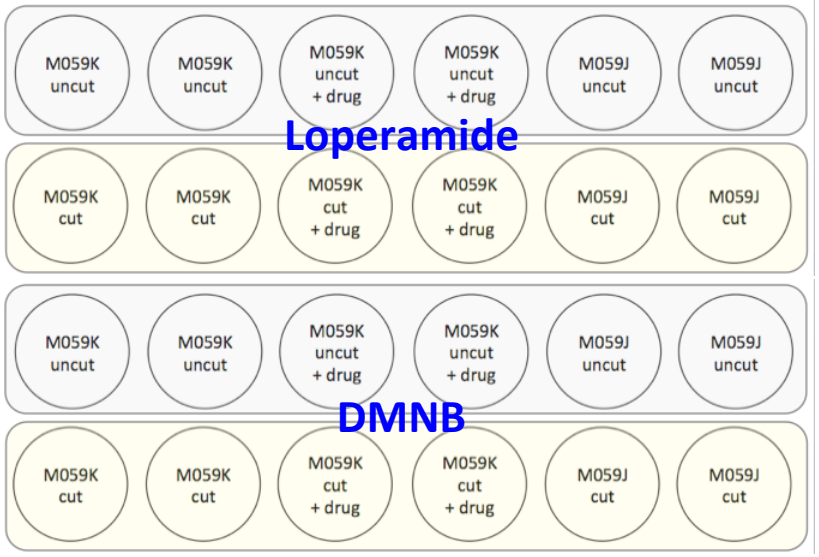
- control



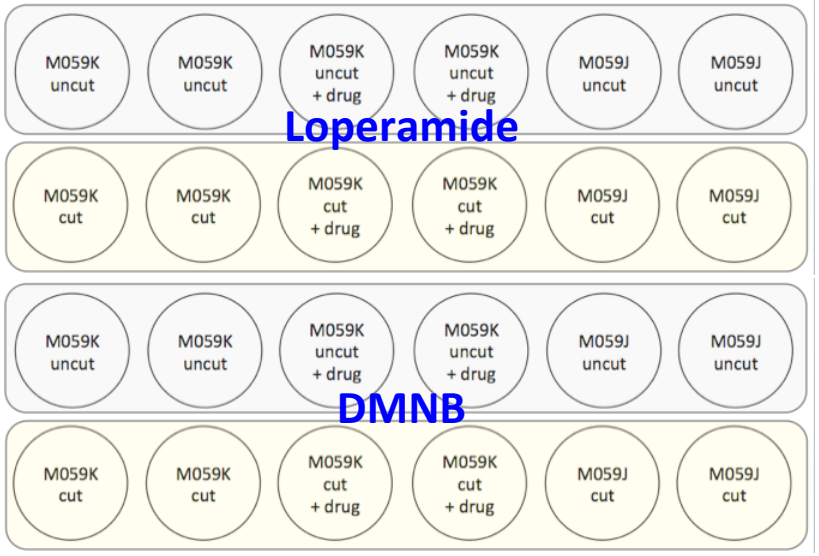
- *PmeI*



- *EcoRI-BglII*

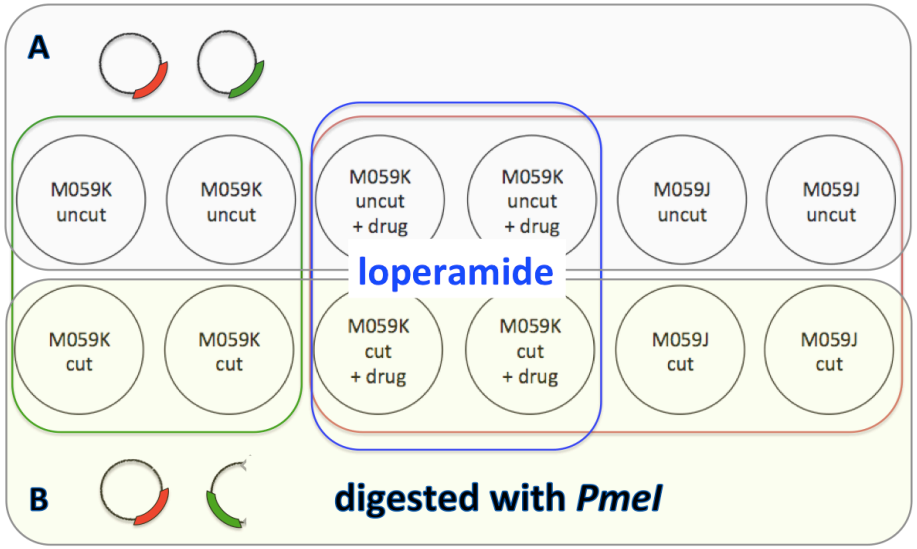


- *PstI-BglII*



Which 12 tubes correspond to *your* experimental conditions?

- Instructor FC sample key.xlsx



Sample #	Sample identity	Group
1	Mock	Control
2	Mock	Control
3	EGFP	Control
4	EGFP	Control
5	RFP	Control
6	RFP	Control
7	EGFP + RFP	Control
8	EGFP + RFP	Control
9	K uncut (A1)	PmeI loperamide
10	K uncut (A2)	PmeI loperamide
11	K uncut w/ drug (A3)	PmeI loperamide
12	K uncut w/ drug (A4)	PmeI loperamide
13	J uncut (A5)	PmeI loperamide
14	J uncut (A6)	PmeI loperamide
15	K cut (B1)	PmeI loperamide
16	K cut (B2)	PmeI loperamide
17	K cut w/ drug (B3)	PmeI loperamide
18	K cut w/ drug (B4)	PmeI loperamide
19	J cut (B5)	PmeI loperamide
20	J cut (B6)	PmeI loperamide
21	K uncut (A1)	PmeI DMNB
22	K uncut (A2)	PmeI DMNB
23	K uncut w/ drug (A3)	PmeI DMNB

