Isolation of human pancreatic islets for DNase-seq

From: Duke ENCODE group
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1) Source of cells: Islets are obtained from organ donors by the National Disease Research Interchange (NDRI). Website:
http://www.ndriresource.org/NDRI_Initiatives/Pancreatic_Islets/31/

2) Lineage of cells: Primary

3) Donor information: Organ donors, male and female.

4) Islets are prepared by NDRI and shipped fresh in CMRL1066 media, 1% human serum albumin, no phenopthalein. Islets are assessed by purity and viability by Dithizone (diphenylthiocarbazone) dye uptake, which allows for simultaneous viability and purity assessment. Islets are counted on 50um grids and the combination of size and staining quality contributes to overall islet quality. Our general criteria is islet size 50-250um with average ~150um diameter, > 70% purity and >90% viability.

4) Islet dissociation into single cell suspension. For DNase-seq experiments, they can be dissociated into single cell (or nearly single cell) suspension using the following protocol.

1) Reconstitute Dispase I (Roche) in 0.4ml dH2O and aliquot at 100ul per tube, final concentration ~0.05U/ul. Store at - 20^0C.
2) Place islets received and 1X PBS, sterile, without Ca+ of Mg+ in 37C incubator until equilibrated (about 30 minutes to 1 hour).
3) Spin down islets 500rpm/5 minutes at RT.
4) Aspirate media
5) Wash with 10.0 ml pre-warmed 1X PBS, spin down islets 500rpm/5 minutes at RT and aspirate supernatant.
6) add 50ul Dispase I stock (0.05U/ul) to 1.0ml pre-warmed 1X PBS and resuspend islets. Transfer islets to 12-well culture dish and incubate at 37C for 30 minutes.
7) Agitate islets with either 2ml sterile pipette (or pasteur pipette).
8) Continue incubation for 30 minutes.
9) Centrifuge 800rpm/5 minutes at RT. Aspirate supernatant.
10) Repeat steps 6-9 until disaggregation is satisfactory (single cell suspension with a small number of multi-cell clumps).

11) Wash with 10.0 ml pre-warmed 1X PBS, spin down islets 800rpm/5 minutes at RT and aspirate supernatant.