M2D6: Data Analysis + Paper Discussion

Announcements:

1) Mod2 Research Paper due **Friday, ~~April~~ 17th at 10pm**

Monday Apr. 20 5pm

Resources you have available:

1) 20.109 Instructional staff
2) WRAP faculty — feedback on prose + structure
3) BE Communications Lab — feedback on flow + clarity

Listen

Introduction

IMRaD Research Paper Structure

Introduction

Big picture

research question

Methods

Results

subheadings

concise

NOT have to be chronological

Discussion

research question

big picture

What section is this?

We next sought to confirm we could detect the expected effects of known chemical inhibitors on DSB repair activity in 384-well microplates, using our screening process flow. To this end, we tested mirin, a recently described inhibitor of the Mre11–Rad50–Nbs1 (MRN) complex (45). We tested mirin along with DMSO controls in 384-well plates, at a range of doses in triplicate. The percentages of RFP- and GFP-positive cells from triplicate samples in wells containing various doses of mirin were normalized to the percentages observed in wells containing DMSO-treated cells for both mNHEJ and HR, respectively.

Finally, the findings that multiple known FDA-approved drugs have activity as DSB repair inhibitors and tumor cell radiosensitizers raise the possibility that these agents can be readily tested in clinical trials as radiosensitizers in the near future. As discussed earlier, GBM tumors would be an ideal target to test such agents, because they are exquisitely radioresistant tumors, and local recurrence is the predominant mode of failure for these tumors (62). Drugs in our hit-list, such as mibefradil, pimozide, and AMN082, are of particular interest for the treatment of brain tumors, because they are known to penetrate the blood brain barrier (75-77).

Emerging evidence indicates that many subpathways exist within both the NHEJ and HR pathways of repair. In particular, NHEJ repair mainly is composed of canonical NHEJ (cNHEJ) and noncanonical NHEJ repair. The latter process has been given many names, including back-up NHEJ (bNHEJ), alternative NHEJ (aNHEJ), and microhomology-mediated NHEJ (MMEJ; ref. 12). This lack of consensus, in part, can be attributed to the fact that specificDSBrepair proteins thatmediate non canonical NHEJ repair remain elusive. The cNHEJ pathway is well defined and results in minimal processing of the DSB ends (13), while the latter process typically results in deletions with local sequence microhomology (14–17). cNHEJ proteins include Ku70/80, DNA-PK catalytic subunit (DNA-PKcs), X-ray repair cross-complementing protein 4 (XRCC4), and ligase IV (13).

Introduction

Emerging evidence indicates that many subpathways exist within both the NHEJ and HR pathways of repair. In particular, NHEJ repair mainly is composed of canonical NHEJ (cNHEJ) and noncanonical NHEJ repair. The latter process has been given many names, including back-up NHEJ (bNHEJ), alternative NHEJ (aNHEJ), and microhomology-mediated NHEJ (MMEJ; ref. 12). This lack of consensus, in part, can be attributed to the fact that specificDSBrepair proteins thatmediate non canonical NHEJ repair remain elusive. The cNHEJ pathway is well defined and results in minimal processing of the DSB ends (13), while the latter process typically results in deletions with local sequence microhomology (14–17). cNHEJ proteins include Ku70/80, DNA-PK catalytic subunit (DNA-PKcs), X-ray repair cross-complementing protein 4 (XRCC4), and ligase IV (13).

- background from other work (scientific)
- acronyms
- references
- motivation
  - last paragraph
    - hypothesis
    - some design
    - teaser of results

Results

0

We next sought to confirm we could detect the expected effects of known chemical inhibitors on DSB repair activity in 384-well microplates, using our screening process flow. To this end, we tested mirin, a recently described inhibitor of the Mre11–Rad50–Nbs1 (MRN) complex (45). We tested mirin along with DMSO controls in 384-well plates, at a range of doses in triplicate. The percentages of RFP- and GFP-positive cells from triplicate samples in wells containing various doses of mirin were normalized to the percentages observed in wells containing DMSO-treated cells for both mNHEJ and HR, respectively.

Some methods flow of data experiments

1 motivates experiment

2 describes data

transition to next experiment.

Discussion:

what results mean

why study is important

Finally, the findings that multiple known FDA-approved drugs have activity as DSB repair inhibitors and tumor cell radiosensitizers raise the possibility that these agents can be readily tested in clinical trials as radiosensitizers in the near future. As discussed earlier, GBM tumors would be an ideal target to test such agents, because they are exquisitely radioresistant tumors, and local recurrence is the predominant mode of failure for these tumors (62). Drugs in our hit-list, such as mibefradil, pimozide, and AMN082, are of particular interest for the treatment of brain tumors, because they are known to penetrate the blood brain barrier (75-77).

suggest future experiment.

limitations

Future:

what is the next step?

* big pic in the sky idea.

