



Single-cell ATAC sequencing

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Date submitted: August 13, 2020

I. Steps in pre-processing

1. Transfer handpicked islets (approximately 5,000 IEQs) into 15 ml conical tube.
2. Add 10 ml of 1xPBS w/o Ca^{2+} , Mg^{2+} (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.

II. Links to kits used in post-processing

1. For nuclei isolation, this protocol is used: [Nuclei Isolation for Single Cell ATAC Sequencing](#). For this protocol we target a 5000 nuclei recovery.
2. Samples are processed for scATAC seq using [Chromium Single Cell ATAC Reagent Kits](#).