

May 9th, 2023

4L vs 5L

[illegible]

11:30 am start
 11:52 am PNAse
 12:03 pm stain count
 12:13 pm count start
 55pm Inarb

7.03 pm sample spin

Hot @ 2:17pm

8pm < everything started >

Hot @ 8:18 pm

Hot sc's @ 8:46pm → 9:16 pm ✓

sc combies @ 8:48pm → 9:03pm

Sample spin @ 8:52

cold sc's @ 9:01 → 9:31

VaT's @ 9:05 → 9:35

Combined spin @ 9:35pm

Cold samples @ 9:46 → 10:16 pm Ish

Sc's Fix Perm @ 9:51 → 10:01 → 10:11

Rbc ly se @ 22:18 → 21

Spin @ 10:16

Samples Fix @ 10:32 → 42 → 52

Intra sc's @ 10:51 → 11:31pm

Samples 1st perm @ 10:53

2nd spin ✓

Beads + 1ml PBS spin 500g 5min @ 11:04pm

Samples Intra @ 11:15 → 11:55

bead sc's first set 11:30 → 12:00 am

2nd set 11:43 → 12:13 am

Spin @ 00:02

Spin @ 00:13

Cell sc's done @ 11:46pm

Final cell spin @ 23:58 pm

00:24 am FixPerm Beads → 34 → 44
 36 ✓

1ml FBS

36

top
 17409/pl
 $a_1 < 10.7 \text{ pl} > 1.5 \text{ ml}$
 8.6 E6 12.6

top
 1500 c/pl
 $a_2 < \text{pl} > 1.5 \text{ ml}$
 11.5 E6 16.5

12.9

17.3

394-6
 SW9N a-3
 SW9P a-4

.6/10.5

$$\frac{30.15}{3 \text{ ml}} = 10.5 \text{ c/ml}$$

$$\begin{array}{r} 31 \\ \times 6 \\ \hline 186 \end{array}$$

$$\begin{array}{r} 5 \\ \times 6 \\ \hline 30 \end{array}$$

$$3/10.5 = 286 \mu\text{l}$$

10.5 wells

dil			PMA
0	0	0	0
0	0	0	0

$$\begin{array}{r} 30 \\ \times 3 \\ \hline 90 \text{ ml} \rightarrow \end{array}$$

$$\begin{array}{r} 500 \\ \times 3 \\ \hline 1500 = 6 \mu\text{l} \end{array}$$

$$\begin{array}{r} 500 \\ \times 72 \\ \hline 3500 \text{ Mpl} \end{array}$$

Ex-vivo gd-CK: PMA-ionomycin									
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	2	Intracellular 40min @RT	2
1	V450	BV421	PD1			2	4		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD27			1.5	3		
4	B530	Alexa 488	Vδ2			1.2	2.4		
5	B710	PerCPeF710	CD3			1	2		
6	Y590	PE	CD56			0.5	1		
	V615	PE-Dazz							
7	Y780	PE-Vio770	IFNγ					0.6	1.2
8	R670	Alexa 647	TNFα					2	4
9	R780	APC-Fire750	CD45RO			2	4		
Antibody Total						8.2	16.4	2.6	5.2
PBS						12.3	24.6	17.9	35.8
Pipette draw volume / sample						19.5		19.5	

002
 1/10/002
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 1/10/002
 002
 1/10/002