just gate cells

goal: cells

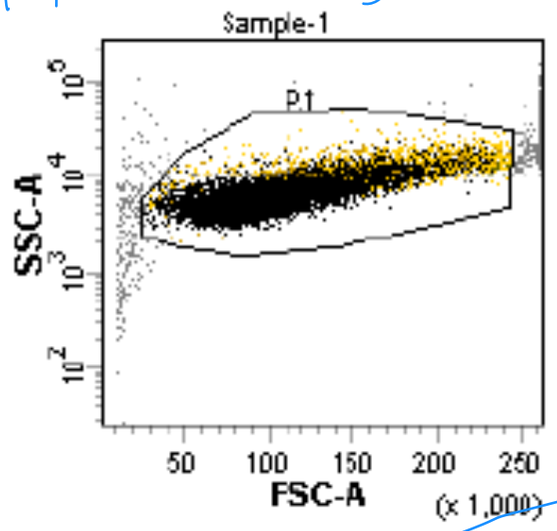
aggregates

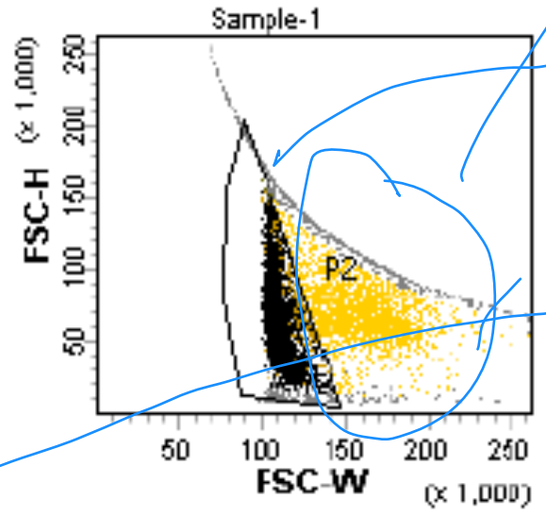
garbage

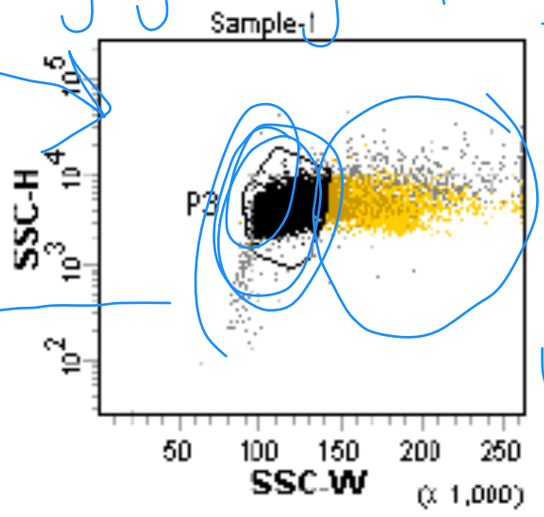
goal: live cells Topro3

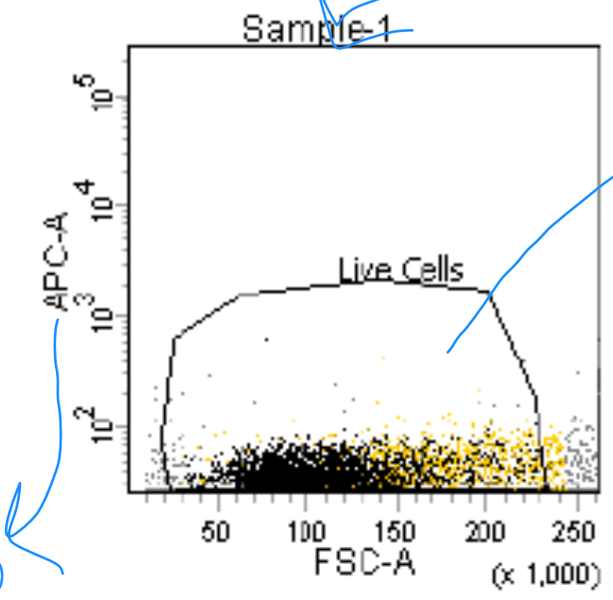
grün

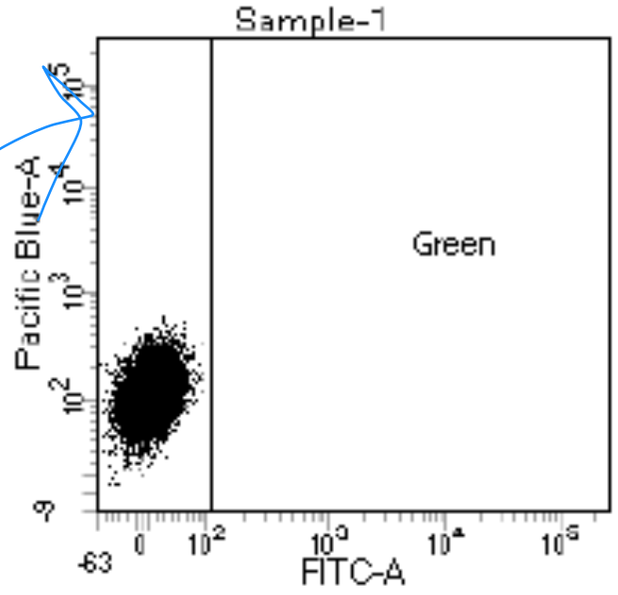
BD FACSDiva 8.0

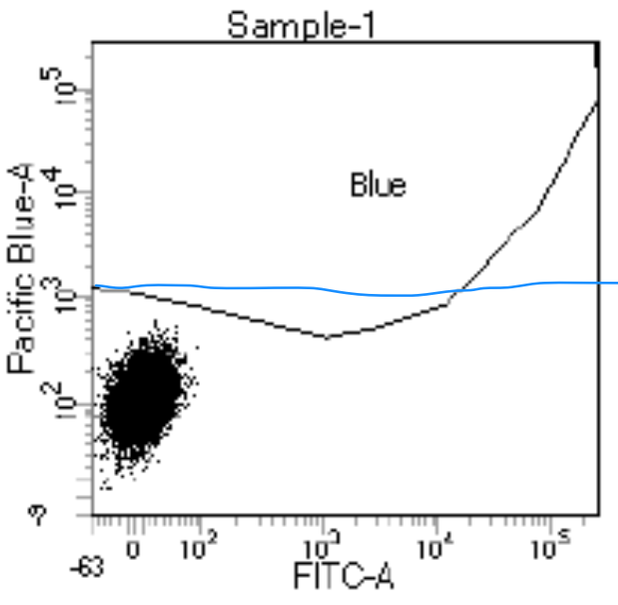


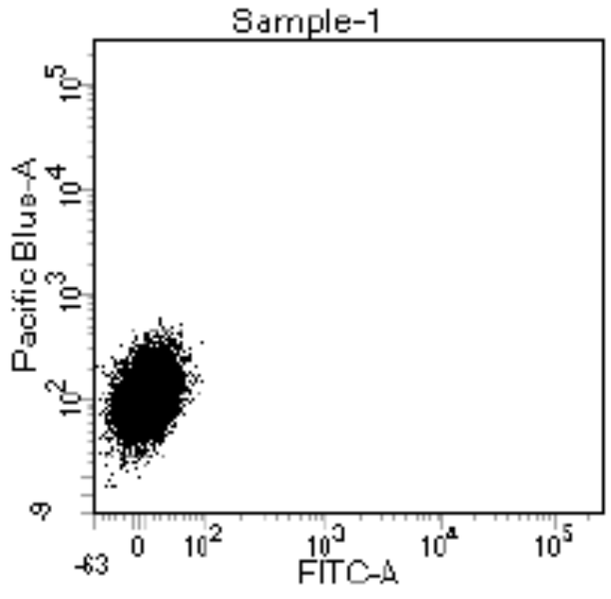


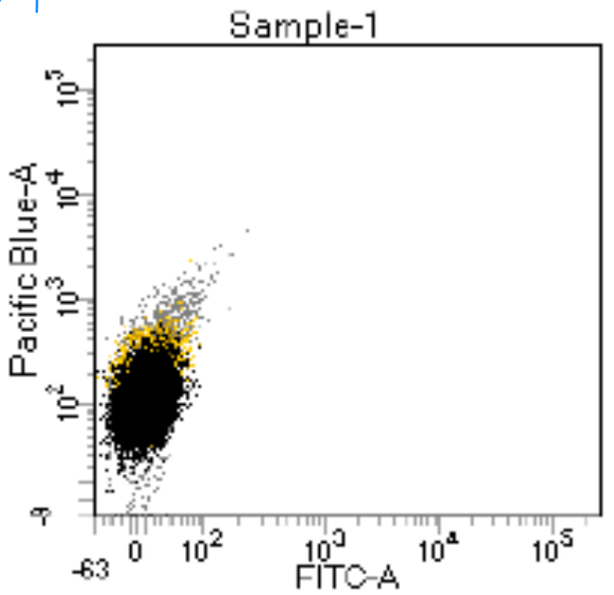










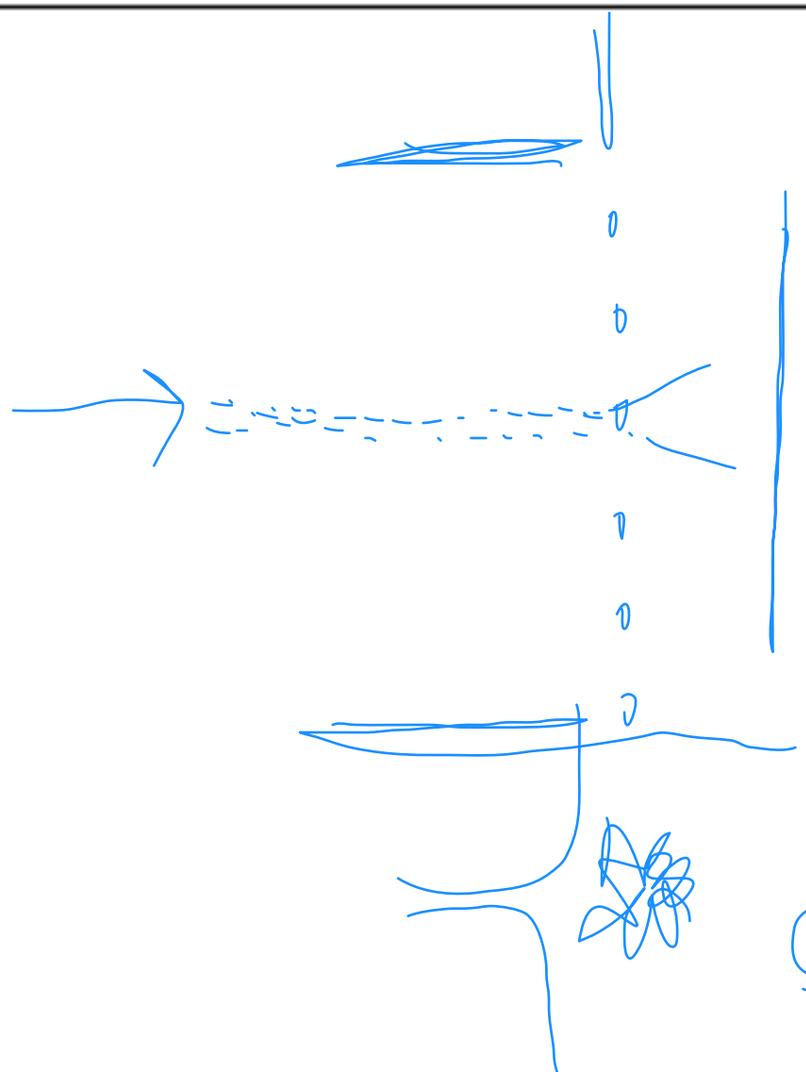


Tube: 1

Population	#Events	%Parent	%Total
All Events	13,284	#####	100.0
P1	12,439	93.6	93.6
