- Announcements
- Pre-lab Lecture
  - Review central question(s)
  - Flow cytometry gating
  - [C401] dose response workflow
  - Tips for flow cytometry prep
  - Today in Lab (M2D6)

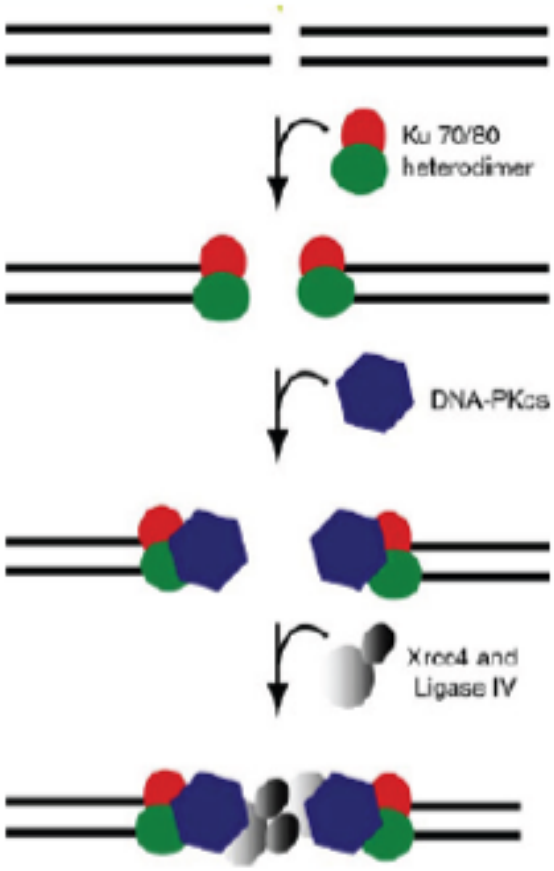
Announcements

- Module 1 revision due just now
  - give a heads up if you are taking a late day
- Reflection 2 also due
  - take the time you need to be thoughtful… but not so much time that you forget the experience

ideally: on Stellar + hard copy

Canonical NHEJ Pathway:

baseline/WT: K1



Ku70
Ku80 ~~X~~ xrs6

DNA-PKcs
↓
C401 (w/K1)

Xrcc4

Ligase IV

Quick review:

