Protocol

Epigenomic, cistromic, and transcriptomic profiling of primary kidney tubular cells

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Abstract

Spatiotemporal regulation of gene expression is essential for maintaining cellular homeostasis throughout kidney development and disease progression. Transcription factors (TFs) and epigenetic modifications play pivotal roles in controlling gene expression. Profiling chromatin modifications across the genome, along with the distribution and target regulation by TFs in specific kidney cell types, is crucial for understanding the dynamic changes in gene expression. Here, we presented a comprehensive workflow for epigenomic, cistromic, and transcriptomic analyses of primary kidney tubular cells. Specifically, our methodologies included the isolation of primary kidney tubular epithelial cells, RNA extraction, assay for transposase-accessible chromatin using sequencing, ultra-low-input micrococcal nuclease-based native chromatin immunoprecipitation, cleavage under targets and release using nuclease, and subsequent bioinformatic analysis. This protocol provides a methodological framework for investigating the roles of TFs and epigenetic modifications in kidney development and diseases.

Keywords: Epigenomic, Cistromic, Transcriptomic, Kidney disease

1. INTRODUCTION

Cellular homeostasis is largely governed by transcription factor (TF)-controlled gene expression programs [1]. TFs bind to short, specific DNA sequences known as motifs, which are crucial for activating or repressing downstream target genes essential for normal cellular functions and responses to stimuli [1,2]. Epigenetic modifications, including chromatin accessibility, DNA methylation, and histone modifications, establish appropriate chromatin states in a context-dependent manner. These modifications facilitate TF binding and subsequent gene expression [3,4]. Importantly, TFs operate in a cell-type- and spatiotemporal-context-specific fashion. Disruptions in TF activity can lead to developmental anomalies and contribute significantly to the progression of renal diseases [5-7].

Chromatin immunoprecipitation coupled with sequencing (ChIP-seq) is widely used to identify the genomic locations of TFs and epigenetic modifications [8-10]. However, ChIP-seq typically requires a substantial amount of cells, and the use of formaldehyde for cross-linking can reduce signal-to-noise ratio, posing challenges for its application in rare or specific cell populations [11]. This limitation is particularly problematic given the kidney's complex tissue architecture, which comprises over 40 distinct cell types, each being characterized by unique transcriptional profiles [12]. For histone modifications, these challenges can be mitigated using a non-crosslinked method known as ultra-low-input micrococcal nuclease-based native chromatin immunoprecipitation (ULI-NChIP). However, this technique is unsuitable for profiling TFs [11]. An alternative method, Cleavage Under Targets and Release Using Nuclease (CUT&RUN), offers a high-resolution genomic approach for mapping TF binding sites [13]. Its advantages include lower cell requirements, higher spatial resolution, a simplified experimental workflow, preservation of natural protein-DNA interactions, and high sensitivity for detecting low-abundance

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TF binding events [13-15]. Collectively, these benefits render CUT&RUN a powerful tool for exploring the binding dynamics of epigenetic factors and TFs across the genome.

In this study, we introduced a comprehensive workflow for the epigenomic, cistromic, and transcriptomic analysis of primary kidney tubular cells. This protocol includes specialized steps for isolating kidney tubular epithelial cells using lectin or agglutinin-based separation techniques, optimizing RNA extraction from limited cell quantities. In addition, the workflow encompasses the application of assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) for analyzing chromatin accessibility [16], ULI-NChIP for profiling histone modifications, and the use of CUT&RUN to map the binding sites of TF, followed by bioinformatic analysis. Our methodology effectively addresses the aforementioned challenges, allowing for the use of minimal cell numbers while preserving essential cell-type-specific information. This approach facilitates the functional analysis of TFs and epigenomic modifications involved in kidney development and disease progression, providing valuable insights into the molecular mechanisms underlying these processes.