P2	10,033	80.5	80.0
P3	10,214	98.1	76.9
Live Cells	10,201	99.9	76.8
Blue	0	0.0	0.0
Green	0	0.0	0.0
Both	0	0.0	0.0

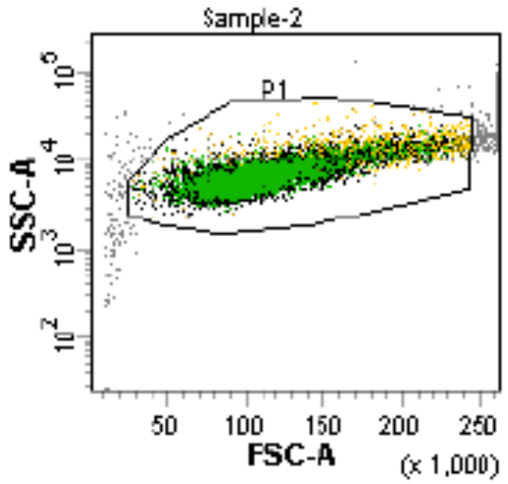
Experiment Name: 20-109 Lab 20150402
Plate Name:
Specimen Name: Sample
Tube Name: 1
Record Date: Apr 2, 2015 3:33:43 PM
$OP: IsaacChaim
GUID: 8f037a3a-f013-44b0-a23d-93617...

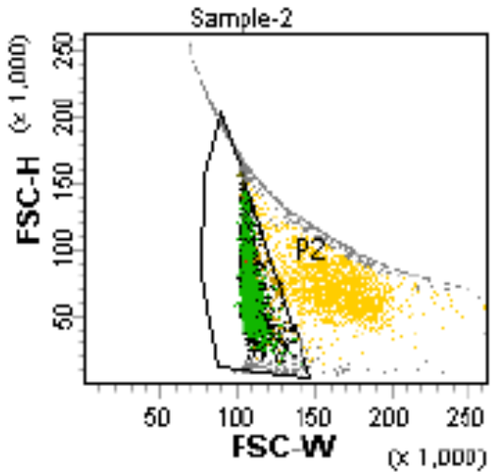
Population	#Events	%Parent	FITC-A Mean	FITC-A Median	Pacifi... Mean	Pacifi... Median
Live Cells	10,201	99.9	6	6	116	106
Blue	0	0.0	####	####	####	####
Green	0	0.0	####	####	####	####
Both	0	0.0	####	####	####	####

