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Single-cell ATAC sequencing

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ABSTRACT

Interrogating cell-type specific chromatin accessibility can reveal cis-regulatory elements linked to downstream gene expression patterns. Assay for Transposase-Accessible Chromatin using Sequencing (ATAC-Seq) is a technique that assays genome-wide chromatin accessibility and can reveal nucleosome positioning patterns, map enhancer and promoter regions, or reveal transcription factor binding sites. Single-cell Assay for Transposase-Accessible Chromatin using Sequencing (scATAC-seq) adapts the bulk ATAC-sequencing protocol to separate individual nuclei and assay chromatin state with single-cell resolution. This technique allows for resolution of gene accessibility patterns within specific pancreatic cell types such as α-cells, β-cells, and acinar cells.

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EXTERNAL LINK

https://hpap.pmacs.upenn.edu/explore/workflow/islet-molecular-phenotyping-studies?protocol=5

PROTOCOL CITATION

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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 scATACseq, do the final resuspension in 0.04% BSA in PBS as is consistent with the instructions for using fresh cell in the Nuclei Isolation Protocol.

Links to kits used in post-processing

2 **1.** For nuclei isolation the protocol used is: Nuclei Isolation for Single Cell ATAC Sequencing. We target a 5000 nuclei recovery for this protocol.

2. Samples are processed for scATAC seq using Chromium Single Cell ATAC Reagent Kits.

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