CyTOF STAINING FOR HUMAN ISLETS

I. Single cell isolation from human islets

1. Transfer handpicked islets (approximately 10,000 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 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.

II. CyTOF barcoding and labelling

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, 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.
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.
8. Wash with perm buffer at 700 xg, 5min.
9. Wash 2x with PBS (700 xg, 5min)
10. Fixation with Fluidigm fix and perm (Fluidigm 201067) +DNA intercalator Iridium (FLuidigm 201192B) at 1:4000 at RT for 1h.
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)
13. alternatively count and resuspend to desired concentration.
14. Analyze on CyTOF at about 300-500 evt/sec