2. MATERIALS

2.1. Chemicals/reagents/others

- 10X Gentle collagenase/hyaluronidase in DMEM (STEMCELL Technologies, 07919)
- EpiCult™-B Mouse Medium Kit (STEMCELL Technologies, 05610)
- Fetal Bovine Serum (VivaCell, C2910-0500)
- Hanks' Balanced Salt Solution (STEMCELL Technologies, 37150)
- Ammonium Chloride Solution (STEMCELL Technologies, 07800)
- Dispase in Hanks' Balanced Salt Solution (STEMCELL Technologies, 07913)
- RPMI 1640 (VivaCell, C3001-0500)
- 5 mg/mL Deoxyribonuclease I (Sigma Aldrich, D4513)
- Trypsin-EDTA (0.25%), phenol red (Gibco, 25200056)
- Falcon® 40 μm Cell Strainer (Corning, 352340)
- Biotinylated Lotus Tetragonolobus Lectin (Vector Laboratories, B-1325)
- Biotinylated Dolichos Biflorus Agglutinin (Vector Laboratories, B-1035)
- CELLection™ Biotin Binder Kit (Invitrogen, 11533D)
- TRIzol™ Reagent (Invitrogen, 15596026CN)
- Concanavalin-coated magnetic beads (Bangs Laboratories, BP531)
- Spermidine (Sigma Aldrich, S0266)
- Roche Complete Protease Inhibitor EDTA-Free tablets (Sigma Aldrich, 5056489001)
- Nuclease-Free Water (Invitrogen, AM9937)
- 1 M HEPES (Gibco, 15630106)
- 5 M Sodium chloride solution, RNase-free (Invitrogen, AM9759)
- 1 M Manganese chloride solution (Sigma Aldrich, M1787)
- 1 M Magnesium chloride solution (Invitrogen, AM9530G)
- 1 M Calcium chloride solution (Sigma Aldrich, 21115)
- 1 M Potassium chloride solution (Sigma Aldrich, 60142)
- 8 M Lithium chloride solution (Sigma Aldrich, L7026)
- 0.5 M Ethylenediaminetetraacetic acid, pH 8.0, RNase-free (Invitrogen, AM9261)
- 0.2 M Ethylene glycol-bis(β-aminoethyl ether)-N,N,N,N-tetraacetic acid (Sigma Aldrich, E3889)
- N,N-Dimethylformamide (Sigma Aldrich, 227056)
- Nuclease micrococcal from *Staphylococcus aureus* (Sigma Aldrich, N3755)
- Bovine serum albumin (Solarbio, A8020)
- Digitonin (EMD Millipore, 300410)
- Concanavalin-coated magnetic beads (Bangs Laboratories, BP531)
- Pierce™ Protein G Magnetic Beads (Thermo Scientific, 88848)
- Sodium dodecyl sulfate (Sigma Aldrich, L4509)
- Sodium Deoxycholate (Sigma Aldrich, S1827)
- 10 mg/mL RNase A, DNase, and protease-free (ThermoFisher Scientific, EN0531)
- 20 mg/mL Glycogen (Invitrogen, R0551)
- Phenol-chloroform-isoamyl alcohol 25:24:1 (Sigma Aldrich, 77617)
- Chloroform (Sigma Aldrich, C2432)
- Proteinase K (Transgen, GE201-01)
- QIAquick PCR Purification Kit (QIAGEN, 28104)
- VAHTS Universal DNA Library Prep Kit (Vazyme, ND607)
- TruePrep DNA Library Prep Kit (Vazyme, TD501)
- VAHTS DNA Clean Beads (Vazyme, N411)
- Qubit™ RNA IQ Assay Kits (Invitrogen, Q33221)
- Qubit™ dsDNA Quantification Assay Kits (Invitrogen, Q32851)
- Agilent High Sensitivity DNA Kit (Agilent, 5067-4626).

Cell Buffer 1	
Materials	**Volume**
Phosphate buffered saline	49.5 mL
10% BSA (w/v)	500 μL
Total	50 mL

Cell Buffer 2	
Materials	**Volume**
Cell Buffer 1	9.96 mL
0.5 M EDTA	40 μL
Total	10 mL

Cell Buffer 3	
Materials	**Volume**
RPMI 1640	9.84 mL
Fetal bovine serum	100 µL
1 M $CaCl_2$	10 µL
1 M $MgCl_2$	50 µL
Total	10 mL

ATAC Lysis Buffer	
Materials	**Volume**
1 M Tris-HCl (pH 7.5)	500 µL
5 M NaCl	100 µL
1 M $MgCl_2$	167 µL
IGEPAL CA-630	50 µL
Nuclease-free Water	49.183 mL
Total	50 mL

