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

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

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🌐 Single-cell RNA sequencing V.1

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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.

Note

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Keywords: Single-cell RNA sequencing, HPAP, HIRN

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.