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10X Genomics Single-Nucleus Assay of Transposase Accessible Chromatin-Sequencing for Epigenetic Profiling of Adult Human Tissues

Forked from 10X Genomics Single-Nucleus RNA-Sequencing for Transcriptomic Profiling of Adult Human Tissues

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ABSTRACT

10x Genomics Single Cell ATAC (v1) sequencing is a microdroplet-based method that allows for the effective capture, sequencing and profiling of accessible chromatin in single nuclei. Chromatin accessibility is a major determinant of gene regulation, defining the transcriptional regulatory networks that determine cellular identity and function as well as additional biological processes (e.g., differentiation, proliferation, development and responses to the extracellular environment). Chromatin accessibility sequencing (ATAC-seq) provides insight into the upstream regulatory landscape associated with open and accessible chromatin regions.

References
1. Lake et al. (2016). Science, doi:10.1126/science.aaf1204.
2. Lake et al. (2018). Nature Biotechnology, doi:10.1038/nbt.4038.
3. Lake et al. (2019). Nature Communications, doi:10.1038/s41467-019-10861-2.
4. Chromium Single Cell ATAC Reagent Kits User Guide v1 (Rev B) CG000168, support.10xgenomics.com.
5. Lake and Zhang. Nuclei Isolation Protocol Applicable to Adult Human Kidney Tissues, kpmp.org/researcher-resources.

DOI

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EXTERNAL LINK

http://genome-tech.ucsd.edu/ZhangLab/

PROTOCOL CITATION

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FORK NOTE

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KEYWORDS

Kidney, KPMP, Single Nucleus, RNA Sequencing, 10X Genomics

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GUIDELINES

Full protocol is from 10X Genomics. All modifications are to the original protocol (Chromium Single Cell ATAC Reagnets Kits v1 User Guide (Rev D) CG000168, support.10xgenomics.com)

MATERIALS TEXT

MATERIALS

Chromium Single Cell ATAC Library **10x Genomics Catalog #PN-1000110**

Chromium Chip E Single Cell ATAC Kit 48 rxns **10x Genomics Catalog #PN-1000155**

Chromium i7 Multiplex Kit N Set A 96 rxn **10x Genomics Catalog #PN-1000084**

Nuclease-free water
Ethanol (200 proof)
Dynabeads MyOne Silane
SPRI select reagent set
50% glycerol
10% Tween 20
Qiagen buffer EB
Qubit dsDNA HS Assay Kit
PCR strip tubes with flat cap
LoBind 1.5 ml tubes
Qubit Assay tubes

Isolate Nuclei

1 Prepare nuclei according to the protocol "Isolation of single nuclei from solid tissues" steps 1-14.
dx.doi.org/10.17504/protocols.io.ufketkw

2 Resuspend nuclei in **100 µl** to **1 mL** of 1X Diluted Nuclei Buffer (volume depends on target concentration)

3 Count nuclei (e.g., BioRad T20 Cell Counter)

4 Check nuclei integrity under fluorescent microscope using DAPI channel. Nuclei should appear distinct, with rounded borders and the majority occurring as singlets. High clumping rates would indicate damaged nuclei and would require

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re-filtering using 30-µm CellTrics filter or exclusion.

5 Dilute nuclei stock to be in the 1000-3200 nuclei per uL range in **5 µl** total volume using the 1X Diluted Nuclei Buffer.

If nuclei concentrations fall below the target range, then centrifugation can be performed to increase nuclei concentrations to be within range

- Start with nuclei stock in PBS with 0.1% BSA. Add 5-10X volumes of 1X Diluted Nuclei Buffer (e.g. add 1 mL if nuclei stock is 100 uL)
- Spin 900xg for 10 minutes (use 500xg if total #nuclei > 100k)
- Resuspend in an appropriate volume of the 1X Diluted Nuclei Buffer.
- Use 2-5 uL to count; 5 uL for loading
-

QC cutoff: minimum of 10,000 nuclei.

Caution: BSA is necessary to avoid clumping and prevent nuclei loss from sticking to the tube during the spin down. DO NOT USE more than 0.01% BSA in nuclei stock

Transposition of Nuclei

6 Prepare transposition mix.

1. Thaw, vortex and centrifuge ATAC buffer and store on ice.
2. Centrifuge briefly ATAC Enzyme and store on ice.
3. Prepare a master mix of **7 µl** ATAC buffer and **3 µl** ATAC Enzyme for each sample. Mix thoroughly.
4. Aliquot **10 µl** of prepared master mix each tube of a PCR 8-tube strip on ice.
5. Add **5 µl** of nuclei suspension for each sample to the tube of master mix on ice.
6. Pipette to mix gently with P-20 set at **10 µl** 6X. Do not centrifuge.

