DNASET March 13th, 2023 2023_ILT 02 Specimen Status Location Conc Date **Tasks** Volume Ly Ly+Mon Total 3E+6 .3E+6BOX12 A HU F Inf070-7 4300 3,54 1.77 11 RWKA 44.801 2,47 a-3 3 4.94 Box 9B Inf374-4 HEU-108 860011/ 7.7/ 2 3,85 5.45 SWY 1911 (4) 10.9 Box 2A Inf039-2 HEU-h 7 7.28/ 2 25 pl 3.64 6.56 clund (5) 13.12 Adult ND050 8.18/ 2086 1-13-23 2 Hermal, 4.09 5.93 (1) 11.86 Adult NDOOG 4200/11 15EG 1-20-23 11.74 2 5.87 7,51 2 15.02 INNOUN IN 070 11.29 1 (3) 6.51 9.37 6.51/9.4 70 1640 JP Inf 37 408 SVHZ 3.46 VI 7.49/11 a 7.49 11.0 10 May Informa RUT S 9.58/ 8.78 13.0 (0) 8.13/13 8:04 am Gran deaning 2nd sel: Too concentrated (3+3=9+1.5 8:18am specimen pull 8:28 an Than 8:46 am Inf 070 Spin 10.05 14.34 00000 0 miller 9:03 am DVASC (2.5) Inf 374 19:14 un 15.19 stain for count / 21.90 0 0 0 0 (3 md/m) 12 ft 0 Moioram 2nd than: Inf 039 16,06 26.20 23000 (4million) 16:09 am spin 10:23 am PNtor UE7.2 VE72 ND050 16:29 count stam 10:47 count done (2.1> NDOOG 000 <2.747 11:43 at holding cons conc@3ml 11:36 am c01072 3M 2.5 1.5 M Inf 070 = 3.35 12:09 pm Added PMA \$769× 298) mto mobater Inf374 = 5.06 191 6:09 pm -296 592/1 Info39 = 4.35 689/1 Combination co1072 229 Ab PEPC4:43 pm 733/1 244 N 2050 Prepped @ 5:56 pm NDOOF 20.5 Sample Spin@ 6:19 pm 20.5 15 14+6=20 4PE 6:36pm -> 54pme x .300 12.0 mls 184.5735 6.000 184. 5 PBS Se's aliquoted & spring @ Gittan 40 NFIZ mls RBC 9.6 Va.72 lyse 7:17pm Het samples 5pl in 12.5mls 120 Va24 July -162,9 pB5 -> 41 pm

Tetr @ 8:cgpn - 18:49 pm 4ºc Scis @ 7:41pm -7 8:11pm Samples Spin 1400rpm Gnine 7:52pm Sch Strken @ 8:28 pm -> 8:38pm -> 8.48pm

37°c Ses @ 7.76pm > 56pm

Samples Colde 9:08pm > [38pm]

1st se Remumble Mus pm & min

2nd se Remumble 09:20pm (1500 G.m.)

Most se's 30pl PAT @ 9:41pm

Rbe 1/se samples 09:45pm

Samples final FBS mashe 9:50pm

Sc miras @ 9:57 > [10:37pm]

Samples Fix Ram @ 10:05 > 15 - 7 25pm

Combined Remubsh @ 10:44pm

Combined Remubsh @ 10:44pm

Combined Remubsh @ 10:44pm

Combined Remubsh @ 10:44pm

LSH 7 619.57

Samples into intercellular @ 10:58 _ > 11:38pm

Final spin 11:41pm

Dene @ 11:52 pm, colls resispended in 50 pl

416 hrs 7

+250pl x 12 3 ml +24pl

(5) +2/1 PMN 1.25 ml (TIO/1) 83/1 < 3.5 CHI 290.5 different
+ 2.31/1 find

" olok colors"

