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Human Dorsal Root Ganglion spatial ATAC-seq protocol

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PRECISION Human Pain ...

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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 spatial ATAC-seq on fresh-frozen human dorsal root ganglion.

Materials

Key materials

- OCT - Fisher HealthCare (Cat. No. 23-730-571)
- SuperFrost Plus charged slides - Fisher Scientific (Cat. No. 12–550-15)

Service

- Spatial ATAC-seq technology product (AXO-0310); Microfluidic barcoding system.

Additional materials and equipment required

- Dry ice
- Stainless steel base mold - Ted Pella (Cat. No. 27276-6)
- Cryostat Leica CM1950

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.

Tissue harversting and storage

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, tissue is fresh frozen on powdered dry ice and stored at -80 °C until processing.

Tissue sectioning

3 Frozen hDRGs are embedded in OCT (Optimal Cutting Temperature embedding medium) in a cryomold by adding small volumes of OCT over dry ice to avoid thawing.

4 Tissues are cryostat sectioned at 10 µm onto SuperFrost Plus charged slides.

5 Slides are briefly warmed to allow the sections to adhere to the slides but are immediately returned to the −20 °C cryostat chamber until sectioning is complete.

6 The slides are removed from the cryostat and stored in a -80 °C freezer until shipment to AtlasXomics for further processing. Samples are shipped on dry ice.

Transposition for Spatial ATAC-seq (AtlasXomics: AXO-0310)

7 Spatial ATAC-seq assay is performed at AtlasXomics (Product code: AXO-0310; https://www.atlasxomics.com/howitworks).

7.1 In brief, tissue is fixed with 0.2% formaldehyde in PBS for 5 min and quenched with 1.25 M glycine for 5 min.

7.2 After fixation, tissue is washed with PBS and nuclease-free water and left to dry.

7.3 Tissue sections are permeabilized for 15 min with a buffer containing 10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.01% Tween-20, 0.1% NP40, 0.001% digitonin and 1%BSA, and washed with a solution containing 10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.1% Tween-20 and 1%BSA.

7.4 Tn5 transposition on the fixed hDRG sections is carried out at 37ºC for 30 min. Transposition reaction buffer contains tagmentation buffer, 0.1% Tween-20, 0.01% digitonin, PBS and Tn5). Transposition reaction is stopped with 40 µM EDTA for 5 min.

8 Spatial barcoding is performed with microfluidics system (Product code: AXO-0310).

8.1 To correlate spatially barcoded accessible chromatin with tissue morphology, images of hDRG sections are acquired (Keyence BZ-X810 at 10X magnification).

8.2 The tissue is lysed using a reverse cross-linking solution containing 200 nM NaCl, 50 mM Tris-HCl pH 8.0, 1 mM EDTA, 1% SDS and 0.4 mg/mL proteinase K at 58°C for 2 h to release DNA fragments, which are then purified and amplified through PCR for library preparation using standard Next Generation Sequencing methods.

8.3 The PCR product is mixed with SPRI magnetic beads and incubated for 10 min. A magnetic stand is used to collect the beads, thereby purifying the final PCR product, which is eluted in nuclease-free water.

8.4 150X150 paired-end sequencing is performed on a NextSeq 2000 with 15% PhiX. A total of 300 million reads per sample are targeted.

Spatial ATAC-seq analysis (AtlasXomics: AXO-0310)

9 Processing of spatial ATAC-seq is perfomed by AtlasXomics.

9.1 Use *Chromap* to trim sequencing adapters, align sequenced reads to the human reference genome GRCh38/hg38 and remove duplicates from fastq files.

9.2 Fragments files containing coordinates of sequenced DNA mapping to barcodes are generated for downstream analysis.

9.3 Use *AtlasXbrowser* to designate pixels on tissue based on microscopy images and to create Seurat-compatible metadata files.

9.4 Use *ArchR* to process fragments files and remove pixels not on tissue.

9.5 At this point of the processing, the .tsv fragments file contains the filtered aligned reads which can be used to proceed with peak calling. We have provided a tissue_positions_list.csv file containing a table with rows corresponding to the 2500

spots on the array, high- and low-resolution images of the tissue, a scale_factors.json file, and experiment metadata. Together, these files can help for further processing of spatial ATAC-seq analysis using *ArchR*.

A brief description of some of the provided files is shown below.

tissue_positions_list.csv

Columns on the file tissue_positions_list.csv correspond to:

- Column 1: barcode
- Column 2: in_tissue binary status indicating if the spot falls on a tissue spot
- Column 3: array row
- Column 4: array column
- Column 5: the row pixel coordinate of the center of the spot in the full resolution image
- Column 6: the column pixel coordinate of the center of the spot in the full resolution image

scale_factors.json

Parameters in the scale_factors.json file correspond to the relative scales of the supplied image and the array barcode features. Four aspects are provided:

- fiducial_diameter_fullres: Number of pixels spanning the diameter of a fiducial spot in the original, high-resolution image
- spot_diameter_fullres: Number of pixels spanning the diameter of a spot in the original, high-resolution image
- tissue_highres_scalef: Scaling factor to convert pixel positions in the original, full-resolution image to pixel positions in the provided high resolution image (.png file)
- tissue_lowres_scalef: Scaling factor to convert pixel positions in the original, full-resolution image to pixel positions in the provided low-resolution image (.png file)

Protocol references

Deng Y, *et al.* (2022) Spatial profiling of chromatin accessibility in mouse and human tissues. *Nature* 609(7926):375-383.

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