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 We use this protocol and it's working

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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.

Note

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Steps in pre-processing

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 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

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 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](#).