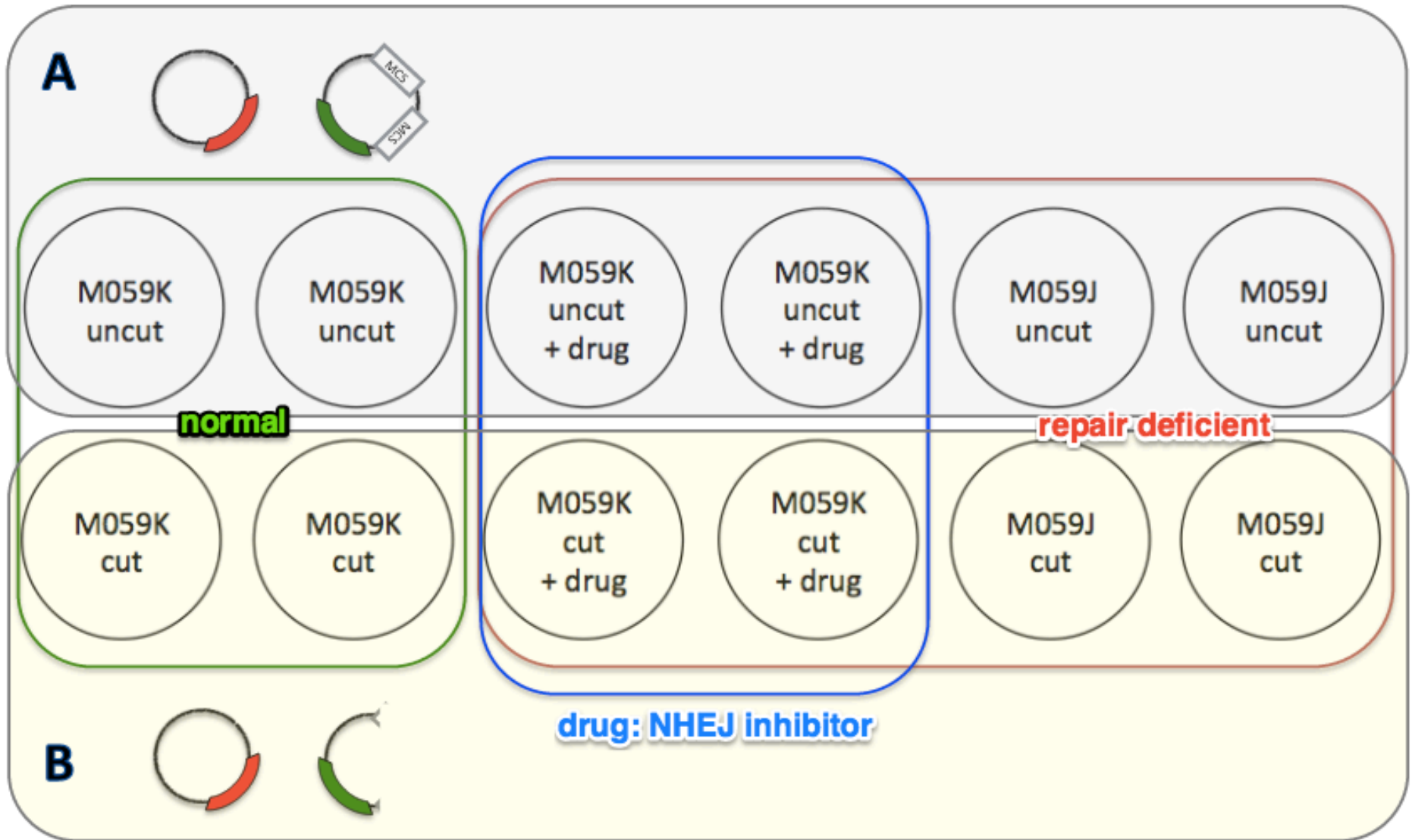


# M2D6: DNA repair assays

04/01/2016

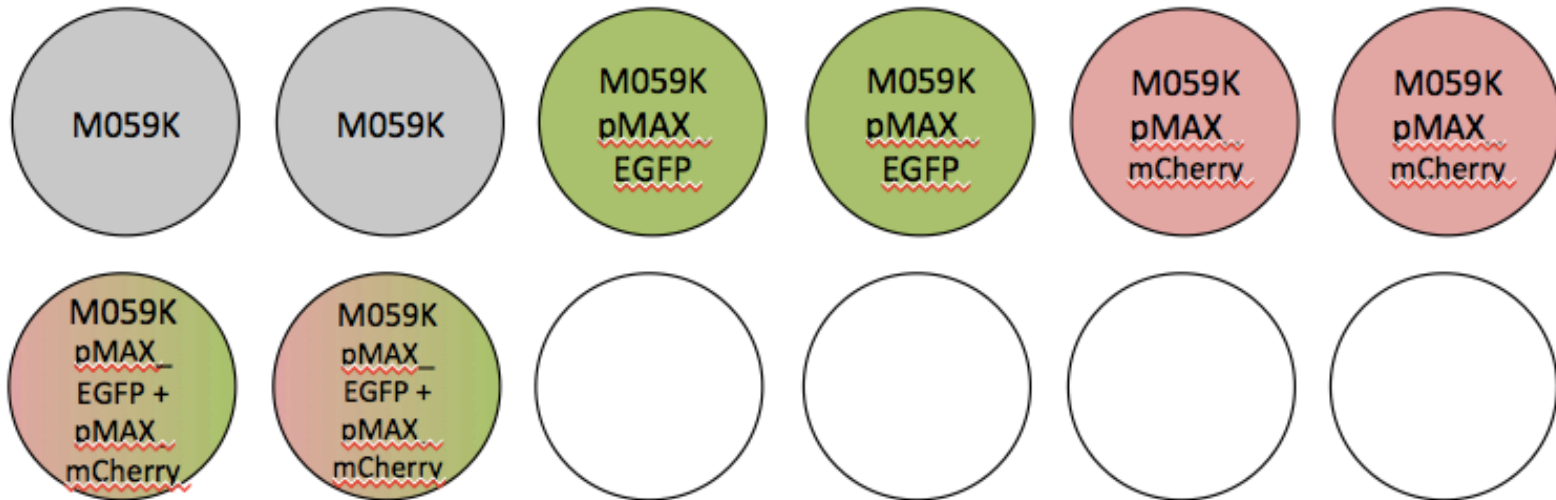


