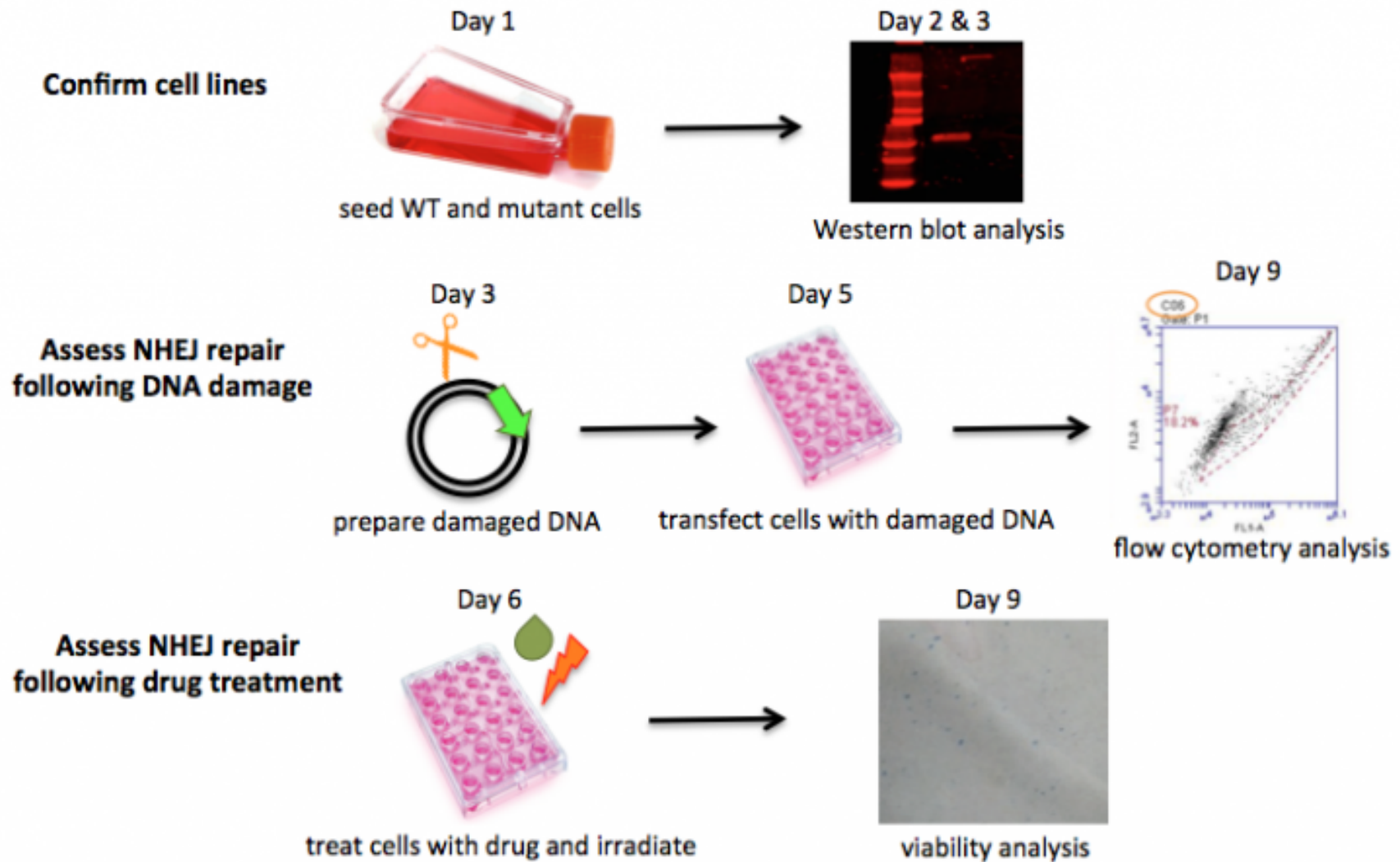


M2D7: Flow cytometry Data Analysis

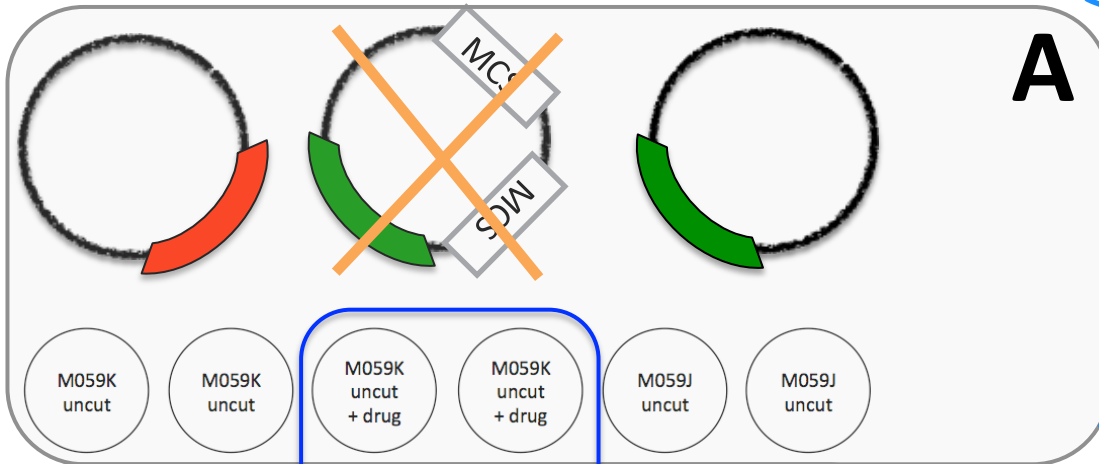
04/05/16

1. Pre-lab discussion
2. Data Analysis!