A) intact BFP+GFP → 1) max fluor/repair

B) cut BFP + ; GFP → 2) B:G ratio

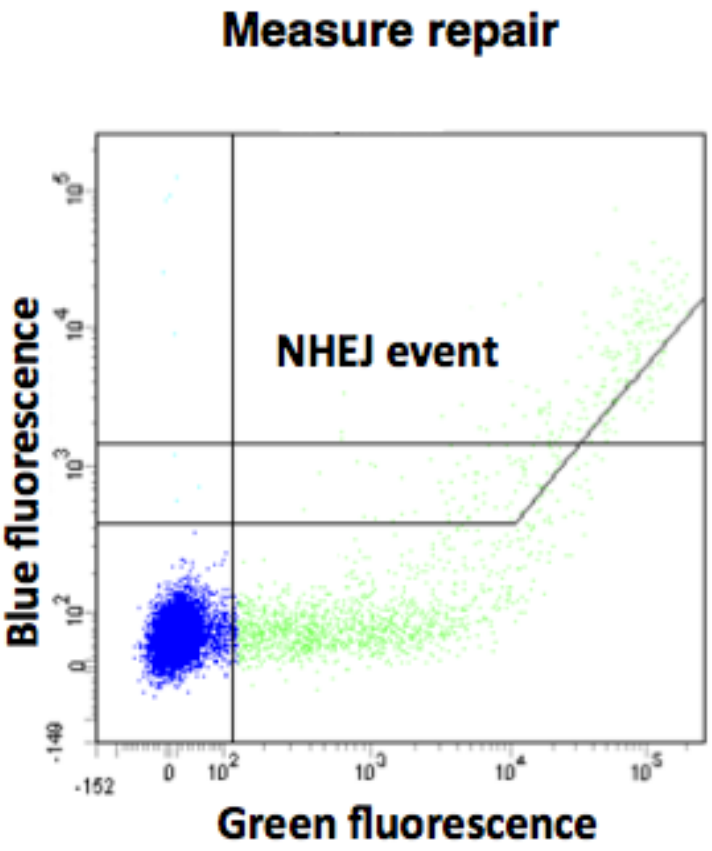
measure repair t'xn control

★ effect of topology (cut) on NHEJ repair efficiency baseline - K1 ↓

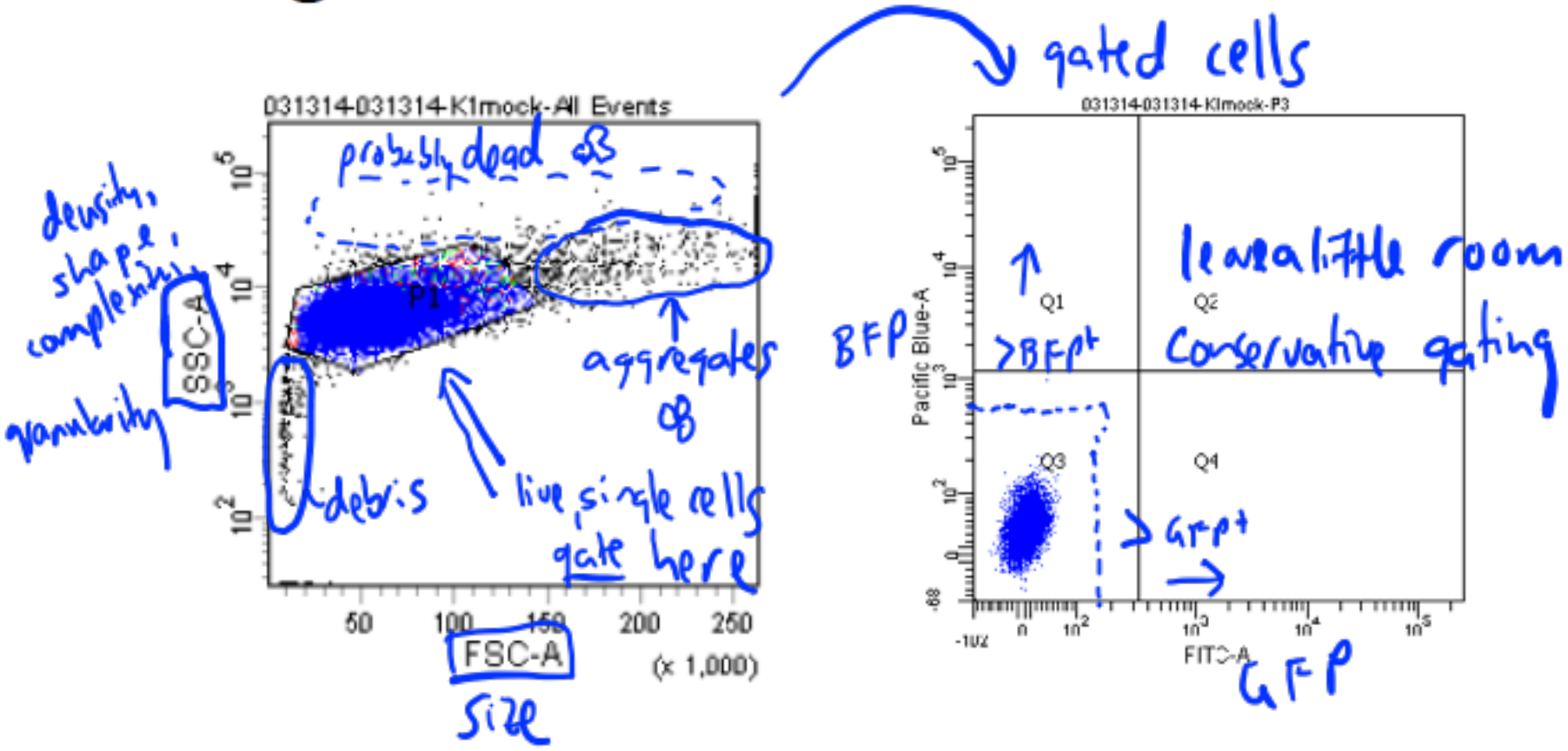
★ roles of Ku80, DNA-PKcs in repair. equivalent involvement?

