M2D6: Data Analysis + Paper Discussion

Announcements:
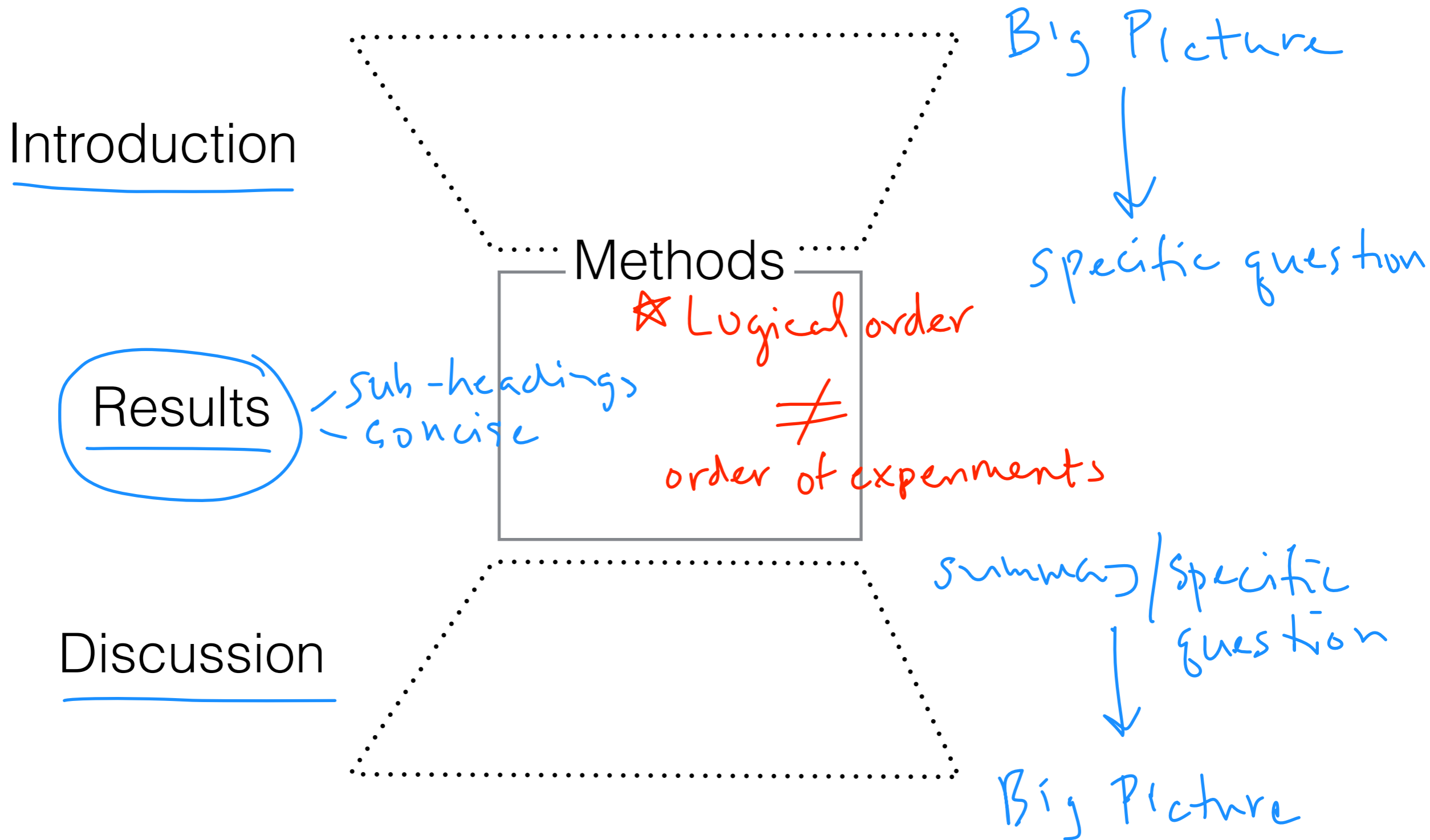
Monday 4/20 @ 5pm

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

Resources you have available:

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

IMRaD Research Paper Structure

Introduction

Methods

Results

Discussion

Big Picture

↓

Specific question

★ Logical order

≠

order of experiments

- Sub-headings
- Concise

summary/specific 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.
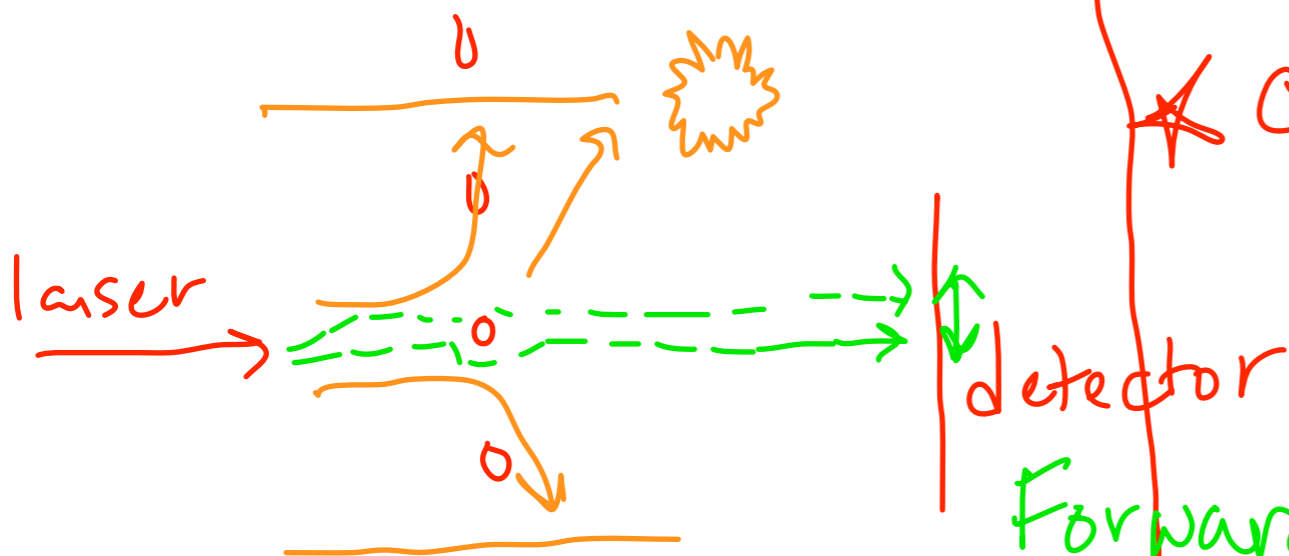
Finally, the findings that multiple known FDA-approved drugs have activity as DSB repair inhibitors and tumor cell radiosensitizers raise the possibility that these agents can be readily tested in clinical trials as radiosensitizers in the near future. As discussed earlier, GBM tumors would be an ideal 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).

