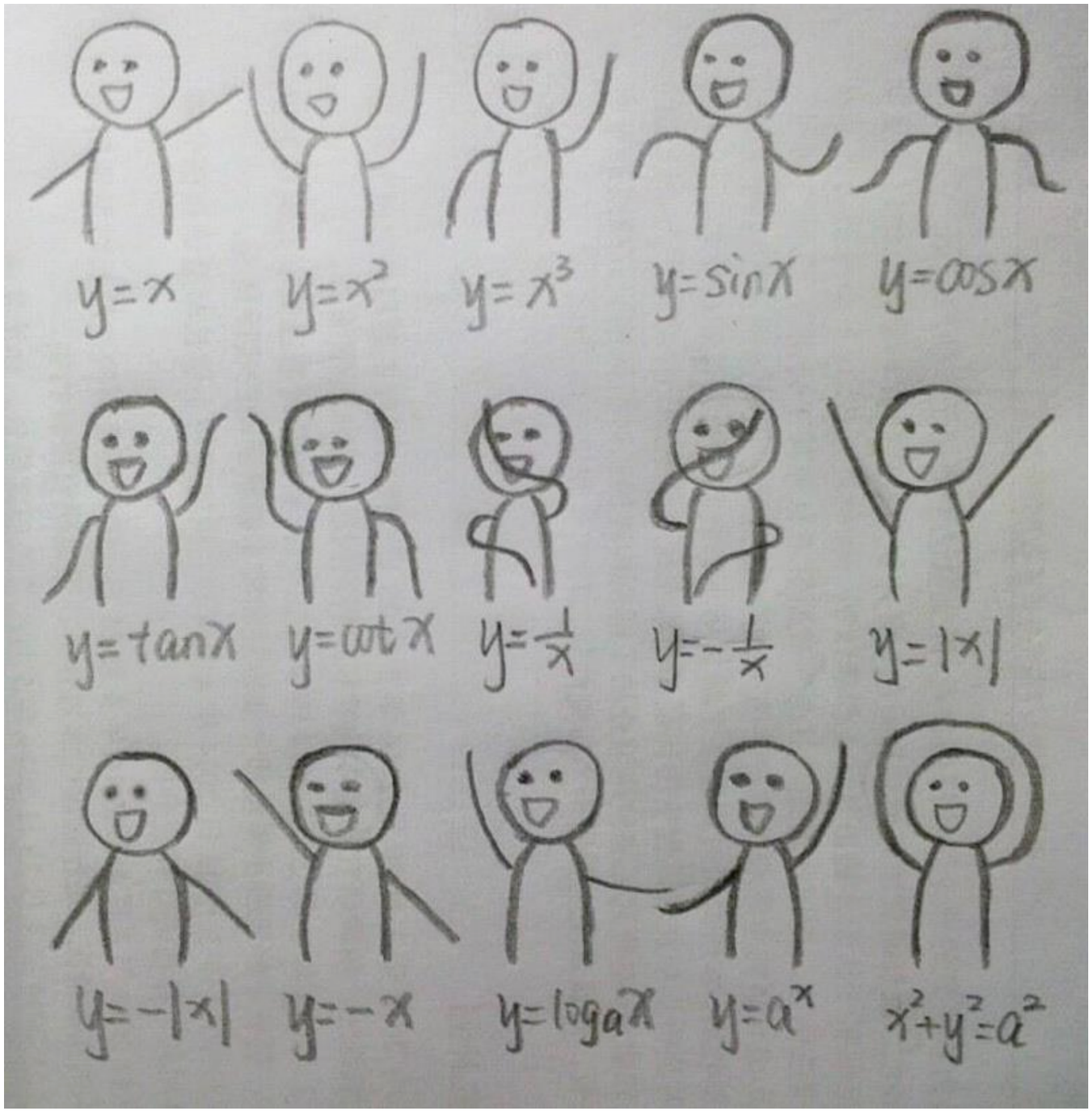
M2D6: DNA repair assays

Do the math dance!



Source: PhilipsShiu

Today we do the tissue culture dance!