# Recall your experimental plate from M2D5

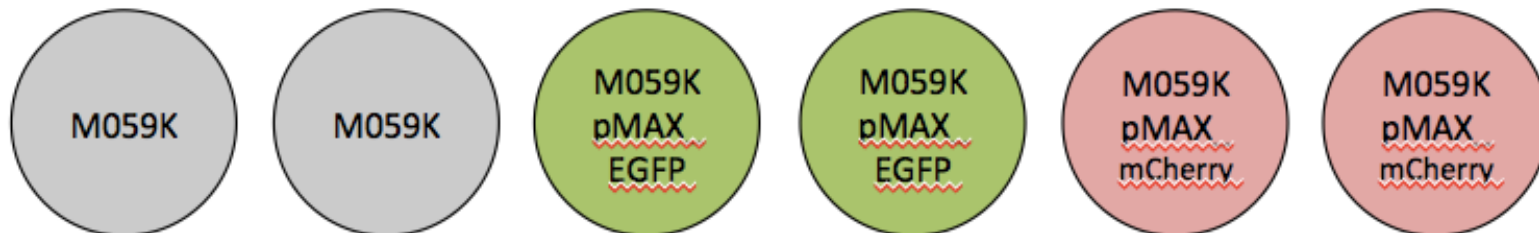