✱ Motivation
✱ Background – define terms

✱ citations

↓ Hypothesis + preview of your
results

Results

past tense
* 1st person OK → don't overdo it

motivation for specific experiment

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.

← exactly what was done

← describes data

1) Motivating sentence → topic sentence Why?

2) What you did → what you found

3) Summary → In sum, it was ....

Discussion

- Interpretation here

- puts the data in future context

- <u>Why</u> are the data important

- Admit your faults

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).

⭐ Close with a pie-in-the-sky statement

laser

detector

Forward Scatter ∝ size of cell

SSC = side scatter = granularity ∝ health

BD FACSDiva 8.0
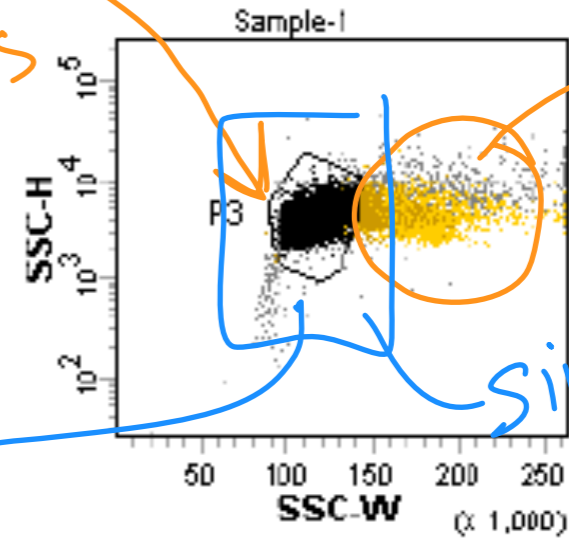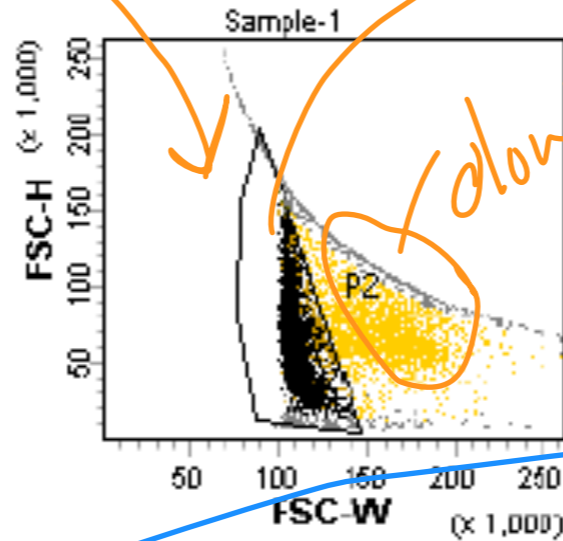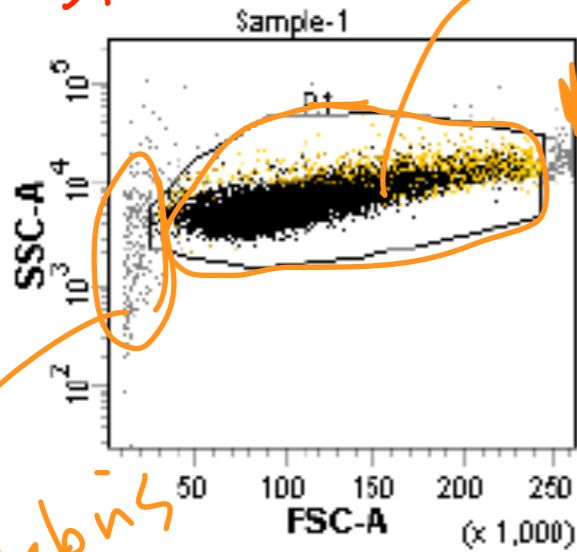
Annotations on flow cytometry gating plots:
- untransfected
- doublets
- dust cell debris
- single cells
- TOPRO3 (for red fluor.)
- Live Cells
- +GFP (GFP)
- +BFP (Blue)
- Green
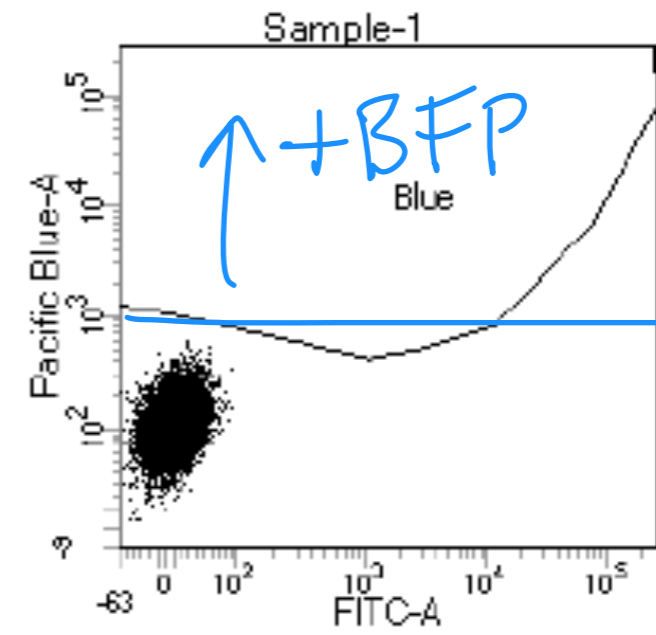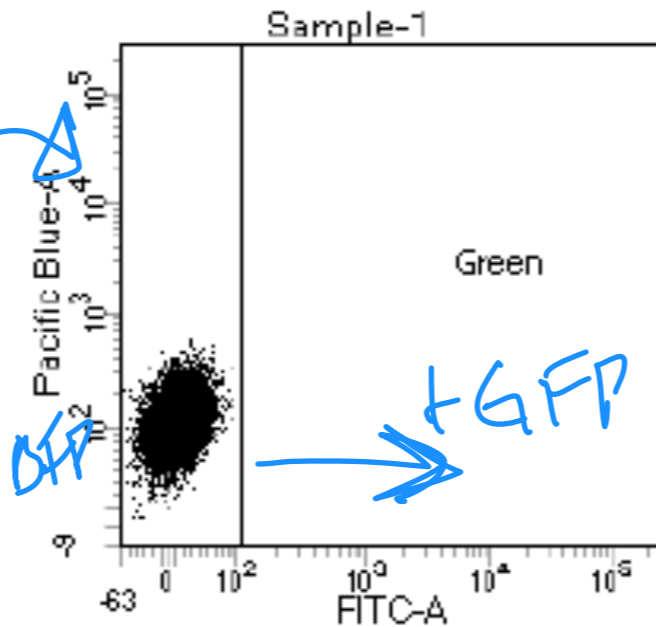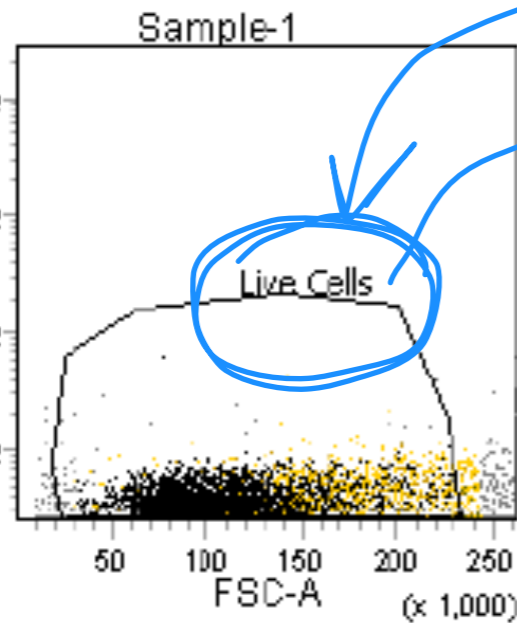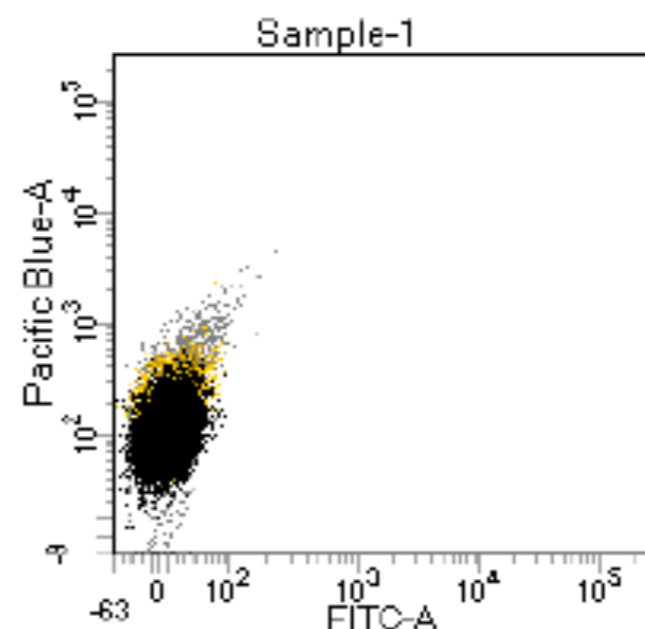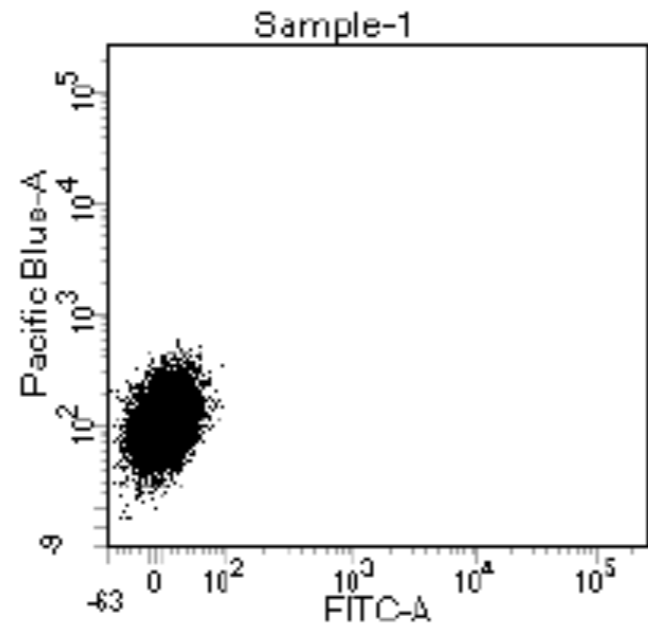
Tube: 1

