October 19th, 2024

PDI PE vs. RB613
(spectral Ghost chase) ↙ to 4Eq

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
Inf136 a2	HU S74T					1		16E6	↑3.0
ND006			10E7	8/22/24		1		10E6	↑1.5

by DNAse 30µl

Saturday, oct 19th:

Thaw @ 3:02 pm
DNAse @ 3:27 pm
Stain for count @ 3.34
At rest @ 4:04 pm
Stim start @ 22:17 pm (5µl/mL SEB)
Golgi Added @ 00:10 am

PDI PE — PDI RB613 — unst — SEB?
Cord ○ ● ○ ● ● ● ● ● ○
Adult ○ ● ○ ● ● unst SEB ○ media?

Δ? AF ~ impact TBD

FBS lot 1025440 for R10

Spectral Ghosts
Cocereba Gates for all 8 specimens
push Terminal nodes
Contrast Ab#1 vs Ab#2
for that condition & specimen
return nodes Δ expressed above
a cutoff.
↳ derive ~~best complexity~~ marker configs
derive for both configurations
the theoretical complexity
& visualize Δ areas
shaded overlap
⊙→ pinpoint the conflicts

Saturday Oct 20th
12:27 spindown
12:54 pm Ab spin @ 3 min 13000 rpm
Abs done @
Calc @ 1:40 pm
2:35 sc aliquots spinning
L/D @ 2:54 pm → 3:10 pm spin @ 3:24 pm
3:01 pm Hot cls → 3:31 pm spin @ 3:34 pm
3:09 pm cold sc's → 3:39 pm
~~3:4 3:27 Full Hot → 3:57 pm~~
3:54 pm sc's Fix → :04 → :14 → 4:24 pm
4:10 pm Full cold → 4:40 pm
4:43 pm RBC lyse → 4:46 pm @ 4:49 spm
4:55 intra sc's → :35 ✓
17:00 → 5:15 pm Strept spin @ 5:16 pm
Main set sub done @ 5:06 pm
5:25 FS. Fix → 35 → 45 → 55 pm
5:43 pm intrac 4th wash

Wash #1
spin @ 4:28 pm
2nd @ 4:41 pm

1st f.s wash @ 5:54 pm
2nd @ 6:02 pm
6:13 pm Intrac → 6:53 pm
3rd wash @ 6:53 pm
Done 7:13 pm

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30 sc's + 2 + 2 (extras, Bead test)
+ 3 unstained

37 − 6 = 31 single × .750 = 31 800 → 24.8
6 cell × .750 = 6 1.600 → 9.6

FBS

48.0

82.4 (wash)

8 × 3 = 2.4 RR
300
+2.7 H_2O

2~~B~~ ctrl
11

36 ctrl units
10 SEB units

~~3~~ ~~sc's~~ ~~2~~
~~3~~ ~~sc's~~

8 f.s = 8.0 = 48.0 +
27.8
85.8 FBS-PBS

9 × 800 = 7.2 ml
LD
NIR

45
× 80
3,600 ml 0.4% PFA-PBS

37 × .4 = 15.6
8 .8 6.4
22.0
ml Fix 6+18

Sunday

7:16 pm: Acquisition prep
7:18 pm Start 180 ul in 95 ul
Both good (more debris in cord) w/ few monocytes !!

¿Vd2 pop?

SEB Inf 136 dry blew dead cells @ bottom

Shutdown Clean @ 8:51 pm

Some chunkyness in CD6 a acquisition

¿2 Δ BV450's.... ??? what? ¿Erickson?

// Weak IFNg today? //

Non-specific + low-staining + Bioscience Nothing

TNFa ✓ signal ... ditto CD62

Δ fluidics ~ laser delay

Δ especially w/ Cy5 PE

Very Δ zombie w/ cord 10^6 vs 10^5

Cord Monocytes have a Y7 R5 platform of AF.

Fixed BV421 gating

13.6 ~~similarity~~ ~~matrix~~ complexity index
→ 13.55 PE
→ for RB613 13.56

The old beads appear to still work?

Unstained ctrl has R5/R8... / ugh...
→ Luminaga it...