ATAC Reaction Buffer	
Materials	**Volume**
1 M Tris-HCl (pH 7.5)	500 µL
1 M $MgCl_2$	250 µL
Dimethylformamide	5 mL
Nuclease-free Water	44.25 mL
Total	50 mL

ULI Lysis Buffer	
Materials	**Volume**
NP40	250 µL
Tween-20	250 µL
10% SDS	500 µL
Nuclease-free Water	49 mL
Total	50 mL

ULI MNase Buffer	
ULI MNase Buffer	**ULI MNase Buffer**
1 M Tris-HCl (pH 8.0)	5 mL
1 M $CaCl_2$	100 µL
Nuclease-free Water	44.9 mL
Total	50 mL

ULI Stop Buffer	
Materials	**Volume**
1 M Tris-HCl (pH 8.0)	5.5 mL
0.5 M EDTA (pH 8.0)	5.5 mL
Nuclease-free Water	39 mL
Total	50 mL

ULI 2X RIPA Buffer	
Materials	**Volume**
5 M NaCl	2.8 mL
Triton X-100	500 µL
10% SDS	500 µL
Sodium deoxycholate	0.1 g
0.1 M EGTA (pH 8.0)	2.5 mL
Nuclease-free Water	43.7 mL
Total	50 mL

ULI RIPA Buffer	
Materials	**Volume**
1 M Tris-HCl (pH 8.0)	500 µL
5 M NaCl	1.4 mL
Triton X-100	500 µL
10% SDS	500 µL
Sodium Deoxycholate	0.05 g
0.5 M EDTA (pH 8.0)	100 µL
Nuclease-free Water	47 mL
Total	50 mL

Add Proteinase inhibitor before use.

ULI LiCl Buffer	
Materials	**Volume**
1 M Tris-HCl (pH 8.0)	500 µL
NP40	250 µL
0.5 M EDTA (pH 8.0)	100 µL
Sodium Deoxycholate	0.25 g
8 M LiCl	1.5625 ml
Nuclease-free Water	47.59 mL
Total	50 mL

Binding Buffer	
Materials	**Volume**
1 M HEPES-KOH (pH 7.9)	1 mL
1 M KCl	500 µL
1 $CaCl_2$	50 µL
1 $MnCl_2$	50 µL
Nuclease-free Water	48.4 mL
Total	50 mL

Wash Buffer	
Materials	**Volume**
1 M HEPES (pH 7.5)	1 mL
5 M NaCl	1.5 mL
2 M Spermidine	12.5 µL
30% BSA (w/v)	167 µL
Nuclease-free Water	47.32 mL
Total	50 mL

Add Proteinase inhibitor before use.

Digitonin Buffer	
Materials	**Volume**
5% Digitonin	400 µL
Wash Buffer	39.6 mL
Total	40 mL

Add Proteinase inhibitor before use.

Antibody Buffer	
Materials	**Volume**
0.5 M EDTA	1 µL
Digitonin Buffer	249 µL
Total	250 µL

Add Proteinase inhibitor before use.

2X Stop Buffer	
Materials	**Volume**
5 M NaCl	40 µL
0.5 M EDTA	40 µL
0.2 M EGTA	20 µL
20 mg/mL RNaseA	2.5 µL
20 mg/mL Glycogen	2 µL
10 ng/mL Spike-in DNA	1 µL
Nuclease-Free Water	894.5 µL
Total	1 mL

2.2. Equipment

- Biological Safety Cabinet (Esco Labculture, AC2)
- Centrifuge (Eppendorf, 5424R and 5810R)
- Thermomixer (Hangzhou Allsheng Instruments, MS-100)
- Vortex mixer (Kylin-Bell Lab Instruments, VORTEX-5)
- Incubator shaker (Shanghai Zhicheng, ZWY-240)
- General rotator (Thermo Scientific, 11-676-341)
- PCR machine (Applied Biosystems, 4484073)
- Microvolume UV-Vis Spectrophotometer (Thermo Scientific, NanoDrop One)
- Qubit 4 Fluorometer (Invitrogen, Q33226)
- Bioanalyzer (Agilent, 2100).