Population	#Events	%Parent	%Total
All Events	13,284	####	100.0
P1	12,439	93.6	93.6
P2	10,633	85.5	80.0
P3	10,214	96.1	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

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	####	####	####	####

BD FACSDiva 8.0
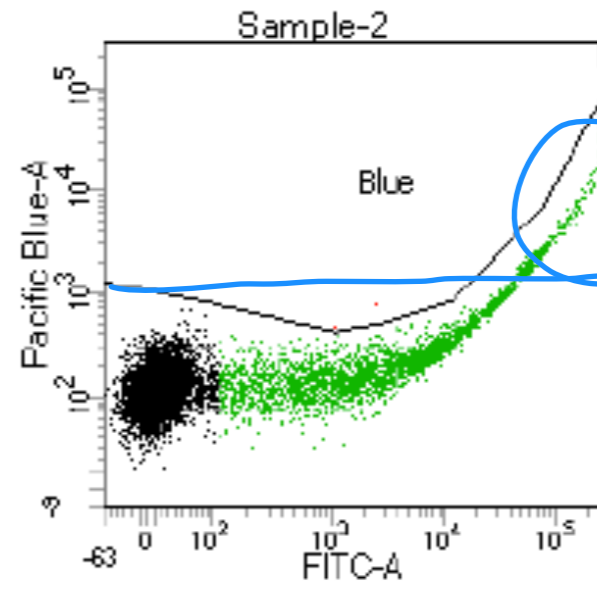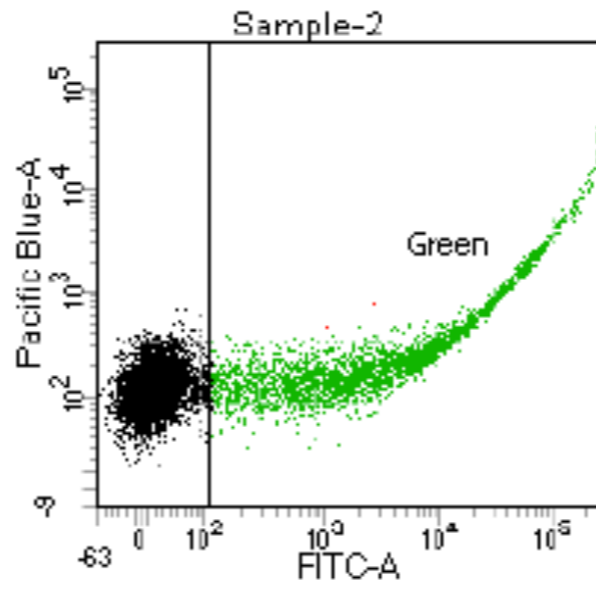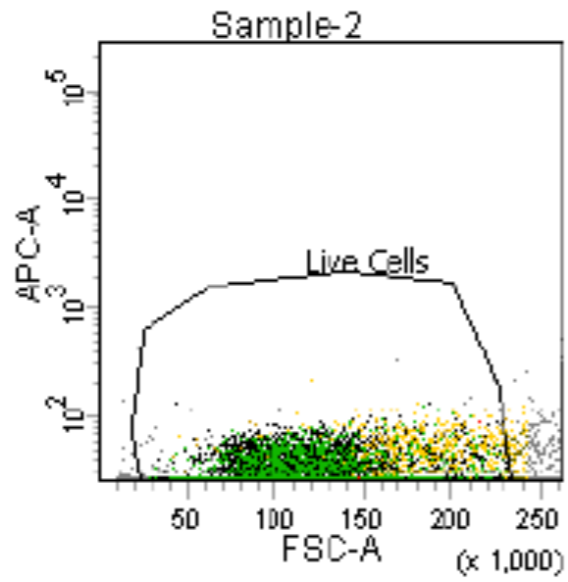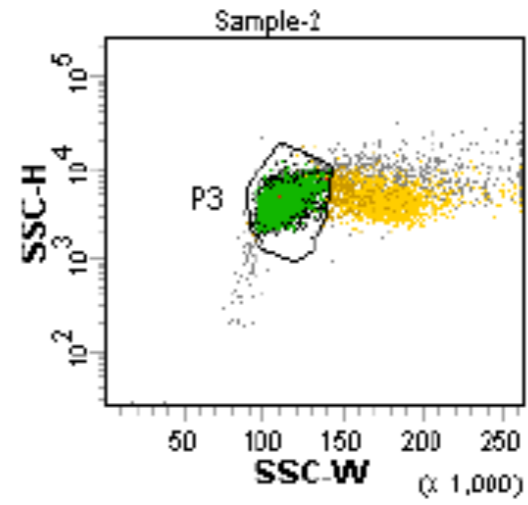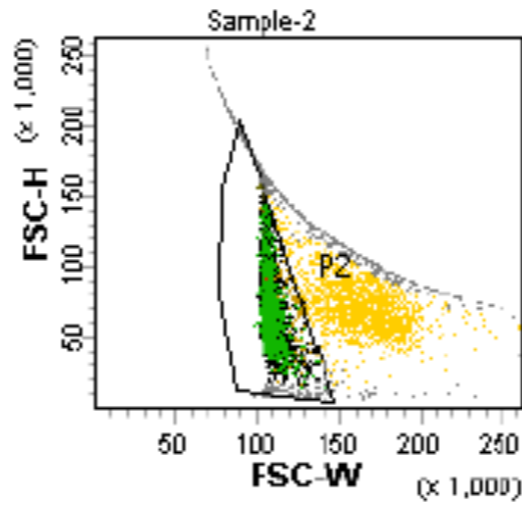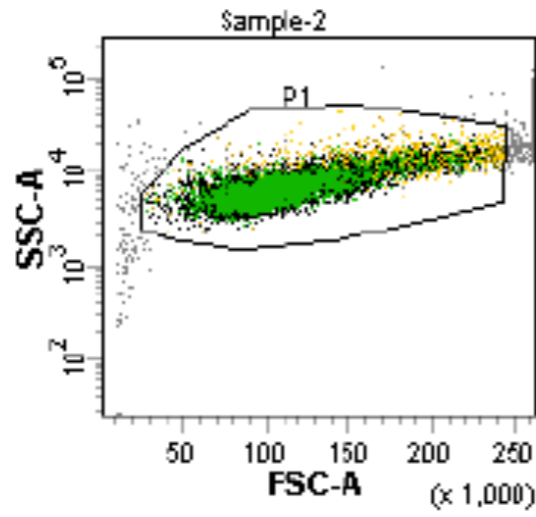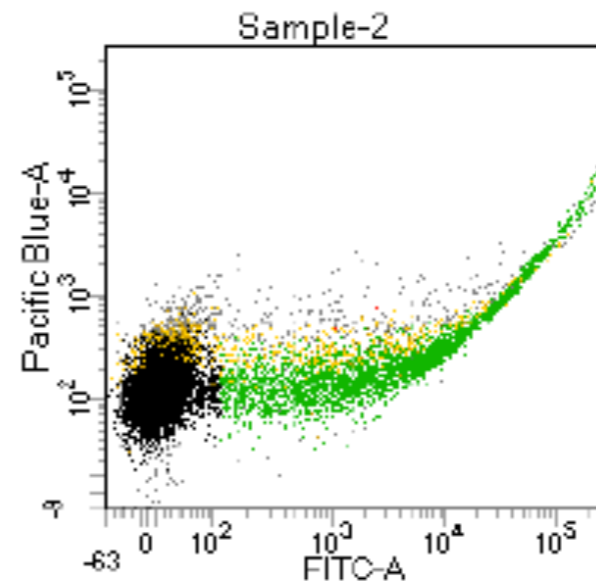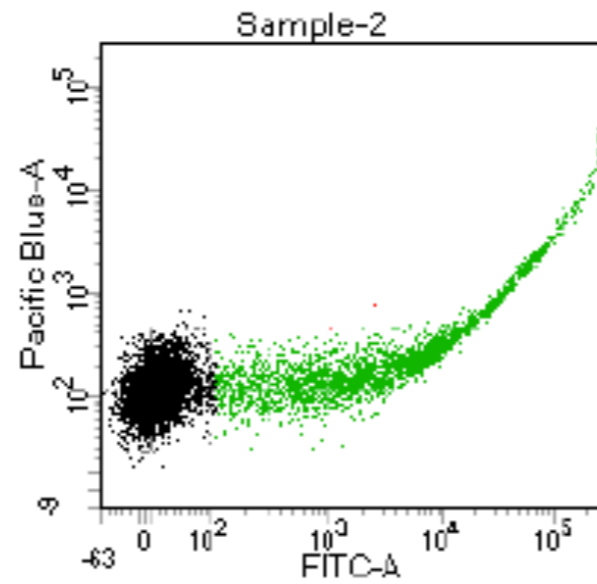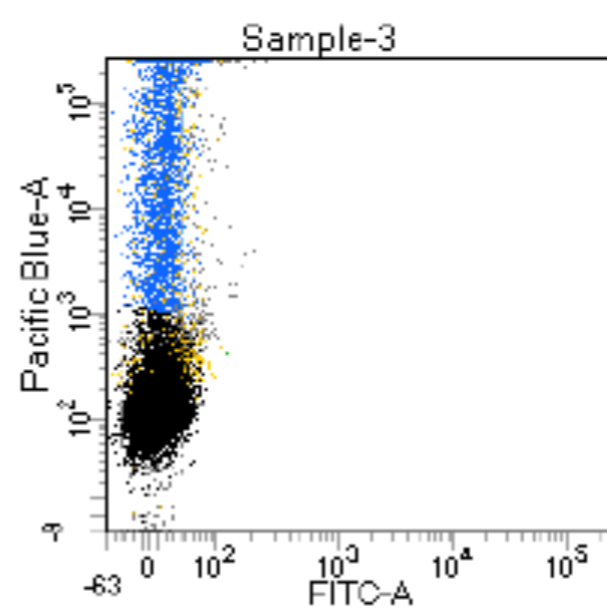