Announcements:

Mod1 Revision due tomorrow

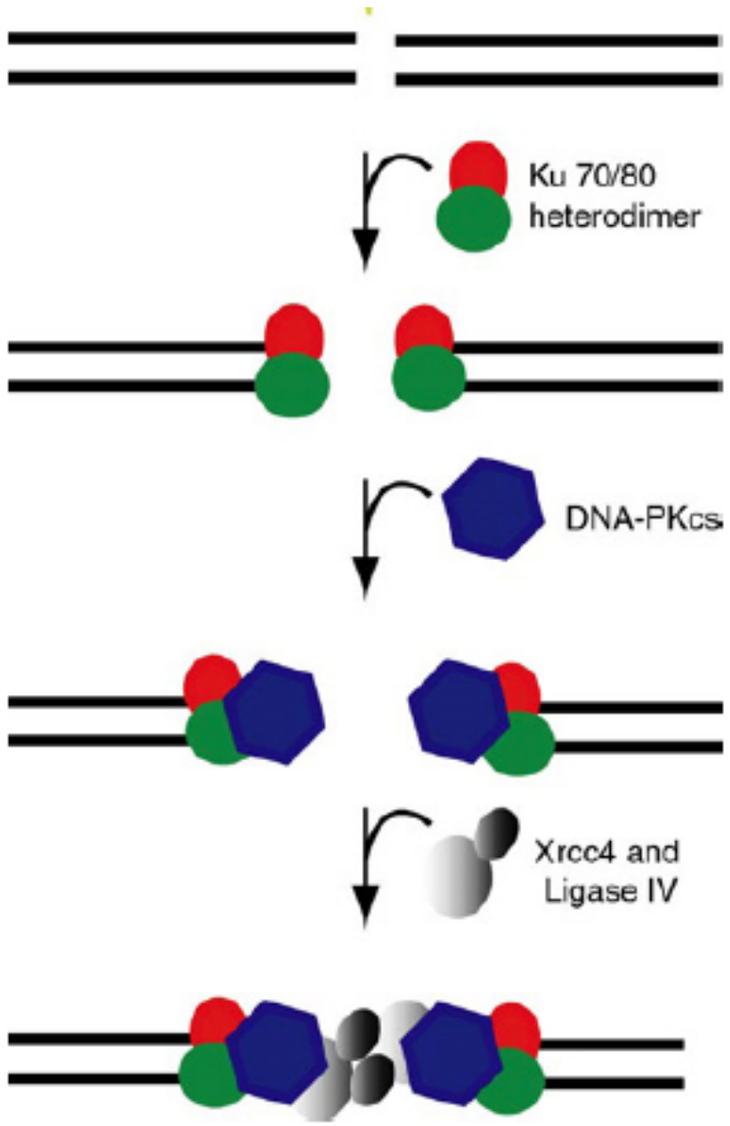
Reflection #2 - don't forget about it

Canonical NHEJ Pathway:

1) K1

2) xrs6

3) K1 + C401



Ku70

Ku~~80~~ xrs6

DNA-PKcs

T

C401

Xrcc4

Ligase IV

Quick review:

A) Intact BFP + Intact GFP

B) damaged BFP + Intact GFP

damage sensor

transfection control

*) Effect of topology on NHEJ

*) Involvement of Ku80 & DNA-PKcs in those repairs

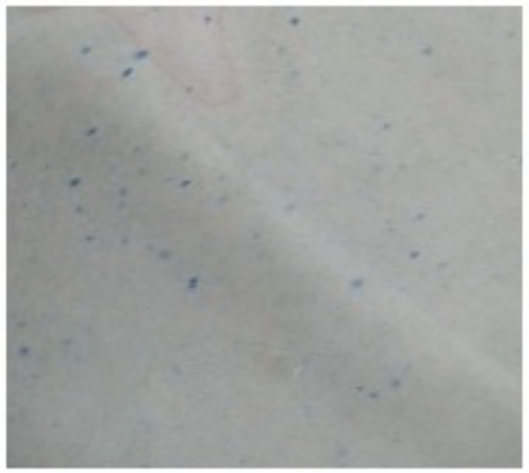
How will we know that the inhibitor works?