2.3. Software packages

- Bowtie2 (Version 2.3.4.1)
- Hisat2 (Version, 2.1.0)
- SAMtools (Version 1.7)
- Deeptools (Version 3.4.3)
- HOMER (Version 4.11)
- FeatureCounts (Version 1.6.0)
- Integrative Genomics Viewer (Version 2.3.34).

3. PROCEDURES

The experimental workflow for RNA-seq and CUT&RUN-seq in LTL- or DBA-positive cells is shown in Figure 1, and the procedures are detailed as follows.

3.1. Prepare kidney single-cell suspension

1. Freshly extract kidney tissues from the mouse and finely minced them using scissors and scalpel.
2. Transfer the minced tissues into a clean 15 mL centrifuge tube.
3. For each 50 mg of kidney tissues, digest with 1 mL of gentle collagenase/hyaluronidase in 9 mL of EpiCult™-B Mouse Medium (2% FBS is added before use).
4. Incubate and rotate at 90 rpm for 1 h at 37°C.
5. Centrifuge at 350 g for 5 min at 4°C and discard the supernatant.
6. Resuspend above cell pellets in 1 mL of ice-cold HBSS and 4 mL of ammonium chloride solution supplemented with 2% FBS, then centrifuge at 350 g for 5 min at 4°C and discard supernatant to remove red blood cells.
7. Add 2 mL of pre-warmed 0.05% Trypsin-EDTA (diluted in PBS) and incubate at 37°C for 2 min.
8. Quench the Trypsin activity by adding 10 mL HBSS (containing 2% FBS).
9. Centrifuge at 350 g for 5 min at 4°C and discard the supernatant.
10. Add 2 mL of pre-warmed 5 U/mL Dispase supplemented with 20 µL of DNase I solution and pipette the samples for 1–2 min.
11. Centrifuge at 350 g for 5 min at 4°C and discard the supernatant.
12. Add 10 mL of ice-cold HBSS (containing 2% FBS) and filter the cell suspension through a 40 µm strainer into a clean 50 mL tube.
13. Centrifuge at 350 g for 5 min at 4°C and discard the supernatant.

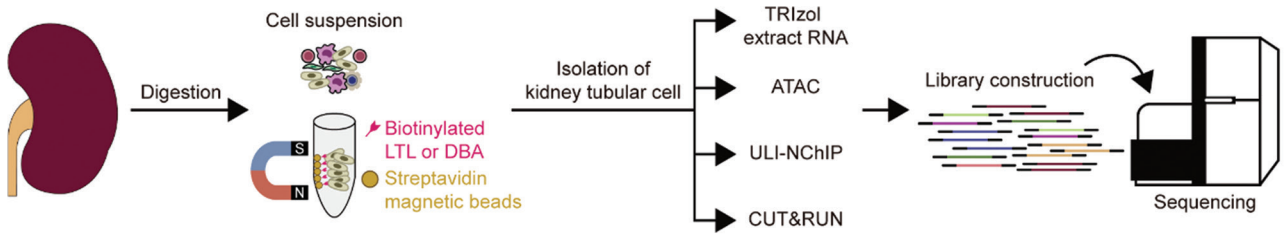

Figure 1. Workflow for epigenomic, cistromic, and transcriptomic analyses in primary kidney tubular cells. A schematic diagram showing the experimental procedure, including LTL- or DBA-positive cells isolation, total RNA extraction, ATAC, CUT&RUN, ULI-NChIP, library construction, and next-generation sequencing.
ATAC: Assay for Transposase-Accessible Chromatin; CUT&RUN: Cleavage Under Targets and Release Using Nuclease; ULI-NChIP: Ultra-low-input micrococcal nuclease-based native chromatin immunoprecipitation.