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Billy Sequency Red Invanidation Invanidat	700			700	50.0	700		Stain	Brilliant	203	14.5		10 Media	70		STAINED CONTROLS !!!	And UNS
Filty Single-concert December Colorate Color	126			175.0	12.5	266		y Total	Antibod	84	6.0		ntibody Total	A			
Filter Surject-control and off Unministed Favordoone Marker						22.4							НТ2	CD38	APU/FIFE SID		
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Filter Supple-control Red cert Function of the cert Function									<1:2500>				N/A	1/0	ADC (Elec TEC	R7	28
										84	6.0		н4АЗ	E/OTO3	Zambia Nin	R6	27
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Filter Single color Red color Red color	7.0			21.0	1.5								300	CD16	APC	R1	24
Filter Single color(a) Red of Unmining and Parameter Marker Clone Cl	35.0												PD1.3.1.3	PD1	PE-vio770	813	23
Filter Single color (a) Red of Unmining and Pulsays Cone	7.0	0.5	300			70.8							MAb11	TNFa	PerCP-Cy5.5	810	22
Filter Single color(ul) Ref crit Unmining crit Munochronne (Balu/says Cobes). SXII Cone (Rit) (R						10.8							M-A251	CD25	PE-Cy5	88	21
Filtar Single-color(u) Red cit Uninning and Ingenic Clore Clo	7.0	0.5				160							M-A261	CD26	PE-CF594	400	20
Filter Single color(u) Red clit Univier ctr Paris Pari	7.0	0.5		10.0	7.1	21	'n						1D11	NKG2D	PE	0.2	1
Files Single color (a) Red crit Name Author Marker				100	1								SK7	CD3	Spark blue 550	22 8	5 6
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Filter Single color(u) Red ctrl Unmining ctrl Routonine Marker Clone Vial Lot String Vial Lot String Vial Lot Name Vial Lot String Vial Lot Name Vial Lot	21.0					21	1,7						11A9	CCR6	BV786	S vis	1 6
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Filter Single color (u) Ref crit Unniving ctrit Fluorochome Marker Cone Val Lot # 14 (nt) 15 min Teiramer 40 Hossian Marker Cone Walcot # 14 (nt) 15 min Min @RT 20min @37c 20min @4c Walcot # 20min @4c Walcot Walcot # 20min @4c Walcot Walcot # 20min @4c Walcot Walc	7.0	0.5				21	1.5						A019D5	CD127	BV421	VI	00
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Filter Single color (u) Ref ctrf Unmining ctrf Fluorochrome Marker Clone Vallot # 14	7.0	0.5		9.8	0.7								1C6/CXCR3	CXCRB	BUV737	U14	6
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Filter Single color (ul) Ref ctrl Unmixing ctrl Fluorochrome Marker Cone Vial Lot # Strimit Vial Lot # Strim				9.8	0.7					1			FN50	CD69	BUV563	UV9	ω
Filter Single color(ul) Ref ctrl Unmixing ctrl Rivorochrome Marker Clone Vial Lot# Stimili 14 (RT) min @RT 30min @37C 14 30min @4C 14 30min @4C 14 30min @4C 14 16.8 Fix/Perm Processing 14 14 15 16.8 RBC Lyse, Spiked 30 min and then a spiked 30 min and then are spiked 30 min and the spiked 30 min and then are spiked 30 min and the spiked 30													RPA-T8	CD8	BUV496	UV7	2
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	14	Spiked 30 min			ColdStain 30min @4C		HotStain 30min @37C	Tetramer 40 min @ RT				Vial Lot#	Clone	Marker		Filter Single color (uI) Ref ctrl name	#

Simplified Protocol

Thew cells, DNAse, count.

Collect, count, aliquot cells 2.3.0E+6 Cells R10 / 5ml polystyrene tube
Bring volume upto 1 ml R10, add 2 ul PMACCirl and CD107a

Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300 pm 8min 800 ui of Livebead mix (1:2500) @RT for 15min Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min Wadh 4 HotStain 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 30min Add 300-500 ul 1x RBC Lysis for 3 minutes Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