#	Filter	Single color (u)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial lot #	During stim!!!	6	L/D 15 min (RT)	Tetramer 40 min @ RT	HisStain 30min @ 37C	6	Coldstain 30min @ 4C	6	RBC Lys. Perm	Spiked 30 min @RT	6
1	UV2				BUV395	CD261	SK11												
2	UV7				AF	AP-UV6													
3	UV9				BUV496	CD8	RPA18												
4	UV10				BUV563	CD69	FN50												
5	UV11				BUV615	CCR4	101												
6	UV14				BUV661	VC2	86												
7	UV16				BUV737	CCR3	1C6/CCR3												
8	UV1				BUV805	CD4	5G3												
9	UV3				BUV421	CD127	AD19D5												
10	UV5				Pacific Blue	CD14	M5E2												
11	UV7				Pacific Blue	CD19	S2SCL												
					BUV480	CD161	HP-3G10												
					BUV510	CD45RA	H100												
12	UV10				BUV605	CD46	5JH11												
13	UV11				BUV650	CCR7	G04H7												
14	UV13				BUV711	CD7	M-1701												
15	UV14				BUV750	IFNγ	B27												
16	UV15				BUV786	CCR6	11A9												
17	B2				ETC/AF488	VC2/VC2id	6B11												
18	B3				Spark Blue 550	CD3	SK7												
19	B4				PE	NG2D	1D11												
20	B6				PE-CF594	TNFα	MAB11												
21	B8				PE-CY5	CD25	M-A251												
22	B10				Pacific-CY5.5	CD26	PD1 3.1.3												
23	B13				PE-UV770	PD1	308												
24	R1				APC	CD16	SC10												
25	R2				Alexafluor647	VC2/VC2id	H4A3												
26	R4				APC-7700	CD174	N/A												
27	R6				Zombie NIR	L/D	N/A												
28	R7				APC/Fire 750	CD27	O23												
29	R8				APC/Fire 810	CD38	H172												
Antibody Total									6.0	36	Antibody Total	17.8	107	12.5	75.0	8.3	49.8		
R10 Media									14.5	87	Brilliant Stain	50	300	50.0	300	12.2	73.2		
Pipette draw volume /sample									19.5		Pipette draw volume /sample	65		59.5		19.5			
And UNSTAINED CONTROLS !!!																			

Simplified Protocol

Thaw cells, DNase, count.

Collect, count, aliquot cells 2.30E+6 cells R10 5ml polypropylene tube
Bring volume upto 1 ml R10, add 2 ul PI/ACtH and CD107a
Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 8min
800 ul of Live/Dead mix (1:2500) @RT for 15min
Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add HisStain mix, incubate @37C for 30 min
Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Add Tetramers, incubate @RT for 10 min
Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Add ColdStain mix, incubate @4C for 20min
Add 300-500 ul 1x RBC Lysis for 3 minutes
Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

300 ul BD FixPerm, incubate @4C for 20min
(vortex every 10 minutes)

First PermWash: 1 ml PermWash 1500 rpm 6 min
Second Perm Wash: 1 ml PermWash 1500 rpm 6 min

Add Intracellular Stain, incubate @RT for 40min
First PermWash: 2 ml PermWash 1500 rpm 6 min

Resuspend in 70 ul 0.4% PFA-PBS
Cap tubes, wrap rack in foil, store at 4°C

$$1.2 \times 6 = 7.2$$

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IFNγ cells spread
241 copies

$$\frac{1.2}{1.2} = 1.0$$

$$1.2 \times 1.0 = 1.2$$

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21/ 20/20
 21/ 20/20

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4L Acquisition start (QC passed @ 2 PM)

6:08 PM start (40 μ L).

event rate 3,160 \rightarrow about 57
time: 1:15 \rightarrow 278,840 events

the abort rate aligns w/ the noise below axis
 \nwarrow / averaged over time

$\langle e \rangle$ la vie \rightarrow

< AF488 CD3 only 5000 events >

Se's @ 7:32 PM

Laser in early cut looks off. \uparrow @ 7:37

Samples @ 6,000 events/sec \rightarrow about 100
5000 cells

Done core 4L samples 7:49 PM

5L Start @ 8:12 adj to ^{PSC} 61 ^{SSC} 334 ^{SSC-B} 327 to match the 4L
46,472 for RNA zombie adjust threshold to 159000

20:23 start 2850 events \sim 31 μ L/min
33 abort.

\rightarrow ~~40~~ 30 μ L residual

Se's done @ 9:29 PM

Initial SL attempt on Zombie ctrl

Zombie ctrl is off (detected gate off)

202 similarity metric !!

155 for SL

Yep checked cells 11:27 am

→ 5L unmix cells 162 for compIndex

DT R T cell Panels

(FSC, 150000)

Complexity Index

13.9 on a 5L

PMA unmix ~~CD3~~ for Dacile

13.39

4L →

Initial unmix done @ 12:15 pm

SSC-A vs SSC-B A 4L vs 5L

mon ↑ SSC upon PMA

Per CP-GS.5

S-153

E-5201

E-058

h-248

E-6801

L-458

2-525

→ 7-558

h-158

1-0501

9-28-b

Discard

4-04

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