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Human Dorsal Root Ganglion bulk ATAC-seq protocol

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Úrzula Franco-Enzástiga[1], Theodore Price[1]

[1]University of Texas at Dallas

Úrzula Franco-Enzástiga
University of Texas at Dallas

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We use this protocol and it's working

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Abstract

In this protocol, we describe how to perform bulk ATAC-seq on fresh human dorsal root ganglia.

Materials

Key materials

Buffers:

Ice-cold bubbled NMDG-aCSF pH 7.4

- 93 mM NMDG
- 2.5 mM KCl
- 1.25 mM NaH_2PO_4
- 30 mM $NaHCO_3$
- 20 mM HEPES
- 25 mM glucose
- 5 mM ascorbic acid
- 2 mM thiourea
- 3 mM sodium pyruvate
- 10 mM Mg_2SO_4
- 0.5 mM $CaCl_2$
- 12 mM N-acetylcysteine; osmolarity 310 mOsm

Ice-cold nuclei isolation buffer

- 250 mM sucrose
- 25 mM KCl
- 5 mM $MgCl_2$
- 10 mM Tris-HCl pH 8.0
- 0.1% Triton X-100 - Sigma-Aldrich (Cat. No. T8787-50mL)

Materials and equipment required:

- ATAC-seq kit - Active Motif kit (Cat. No. 53150)
- DKW tube for KIMBLE® KONTES® Potter-Elvehjem Tissue Dounce homogenizer, Size 19, 1 mL (Cat. No. 885752-0019) with PTFE pestle
- Sterile 40-µm cell strainer - CELLTREAT (Cat. No. 229481)
- Costar® 2 mL tube - Corning (Cat. No. 3213)
- Ultrapure DNase/RNase Free Distilled Water - Thermo Scientific (Cat. No. 10977015)
- Centrifuge 5702R - Eppendorf® (Cat. No. 05-414-126)
- Magnetic separator - 10X (Cat. No. 120250)
- Trypan blue - Gibco (Cat. No. 15-250-061)
- ThermoMixer C - Eppendorf® for 1.5 mL tubes (Cat. No. EP5382000023)
- NextSeq 2000 sequencer
- Bonn scissors - Fine Science Tools (Cat. No. 14184-09)
- Dumont #5 Forceps - Fine Science Tools (Cat. No. 11252-50)
- Microcentrifuge

- Hematocytometer
- 100% ethanol
- Wet ice
- Ice bucket
- 5 cm petri dish
- Pipettes and corresponding pipette tips

Before start

Required PPE: All work must be done wearing appropriate PPE including lab coat and gloves. Proper safety precautions should be taken into account while working with and disposing human tissue. The use of sterile tools and proper cleaning and sterilization before and after every use is recommended.

Tissue harvesting and cleaning procedure

1 Surgically excised lumbar L4 or L5 human dorsal root ganglia (hDRGs) are obtained from organ donors at Southwest Transplant Alliance (STA) at 2 h post cross-clamp.

2 Right after dissection, hDRGs are transported in cold NMDG-aCSF pH 7.4 (see solution components in Materials) bubbled with carbogen gas (95% O_2, 5% CO_2) in wet ice to the laboratory.

3 A petri dish containing ice-cold NMDG-aCSF pH 7.4 is used to place the hDRG, ensuring the petri dish remains on ice throughout the cleaning process.

4 Bonn scissors are used to remove fat and connective tissue surrounding the DRG. Forceps are used to remove dura by pulling it away from the hDRG.

Nuclei isolation

5 Cleaned hDRG is placed in an eppendorf tube containing 2 mL of ice-cold nuclei isolation buffer (see solution components in Materials).

5.1 hDRG is chopped into small pieces using scissors.

5.2 The total volume is transferred to a Dounce homogenizer. Homogenization requires 5 strokes of the loose pestle and 15 strokes of the tight pestle applied to the tissue.

5.3 The homogenate is transferred to a conical tube (2 mL) and centrifuged at 100 x g for 8 min at 4 °C.

5.4 The supernatant is carefully removed without disrupting the soft pellet.

5.5 The pellet is resuspended in 2 mL of triton-free nuclei isolation buffer.

5.6 The resuspended pellet is centrifuged at 100 x g for 8 min at 4 °C.

5.7 The supernatant is removed without disrupting the pellet. At this step most of the pellet are nuclei.

6 Nuclei are resuspended in 2 mL of nuclei isolation buffer without triton and filtered through a 40-µm cell strainer.

Nuclei counting

7 The number of isolated nuclei is counted using a hematocytometer with 0.4% trypan-blue (1:1 ratio) to stain the nuclei.

8 A total of 100,000 nuclei are aliquoted and centrifuged at 500 x g for 10 min at 4 °C. The supernatant is removed to proceed to tagmentation.

Tagmentation

9 Tagmentation is performed by following the instructions provided in the Active Motif kit (https://www.activemotif.com/documents/2182.pdf) (Cat. No. 53150) from the Tagmentation Reaction and Purification section until the end of the protocol. 50 µL of Tagmentation Master Mix (Page 5 of the protocol), prepared right before starting the tagmentation step, are added to each sample.

10 The tagmentation reaction is incubated at 37 °C for 30 min in a thermomixer at 800 rpm.

Tagmented DNA purification

11 250 µL of purification binding buffer and 5 µL of 3 M sodium acetate are added.

12 DNA purification columns are employed to isolate the DNA.

13 Tagmented DNA is eluted in 35 µL of DNA elution buffer.

Library preparation

14 The amplification of tagmented DNA through PCR is carried out using i7-and i5-Illumina's Nextera-based adapters as per the provider's instructions (Cat. No. 53150).

ATAC sequencing

15 Pair-end 75 cycle sequencing reads are acquired on the NextSeq 2000 sequencer. A total of 100 million reads are intended per sample.

Processing of ATAC-seq data

16 Raw bulk ATAC-seq data undergo a process of pre-alignment quality control, read alignment to the reference genome, and post-alignment processing as described below.

16.1 Use *FastQ* to verify the initial quality of raw sequencing using fastq files.

16.2 Use *TrimGalore* to remove adapters and verify quality of trimming using *FastQ.*

16.3 Use *Bowtie2* to map paired-end reads to the reference genome GRCh38/hg38.

16.4 Use *Samtools* to sort and filter reads by removing unmapped and low quality reads, and *Bamtools* to remove mitochondrial DNA reads.

16.5 Remove duplicates using *Picard.*

17 At this point of the processing the .bam file contains the filtered aligned reads which can be used to proceed with peak calling.

Protocol references

Buenrostro JD, Wu B, Chang HY, & Greenleaf WJ (2015) ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. *Curr Protoc Mol Biol* 109:21 29 21-21 29 29.

Valtcheva MV*, et al.* (2016) Surgical extraction of human dorsal root ganglia from organ donors and preparation of primary sensory neuron cultures. *Nat Protoc* 11(10):1877-1888.

Mich JK*, et al.* (2021) Functional enhancer elements drive subclass-selective expression from mouse to primate neocortex. *Cell Rep* 34(13):108754.