3. (Paper Discussion moved to Tues. April 12th)

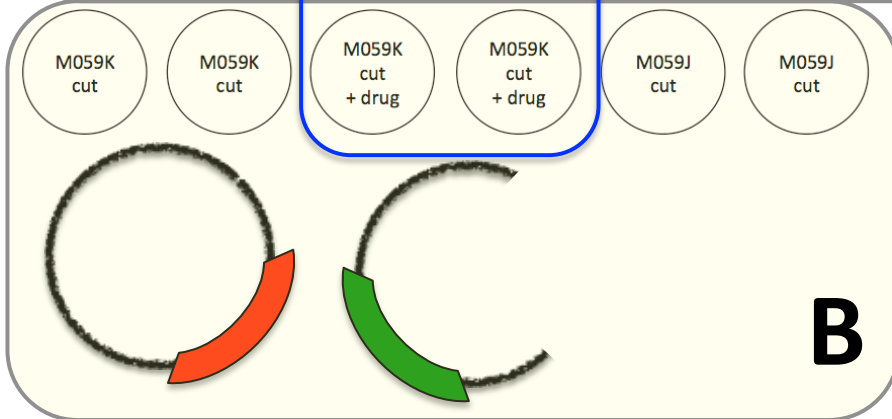
Mod 2 experimental overview



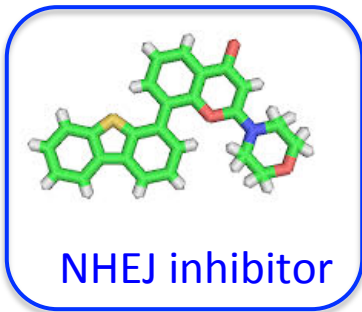
What experiments did we carry out?