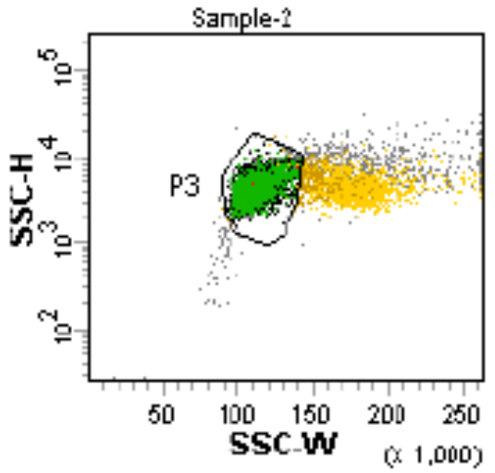


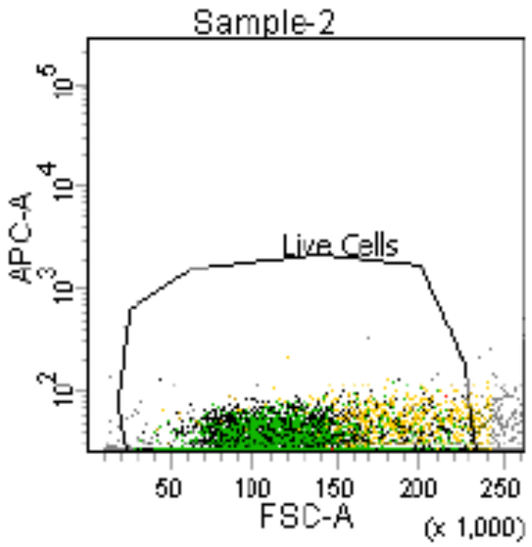
only GFP!