3.2. Enrichment of kidney tubular cells

1. Transfer 25 μL of Biotin Binder Dynabeads (from CELLection™ Biotin Binder Kit) into a clean 1.5 mL centrifuge tube.
2. Resuspend the Dynabeads in 1 mL of Cell Buffer 1.
3. Place the tube in a magnet for 1 min and discard the supernatant.
4. Repeat steps 2.2 to 2.3 once and resuspend the Dynabeads with 25 μL of Cell Buffer 1.
5. Resuspend 10^7 of cells in 1 mL of Cell Buffer 2.
6. Repeat step 2.3.
7. Wash the cells once more by repeating steps 2.5 to 2.6.
8. For proximal tubular cells, add 10 μg of Biotinylated Lotus Tetragonolobus Lectin.

For distal tubular cells, add 10 μg of Biotinylated Dolichos Biflorus Agglutinin.

1. Rotate on a rotator at 4°C for 15 min.
2. Wash cells twice by repeating steps 2.5 to 2.6.
3. Resuspend cells with 1 mL Cell Buffer 2 and mixed with 25 μL of pre-washed Biotin Binder Dynabeads (from step 2.4).
4. Rotate on a rotator at 4°C for 30 min.
5. Wash the cells twice with Cell Buffer 1 by repeating steps 2.2 to 2.3.
6. Resuspend the bead-bound cells in 200 μL of pre-warmed Cell Buffer 3 (at 37°C).
7. Add 4 μL of release buffer (from CELLection™ Biotin Binder Kit).
8. Rotate on a rotator at room temperature for 15 min.
9. Pipette the cell suspension 10 times to maximize cell release.
10. Place the tube in a magnet for 1 min and transfer the cell suspension into a new 1.5 mL centrifuge tube.

3.3. Total RNA extraction of primary kidney tubular cells

1. Centrifuge the purified tubular cells at 350 g for 5 min at 4°C and discard the supernatant.
2. For each sample, count and transfer 10^5 of the purified cells into a new 1.5 mL centrifuge tube.
3. Wash the cells twice by adding 1 mL of ice-cold PBS (containing 2% FBS), then centrifuge at 350 g for 5 min at 4°C and discard the supernatant.
4. Resuspend the cell pellets in 1 mL of TRIzol™ Reagent.
5. Mixed with 200 μL of chloroform by vortex for 15 s.
6. Incubate at room temperature for 5 min.
7. Centrifuge at 12000 g for 15 min at 4°C.
8. Carefully transfer the aqueous phase to a new 1.5 mL tube.
9. Add 500 μL of cold isopropanol (-20°C) and 1 μL of glycogen.
10. Place the tube on ice for 10 min.
11. Centrifuge at 12000 g for 15 min at 4°C and carefully remove the supernatant.
12. Wash the RNA pellet twice by adding 1 mL of cold 70% ethanol (-20°C), then centrifuge at 12000 g for 5 min at 4°C and carefully remove the supernatant.
13. Dry the RNA pellet for 2 min and dissolve the total RNA in 20 μL of nuclease-free water.
14. Measure the RNA concentration on NanoDrop. Measure the RNA integrity and quality on Qubit 4 Fluorometer using the Qubit™ RNA IQ Assay Kits.
15. After library construction, subject the samples to next-generation sequencing on an Illumina platform.

3.4. ATAC-seq in primary kidney tubular cells

1. Count and transfer 10^4 of the purified tubular cells (from step 2.18) into a 1.5 mL tube.
2. Resuspend the purified cells in 1 mL of ice-cold PBS.
3. Centrifuge at 500 g for 5 min at 4°C and remove the supernatant.
4. Resuspend cells in 50 μL of ATAC Lysis Buffer and place the tube on ice for 10 min.
5. Centrifuge at 500 g for 5 min at 4°C and discard the supernatant.
6. Resuspend cells in 50 μL of ATAC Reaction Buffer supplemented with 1 μL of Tagment Enzyme (from TruePrep DNA Library Prep Kit).
7. Place the tube on a Thermomixer. Incubate and rotate at 900 rpm for 30 min at 37°C.
8. Extract and purify the DNA using QIAquick PCR Purification Kit.
9. Measure the DNA concentration on Qubit 4 Fluorometer using Qubit™ dsDNA Quantification Assay Kits.
10. Mix purified DNA thoroughly with the following materials (from TruePrep DNA Library Prep Kit) required for the PCR reaction.
11. Briefly centrifuge the mixture and start PCR reaction according to the following process.
12. Purify the amplified DNA library using VATHS Clean Beads.
13. Measure the DNA concentration on Qubit 4 Fluorometer using Qubit™ dsDNA Quantification Assay Kits. Measure the DNA library quality on the Agilent 2100 Bioanalyzer using Agilent High Sensitivity DNA Kit. Then, proceed to subject the samples to next-generation sequencing on an Illumina platform.