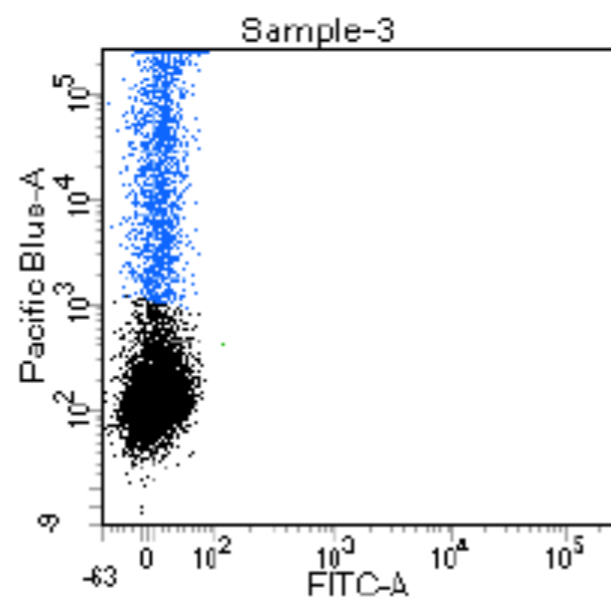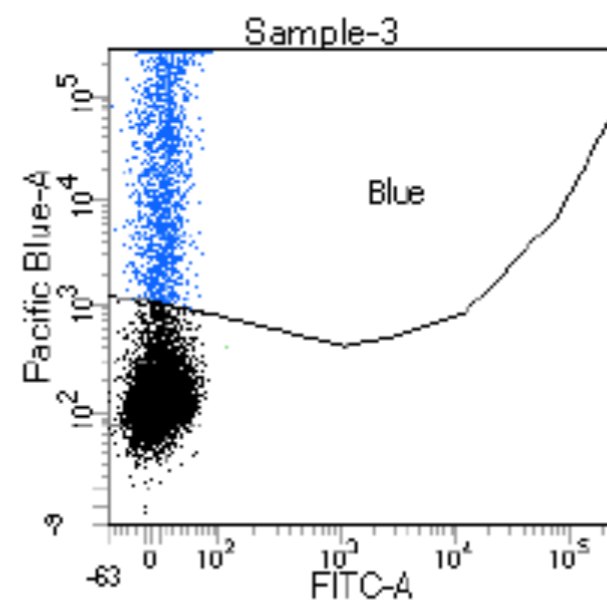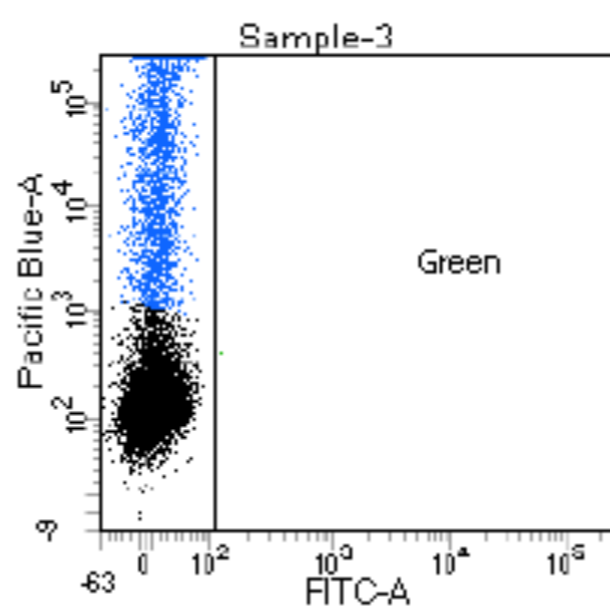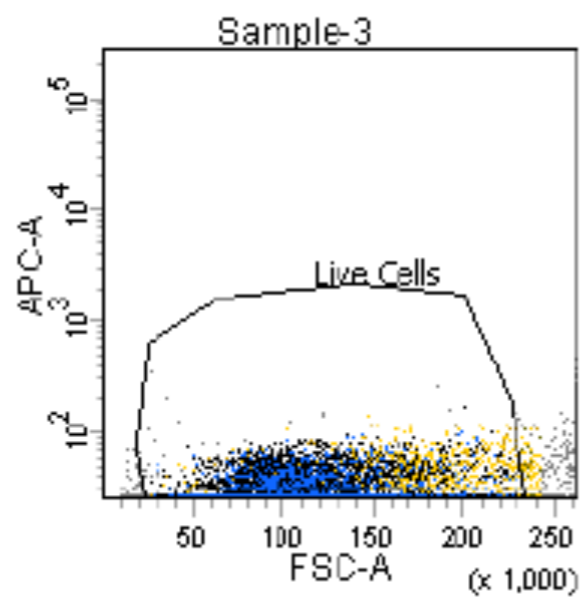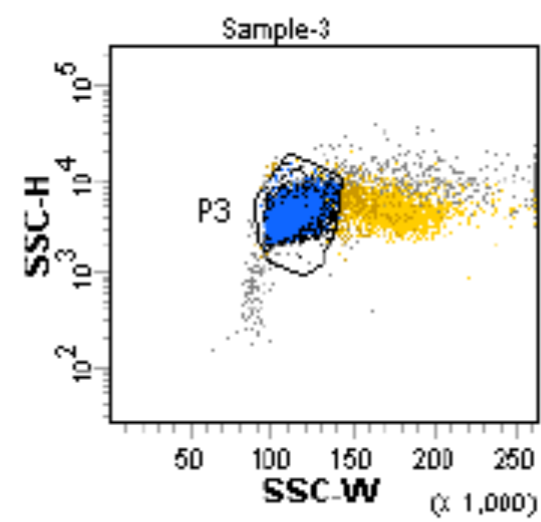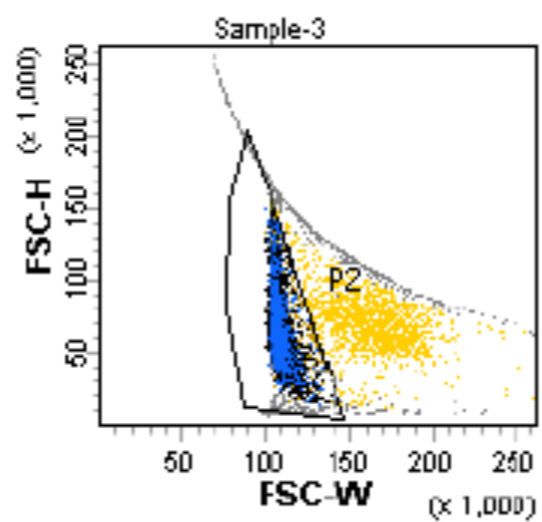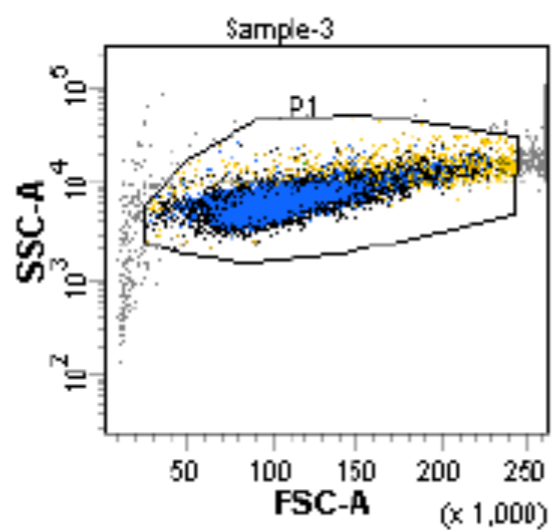
BD FACSDiva 8.0