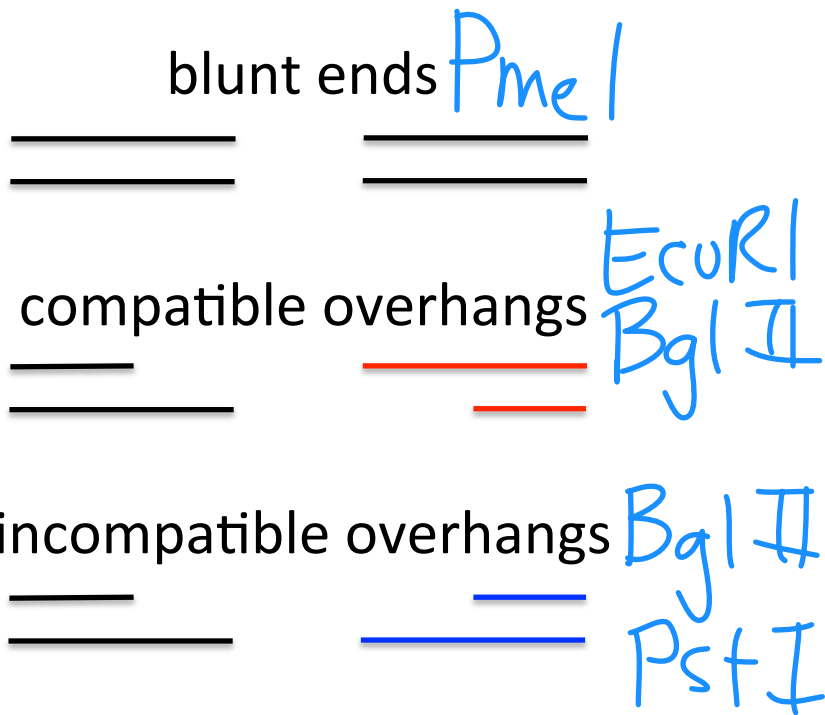
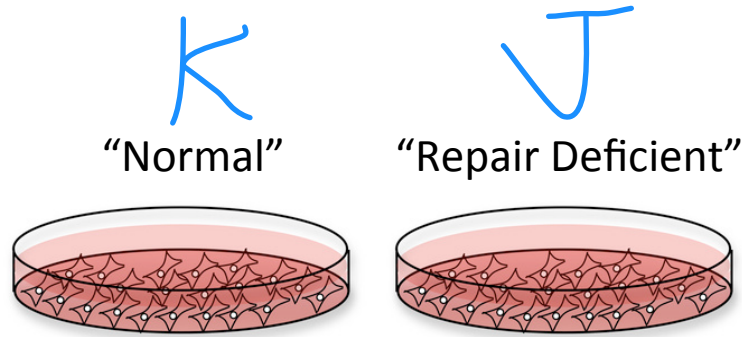
Maximum repair
"intact"



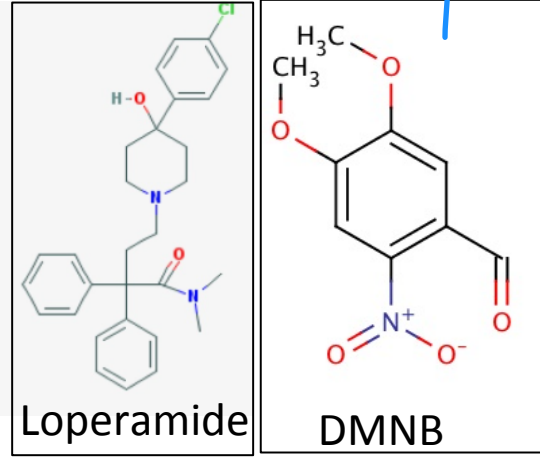
vary depending
on experiment
"damaged"



Experimental Variables



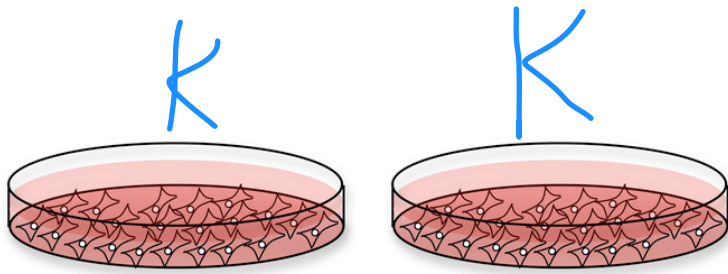
Target.
?
DNApkcs



What questions can we ask with our data?

