Flow CyTOF using single cells from human islets

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Date revised: January 11, 2021

I. Steps in pre-processing

1. Transfer handpicked islets (approximately 5,000 IEQs) into 15 ml conical tube. Add 10 ml of 1xPBS w/o Ca^{2+}, Mg^{2+} (Rockland, MB-008). Centrifuge for 2 min at RT, 180 xg. Aspirate the supernatant.
2. Add 1 ml of warm (37 ^{o}C) 0.05% Trypsin (Invitrogen, 25300054) to the islets. Pipette up and down with p1000.
3. Incubate at 37 ^{o}C for 9 min, or until cells are in single cells. Pipette up and down at t=7 min, 4 min, 2 min, 0 min. Check under microscope towards the end of dissociation.
4. Stop the trypsin reaction by adding 1 ml of 100% FBS (Hyclone, SH3091003) to the disassembled islets and pass cells through BD FACs tube with strainer top (Corning 352235). Use 1 ml of 100% FBS to rinse the tube and pass through the strainer.
5. Transfer cells to 15 ml conical. Centrifuge 4 min, 400 xg.
6. Dump out the supernatant and wash cells with PBS with 10% FBS. Centrifuge for 4 min, 400 xg.
7. Wash the cells with PBS and centrifuge for 4 min, 400 xg. Dump out the supernatant.
8. Cell counting. Resuspend cells to 1x10^{6}/ml in PBS.
9. Cisplatin staining for live/dead differentiation. Dilute cisplatin stock (Fluidigm 201064) at 1:4000 in PBS. Incubate cells with cisplatin at RT 5 min. Note: Time has to be exact since with time, cisplatin will enter live cell membranes.
10. Wash the cells with PBS+10% FBS.
11. Wash the cells again with PBS.
12. Fixation with 4% PFA at RT for 30 min. Make it fresh every time from 32% PFA (EMS 50-980-495). Note: CyTOF has high demanding for fixation.
13. After fixing, spin at 800g x 4min.
14. Freeze at ~5M/500ul in Mr. Frosty.

II. CyTOF barcoding and labelling

Thaw the cells in 37C quickly, and immediately add the Perm buffer

1. Remove the barcodes (Fluidigm 201060) from -20 ^{o}C freezer and allow them to warm up to RT for at least 10 min.
2. Wash cells 2x with Foxp3 perm buffer (eBioscience, 00-5523-00), 700 xg (2900 rpm), 5min. Note: centrifuge at higher speed after fixation.
3. Resuspend each sample to be barcoded in 800 µl of perm buffer (aiming at 1 ~ 3x10^{6} cells)
4. Resuspend barcodes completely in 100 µl of perm buffer and transfer them to the appropriate samples. Mix the samples immediately and completely (pipette immediately).
5. Incubate for 30 min at RT.
6. Centrifuge cells, wash twice with 2ml of perm buffer. 700 xg. 5 min.
7. Intracellular stain with master mix in perm +1% FBS at 4^{o}C overnight. Master Mix is 100ul/million for each tube.

Master Mix:

Metal Panel	Antibodies	Clone	Catalog	Ratio
89Y	CD45	HI30	Fluidigm 3089003B	500
141Pr	HLA-ABC	W6/32	Fluidigm 3141010C	300
142Nd	SOMATOSTATIN	G-10	sc-55565	4000
143Nd	CD4	RPA-T4	Biolegend 300516	1000
144Nd	EpCAM	9C4	Fluidigm 3144026D	4000
145Nd	C-PEP	3A1	Thermo MA1-22710	400
146Nd	CD8a	RPA-T8	Fluidigm 3146001B	4000
147Sm	GLUCAGON	C-11	santa cruz sc-514592x	400
148Nd	CD16	3G8	Fluidigm 3148004B	500
149Sm	CD45RO	UCHL1	Fluidigm 3149001B	300
150Nd	pan-Cytokeratin	C11	santa cruz sc-8018	2000
151Eu	CD56	NCAM16.2	Fisher Scientific BDB559043	4000
152Sm	PANCREAIC POLYPEPTIDE	gt poly	abcam ab77192	2000
153Eu	CD45RA	HI100	Fluidigm 3153001B	500
154Sm	CD3	UCHT1	Fluidigm 3154003B	8000
155Gd	CD27	L128	Fluidigm 3155001B	1000
156Gd	CD68	Y1/82A	Biolegend 333802	2000
158Gd	PD-1	EH12.2H7	Biolegend 329902	2000
159Tb	Eomes	WD1928	Thermo 14-4877-82	1000
160Gd	PDGFRA	D13C6	Fluidigm 3160007A	4000

161Dy	Tbet	4B10	Fluidigm 3161014B	300
162Dy	Foxp3	PCH101	Fluidigm 3162011A	600
163Dy	CD9	SN4 C3-3A2	eBioscience 14-0098	4000
164Dy	CD49F	GoH3	Fluidigm 3164006B	4000
165Ho	CD19	H1B19	Fluidigm 3165025B	500
166Er	CD44	BJ18	Fluidigm 3166001B	1000
167Er	CD11c	Bu15	Biolegend 337202	4000
168Er	Ki67	B56	Fluidigm 3168007B	600
169Tm	CTLA4	BN3	Biolegend 369602	1000
171Yb	PDX1	gt poly	santa cruz sc-14664	1000
172Yb	pS6	N7-548	Fluidigm 3172008A	2000
173Yb	HLA-DR	L243	Fluidigm 3173005B	1000
174Yb	ST8SIA1	ms IgM	Grumpy lab	1000
175Lu	CD14	M5E2	Fluidigm 3175015B	500
176Yb	Ghrelin	gt poly	santa cruz sc-10368	4000
170Er	P16	2D9A12	abcam ab54210	200

8. Wash with 2x perm buffer at 700 xg, 5min.
9. Wash 2x with PBS (700 xg, 5min)
10. DNA intercalator Iridium (FLuidigm 201192B, aliquoted in pcr tubes) at 1:4000 at RT for 1h in buffer to stain DNA is 2% PFA in PBS with Iridium.
11. Right before running samples on CyTOF2, wash (2x) with MilliQ H2O (700 xg, 5min)
12. Resuspend in MilliQ H_2O. Adjust cell concentration to about $5x10^5$/ml (expect lose 40% of the starting cells at this step)
    a. Alternatively, count and resuspend to desired concentration.
13. Analyze on CyTOF at about 300-500 evt/sec.