# Additional controls

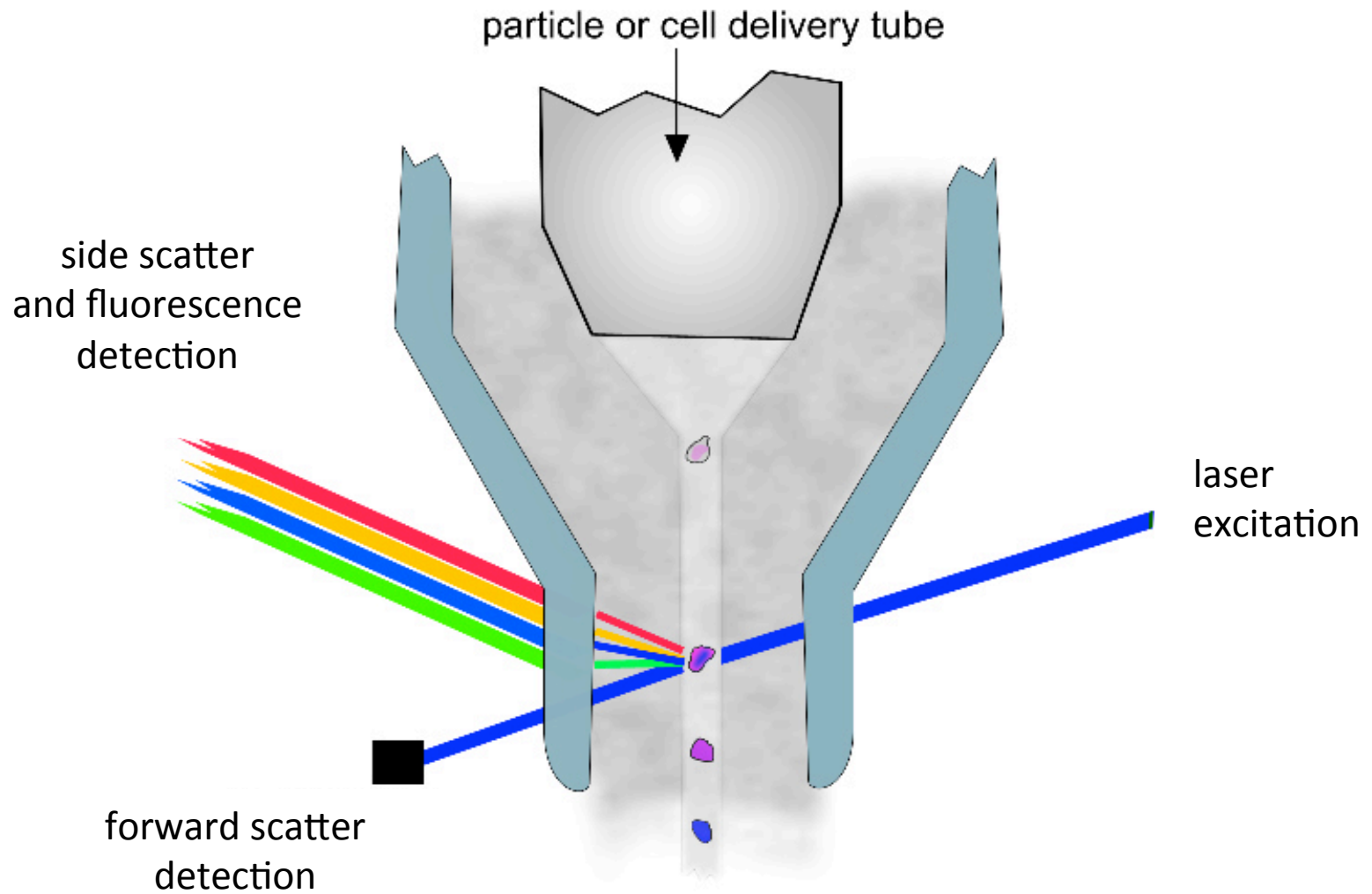
- by instructors:



- by you today for demo:

