KAPP-Sen TMC: Dissociation of Pancreatic Islets (non-recovered) Protocol

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Cellular Senescence Network (SenNet) Method Development Community

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ABSTRACT

The dispersed samples were shipped cold from **PRODOLABS**.
Prior to scRNA-seq, dispersed samples from brain dead donor's pancreatic acinar and ducts were dissociated as follows.

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Protocol status: Working
We use this protocol and it's working

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Abstract

1 The dispersed samples were shipped cold from **PRODOLABS**.
Prior to scRNA-seq, dispersed samples from brain dead donor's pancreatic acinar and ducts were dissociated as follows.

Cell Dissociation with TrypLE

2 NOTE: Before beginning cell dissociation coat all the materials (pipettes, tubes, etc.) with stopping media to prevent sticking. Stopping Media: DMEM + 10% FBS + 1:100 Glutamate.
1. Distribute specimens into 50 ml conical tubes, try to make similar cell concentrations across all tubes. *** Receive acinar in ~300 ml, should distribute into 12 tubes with 25 ml each***
2. Centrifuge acinar samples for 2 min at 230 g and 4C. Leave duct samples to settle without disturbance.
3. Wash samples 3X with 25 ml PBS.
4. Add 10 ml TrypLE E and incubate at 37C, mixing with a pipette approximately every 5 min.
    i. Acinar: in TrypLE E at 37C for approximately 30 mins.
    ii. Ducts: in TrypLE E at 37C for approximately 15 mins.
5. To stop reaction, add 30 ml per tube of stopping media.
6. Centrifuge acinar and ducts at 1300 rpm, 4C, 2 mins.
7. To stop: add 30 ml per tube of stopping media.
8. Centrifuge acinar and ducts at 1300 rpm, 4C, 2 mins.
9. Aspirate TrypLE E and wash 1X with stop media. Aspirate then resuspend in stop media to (Acinar) 5 ml or (Ducts) 2 ml.
10. Transfer cell suspension through 70 µm filter then combine to 2 ml (Acinar) and 1ml (Ducts). Filter again through a 40 µm Flowmi.
11. Count cells using AO/PI (acridine orage/propidium iodide) Cell Viability Kit for Luna-FL automated cell counter.
12. Proceed to cell fixation.

Fixation of Cells & Nuclei for Chromium Fixed RNA Profiling

3 Cells were fixated prior to scRNAseq according to https://dx.doi.org/10.17504/protocols.io.[...]/v1