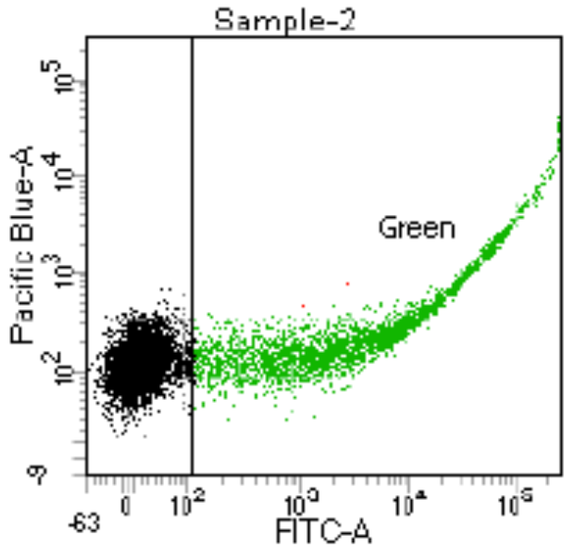
BD FACSDiva 8.0

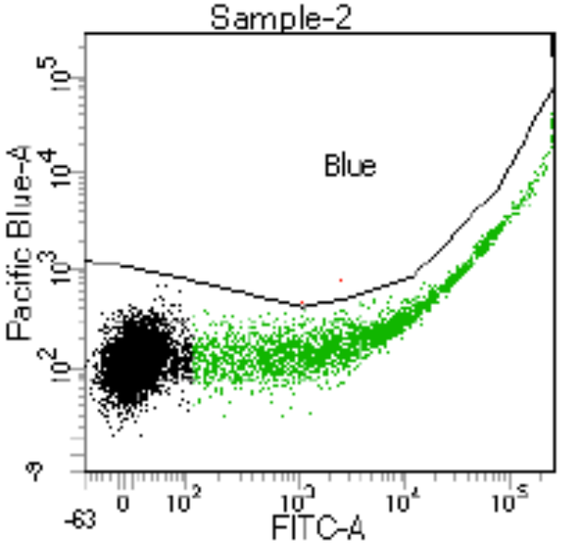


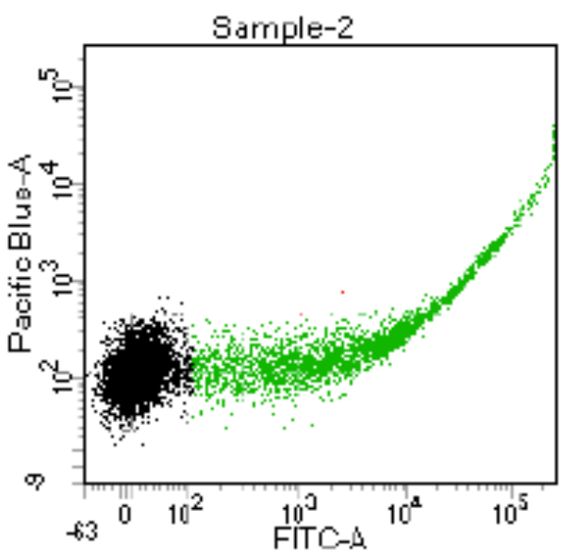


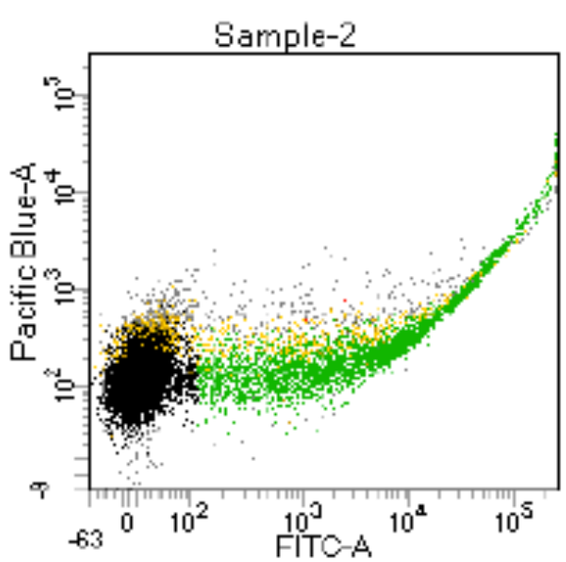






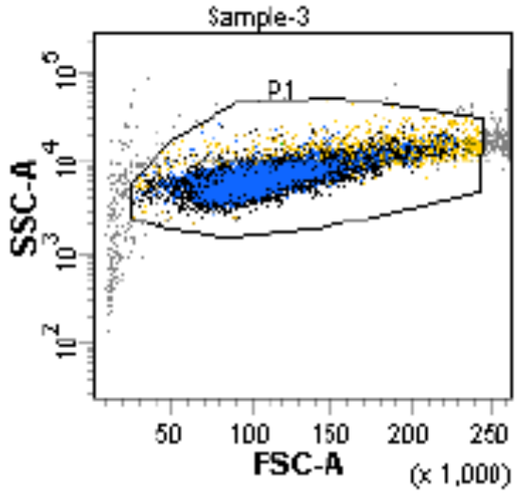


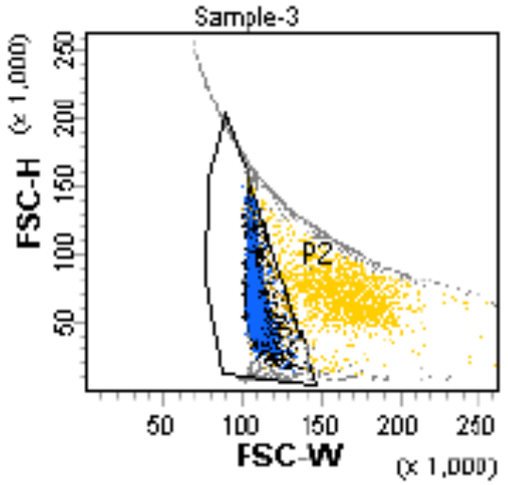


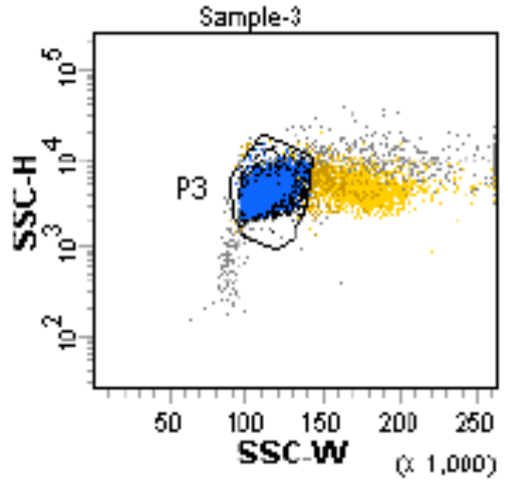


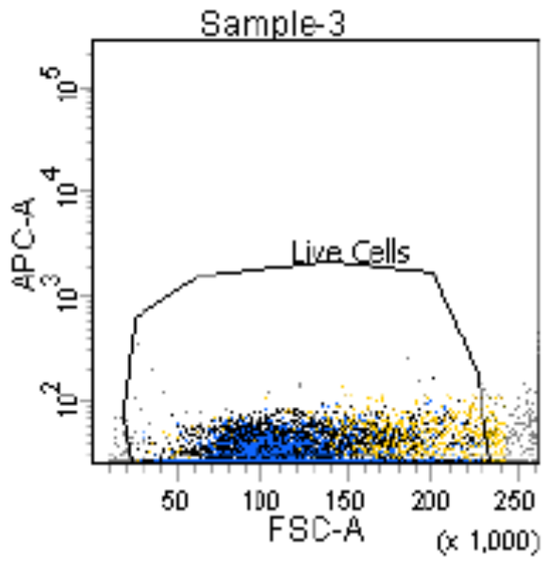
Only BFP.