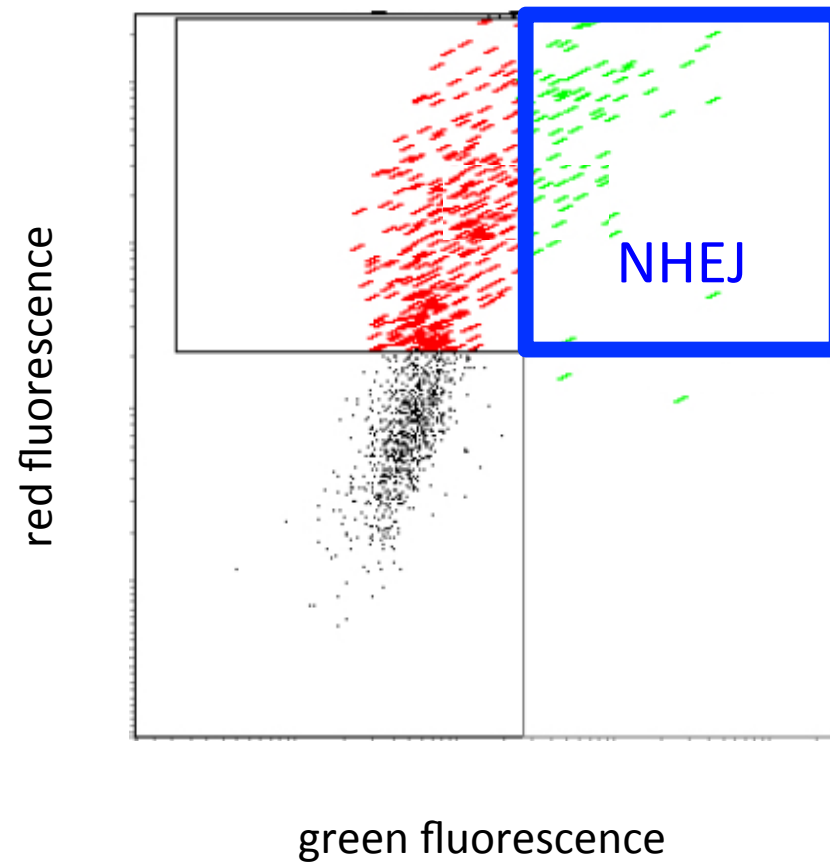


# Flow cytometry



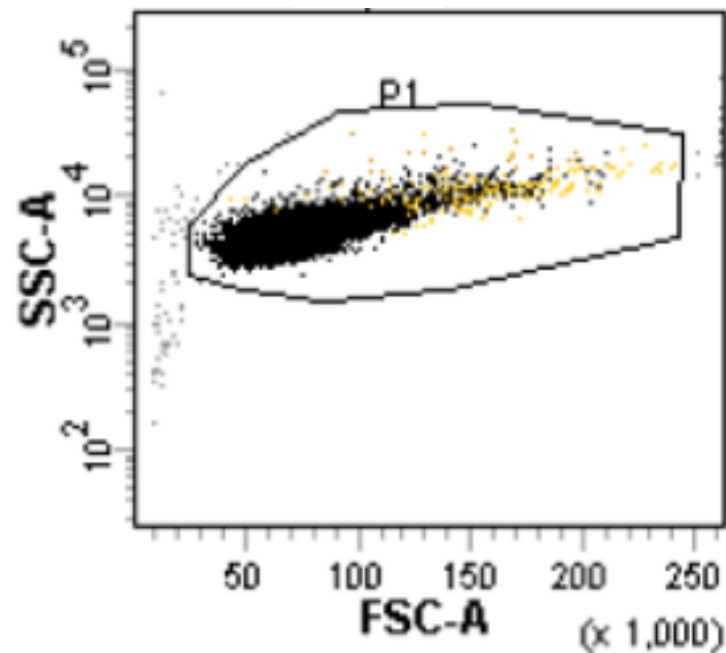
# Analysis of flow cytometry data

- Several “gating” steps precede NHEJ repair capacity calculations