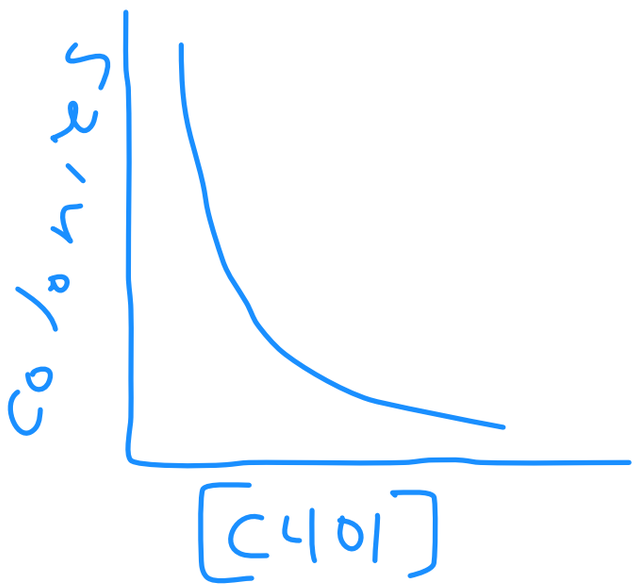
Day 6

Plate irradiated K1 with varying [C401]

DAY 7

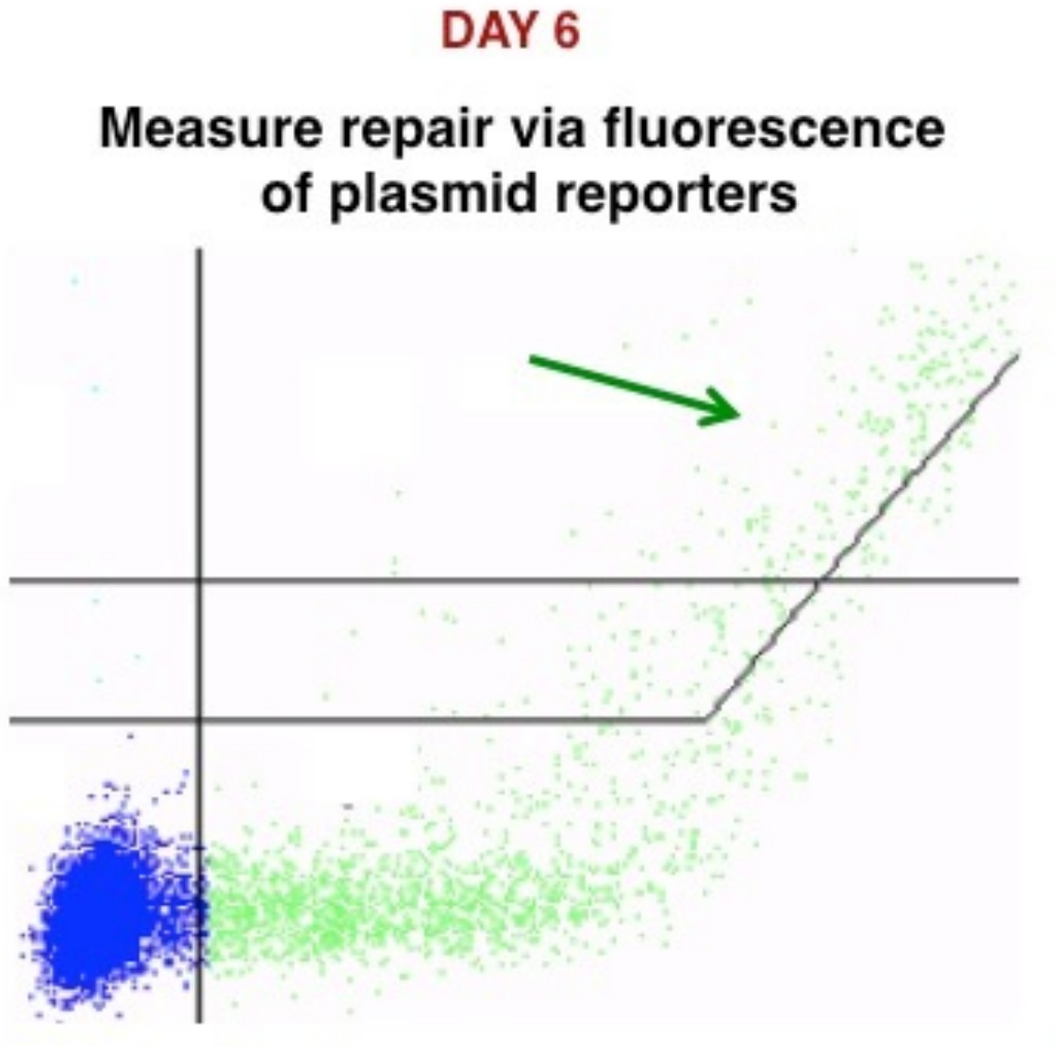
Stain for colonies





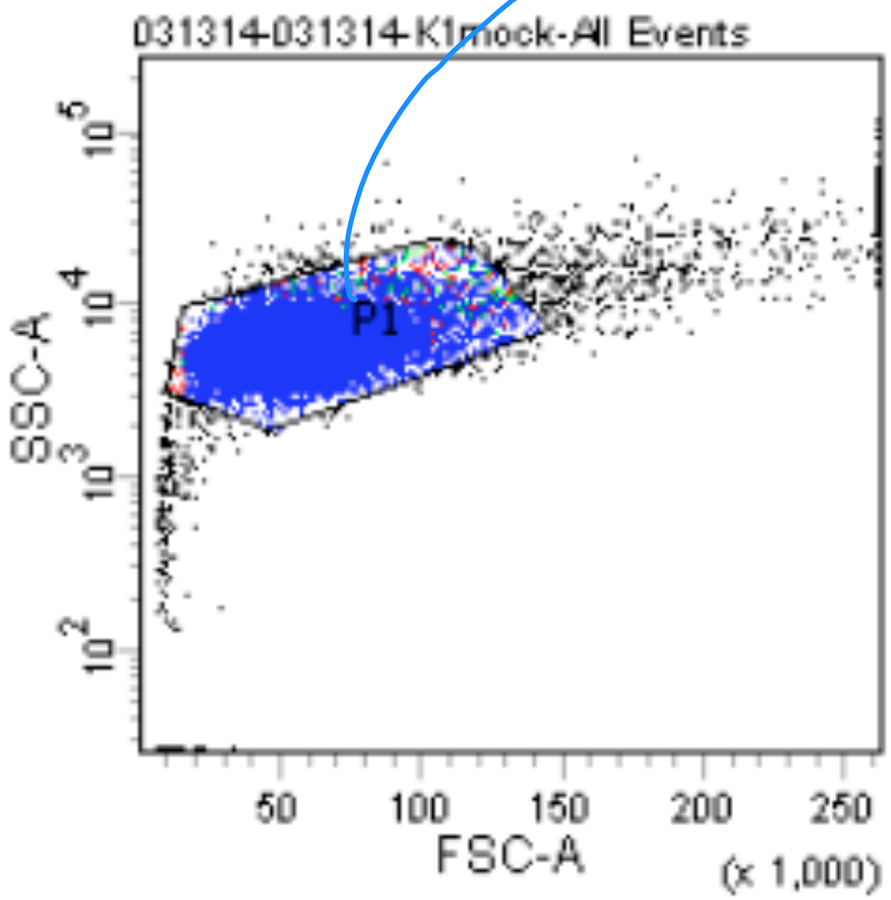
[C401], μM	Protocol
ϕ	1) Inhibitor added @ 5pm last night
0.1	2) Shannon will lift cells
0.5	3) Carrie will Zap them (4Gy)
1	4) You will count
5	5) Each team → 1 6-well plate with each [C401]
10	

Flow cytometry: There are a lot of steps before we get to this plot!