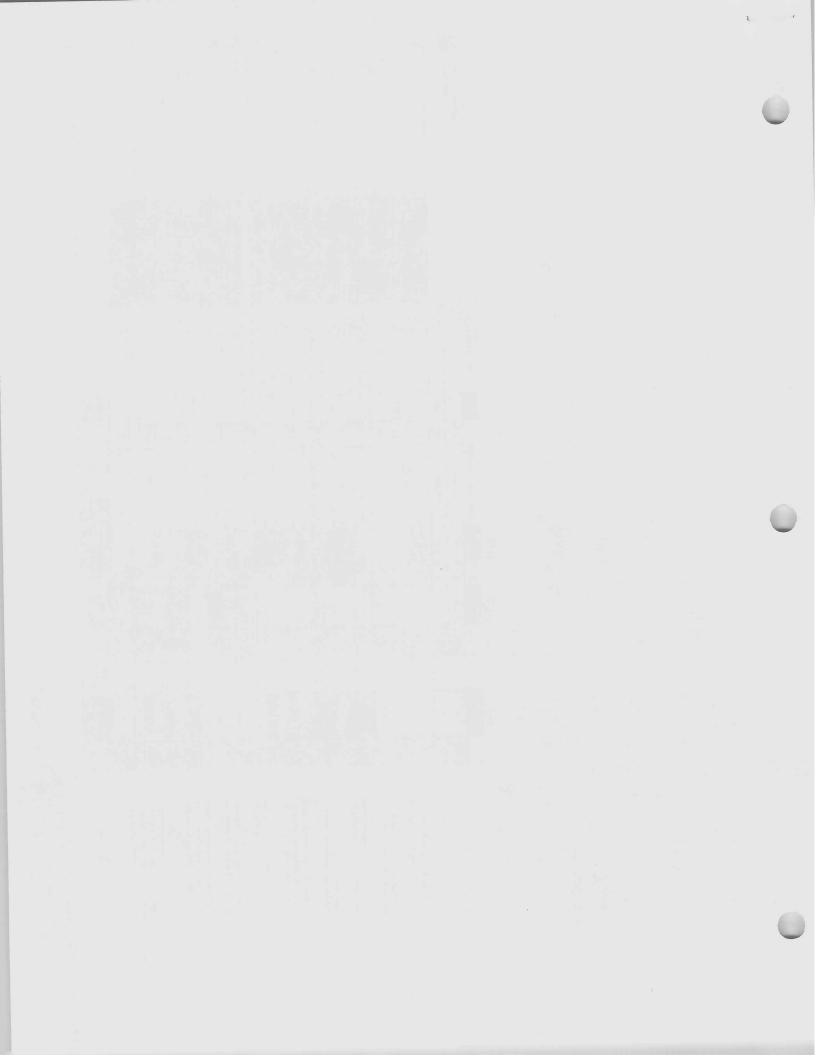
300 ul BD FixPerm, incubate @ 4C for 20min (vortex every 10 minutes)

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 mi PermWash 1500 rpm 6 min
2 mseuspend in 70 ul 0.4% PFA-PBS
Cap tubes, wrap rack in foil, store at 4°C

+ 1 coal sc + 2 cmbre (cd

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ILT & NK SFC Panel

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3/13/2023

45 PM sc acquisition 30 pl, 30 quo events Sels all NDOOR or Inf374 ... - EDIC7a NDOSO PMA 1:45 PM scaequation 26.00 pl/min ~ (60,000 css

Yes Fixed Perned

CD69 NDCOG ... TBD yes BUVGIS ... alternate stain? CO3/beads ... : or bull pop regative. .. erough exerts error heels

1/2 hour to get to corel no instaineds)

I hour mark all Sc's - 3 unstained (had add 2 ref, so essentially the !!) There's a bit more pronounced Blue peak to for ND050 atr 1 unshamed

50's done @ 2:51 pm/

2:56 unstanced sample 1:56 \$ 850,000/1,000,000 recovery

Does incorporating in AF into se signal make more difficult to separate? or does force separation @ the more destructure sections?

1039 more dead cells, especially after AMA-ion omyorn/

Samples @ 3:12 pm]] Done @ 3:52 pm (2:07:00 minutes)

Initial unmexing: NDOOG or Inf 374 sols General AF extrad 7 9