From Prof. Samson Lecture 1:

How efficiently does DNA repair via NHEJ act on DNA damage with different damage types?



blunt ends



compatible overhangs

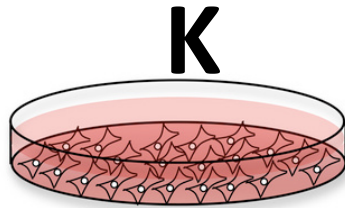
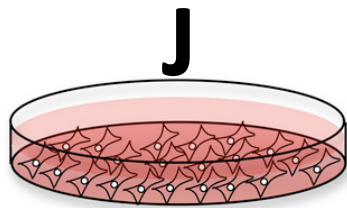


incompatible overhangs

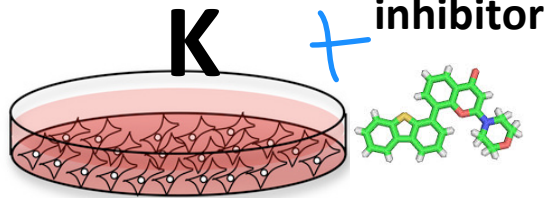
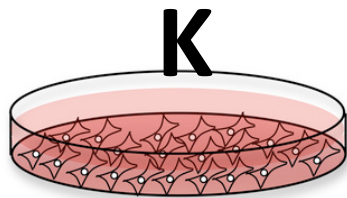


- need whole data set
- statistical analysis
Req for report!

What questions can we ask with our data?

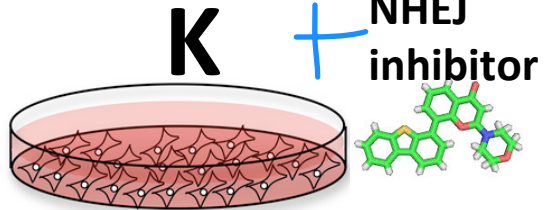
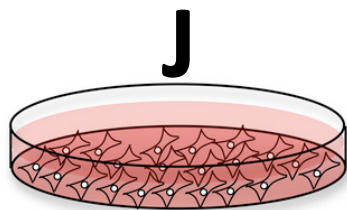


How does absence of DNA-pk influence NHEJ in our assay?



NHEJ inhibitor

Does pharmacological inhibition of NHEJ machinery in M059J/K cell lines reduce NHEJ in our assay?

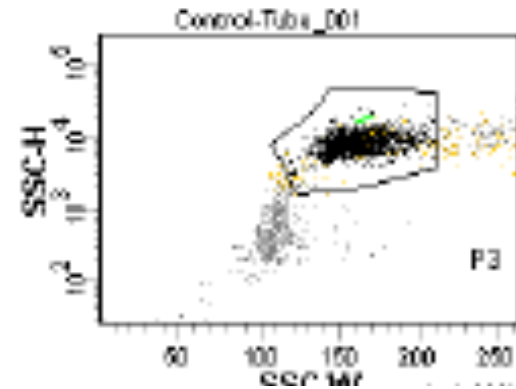
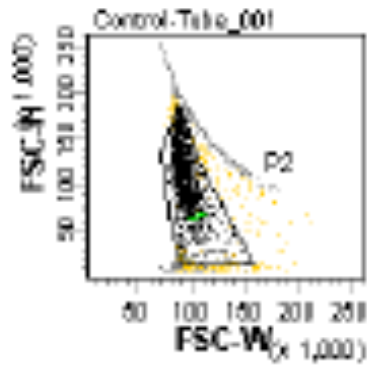
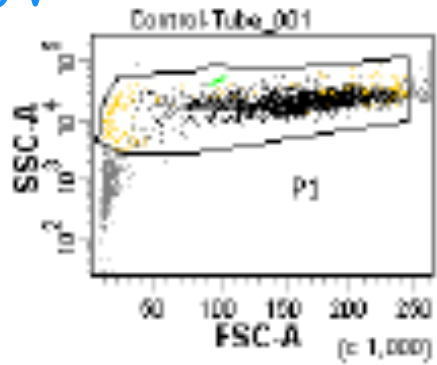


NHEJ inhibitor

Is there a difference between no DNAPk expression and inhibition of NHEJ in our assay?

