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Single-cell RNA sequencing V.2

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Human Islet Research Network

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ABSTRACT

Single-cell RNA sequencing (scRNA-seq) allows for transcriptional profiling of individual cells within a heterogenous sample. This protocol describes a method for performing scRNA-seq using handpicked pancreatic islets from organ donors.

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EXTERNAL LINK

https://hpap.pmacs.upenn.edu/explore/workflow/islet-molecular-phenotyping-studies?protocol=4

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Steps in pre-processing

1 **1.** Transfer handpicked islets (approximately 5,000 IEQs) into **15 mL** conical tube.

2. Add **10 mL** of 1xPBS w/o Ca2+, Mg2+ (Rockland, MB-008). Centrifuge for 2 min at RT, 180 xg. Aspirate the supernatant.

3. Add **1 mL** of warm (**37 °C**) 0.05% Trypsin (Invitrogen, 25300054) to the islets. Pipette up and down with p1000.

4. Incubate at **37 °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.

5. Stop the trypsin reaction by adding **1 mL** of 100% FBS (Hyclone, SH3091003) to the dissociated islets and pass cells through BD FACs tube with strainer top (Corning 352235)

6. Use **1 mL** of 100% FBS to rinse the tube and pass through the strainer.

7. Transfer cells to **15 mL** conical. Centrifuge 4 min, 400 xg.

8. Remove the supernatant and wash cells with PBS with 10% FBS. Centrifuge for 4 min, 400 xg.

9. Wash the cells with PBS with 10% FBS and centrifuge for 4 min, 400 xg. Remove the supernatant.

10. Count cells using a countess chamber.

11. For the scRNAseq, do the final resuspension in 10% FBS in PBS and adjust the volume to make the final suspension 1000 cells/microliter. Filter the cells one more time prior to loading them onto the 10X Genomics chip.

Links to Kits used in post-processing

2 **1.** Older HPAP samples (specifically donors HPAP-001 to HPAP-019) were processed using C1 Single-Cell mRNA Seq HT IFC and Reagent Kit v2 (product ID: 101-4964) which has been discontinued.

2. All current samples (HPAP-019 onwards) were processed using the Chromium Single Cell 3' Reagent Kit.

3. Unfortunately, since 10x Genomics is going to stop manufacturing the above kit, we will be using the new kit Chromium Next GEM Single Cell 3' Reagent Kits v3.1. For this protocol we target a 5000 cell recovery.

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