Less so for ND006

Samples @ 8:25

33 ul/min
Med 7.5 K events/sec

~1.3 M cells 200 abort rate

Cord run 80 rate

8.2 10.9K 438

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αβ T cell SFC panel

#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!!	8	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	8	ColdStain 30min @4C	8	4°C for 15 min	RBC Lyse, then Fix/Perm	Intranuclear Stain 40 min @RT	8
1	UV2	BUV395	CD62L	(DREG-56)	1							1.2	9.6				
	UV7																
2	UV9	BUV563	CD69	(FN50)	2												
3	UV10	BUV615	CCR4	(1G1)	2					1.8	14.4					0.8	6.4
4	UV11	BUV661	Vδ2	(B6)	2							0.5	4			0.1	0.8
5	UV14	BUV737	CD38		1							0.5	4				
6	UV16	BUV805	CD4	(SK3)	1					1.5	12					0.7	5.6
7	V1	BV421	CD127		1					1.5	12						
8	V3	Pacific Blue	CD14	(M5E2)	2							1.5	12				
	V3	Pacific Blue	CD19	(HIB19)								1.5	12				
9	V5	BV480	CD161	(REA631)	1					1.5	12			Strep <0.4>			
10	V7	BV510	CD45RA	(Hi100)	1							0.5	4				
	V8	BV570															
11	V10	BV605	CXCR5	(J252D4)	2					2.5	20						
~~12~~	V11	BV650															
13	V13	BV711	Vα7.2		2							1.2	9.6				
14	V14	BV750	IFNγ	(B27)	2											1.5	12
15	V15	BV786	CCR6	(11A9)	1					1.2	9.6						
16	B2	Alexa Fluor 488	FoxP3		Beads											3	24
17	B3	Spark Blue 550	CD3	(SK7)	1							1.2	9.6			0.5	4
18	B4	Spark Blue 574	CD8		1							1.0	8				
19	B6	RB613	PD1		1							1.0					
20	B9	PerCP-Cy5.5	CD26	(M-A261)	1											1.8	14.4
21	YG1	PE	PD1	(EH12.2H7	1							1.0					
22	YG3	PE-Dazzle 594	TNFα	(MAB11)	2											1	8
23	YG5	PE-Cy5	CXCR3		2							~~1.0~~ 0.0	8 64				
24	YG8	PE-Fire 744	CD25		2					1.2	9.6						
25	YG9	PE-Vio 770	HLA-DR	(L243)	1							0.6	4.8				
	YG10	PE-Fire810															
26	R1	APC	CD39	(A1)	1					1.2	9.6						
27	R2	Alexa Fluor 647	IL2	(MQ1-	2											1.2	9.6
28	R4	APC-R700	CD107a	(H4A3)	Supernatan	1.2	9.6										
29	R6	Zombie NIR	L/D		2			<1:2500>									
30	R7	APC-Fire 750	CD27	(O323)	1							1.5	12				
31	R8	APC-Fire 810	CCR7		1					1	8						
And UNSTAINED CONTROLS !!!				Antibody Total		1.2		Antibody Total		13.4	107.2	14.2	113.6			10.6	84.8
				R10 Media		19.3		Brilliant Stain		26.8	214.4	28.4	227.2			21.2	169.6
				Pippette draw volume		19.5		Pippette draw		37.2		39.6				28.8	

Wash with 2 ml PBS, spin down 1300rpm 8min
800 ul of LiveDead mix (1:2500) @RT for 15min
Wash 1.5 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add CD161 biotin antibody for 10 minutes at RT

Add HotStain mix, incubate @ 37C for 30min
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

Add ColdStain mix, incubate @ 4C for 30min

Add 300-500 ul 1x RBC Lysis for 3 minutes
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

Add Streptavidin Mix, incubate @ 4C for 15min
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

0.8 ml Nuclear FixPerm, incubate @ 4C for 30min
(vortex every 10 minutes)

First PermWash: 1.5 ml NuclearWash 1500 rpm 6 min

Second Perm Wash: 1.5 ml NuclearWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min
First PermWash: 1.5 ml NuclearWash 1500 rpm 6 min
Second Perm Wash: 1.5 ml NuclearWash 1500 rpm 6 min

Resuspend in 80 ul 0.4% PFA-PBS
Cap tubes, wrap rack in foil, store at 4°C

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