Slide from Shannon H

Lots of steps to get from here to there



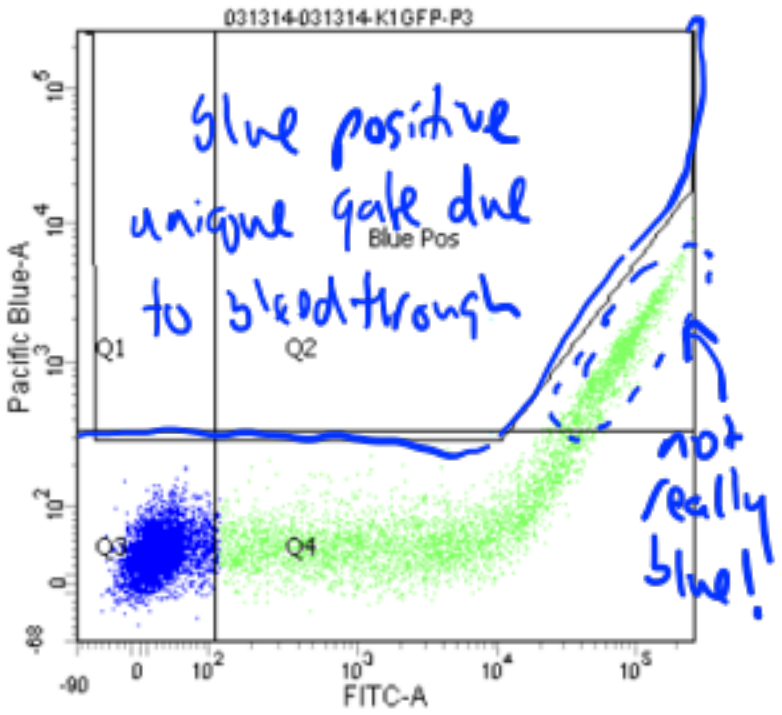
Negative control: "mock" treatment



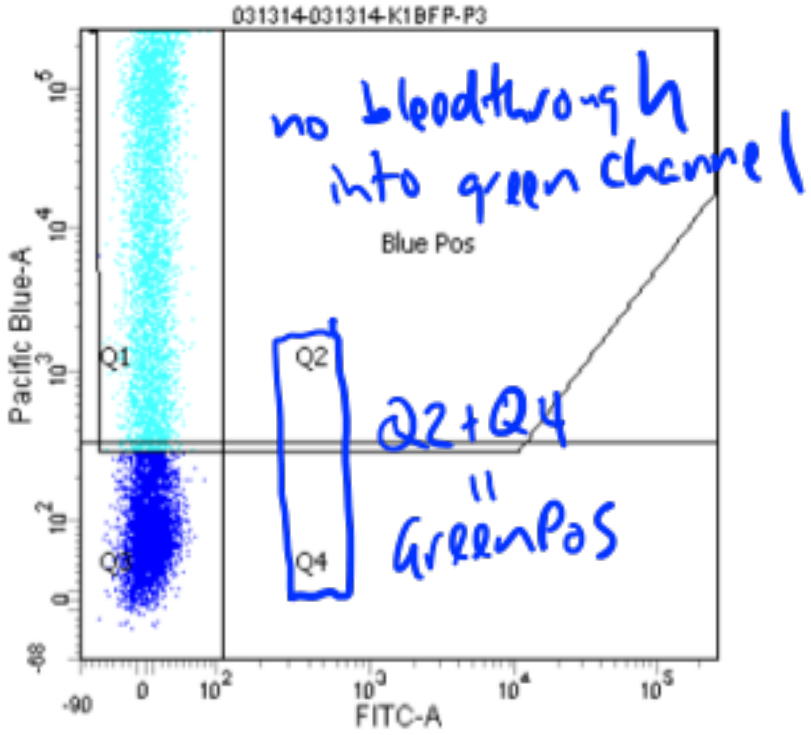
adjust voltages: population "onscreen"
adjust gates: select population of interest