7 Isothermal incubation 1h

1. Incubate in a thermcycler at **37 °C** for **01:00:00** with lid set at **50 °C** .

GEM Generation and Barcoding

8 Prepare Reagents for use

1. Equilibrate gel beads to room temperature for **00:30:00** .
2. Thaw, vortex, and centrifuge Barcoding Reagent and Reducing Agent B. Verify no precipitate in Reducing Agent B.
3. Centrifuge Barcoding Enzyme briefly before adding to Master Mix.

9 Prepare Master Mix

1. Prepare Master Mix on ice (follow the recipe from the User Guide). Pipette mix 15X and centrifuge briefly.
2. Add **65 µl** Master Mix to each tube of transposed nuclei on ice.

10 Load Chromium Single Cell E Chip

1. Assemble Chromium Chip E in a 10X Chip Holder.
2. Must load chip in order according to row label for microfluidic channels to work properly: Row 1 → Row 2 → Row 3

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3. Make sure NO bubbles are introduced while loading chip.
4. Dispense 50% glycerol solution into unused Chip Wells (if <8 samples used per chip)
    a. **75 µl** into unused wells in Row 1
    b. **40 µl** into unused wells in Row 2
    c. **240 µl** into unused wells in Row 3

5. Load **75 µl** Master Mix and Transposed Nuclei Suspension into the bottom center of each well in Row 1 without introducing bubbles.

6. Snap the Gel Bead strip into a 10X Vortex Adapter. Vortex **00:00:30** . Remove the Gel Bead strip and flick sharply downward to ensure maximum recovery. Confirm there are no bubbles at the bottom of the tubes and liquid levels look even.

7. Load **40 µl** gel beads in Row 2. Only puncture the foil seal for gel bead tubes being used. Dispense slowly and without introducing bubbles.

8. Load **240 µl** partitioning oil into each Row 3 by pipetting two aliquots of **140 µl** .

9. Attach 10X gasket. Align the top-notch. Ensure gasket holes are aligned with the wells. Avoid touching the smooth gasket surface. Do not press down on the gasket.

11 Run the Chromium Controller

1. Press the eject button on the controller to eject.
2. Place the assembled chip with the gasket in the tray. Press the button to retract the tray.
3. Confirm the program on screen. Press the play button.
4. At completion of the run (~ **00:07:00**), the controller will chime. Proceed immediately to the next step.

12 Transfer GEMs

1. Chill strip tubes on ice.
2. Press the eject button to remove the chip.
3. Discard the gasket. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees.
4. Check the volume in rows 1-3. Abnormally high volume in any well indicates a clog.
5. Slowly aspirate **100 µl** GEMs from the lowest points of the Recovery Wells without creating a seal between the pipette tips and the wells.

> GEMs should appear opaque and uniform across all channels. Excess partitioning oil (clear) in the pipette tips indicates a potential clog.

6. Slowly dispense (~ **00:00:20**) GEMs into the tube strip on ice with the pipette tips against the sidewalls of the wells.

13 GEM Incubation

1. Incubate in a thermocycler to perform linear amplification.
2. Store at **4 °C** for up to **72:00:00** or at **-20 °C** for up to a week. Or proceed to the next step.

14 Post GEM Incubation Cleanup - Prepare Reagents for use

1. Thaw, vortex, and centrifuge a tube of Reducing Agent B.
2. Thaw Cleanup Buffer at **65 °C** for **00:10:00** with shaking at max rpm then cool to room temperature.

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15 Post GEM Cleanup --Dynabeads

1. Add **125 µl** Recovery Agent to each sample at room temperature. DO NOT MIX. Wait **00:01:00** .
2. Carefully aspirate **125 µl** of pink oil phase from the bottom of the tube. DO NOT aspirate any aqueous sample.
3. Prepare Dynabeads Cleanup Mix (follow the recipe from the User Guide)
4. Vortex and centrifuge mixture and add **200 µl** to each sample. Pipette 5X to mix.
5. Incubate at room temperature for **00:10:00** ; pipette mix again **00:05:00** into incubation.
6. Prepare Elution Solution I (follow the recipe from the User Guide). Vortex and centrifuge briefly.
7. Place the sample on a magnetic separator and remove the supernatant.
8. Wash twice with **300 µl** freshly prepared 80% ethanol.
9. Elute with **40.5 µl** Elution Solution I.
10. Transfer **40 µl** to a new strip tube.