NHEJ repair frequency calculations

Population	#Events	%Parent	FITC-A Mean	FITC-A Median	PE-Cy5-5... Mean	PE-Cy5-5... Median
Live Cells	561	90.2	44,041	2,321	19,774	1,744
Red Cells	136	24.2	161,162	173,621	76,612	33,952
Green Cells	225	40.1	107,665	55,861	47,414	9,680

1. raw signal

   RAW_{EGFP} = (% green cells) * (median green fluorescence intensity)

2. normalized EGFP expression

   $NORM = RAW_{EGFP} / RAW_{mCherry}$

3. NHEJ repair value

   $NHEJ = NORM_{EGFP.damaged} / NORM_{EGFP.intact} = NORM_B / NORM_A$

   x100 for percentage (%)

Crunch numbers!

In Instructor FC spreadsheet.xlsx

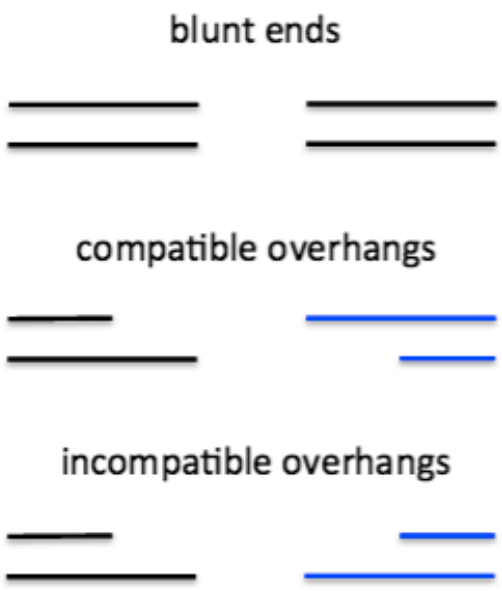
- Identify relevant columns
- Relabel headers
- Calculate % NHEJ
  - 3 x 2 per team: duplicates of K, K+drug, and J

D	P	Q	R	S	T	X	Z	AB	AE	AF	AG	AH	AI
Tube Name					Red Cells %F	Red Cells PE-C	Green Cells %F	Green Cells FITC-A Median					
Tube Name	**Cell line**	**Drug**	**Enzymes**	**DNA**	**% pos. red**	**median red**	**% pos. green**	**median green**	**% pos red * MFI**	**% pos green * MFI**	**norm. G/R**	**NHEJ**	**%NHEJ**
Tube_007	K			GFP-mCherry	24	33952	40	55861	821638.4	2240026.1	2.73		
Tube_008	K			GFP-mCherry	25	43871	43	57852	1088000.8	2476065.6	2.28		
Tube_009	K	---	---	GFP-mCherry	22	27058	37	48129	581747.0	1761521.4	3.03		
Tube_010	K	---	---	GFP-mCherry	26	35728	41	55536	932500.8	2271422.4	2.44		
Tube_011	K	loper.	---	GFP-mCherry	29	48576	45	90575	1408704.0	4057760.0	2.88		
Tube_012	K	loper.	---	GFP-mCherry	26	37318	40	74934	977731.6	2982373.2	3.05		
Tube_013	J	---	---	GFP-mCherry	9	24469	19	22513	207986.5	429998.3	2.07		
Tube_014	J	---	---	GFP-mCherry	15	35077	21	41955	512124.2	897837.0	1.75		
Tube_015	K	---	PmeI	GFPcut-mCherry	35	32542	12	24115	1148732.6	291791.5	0.25	0.084	8.4
Tube_016	K	---	PmeI	GFPcut-mCherry	30	36955	14	15578	1119736.5	213418.6	0.19	0.078	7.8
Tube_017	K	loper.	PmeI	GFPcut-mCherry	34	52908	13	16680	1793581.2	223512.0	0.12	0.043	4.3
Tube_018	K	loper.	PmeI	GFPcut-mCherry	33	31003	13	12564	1019998.7	167101.2	0.16	0.054	5.4
Tube_019	J	---	PmeI	GFPcut-mCherry	12	14575	1	11928	170527.5	8349.6	0.05	0.024	2.4
Tube_020	J	---	PmeI	GFPcut-mCherry	18	11962	0	---	215316.0	#VALUE!	#VALUE!	0.000	0.0

What questions can we ask with our data?

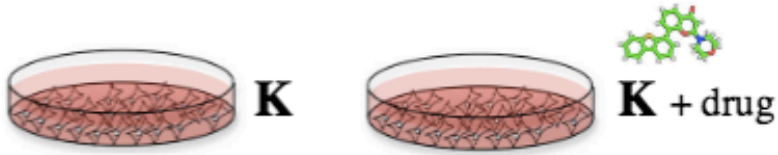
Examples:

- How efficient is NHEJ at repairing different types of double-stranded breaks (DSBs)?

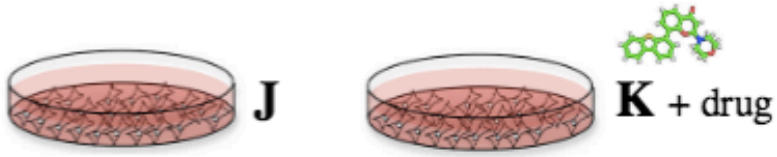


- **Does loss of DNA-PKcs affect NHEJ efficiency?**

- **To what extent does this drug affect NHEJ?**



- **Is there a difference between lack of DNA-PK and drug inhibition?**



etc... Ask your own questions.