# 1. Determine the relevant cell population

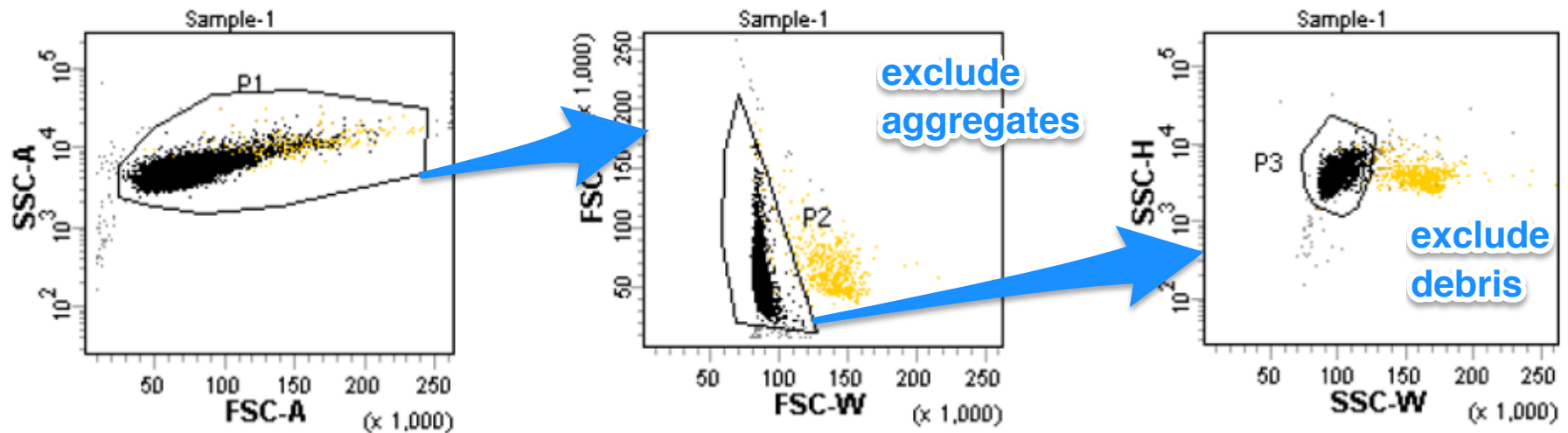
- Use “mock” transfected M059K
  - no plasmid DNA in lipofection = no fluorescence



- FSC, forward scatter: [size](#)
- SSC, side scatter: [shape](#)