BD FACSDiva 8.0
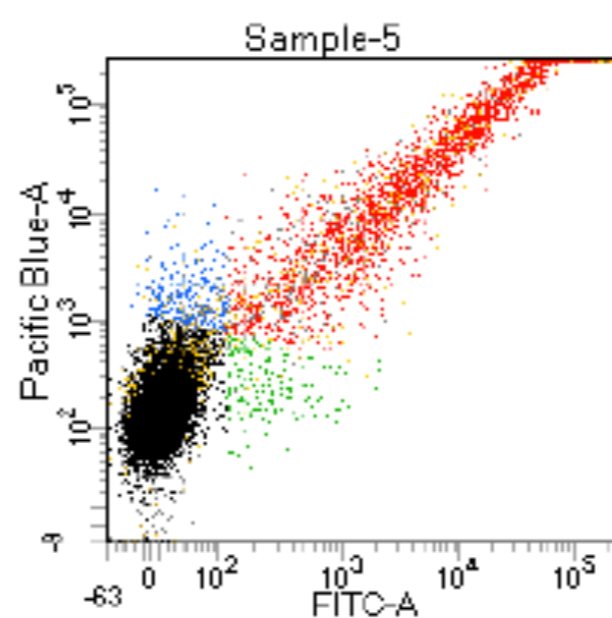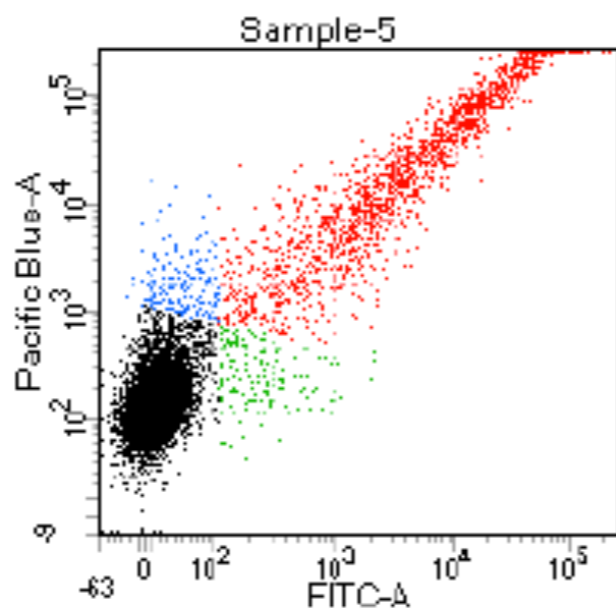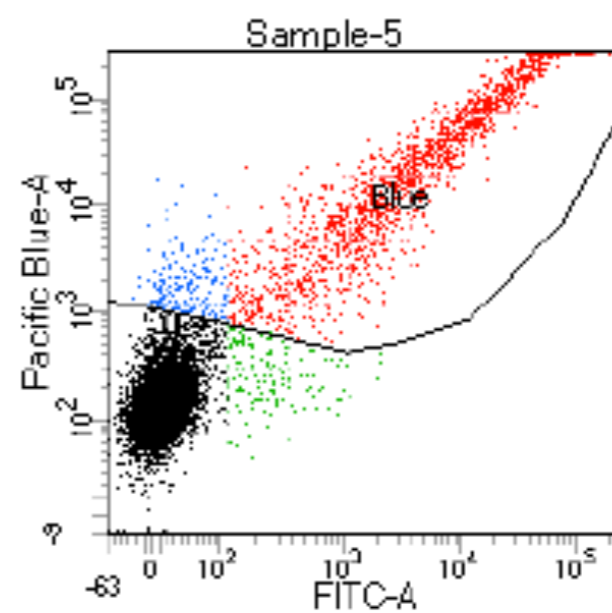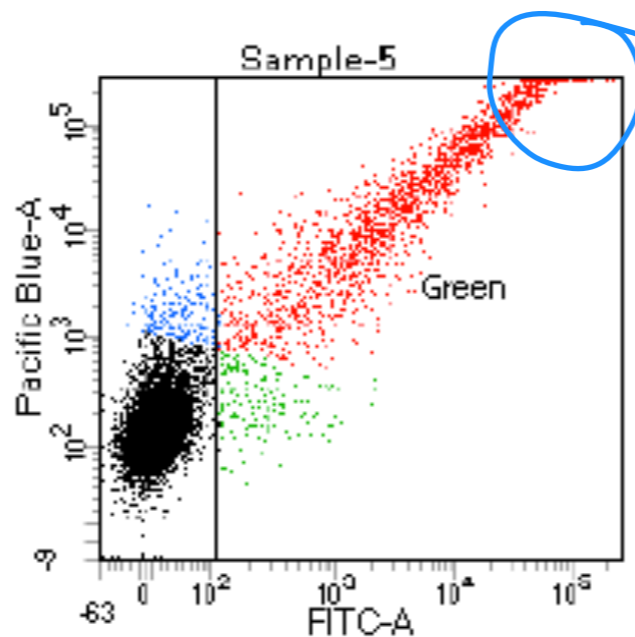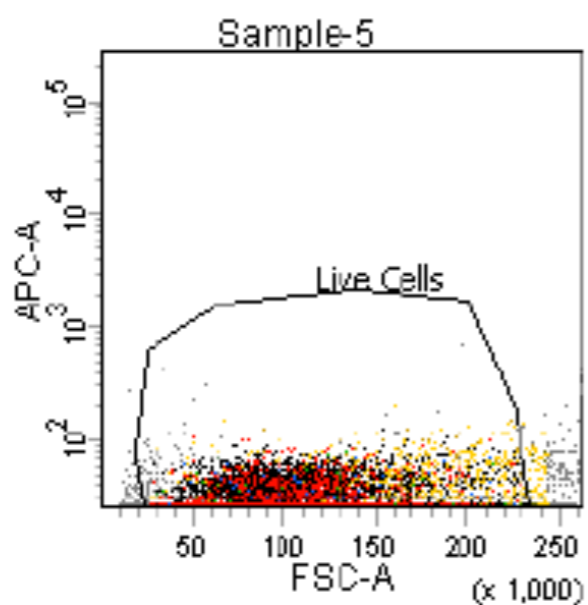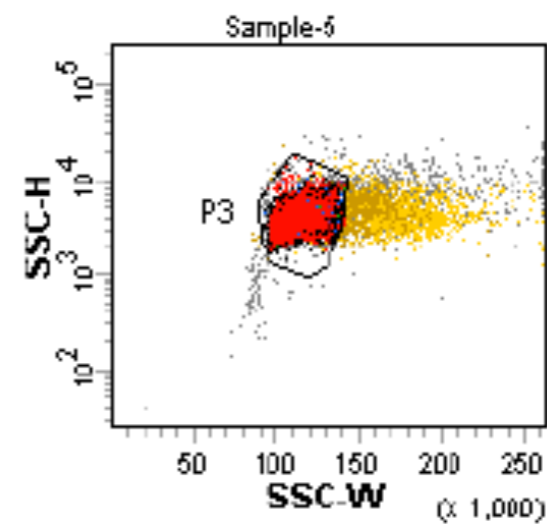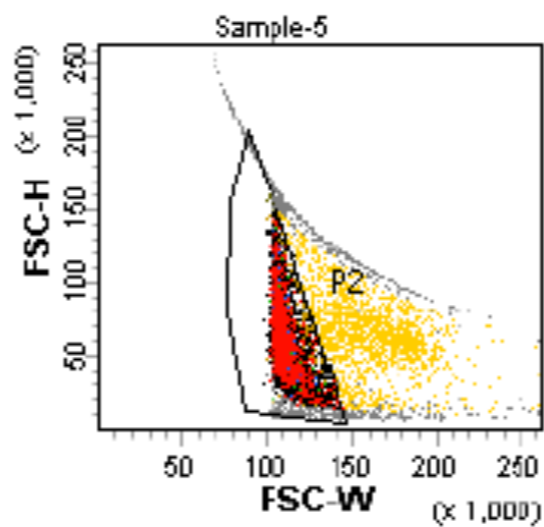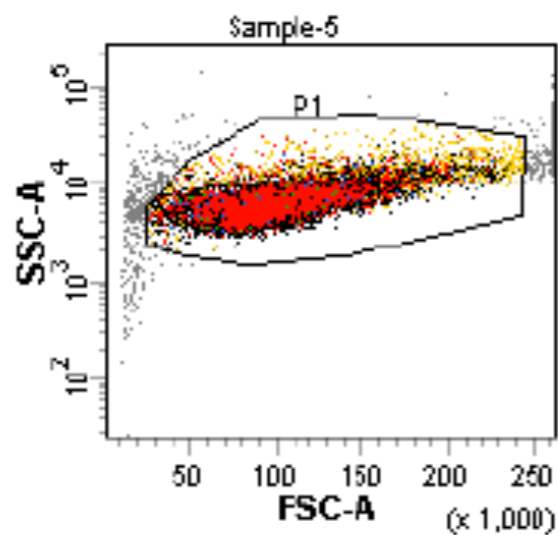
Tube: 5