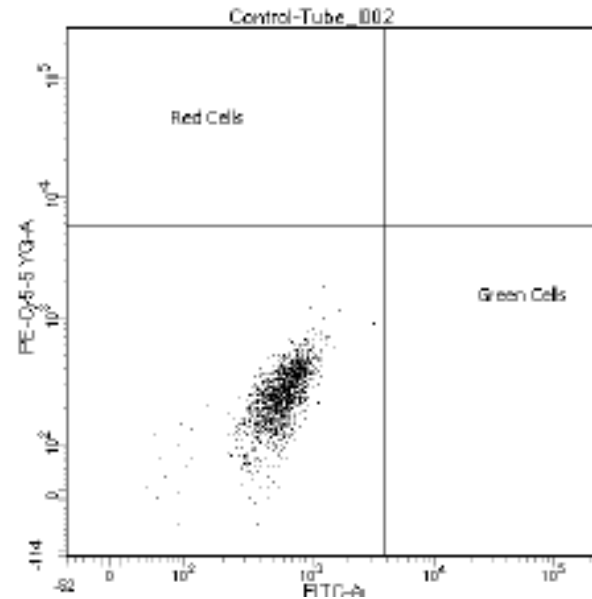
Mock = transfected
Our data:

No pNA

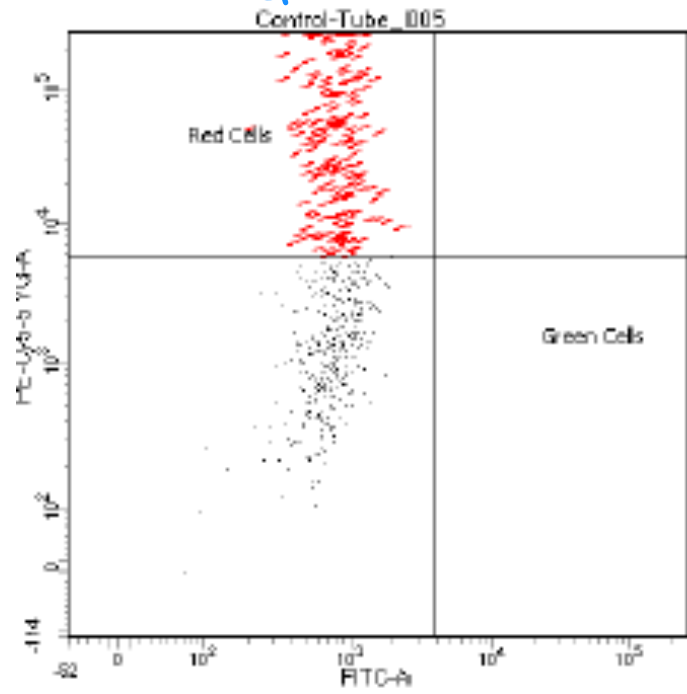


SSC
FSC

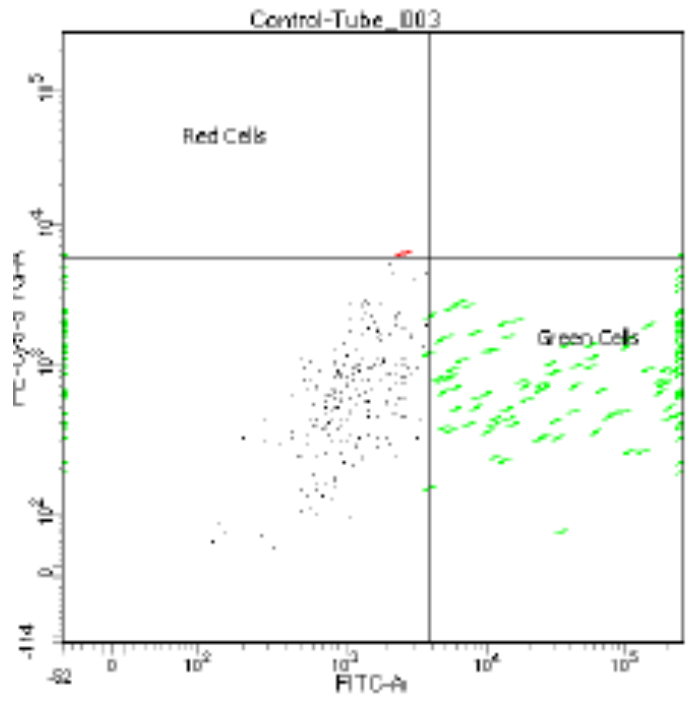
relevant: healthy
Single cells



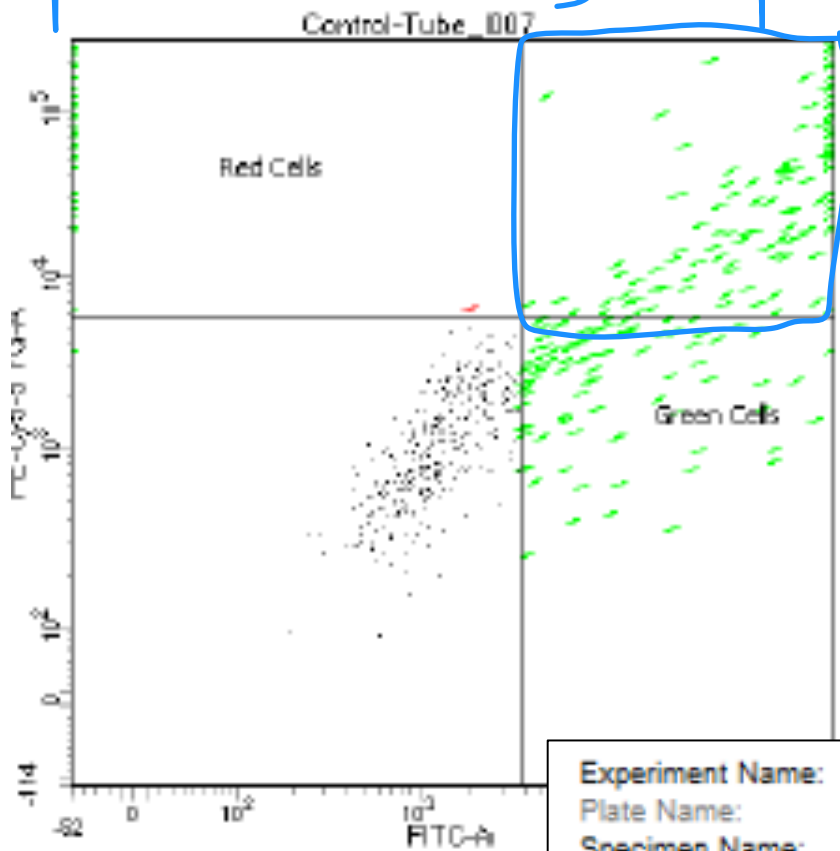
pMAX-mcherry
alone



pMAX-EGFP
alone



pMAX mCherry + pMAX-EGFP



Red + green

Experiment Name:	20_109_Samples_4-4-16					
Plate Name:						
Specimen Name:	Control					
Tube Name:	Tube_007					
Record Date:	Apr 4, 2016 5:14:48 PM					
SOP:	IsaacChaim					
GUID:	a8c801b7-4609-472e-86fc-d84581ed597f					
Population	#Events	%Parent	FITC-A Mean	FITC-A Median	PE-Cy5-5... Mean	PE-Cy5-5... Median
Live Cells	581	90.2	44,041	2,321	19,774	1,744
Red Cells	136	24.2	161,162	173,621	78,612	33,952
Green Cells	225	40.1	107,665	55,861	47,414	9,680

How to calculate % NHEJ:

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	138	24.2	161,162	173,621	76,612	33,952
■ Green Cells	225	40.1	107,665	55,861	47,414	9,680

- 1) Calculate RAW data: $(\% \text{ GFP})(\text{Mean F.I.}) = \text{RAW}_{\text{egfp}}$
- 2) Calculate the Normalized (NORM) data:
 $(\text{RAW}_{\text{egfp}} / \text{RAW}_{\text{mCherry}}) = \text{NORM}$
- 3) Calculate NHEJ Repair value:
 $(\text{NORM}_{\text{egfp.damaged}} / \text{NORM}_{\text{egfp.intact}})$