# 1. Determine the relevant cell population

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

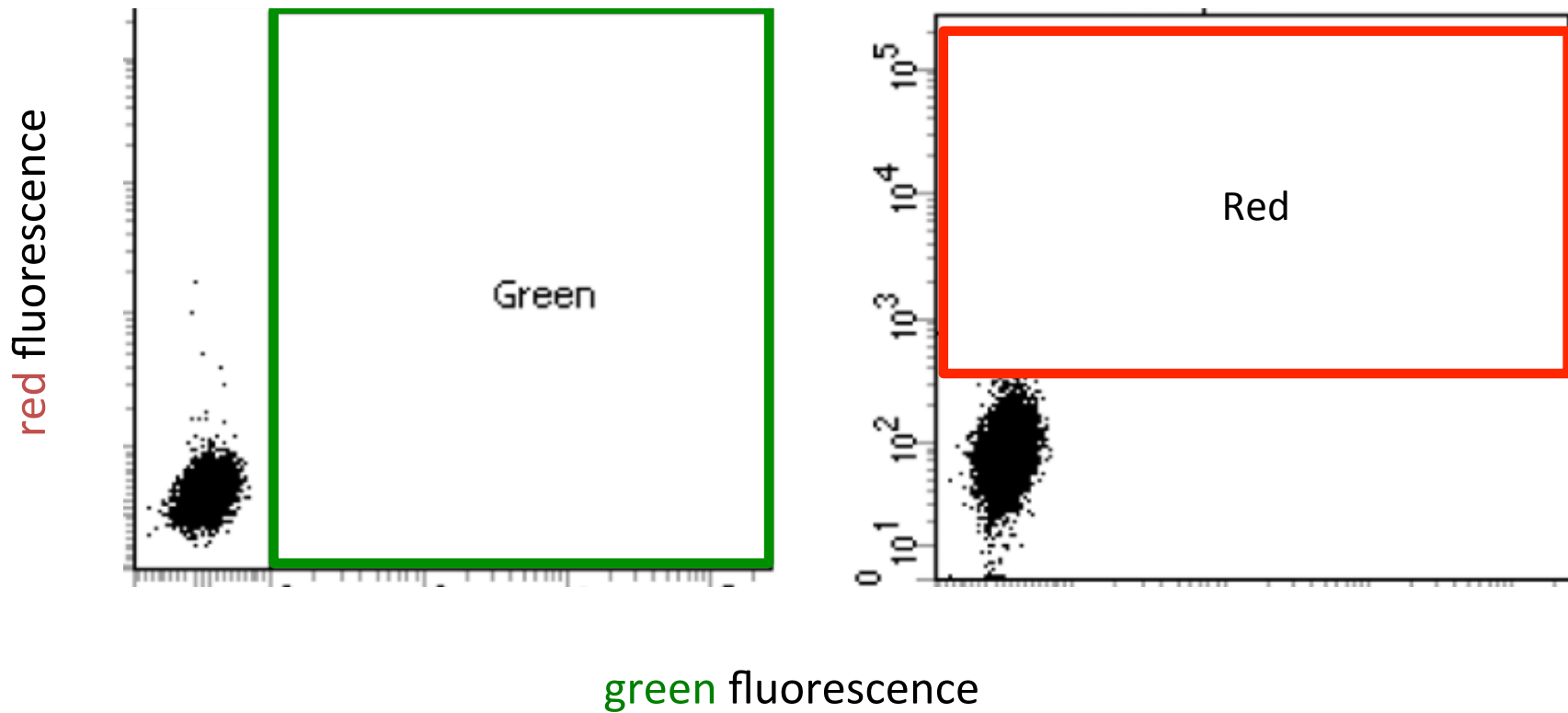


- FSC-H, forward scatter height (amplitude)
- FSC-W, forward scatter width (duration)