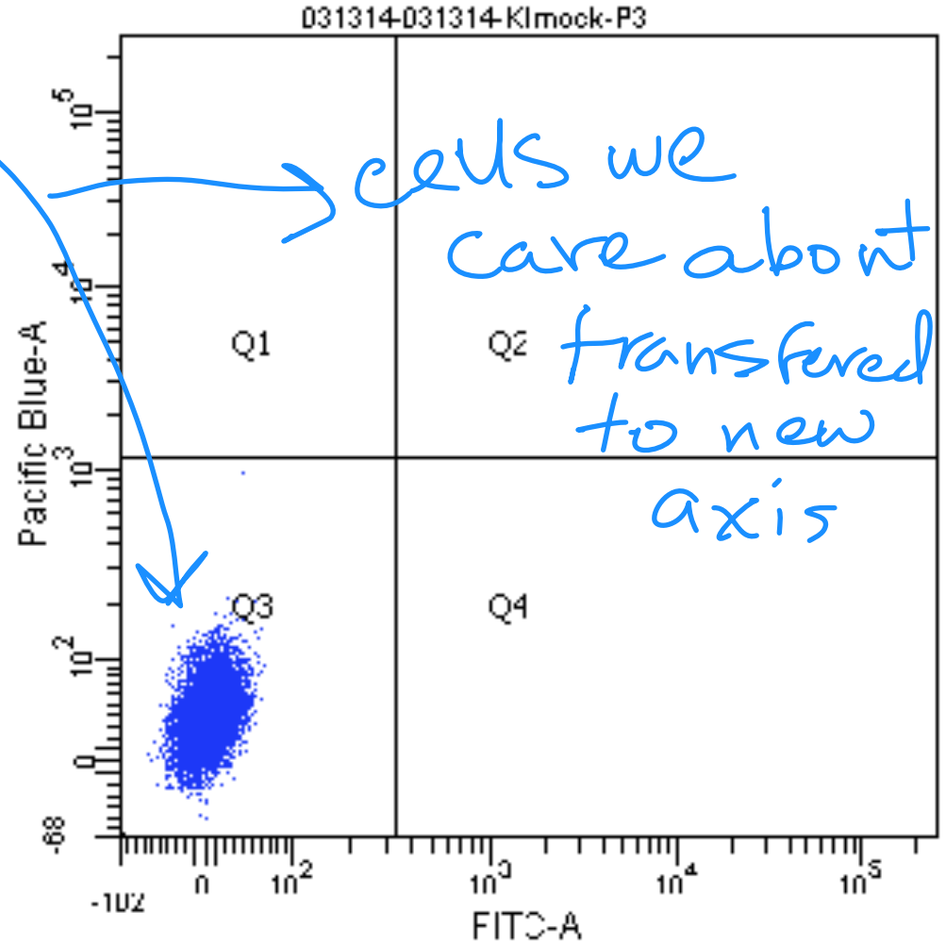
*Remember; all measurements are done @ same time