Components for the PCR reaction	
Materials	**Volume**
Purified DNA	20 μL
5 x TAB	10 μL
PPM	5 μL
P5 Primer	5 μL

P7 Primer	5 μL
TAE	1 μL
Total	50 μL

PCR reaction process			
Steps	**Temperature**	**Time**	**Cycle**
I	72°C	5 min	1
II	98°C	30 s	1
III	98°C	10 s	10-15
	63°C	30 s	
	72°C	1 min	
IV	72°C	2 min	1
V	4°C	Hold	-

3.5. ULI-NChIP of histone modifications in primary kidney tubular cells

1. Count and transfer 10^4 of the purified tubular cells (from step 2.18) into a 1.5 mL tube.
2. Centrifuge at 2000 g for 5 min at 4°C and discard the supernatant.
3. Resuspend cells in 20 μL of ULI Lysis Buffer. Incubate on ice for 10 min.
4. Mix cell suspension with 20 μL of ULI MNase Buffer supplemented with 0.02 U of MNase.
5. Incubate at 37°C for 5 min.
6. Immediately place the tube on ice and mix the sample with 5 μL of ULI Stop Buffer.
7. Add 45 μL of ULI 2X RIPA Buffer.
8. Centrifuge at 14500 g for 15 min at 4°C and transfer the supernatant into a new 1.5 mL tube.
9. Add 40 μL of ULI RIPA Buffer supplemented with 0.5–1 μg of antibody.
10. Rotate on a rotator at 4°C overnight.
11. Add 400 μg of Protein G Magnetic Beads, Rotate on a rotator at 4°C for 2 h.
12. Wash the bead-bound DNA five times, each time with 150 μL of ULI RIPA Buffer. Wash the bead-bound DNA once with 150 μL of ULI LiCl Buffer. Resuspend the beads with 30 μL of nuclease-free water supplemented with 1 μL of Proteinase K (20 mg/mL).
13. Place the tube on a Thermomixer. Rotate at 900 rpm for 90 min at 55°C.
14. Incubate at 72°C for 40 min to quench the activity of Proteinase K.
15. Measure the DNA concentration on Qubit 4 Fluorometer using Qubit™ dsDNA Quantification Assay Kits.
16. After library construction using the VAHTS Universal DNA Library Prep Kit, measure the DNA library quality on the Agilent 2100 Bioanalyzer using Agilent High Sensitivity DNA Kit. Then, proceed to subject the samples to next-generation sequencing on an Illumina platform.

3.6. CUT&RUN-seq of TFs of interest in kidney tubular cells

1. Mix 0.5 μg antibody and 70 ng pA-MNase with 100 μL of Antibody Buffer in a 1.5 mL tube. Place the tube on ice until further use.
2. For each 10^4 of cells, transfer 10 μL of concanavalin-coated magnetic beads into a 1.5 mL tube.
3. Resuspend the concanavalin-coated magnetic beads in 100 μL of Binding Buffer.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Repeat steps 6.3-6.4 once. Resuspend concanavalin-coated magnetic beads in 10 μL of Binding Buffer. Place the tube on ice until further use.
6. Count and transfer 10^4 of the purified tubular cells (from step 2.18) into a 1.5 mL tube. Centrifuge at 350 g for 5 min at room temperature and discard the supernatant.
7. Wash the cells twice by adding 1 mL of Wash Buffer, then centrifuge at 600 g for 3 min at room temperature and discard the supernatant.
8. Resuspend the cells in 100 μL of Wash Buffer supplemented with 10 μL pre-washed concanavalin-coated magnetic beads (from step 6.5) using a vortex.
9. Rotate on a rotator at room temperature for 10 min.
10. Place the tube in a magnet for 1 min and discard the supernatant.
11. Resuspend the bead-bound cells in 100 μL of Antibody Buffer (from step 6.1) using a vortex mixer.
12. Rotate on a rotator at 4°C for 4 h to overnight.
13. Wash the bead-bound cells twice by adding 800 μL of Digitonin Buffer. Then, place the tube in a magnet for 1 min and discard the supernatant.
14. Resuspend the bead-bound cells in 100 μL of Digitonin Buffer using a vortex.
15. Place the tube on ice for at least 5 min to chill the cells to 0°C.