16 Post GEM Cleanup --SPRIselect 3d 0h 7m

1. Perform a 1.2X beads purification with SPRIselect reagent.
2. Incubate sample with beads for **00:05:00** at room temperature
3. Wash twice with **200 µl** freshly prepared 80% ethanol
4. Elute with **40.5 µl** Qiagen Buffer EB. Incubate for **00:02:00**
5. Bind beads to magnet and transfer **40 µl** eluant to a new strip tube.
6. Store at **4 °C** for up to **72:00:00** or at **-20 °C** for up to 4 weeks. Or proceed to the next step.

Library Construction

17 Sample Index PCR

1. Thaw, vortex, and centrifuge a tube of SI-Primer B and a tube of Amp Mix and store on ice.
2. Prepare Sample Index PCR Mix on ice (follow the recipe from the User Guide). Vortex and centrifuge briefly.
3. Add **57.5 µl** Sample Index PCR Mix to **40 µl** sample.
4. Add **2.5 µl** individual Chromium i7 Sample Index N, Set A to each sample.
5. Pipette mix 15X to mix and centrifuge briefly.
6. Incubate in a thermocycler to amplify DNA.

- Use cycle number optimization table for total number of cycles
- Maximum: load 1,600 nuclei - perform 12 cycles

18 Post Sample Index PCR Double Sided Size Selection - SPRIselect 5m

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1. Vortex to resuspend the SPRIselect reagent. Add **40 µl** SPRIselect reagent (0.4X) to each sample and pipette mix 15X.
2. Incubate **00:05:00** at room temperature.
3. Centrifuge briefly
4. Place sample on the magnetic separator. DO NOT discard the supernatant.
5. Transfer **130 µl** supernatant to a new tube strip tube.
6. Vortex to resuspend the SPRIselect reagent. Add **74 µl** SPRIselect reagent (1.14X) to each sample and pipette mix 15X.
7. Place sample on the magnetic separator and remove supernatant. DO NOT discard any beads.
8. Wash twice with **200 µl** freshly prepared 80% ethanol
9. Elute with **20 µl** Qiagen Buffer EB.
10. Bind beads to magnet and transfer **20 µl** eluant to a new strip tube.

Post Library Construction QC:
Quantify library (e.g. using Qubit dsDNA HS assay).
Estimate library size range (e.g. using TapeStation or Bioanalyzer) - expected size range is 170-700bp and including nucleosome pattern of at least 3 different bands/peaks.

Sequencing

19 MiSeq Sequencing - **QC for estimation of library quality and number of nuclei captured, target 10,000 reads per nucleus**

1. Paired End, Single Indexing
   a. Read 1: 50 cycles
   b. i7 Index: 8 cycles
   c. i5 Index: 16 cycles
   d. Read 2: 50 cycles
2. Library Loading
   a. 10X recommended Loading concentration: 11 pM
   b. Optional: 1% PhiX
3. Output
   a. 22-25 million reads

20 NovaSeq Sequencing (target - 50,000-100,000 reads per nucleus)

1. Paired End, Single Indexing
   a. Read 1: 50 cycles
   b. i7 Index: 8 cycles
   c. i5 Index: 16 cycles
   d. Read 2: 50 cycles
2. Library Loading
   a. 10X recommended Loading concentration: 280 pM
   b. Optional: 1% PhiX
3. Output
   a. SP: 650–800 million reads
   b. S1: 1.3–1.6 billion reads
   c. S2: 3.3 –4.1 billion reads
   d. S4: 8-10 billion reads

Cell Ranger Mapping and Analysis Pipeline

a. Generate Sample Sheet

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Use sample sheet generator provided by 10X Genomics to generate a "SampleSheet.csv":Sample Sheet Generator

b. Generate fastq files

Use **mkfastq** command, e.g.:
cellranger-atac **mkfastq** --id=sample1 --run=/IlluminaOutput/Run --samplesheet=Samplesheet.csv

c. Run count for each sample

Use **count** command, e.g.:
cellranger-atac **count** --id=sample1 --reference=/opt/refdata-cellranger-arc-GRCh38-2020-A-2.0.0 --fastqs=/IlluminaOutput/Run/fastq_path --sample=mysample --localcores=8 --localmem=64

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