**Final ATAC-seq protocol for frozen tissues**

The protocol is modified from the omni-ATAC protocol (Corces et al. 2017) and protocol work on cryopreserved nuclei(Halstead, et al., 2020), and Roslin lab protocol worked on muscle (Emily Clark). For more detail of this protocol, please see the Supplementary Protocol, the steps were kept on ice.

1. **Extraction of nuclei from frozen tissues**

A frozen tissue fragment ∼50 mg was placed into a pre-chilled GentleMACS C-tubes containing 2 ml of cold 1× homogenization unstable buffer (HB buffer) (5 mM CaCl2, 3 mM Mg(Ac)2, 10 mM Tris pH 7.8, 320 mM sucrose, 0.1 mM EDTA, 0.1% IgepalCA630, 0.1 mM PMSF and 167 μM β-mercaptoethanol, in water). Using the scissor to cut tissues into small piece, place the C-tube on the GentleMACS dissociator and run the equivalent to ‘E0.1c Tube’ programme twice. Filter the sample through a 70μm corning cell strainer into a 50ml conical falcon tube (conical NOT flat-bottomed) and rinse the C-tube with 1ml 1XHB buffer (+PIC) and put through the filter. Spin the cell solution down at 3000g for 5 minutes, remove the supernatant and resuspend the pellet in 350ul 1XHB buffer. Transfer the sample to a 2ml “Protein Lo-Bind eppendorf tube”. An equal volume (350 μl) of a 50% iodixanol solution (50% iodixanol in 1× HB buffer) was added and mixed by pipetting to make a final concentration of 25% iodixanol. 525 μl of a 29% iodixanol solution (29% iodixanol in 1× homogenization buffer containing 480 mM sucrose) was layered underneath the 25% iodixanol mixture. In a similar fashion, 525 μl of a 35% iodixanol solution (35% iodixanol in 1× homogenization containing 480 mM sucrose) was layered underneath the 29% iodixanol solution. A clearly defined interface should be visible between all three layers. In the swinging bucket centrifuge spin the 2ml tubes for 25 minutes at 3155g at 4oC with no break. A thin “whitish” band should appear between layer 1 and 2(from the top). Collect this band (200μl) and transfer it to a new collection tube. Take an aliquot of 10μl for nuclei purity evaluation (microscope) and counting use the DAPI -> fluorescence microscope -> counting. Check the nuclei intact and count nuclei in blue light and white light. The intact nuclei should look like a peanut, and round. We are using part of nuclei for further experiment; the rest of nuclei can be stored at -80℃ after adding 10% DMSO.

1. **ATAC-seq library generation**

250,000 counted nuclei were then transferred to a tube containing 1 ml of ATAC-seq RSB with 0.1% IgepalCA630. Nuclei were pelleted by centrifugation at 500 r.c.f. for 10 min in a pre-chilled (4 °C) fixed-angle centrifuge. Supernatant have been removed carfully. And the Tagmentation mix (25 μl 2× TD buffer, 2.5 μl transposase (100 nM final), 22.5 μl water) was added directly to the nuclear pellet and mixed by pipetting up and down six times. Transposition reactions were incubated at 37 °C for 60 min in a thermomixer with shaking at 300 r.p.m. Reactions were cleaned up with MinElute PCR purification kit (elute with 10 µL Buffer EB). Measure the concentration before PCR by Qbit. Add 40 μL PCR master mix (25 ul NEBNext 2x PCR MasterMix (M0541, NEB), 16 μL ddH2O, 1 μL 25 μM Primer 1,1 μL 25 μM Primer 2) to 10 μL eluted DNA and cycle as follows: 1 times (5 min 72℃; 30 sec 98℃), 15 cycle (10 sec 98℃ ; 30 sec 63℃; 1 min 72℃), hold 4℃. Purify libraries using MinElute PCR purification kit and measure the concentration by Qbit. Run traces on Agilent Bioanalyzer High Sensitivity DNA chip. Doing size-selection (150-250bp) for libraries by AMPure bead. Measure the concentration by Qbit and Run traces on Agilent Bioanalyzer High Sensitivity DNA chip. Submit the library for test sequencing (NextSeq, paired end 40 bp reads)