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We use this protocol and it's working

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KAPP-Sen TMC: Dissociation of Pancreatic Acinar and Ducts (non-recovered)

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Cellular Senescence Network (SenNet) Method Development Community

KAPP-Sen TM

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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.

Keywords: SenNet KAPP-Sen
TMC

Cell Dissociation with TrypLE

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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.

1.1 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***

1.2 Centrifuge acinar samples for 2 min at 230 g and 4°C. Leave duct samples to settle without disturbance.

1.3 Wash samples 3X with 25 ml PBS.

1.4 Add 10 ml TrypLE E and incubate at 37°C, mixing with a pipette approximately every 5 min.
1. Acinar: in TrypLE E at 37°C for approximately 30 mins.
2. Ducts: in TrypLE E at 37°C for approximately 15 mins.

1.5 To stop reaction, add 30 ml per tube of stopping media.

1.6 Centrifuge acinar and ducts at 1300 rpm, 4°C, 2 mins.

1.7 To stop: add 30 ml per tube of stopping media.

1.8 Centrifuge acinar and ducts at 1300 rpm, 4C, 2 mins.

1.9 Aspirate TrypLE E and wash 1X with stop media. Aspirate then resuspend in stop media to (Acinar) 5 ml or (Ducts) 2 ml.

1.10 Transfer cell suspension through 70 µm filter then combine to 2 ml (Acinar) and 1ml (Ducts). Filter again through a 40 µm Flowmi.

1.11 Count cells using AO/PI (acridine orage/propidium iodide) Cell Viability Kit for Luna-FL automated cell counter.

1.12 Proceed to cell fixation.

Fixation of Cells & Nuclei for Chromium Fixed RNA Profiling

2 Cells were fixated prior to scRNAseq according to https://dx.doi.org/10.17504/protocols.io.x54v9py5zg3e/v1