Nov 26, 2025 Version 1

TDA-MIT: Nuclei isolation for 10X single-nuclei RNA sequencing V.1

DOI
https://dx.doi.org/10.17504/protocols.io.j8nlkyx3wg5r/v1

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Protocol Citation: Ke Zhang 2025. TDA-MIT: Nuclei isolation for 10X single-nuclei RNA sequencing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkyx3wg5r/v1Version created by **Ke Zhang**

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

Created: November 26, 2025

Last Modified: November 26, 2025

Protocol Integer ID: 233583

Keywords: single-nuclei sequencing, TDA, 10x genomics nuclei isolation kit, chromium nuclei isolation kit with rnase inhibitor, nuclei isolation for 10x, chromium nuclei isolation kit, 10x genomics library prep kit, sequencing library preparation, nuclei isolation, nuclei rna, single nuclei, illumina nextseq 550 sequencer, rnase inhibitor, sequencer, nextseq high output cartridge kit, using nextseq

Abstract

This protocol describes the single nuclei sequencing library preparation using the 10X Genomics Nuclei Isolation Kit (Chromium Nuclei Isolation Kit with RNase Inhibitor) with 10X Genomics Library Prep Kit (Chromium Next GEM Single Cell 3' Kit v3.1) followed by sequencing on the Illumina NextSeq 550 sequencer (Illumina) platform using NextSeq High Output Cartridge kits.

Materials

Leica Cryostat
O.C.T. Compound
Chromium Next GEM Single Cell 3′ Reagent Kits v3.1 (PN-1000121, 10x Genomics)
Chromium Nuclei Isolation Kit (PN-1000494, 10x Genomics)
TapeStation 4200, Agilent Technologies

Sample collection

1 p16-3MR mice were generated from Jackson lab (RRID: IMSR_JAX:037045), and skin and lung samples were collected from mice at 2 months (n= 3) and 26 months (n= 3) old.

Tissue cryopreservation

2 Dissected mouse skin and lung samples were placed into OCT-containing plastic molds, frozen on dry ice, and stored at -80°C.

Single-nuclei isolation

3 Single nuclei isolation was conducted according to the 10x Genomics guide (CG000505) using Chromium Nuclei Isolation Kit with RNase Inhibitor (PN-1000494). Briefly, OCT embedded mouse lung and skin tissues were cut into 100 µm slices, and nuclei were isolated from six slices. Lysis buffer was added to the tissue slices, and the tissues were dissociated with a plastic pestle until homogeneous. The mixture was then incubated on ice for 10 minutes. The dissociated nuclei suspension was passed through a nuclei isolation column by centrifugation. Nuclei were pelleted by centrifugation at 500 rcf at 4°C for 3 minutes, followed by the removal of cell debris. The nuclei pellet was washed and resuspended in wash and resuspension buffer, and the suspended nuclei were kept on ice before loading into the 10x Chromium Controller.

Single-nuclei RNA expression library preparation and sequencing

4 Isolated nuclei were processed, and single-nuclei libraries were prepared using the Chromium Next GEM Single Cell 3′ Reagent Kits v3.1 (Dual Index) protocol (10X Genomics), loading 8,000 nuclei per sample. Pooled libraries were then sequenced using NextSeq High Output Cartridge kits and a NextSeq 550 sequencer (Illumina). RNA Libraries were sequenced using the configuration of R1: 28 cycles, R2: 44 cycles, Index1: 10 cycles, Index2: 10 cycles.