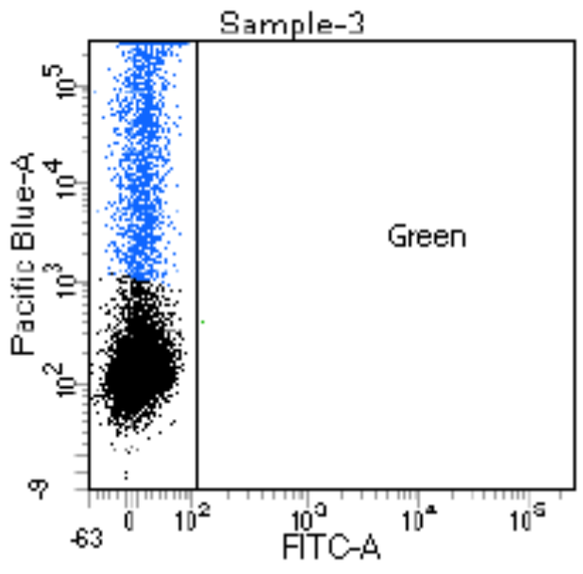
BD FACSDiva 8.0

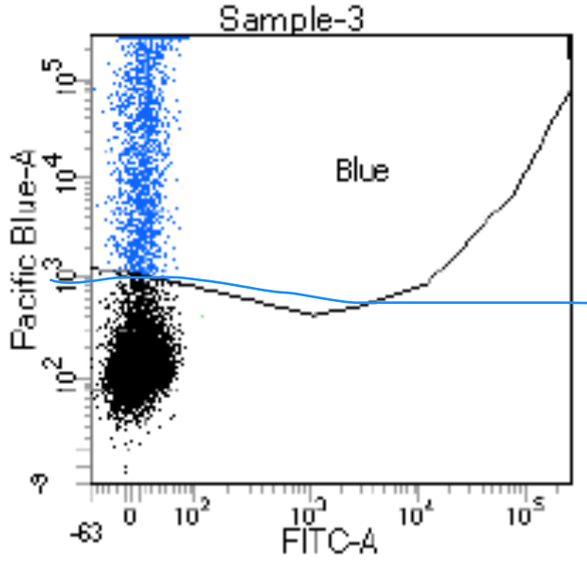


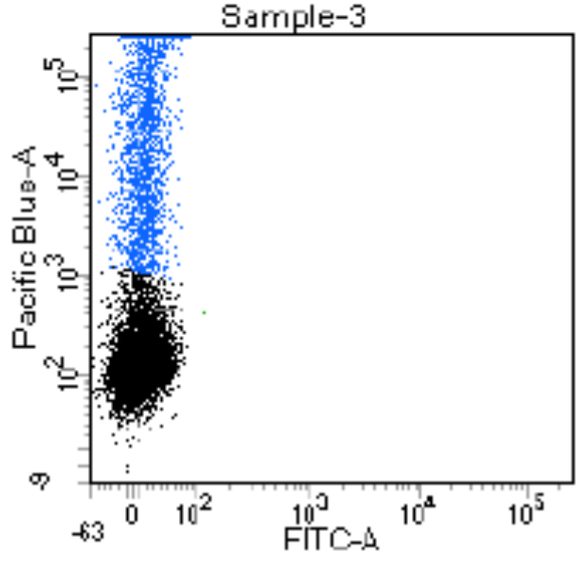


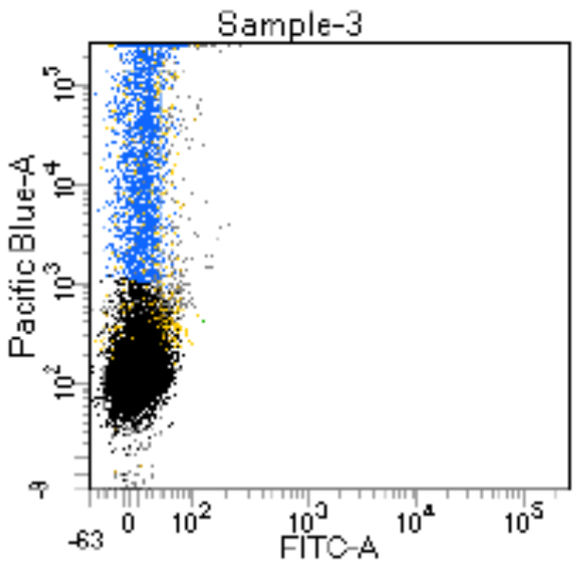






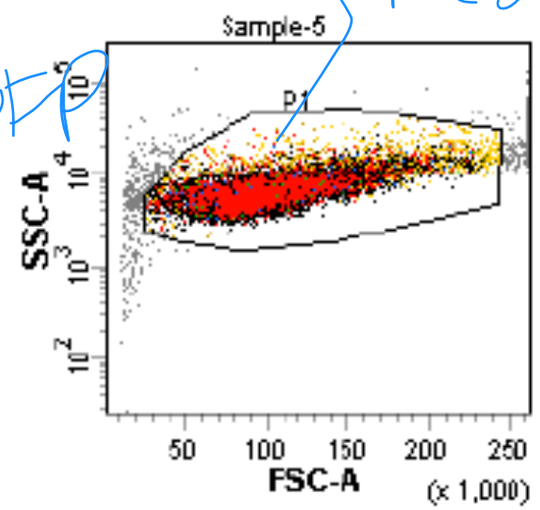


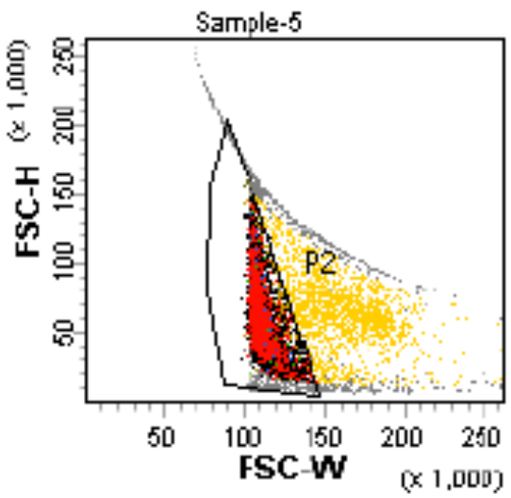


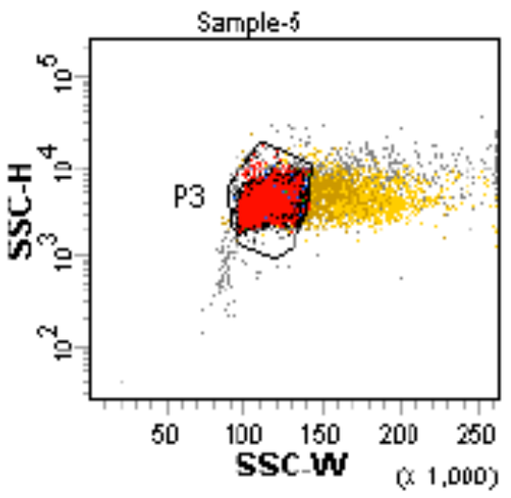


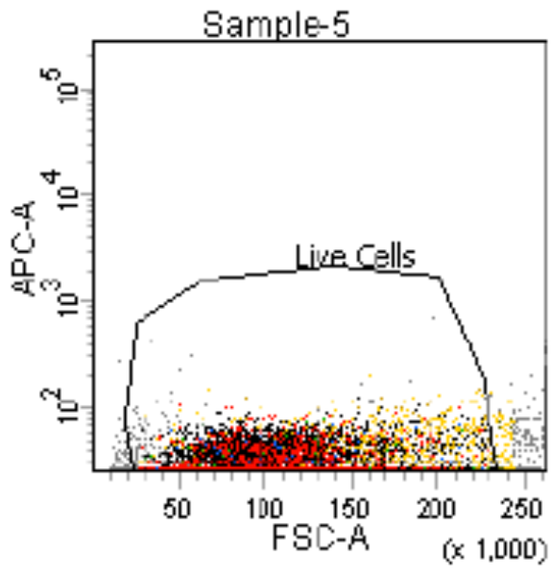
Intact GFP
Intact BFP
repair
Red

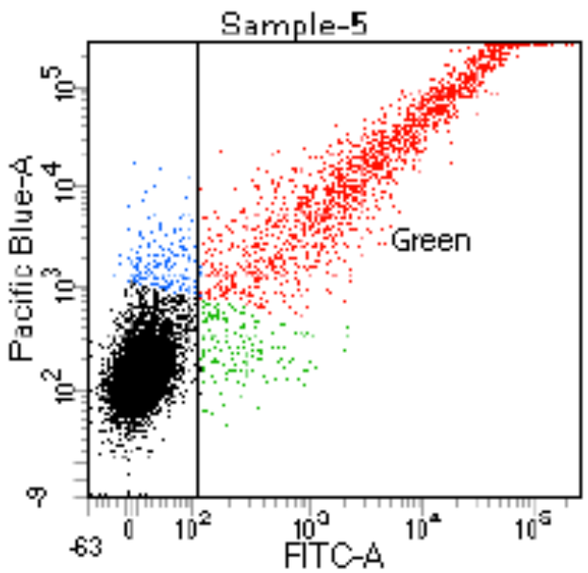
BD FACSDiva 8.0

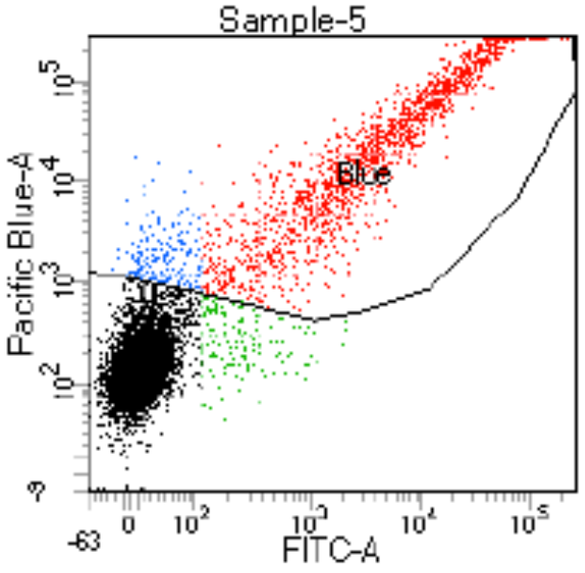


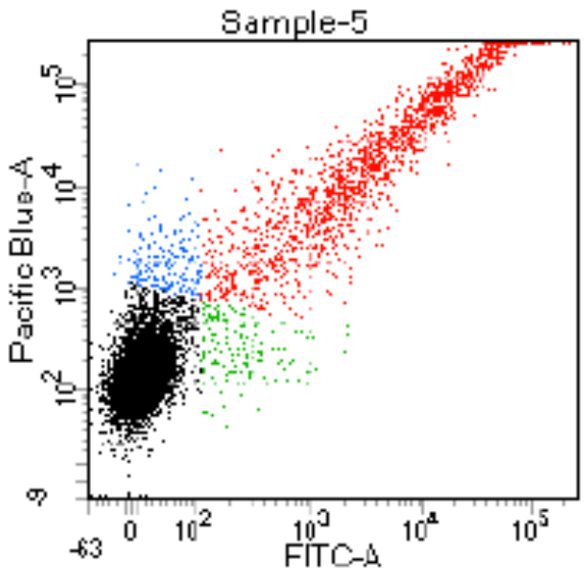


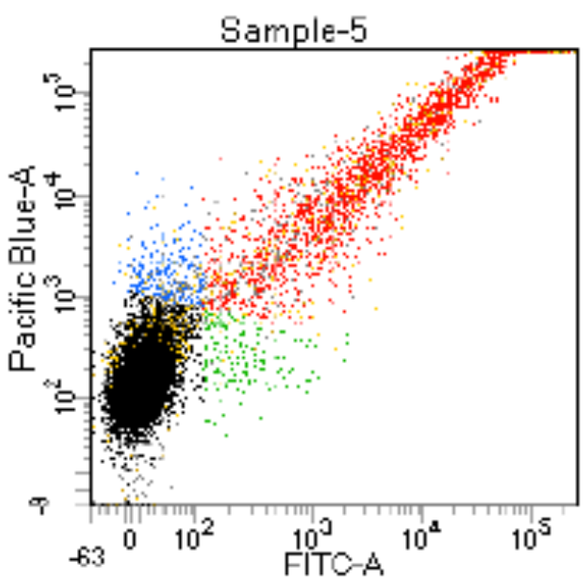












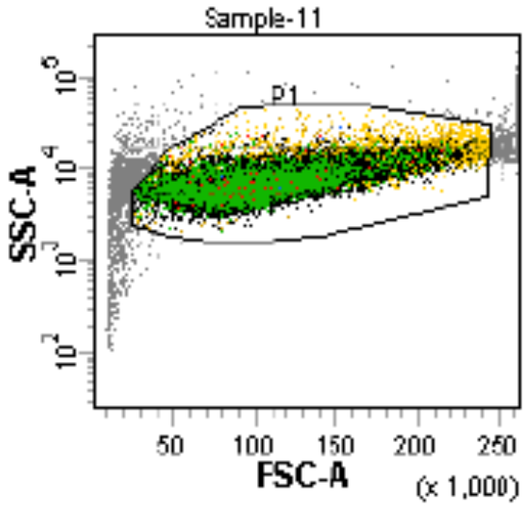
Tube: 5

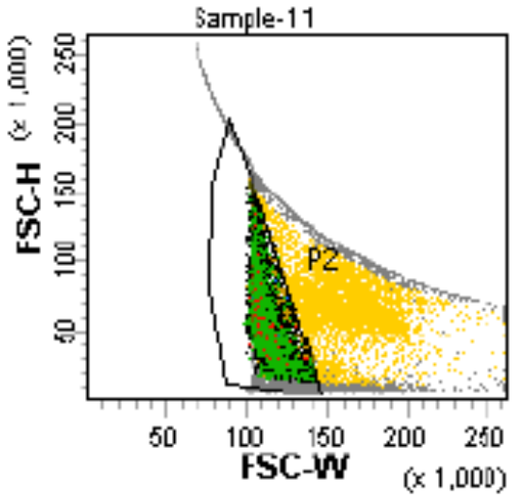
Population	#Events	%Parent	%Total