Tips: It is better to use wet ice.

16. Mix the sample with 2 μL of 100 mM $CaCl_2$ using a vortex mixer.
17. Immediately place the tube back on ice and incubate for 30 min.
18. Mix the sample with 100 μL of 2X Stop Buffer using a vortex mixer.
19. Incubate at 37°C for 30 min.
20. Place the tube in a magnet for 1 min, then transfer the supernatant into a new 1.5 mL tube.
21. Mix the sample with 2 μL of 10% SDS and 2.5 μL of Proteinase K (20 mg/mL).
22. Incubate at 50°C for 1 h.
23. Mix the sample with 200 μL of PCI.
24. Centrifuge at 16000 g for 5 min.

25. Carefully transfer the aqueous phase to a new 1.5 mL tube.
26. Add 200 µL of chloroform and mix it by inverting 8-10 times.
27. Repeat steps 6.24-6.25.
28. Add 1 µL of glycogen (20 mg/mL) and 500 µL of cold ethanol (-20°C).
29. Centrifuge at 16000 g for 15 min at 4°C and discard the supernatant.
30. Wash the DNA pellet twice by adding 1 mL of cold ethanol (-20°C), then centrifuge at 16000 g for 5 min at 4°C and discard the supernatant.
31. Dry the pellet for 5 min and dissolve the DNA in 30 µL of nuclease-free water.
32. Measure the DNA concentration on Qubit 4 Fluorometer using Qubit™ dsDNA Quantification Assay Kits and measure the DNA quality on the Agilent 2100 Bioanalyzer using the Agilent High Sensitivity DNA Kit.
33. After library construction using the VAHTS Universal DNA Library Prep Kit, proceed to subject the samples to next-generation sequencing on an Illumina platform.

3.7. Integrative cistromic and transcriptomic analyses

1. Map RNA-seq reads to the reference genome using HISAT2 with the default options.
2. Count the number of mapped reads on each gene using FeatureCounts.
3. Calculate FPKM by dividing the number of fragments of a gene by the total sequencing depth and then dividing the ratio by the gene length.
4. Generate the bigwig files from RNA-seq by DeepTools and visualized by IGV (Figure 2A).
5. Identify differentially expressed genes (DEGs) using DESeq2 with a fold change >2 and FDR <0.05.
6. For ULI-NChIP-seq, map reads to the mouse reference genome using Bowtie2 with the default options. For ATAC-seq, map reads to reference genome using paraments: --local --very-sensitive --no-mixed --no-discordant -I 25 -X 700. For CUT&RUN-seq, map reads to reference genome using paraments: --local, --very-sensitive-local, --no-unal, --no-mixed, --no-discordant, --phred33, -I 10, and -X 700.
7. Remove unmapped and duplicate reads using samtools-rmdup with the default options.
8. Call peak and annotate the peak using HOMER with the default paraments.
9. Generate the bigwig files from ATAC-seq, ULI-NChIP-seq, and CUT&RUN-seq by DeepTools and visualized by IGV (Figure 2A).
10. Generate columns by using GraphPad with the FPKM values of genes annotated to peaks. (Figure 2B).

4. TROUBLESHOOTING

Potential issues and suggestions			
Item	**Description**	**Causes**	**Suggestions**
I	Low RNA or DNA concentration	Low input or significant loss during processing	Increase the starting amount of cells or reduce the time of washing
II	Low-enriched DNA is eluted after CUT&RUN	Low input or low antibody binding efficiency	Increase the starting amount of cells or use a secondary antibody
III	High background noise in the next-generation data	Low signal-to-background ratio	Remove dead cells or use high-salt digitonin buffer to wash the bead-bound cells