1) Determine the 'relevant' cell population



alive

dead



BFP

cells we care about transfered to new axis

GFP

SSC = side scatter ≡ shape granularity

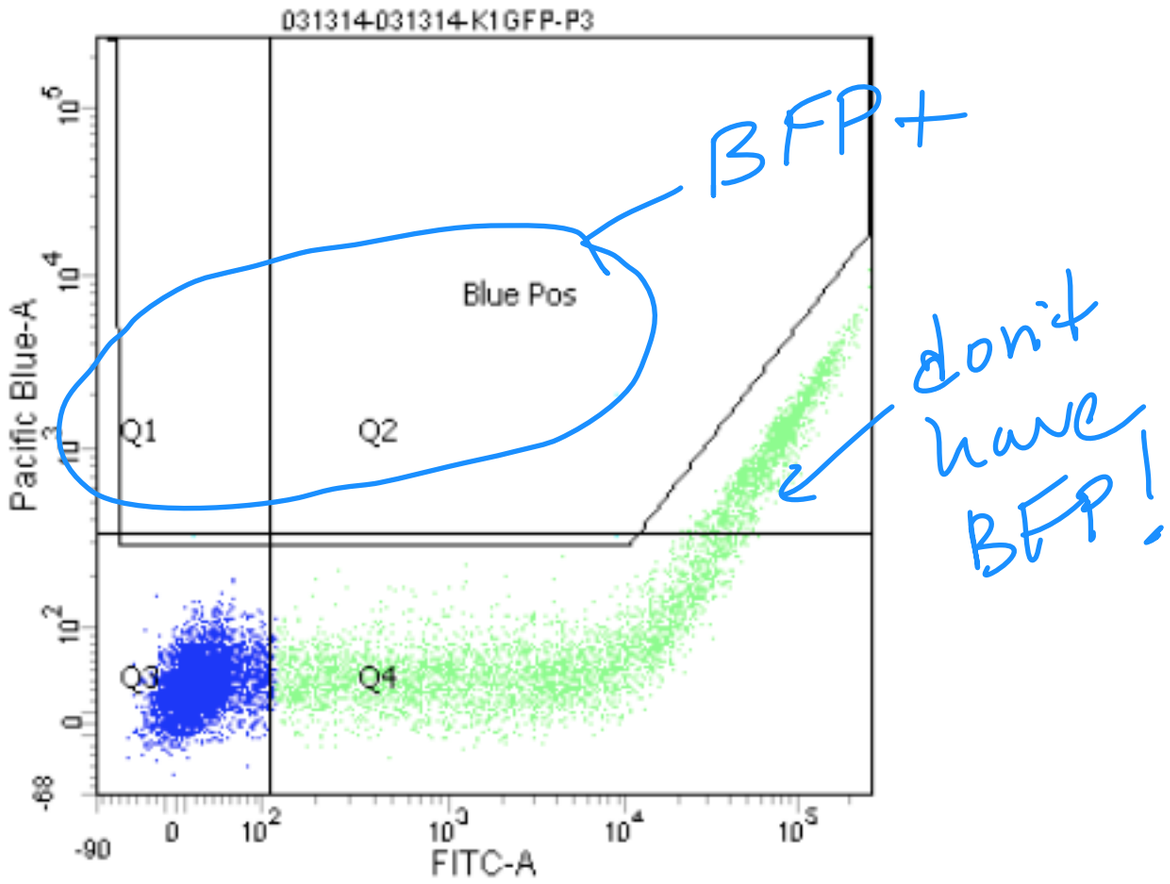
FSC = forward scatter] debris

] good

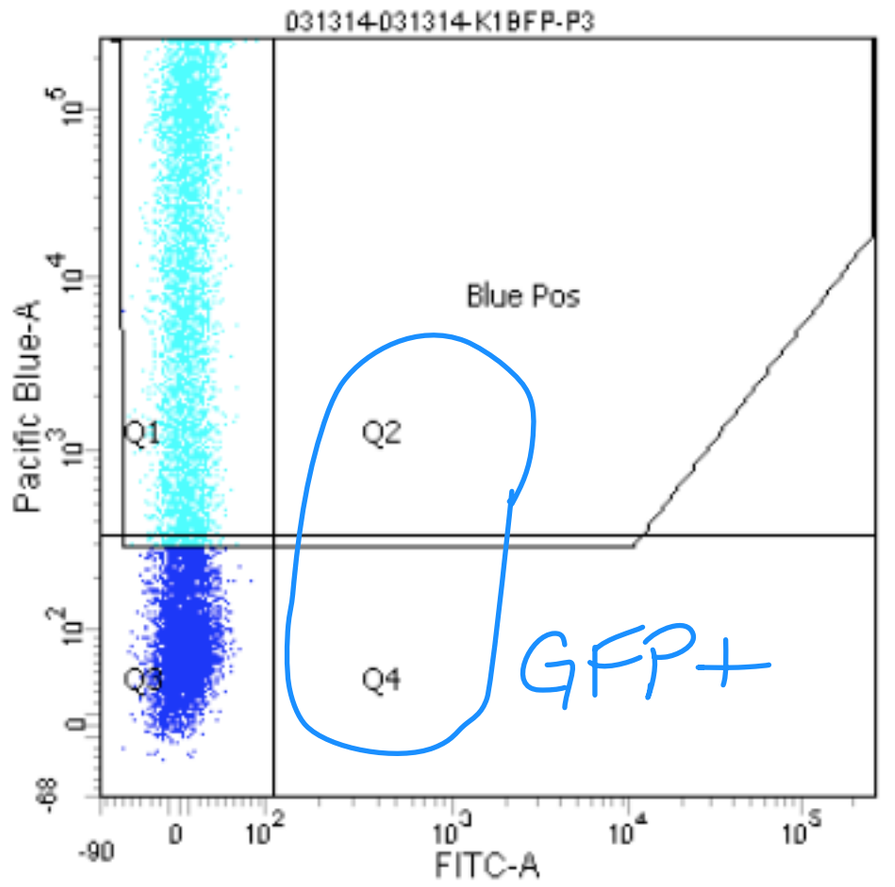
] aggregates

2) Set your negative gate

3) Set 'positive' gates with single color controls

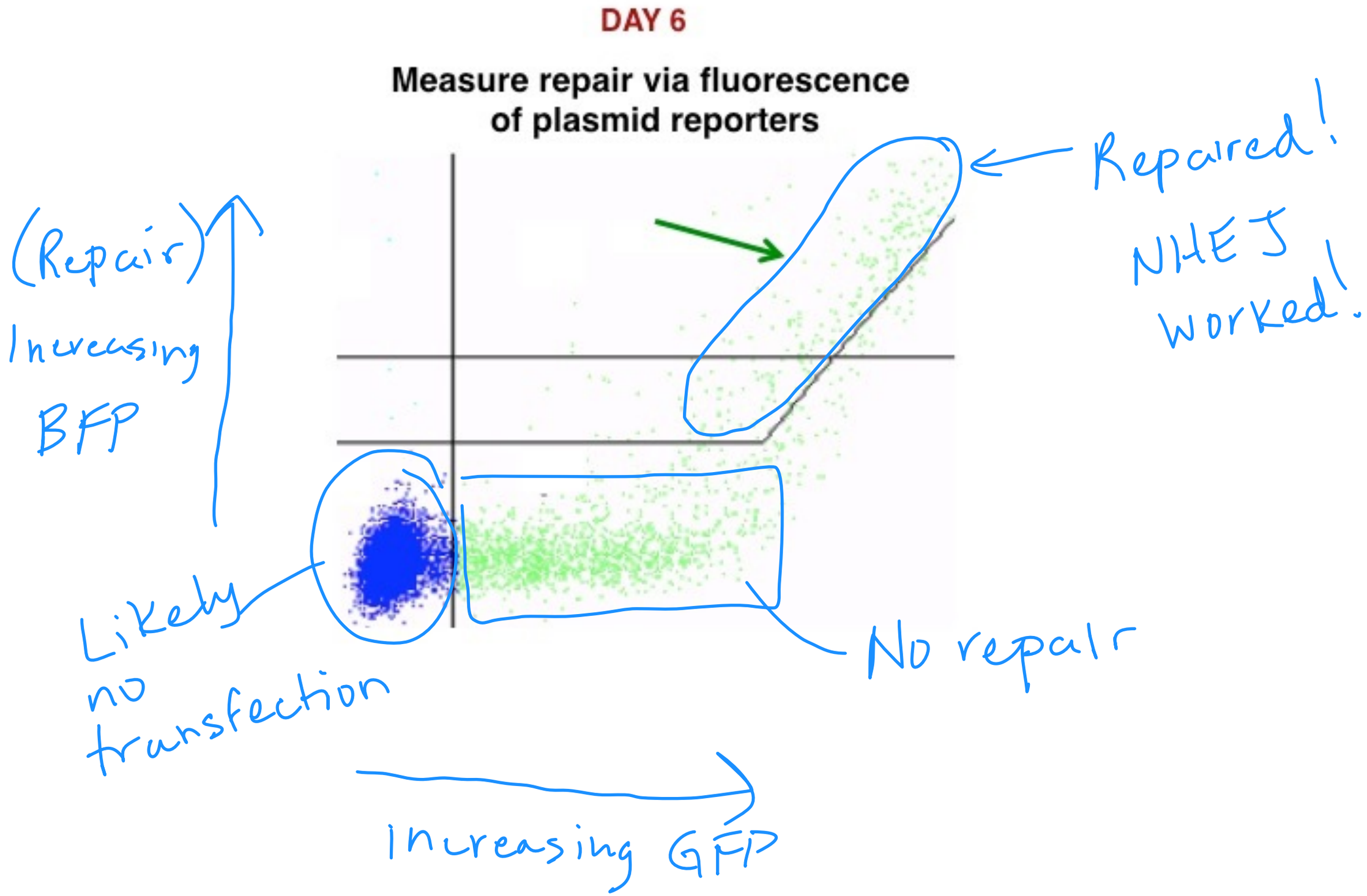


*only GFP positive



*only BFP positive

4) Quantify experimental conditions



Part 1: Hints

1. Aspirating media — clean pipette between conditions.
2. Correct tube labeling is key.

Condition	Red	Orange	Yellow	Green	Blue	Pink
K1 Intact	16	28	40	52	64	76
K1 Intact	17	29	41	53	65	77
C401 Intact	18	30	42	54	66	78
C401 Intact	19	31	43	55	67	79
xrs6 Intact	20	32	44	56	68	80
xrs6 Intact	21	33	45	57	69	81
K1 damaged	22	34	46	58	70	82
K1 damaged	23	35	47	59	71	83
C401 damaged	24	36	48	60	72	84
C401 damaged	25	37	49	61	73	85
xrs6 damaged	26	38	50	62	74	86
xrs6 damaged	27	39	51	63	75	87

3. Mixing is very important — need single cell suspension!
4. Plan your workflow with partner BEFORE you start.

Today in the lab:

1. Red, orange, yellow, green, pink in TC first to prep flow. Blue team will follow in first open hood.
2. Note: it will be crowded! Work at a purposeful pace.
3. You all take a break and I will prep the cells for irradiation. Carrie is coming to get them at 3:30pm.
4. You count the cells while they are getting zapped.
5. *You tell me* how you'd like to plate — please decide as team T/R.