All Events	12,610	#####	100.0
P1	11,831	93.8	93.8
P2	10,466	88.5	83.0
P3	10,010	95.6	79.4
Live Cells	10,000	99.9	79.3
Blue	1,700	17.0	13.5
Green	1,670	16.7	13.2
Both	1,522	15.2	12.1

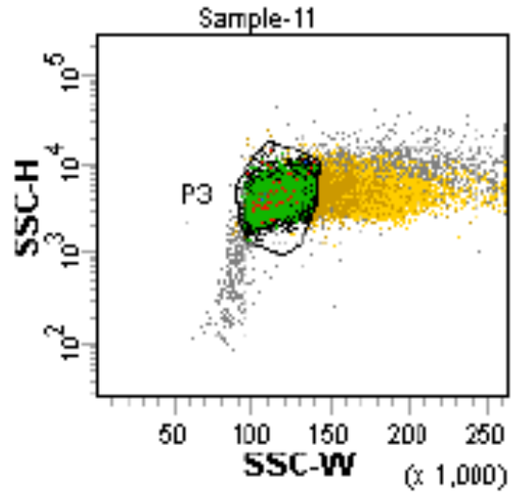
Experiment Name:	20-109 Lab 20150402
Plate Name:	
Specimen Name:	Sample
Tube Name:	5
Record Date:	Apr 2, 2015 3:36:40 PM
$OP:	IsaacChaim
GUID:	1fb4f300-779a-4443-aa40-1e406...

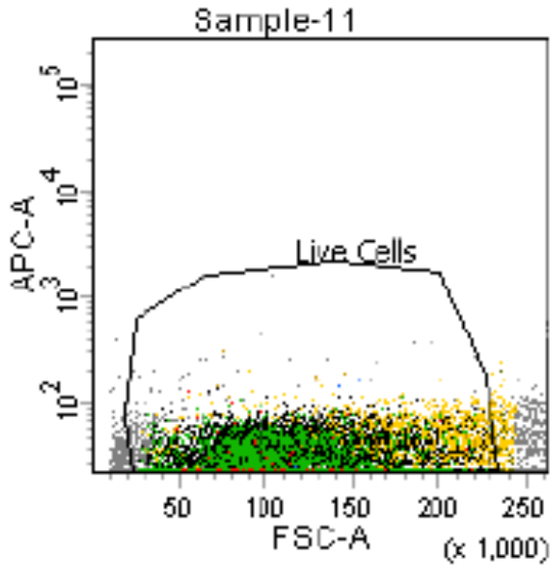
Population	#Events	%Parent	FITC-A Mean	FITC-A Median	Pacifi... Mean	Pacifi... Median
Live Cells	10,000	99.9	1,910	14	8,422	146
Blue	1,700	17.0	11,160	2,583	48,752	14,496
Green	1,670	16.7	11,384	2,721	49,407	15,068
Both	1,522	15.2	12,460	3,527	54,182	19,257

