~~August 17th, 2022~~
Sept 15th, 2022

NK & ILT panel
~~70 SFC Panels #1~~

1:100

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3.0 M ~~1.5E+6~~	.2 M 0.5 E+6
280c/µl NY059	Adult		10^7	12-11-15		3	5.56	7.1	16.7	540µl	36µl
300c/µl NY059	Adult		10^7	12-11-15		3	6.23	7.96	18.7	482µl	32µl
									35.4		

2nd tube melted by dump into 15ml

~12 wells
(3 sc teplicates)

Count @ 12:13pm
count @ 12:26pm

O 100K | O
O 100K | O
O 100K | O
O | O
O | O
O | O
O | O
O | O

O ILT panel (w/ modified intracellular step)
O ILT panel (as is)
O + CD16 on APC ✓
O + CD16 on AF350 ✓
O + CD7 on APC
O + CD7 on BV711
O + CD16 on APC + CD7 on BV711 ✓
O + CD16 on AF350 + CD7 on APC
~~O + CD16 on AF350 + CD7 on BV711~~ ✓

] duplicate, semore, etc.

Monocyte Panel ?

Previously — Single color unstained
NKG2A
CD28
NKG2A CD28
CD127(1)
CD127(2)
+NKG2A
| CD28

Perf
GrzmB
Perf GrzmB
127(1)
127(1)+Perf
127+1 + GrzmB
NKG2A+Perf
NKG2A+GrzmB
Perf+GrzmB+CD127 711 //

8 samples → 21M cells ~~samples~~
+ 3-6M cells sc
24-28
(7M) (run sc controls for gd panels)

22M cells
~~24M cells~~
↓
18M cells + ~~8~~41 sc's//
~200K cells//

//Panel + CD16 AF350
Panel + BUV737 CD27//

"PeacoQC webinar 1-2pm, data planning happened @ this time."

Running ILT panels w/o stimulation (neglible cytokines/degranulation)
- no antibodies
- Yes antibodies

* no controls : smaller ↓ complexity panel
Yes controls : > N×M matrix than actual
* no controls : assumptions violated
Yes controls : = N×M matrix (overestimate?)

// 3:30pm

PMA concentration ~~for 3M cells?~~
OOOO
×.200 = .8
30 mill conc
Ala gd protocol //1.5 million in 500µl
1µl ↑
/~125µl @ end//

Spiked in CD107A
& Cytokines (w/ 10µl Brilliant)

4 • 494 (2.75) → 1.976 ↑4ml 24µl
4 • 441 → 1.765 ↑4ml 24µl

9000
45

#1 → 270µl + 230µl R10 + 1µl PMA
In @ 4:01pm → 6:56pm
5:12pm
5:18pm CD107A spiked in
5:45pm Abs prepped
6:11 reagents prepped

6:15 specimens from 37° pulled ← cris
6:26 spin
6:38 L/D → 6:53
(sc extra tube fullup from both a/d into ILT) 4+9=13
7:00: PMAed distributed, 0.6µl CD107a to 100µl added ~~both reable~~ for CD107 & single color
Tetramers in @ 7:09pm → 7:49pm

August 17th, 2022

~~78 SFC Panels #1~~

RAM for flowJo to cso
↳ IT more strict

Tets out @ 7:49 pm
scls in spin 7:15 pm
PMA-CD107 out @ 8 pm
Scs out wash @ 7:32pm
7:44pm Hot scs in → 8:15 pm
4/D5c in @ 8:13 pm

Samples 37°c in @ 8:16 → :46
(+1µl of new abs to corresponding tubes, would've been better @ 4°c? yes. Did I space that? also yes)
8:19 37°c sc RBC lyse → :31

8:26 pm unstained CD107a RBC lyse →
RBC lyse 4/D5c 52
8:48 pm first cold batch in → 9:18 pm

Samples hot wash → 8:53 → 8:59
9:02 Cold stain samples → 9:32
2nd cold scs in @ 9:11 → 9:41
(Cur 6 scnB extra spin "intracellular" added absent)
9:18-19 RBC lyse cold scs fresh wash @ 9:23 → into waiting
Scs intra + samples @ cold 9:35 rbc lyse. wash @ 9:40 pm
2nd cold rBc lyse @ 9:43 pm
9:55 all @ checkpoint II
9:59 pm samples intras Fix → 09 → 19 / 20 1st wash
scs @ 22:04 → :14 → :24
2nd / 1st perm wash combo @ 22:32
Intracellulars @ 10:59 → 11:40
10:44a 2nd wash in

Bulk scs done @ 11:18 pm

Done @ 11:56 pm

~~August 17th, 2022~~

Sept 16th: Aurora on @ 7:00 AM — ~~PD-SFC Panels #1~~

experiment setup @ 7:31

Δunmixing control selection from experiment menu.

included added extra sc's for gd swap (if needed)

~ since uncertain how many sc's reanch in tubes

remember use monocyte unmixing for CD14

collecting 5000 events for sc's will circle back as needed.

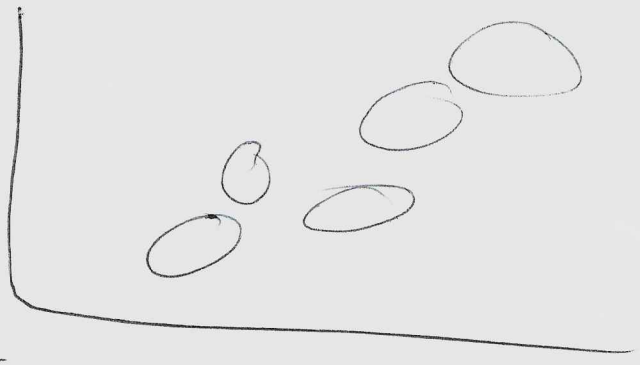
5K PD1 +25000

CD25 +

CxcR3 +2011

AF350 CD16 +50 K

Just acquiring 100µl ~ 1.5M events for samples



Finished @ ~9:45 am