## 2. Set negative gates

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

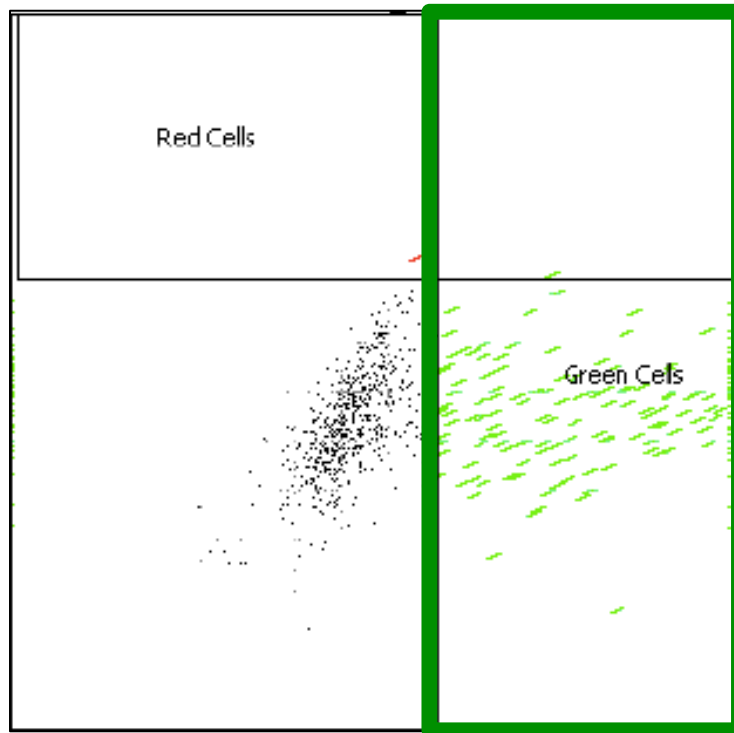
excluding non fluorescent cells



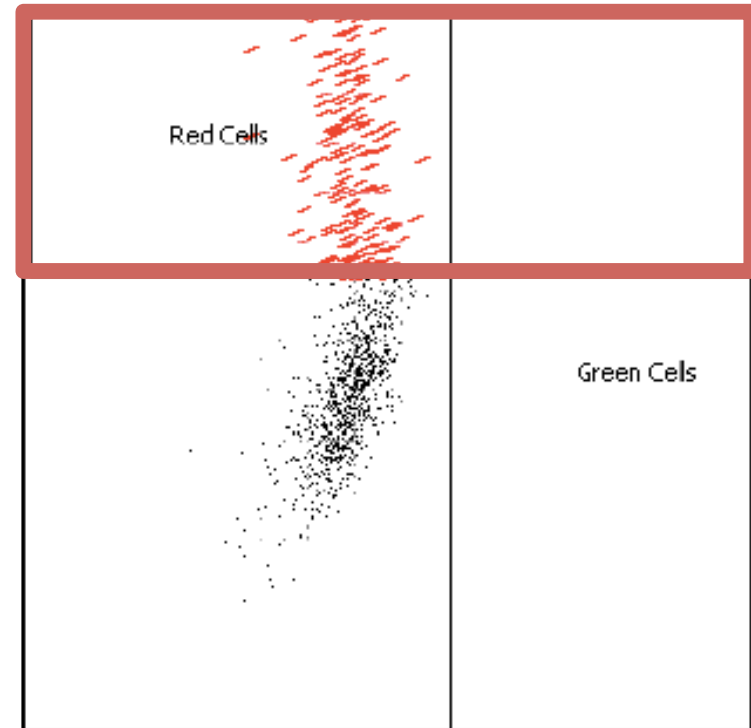


### 3. Set positive gates

- Use M059K transfected with pMAX\_EGFP only



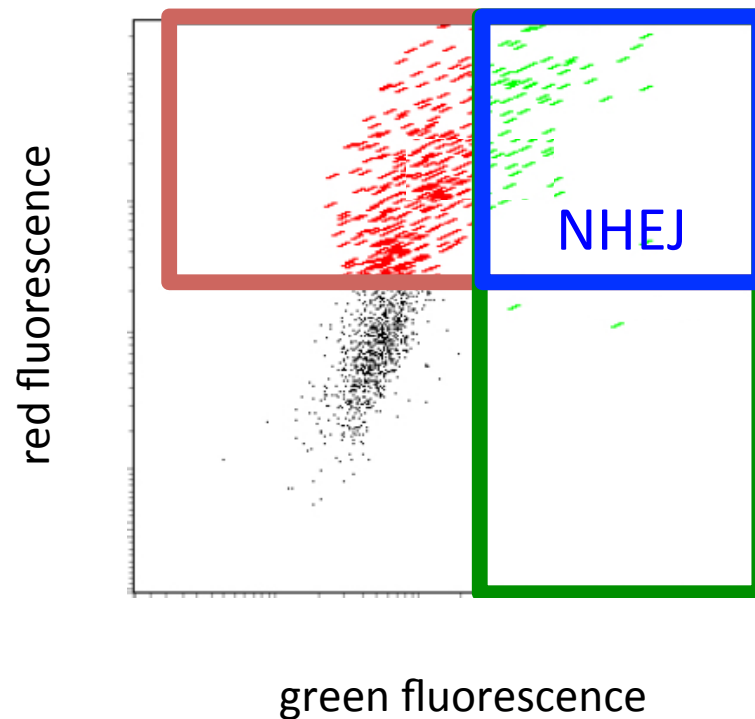
- Use M059K transfected with pMAX\_mCherry only



including fluorescent cells

## 4. Quantify experimental conditions

- M059K with both pMAX\_mCherry and pMAX\_EGFP
  - “maximum repair”
- Cells with pMAX\_mCherry and pMAX\_EGFP\_MCS repaired!



How are you going to present data in M2 research article?

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

## Examples:

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

blunt ends



compatible overhangs



incompatible overhangs

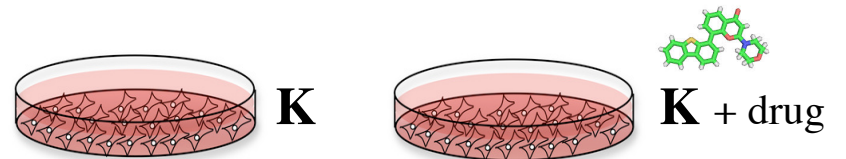


etc... Ask your own questions.