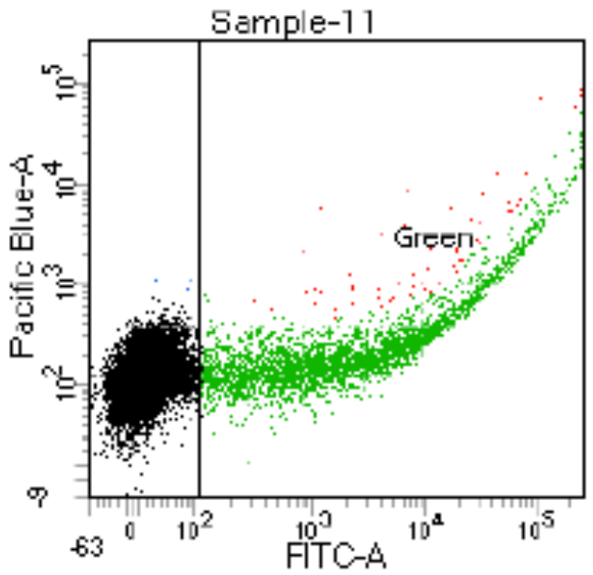
BD FACSDiva 8.0

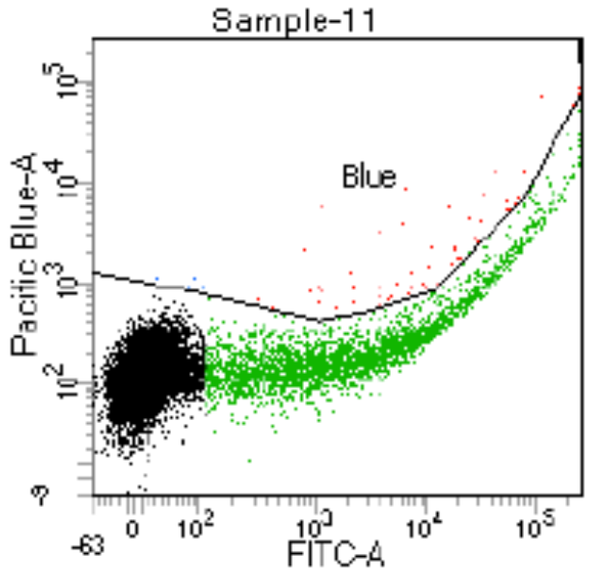


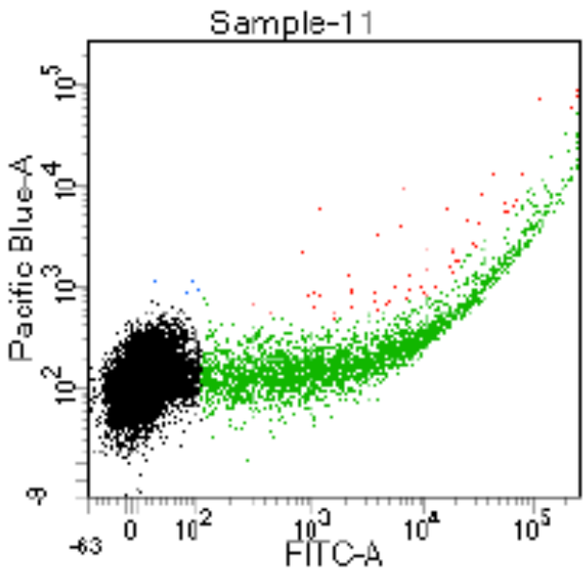


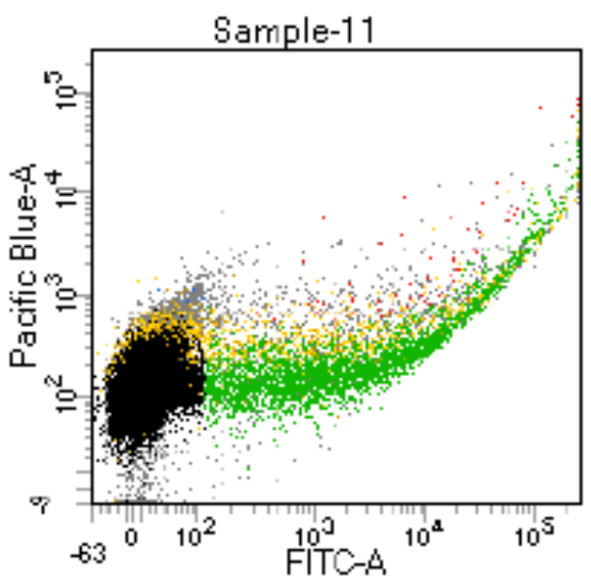












Tube: 11

Population	#Events	%Parent	%Total
All Events	27,158	#####	100.0
P1	24,432	90.0	90.0
P2	20,815	85.2	76.6
P3	20,033	96.2	73.8
Live Cells	20,000	99.8	73.6
Blue	60	0.3	0.2
Green	2,849	14.2	10.5
Both	56	0.3	0.2

Experiment Name: 20-109 Lab 20150402
Plate Name:
Specimen Name: Sample
Tube Name: 11
Record Date: Apr 2, 2015 3:44:03 PM
$OP: IsaacChaim
GUID: d457ec27-a25b-4a61-bec8-a4da...

green blue

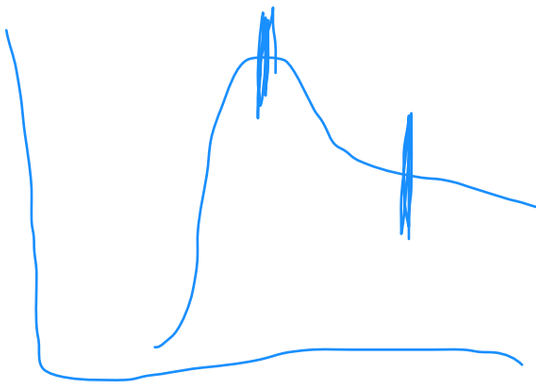
Population	#Events	%Parent	FITC-A Mean	FITC-A Median	Pacifi... Mean	Pacifi... Median
Live Cells	20,000	99.8	1,603	15	215	121
Blue	60	0.3	29,212	8,259	7,445	1,395
Green	2,849	14.2	11,178	1,519	750	175
Both	56	0.3	31,294	10,240	7,505	1,606

How to calculate % NHEJ:

1) Calculate RAW data

(1 BFP)(MFI) = RAW BFP

mean/median fluor intensity



2) Calculate NORM data

$$\left(\frac{RAW_{BFP}}{RAW_{BFP}} = NORM\right)_{damaged}$$

3) Calculate % NHEJ

$$NORM_{damaged} / NORM_{intact} = \%\ NHEJ$$

Today in lab:

2pm — paper discussion with Prof. Samson

~3pm — process your flow cytometry data

by 5pm — send me your spreadsheet