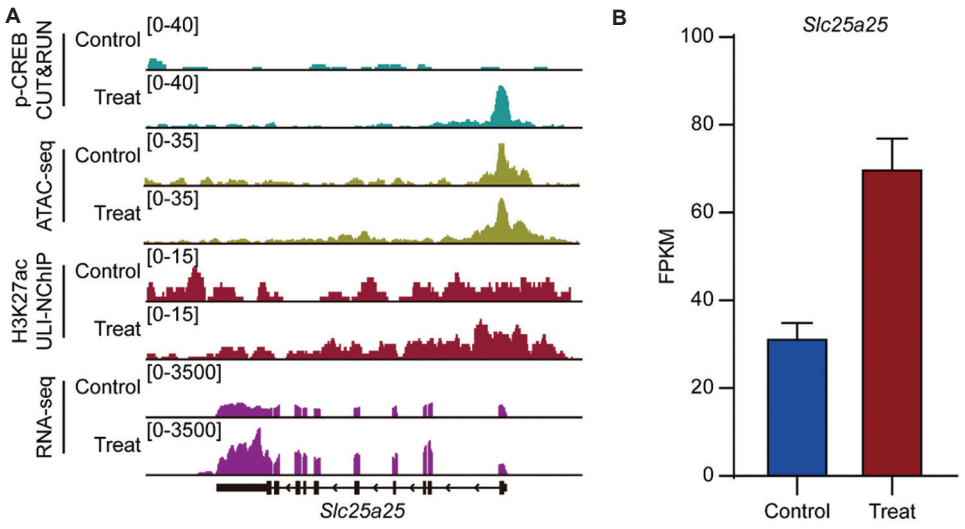

Figure 2. Representative track profiles of CUT&RUN, ATAC-seq, ULI-ChIP-seq, and RNA-seq on the *Slc25a25* gene. (A) IGV images show the binding of a transcription factor (phosphorylated CREB) to the promoter of *Slc25a25*, along with the read density of ATAC-seq, ULI-NChIP of histone modification (H3K27ac) and mRNA-seq in kidney tubular cells in control or treatment group (N = 1). (B) Columns exhibit the FPKM values of *Slc25a25* mRNA in kidney tubular cells in control or treatment group (N = 2). Log_2 Foldchange = 1.416768902, *Padj* = 1.09924624612751E-27. ATAC: Assay for Transposase-Accessible Chromatin; CUT&RUN: Cleavage Under Targets and Release Using Nuclease; ULI-NChIP: Ultra-low-input micrococcal nuclease-based native chromatin immunoprecipitation.

5. DISCUSSION

In this study, we detailed a protocol for conducting ULI-NChIP-seq for profiling histone modifications, ATAC-seq for chromatin accessibility analysis, and CUT&RUN-seq for TF characterization in freshly isolated kidney tubular cells. Recently, while Cleavage Under Targets and Tagmentation (CUT&Tag) technology has been adapted to single-cell applications [17], integrating single-cell CUT&Tag with single-cell transcriptomic data remains challenging [17,18]. Future studies are warranted to overcome technical barriers associated with performing single-nucleus CUT&RUN (or CUT&Tag) and RNA-seq simultaneously in the same cell. Achieving this integration will be crucial for a more in-depth understanding of the molecular mechanisms driving renal biology and pathology. Moreover, longitudinal studies could provide dynamic insights into TF activity and epigenetic modifications throughout kidney disease progression, potentially leading to the discovery of diagnostic markers or therapeutic targets. The integration of these findings with other omics data, such as proteomic or metabolomic data, alongside advancements in bioinformatic tools for handling complex multi-omics integration, could greatly enhance our understanding of regulatory networks in kidney cells and beyond [19]. In addition, improving the efficiency and accessibility of CUT&RUN and other genomic techniques could revolutionize their application in routine laboratory use and large-scale studies, paving the way for new therapeutic approaches and deeper insights into kidney biology and pathology.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Yupeng Chen
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All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All mouse experiments involved in kidney tubular cell isolation in this study were approved by the Ethics Committee of Tianjin Medical University.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA

All the raw sequencing files and processed data used in this protocol were submitted to Gene Expression Omnibus with the accession numbers GSE165416, GSE165417, GSE244997, and GSE244998 (https://ncbi.nlm.nih.gov/geo/).

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