Single-color controls to set "positive" gates

GFP transfection



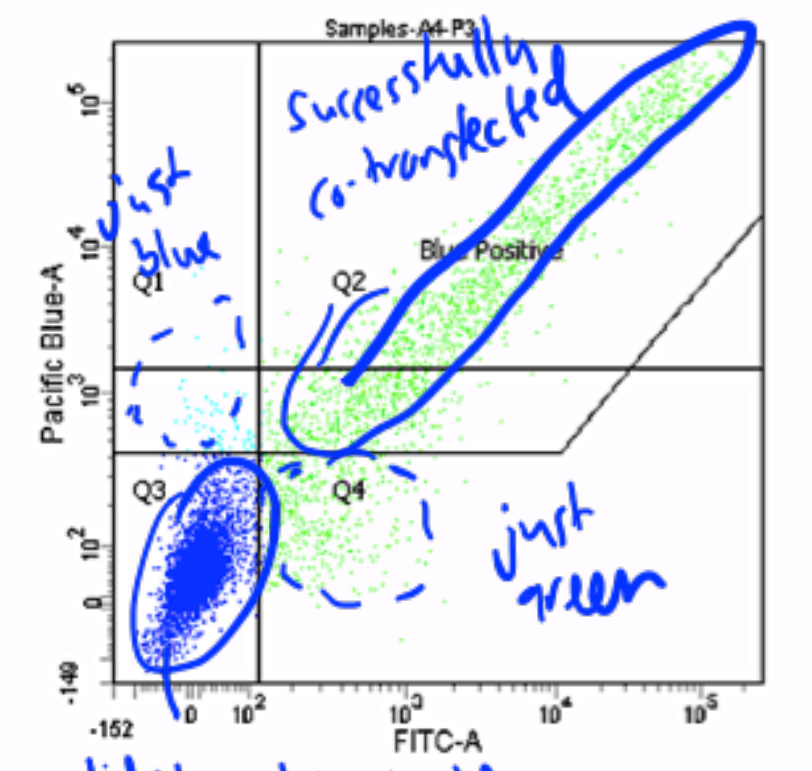
BFP transfection



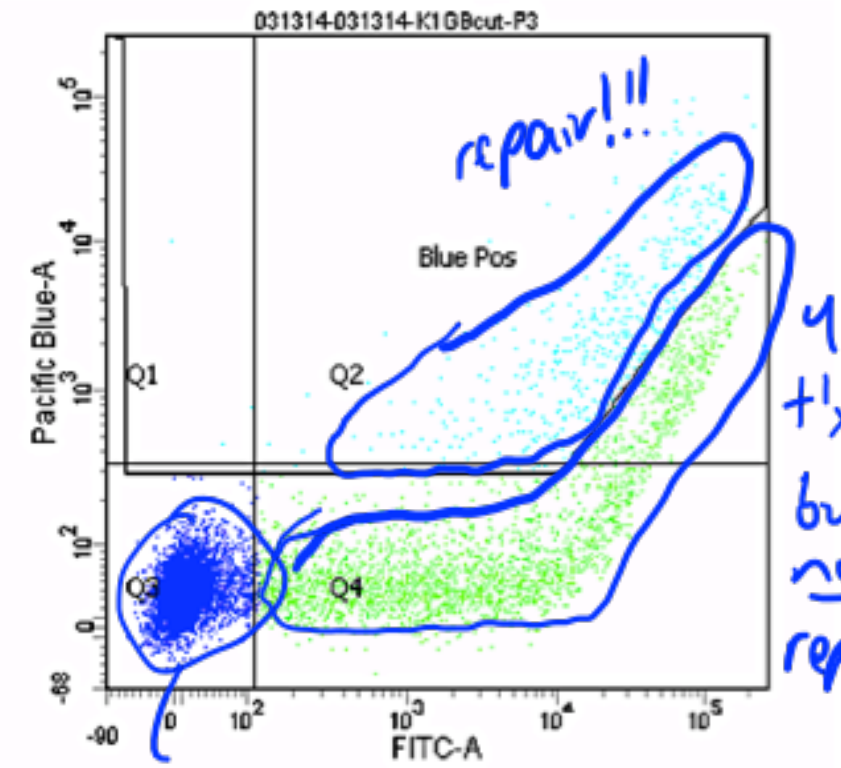
GFP^{+++} cells can be compensated by % subtraction

Dual intact versus repair experiment

GFP/BFP co- transfection



GFP + cut-BFP



[C401] dose response workflow

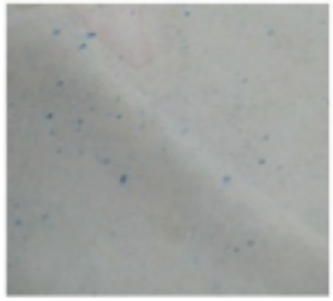
T25s pre-treatment

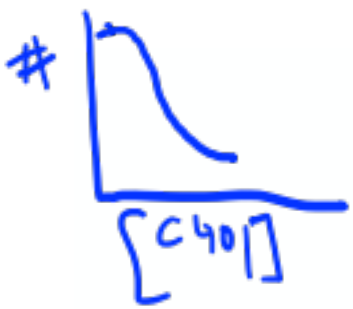
Day 6

Plate irradiated K1 with varying [C401]

DAY 7

Stain for colonies





[C401], µM	Protocol
0	1) inhibitor added @ 5pm Thu
0.1	2) Shannon will detach cells from T25s into 15-conc.
0.5	3) Lizzie will irradiate in the cancials
1	4) you will count
5	5) 1000 cells per well in a 6-well plate per tech
10	

Slide from Shannon H

Hints for flow cytometry prep

- Aspiration technique
  - remove all liquid but don't linger
  - *clean* pipette between conditions
- Label tubes with **correct number** + your color
- Pipet well to mix cells, disrupt aggregates
- Plan a workflow with your partner in advance

Today in Lab (M2D6)

- Note: Shannon and Su will be in TC all afternoon
- Get flow tubes ready: ROYGB teams first! Then PPSW
- Love this phrase: "Work at a purposeful pace" (SKH)
- When *everyone* is done, Shannon will prep the six C401-treated flasks for irradiation
- Meanwhile, y'all make a plan and divide up the labor
- Around 3 pm, Lizzie N from Engelward lab will come irradiate your cells at 4 Gy
- Meanwhile, you count the cells and finalize plating plan – collaborative tissue culture!