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

(Live Cells row circled)

Experiment Name:	20-109 Lab 20150402
Plate Name:	
Specimen Name:	Sample
Tube Name:	5
Record Date:	Apr 2, 2015 3:38:40 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

(Blue and Green rows circled)

MFI

BD FACSDiva 8.0
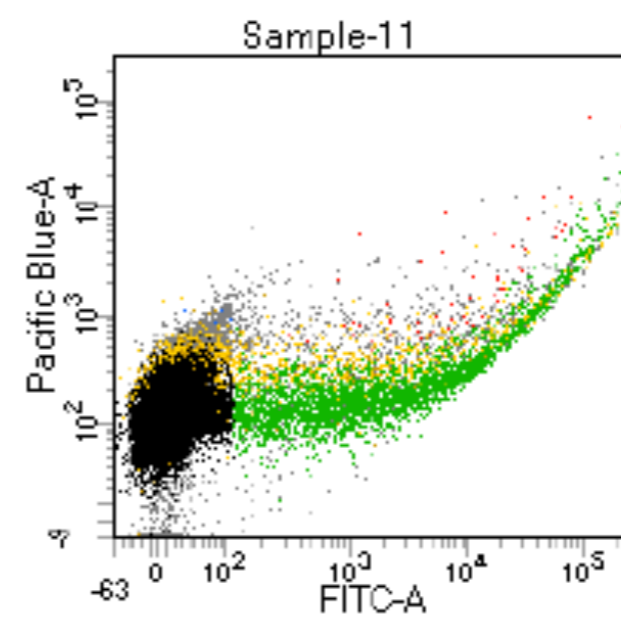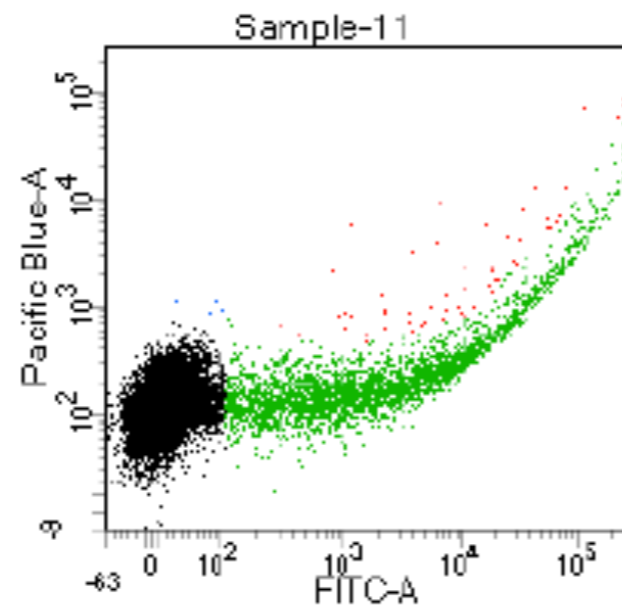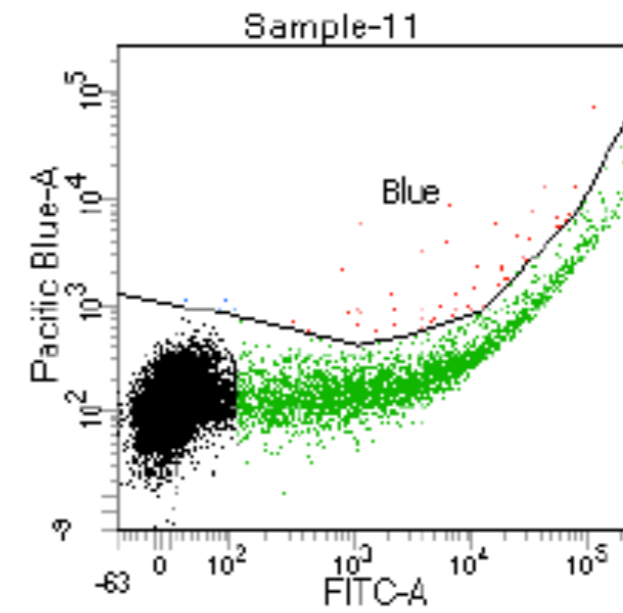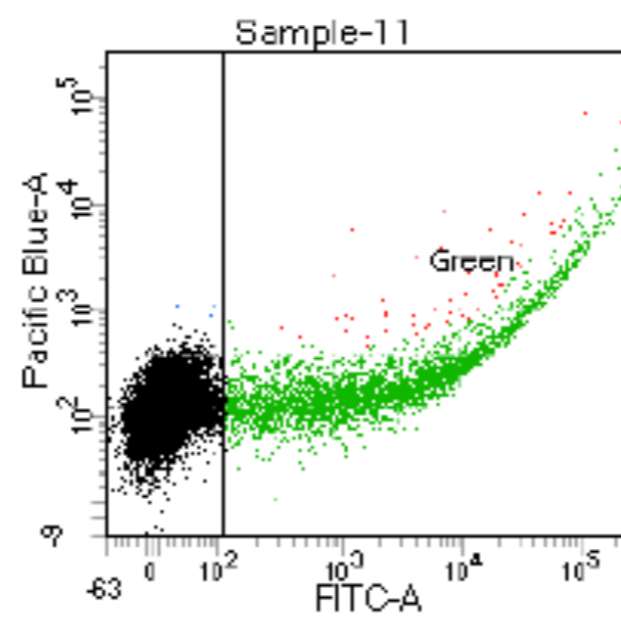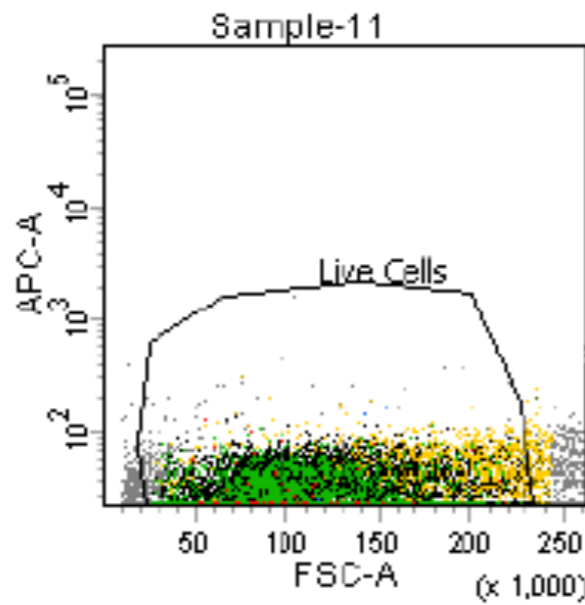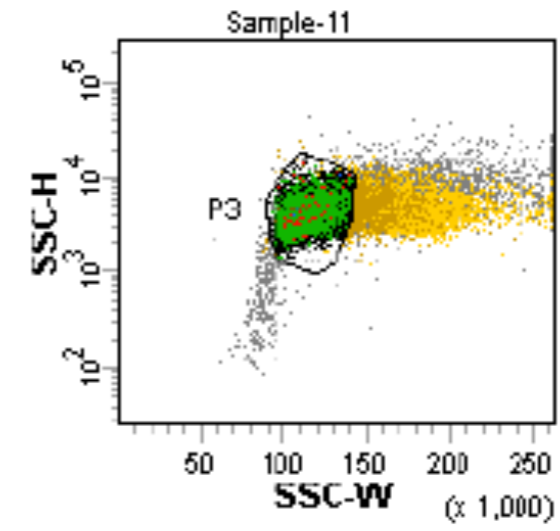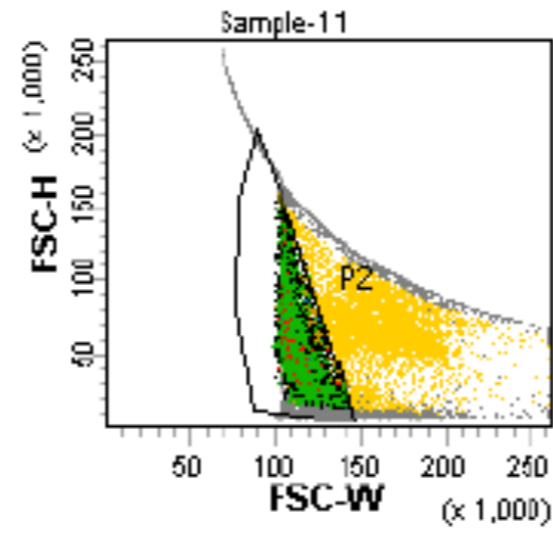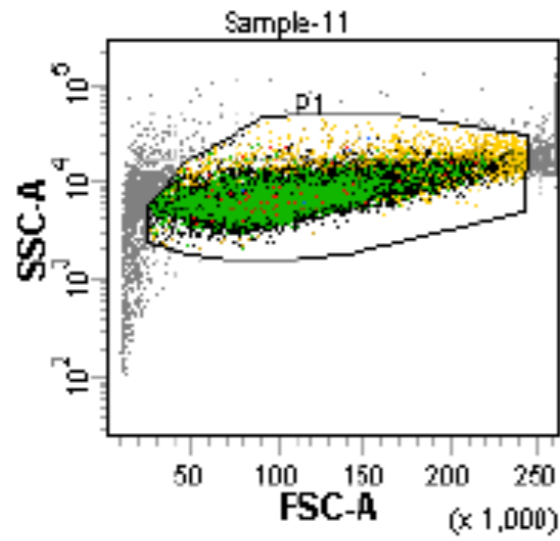
Tube: 11

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...

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	31,254	10,240	7,505	1,898

How to calculate % NHEJ:

1) Calculate RAW data

$$(\% BFP)(MFI) = RAW_{BFP}$$

2) Calculate NORM data

$$\left(\frac{RAW_{BFP}}{RAW_{GFP}} = NORM \right) \quad Damaged$$

3) Calculate % NHEJ $= \dfrac{NORM_{Damaged}}{NORM_{Intact}} \leftarrow$ Most repair

Today in lab:

2pm — paper discussion with Prof. Samson

~3pm — process your flow cytometry data

by 5pm — send me your spreadsheet