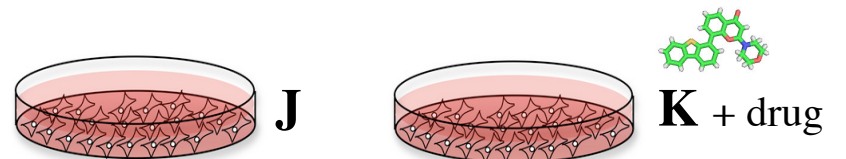
- Does loss of DNA-PKcs affect NHEJ efficiency?



- To what extent does this drug affect NHEJ?



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



# Craft your story carefully!

- Big picture:
  - cancer
  - **immunology**
  - **gene editing**
  - ... (journal club for inspiration)
- Results:
  - group / graph data judiciously (not all on one plot?!)
  - statistical analysis
- Discussion / interpretation
  - do not overreach



One last experiment:

## Evidence that our drug reduces DNA repair *in M059K/J cells*?

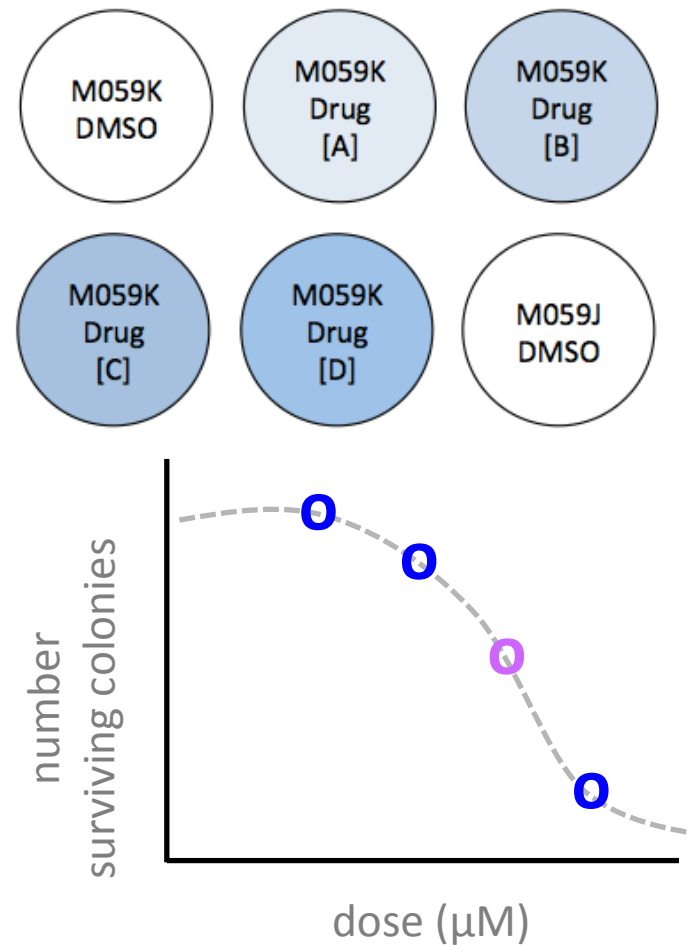
- Seed cells at very low density
  - 250 cells/well only!

After 2 days,

- dose response of NHEJ inhibitor around  $IC_{50}$
- ionizing radiation (4 Gy)

After 8 days,

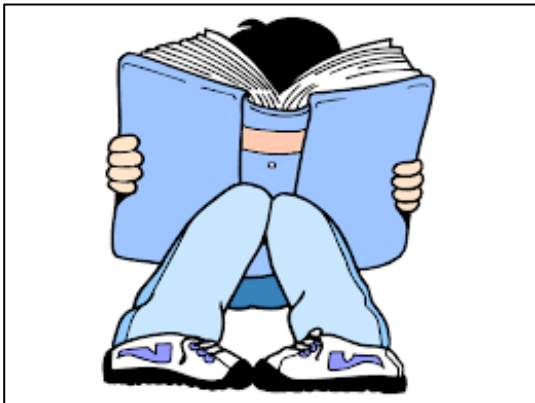
- count surviving cells via colony formation assay



## In lab today, split up:



- Tissue culture room
  - Red, Orange, Blue, Purple
  - Check drug & DMSO calculations with Leslie & Maxine



- Read Dietlein *et al.* paper
  - to be discussed with Prof. Samson on M2D7