UCSD Center for Epigenomics ATAC-seq protocol for cells

(adapted from Buenrostro et al., 2015; PMID: 25559105)

Tissue pulverization

Ensure the samples are kept frozen on dry ice throughout pulverization.

1. Pour liquid nitrogen into a mortar and pestle until frozen.
2. Remove tissue from tube and place into liquid nitrogen with a clean cold spatula.
3. Grind up the sample with liquid nitrogen using the mortar and pestle.
4. Use a clean cold spatula to scoop ground tissue back to the original tube.
5. Place the sample back onto dry ice or directly to -80 °C.
6. Clean the mortar and pestle with 10% bleach and 70% ethanol before using it for the next sample.

Nuclei preparation

7. Transfer 5-15 mg pulverized tissue in a frozen 1.5 ml tube. Amount of tissue needed depends on the tissue type.
8. Immediately resuspend in 1 ml nuclei permeabilization buffer (NPB) and rotate for 10 min at 4°C.

Composition of nuclei permeabilization buffer (NPB):

Reagent	Stock concentration	Final concentration	Amount per 10 ml
BSA (A7906, Sigma)	--	5 %	500 mg
IGEPAL-CA630 (I8896, Sigma)	10 % (m/V)	0.2 % (m/V)	200 µl
DTT (D9779, Sigma)	200 mM	1 mM	50 µl
cOmplete EDTA-free protease inhibitor (05056489001, Roche)	25x	1x	400 µl
PBS (10010-23, Thermo Fisher Scientific)	--	--	9.35 ml

9. Filter nuclei suspension using a 30 µm CellTrics (25004-0042-2316, Sysmex) and spin down for 5 min at 500 g at 4 °C.
10. Resuspend nuclei in cold 50 µl tagmentation buffer (TB) and place on ice. Take an aliquot and determine nuclei concentration using a hemocytometer, e.g. use 5 µl nuclei suspension, add 20 µl TB and 25 µl Trypan Blue Stain (0.4 %, 15250061, Thermo Fisher Scientific)

Composition of tagmentation buffer (TB)

Reagent	Stock concentration	Final concentration	Volume per 10 ml
Tris-acetate (pH=7.8, BP-152, Thermo Fisher Scientific)	1 M	33 mM	330 µl
K-acetate (P5708, Sigma)	3 M	66 mM	220 µl
Mg-acetate (M2545, Sigma)	300 mM	11 mM	367 µl
DMF (DX1730, EMD Millipore)	--	16 %	1.6 ml
Molecular biology water (46000-CM, Corning)	--	--	7.483 ml

11. Adjust nuclei concentration to 2,000-5,000 nuclei/µl (20-50K total) and use 10 µl for tagmentation

Tagmentation and library generation

12. Add 0.5 µl Tagment DNA Enzyme 1 (FC-121-1030, Illumina) mix 5 times by pipetting and incubate 30 min with 500 rpm at 37 °C
13. After completion add 100 µl PB buffer (Qiagen) and 5 µl Na-acetate (3 M, pH = 5.2). Purify using MinElute PCR Purification Kit (28004, Qiagen) and elute in 10 µl EB.
14. Amplify fragments by PCR. Please see "Appendix" for primer sequences.

Reaction setup

Reagent	Volume per sample
Tagmented DNA	10 µl
NEBNext 2x PCR MasterMix (M0541, NEB)	25 µl
i5-primer (25 µM)	2 µl
i7-primer (25 µM)	2 µl
Molecular biology water (46000-CM, Corning)	11 µl

Temperature profile

Step 1: 72°C for 5 min
Step 2: 98°C for 30 s
[Step 3: 98°C for 10 s
Step 4: 63°C for 30s
Step 5: 72°C for 1 min]
(Repeat steps 3-5 for a total number of 8 cycles)
Step 6: keep at 12°C.

15. Add 250 µl Buffer PB (Qiagen) and 10 µl Na-acetate (3 M, pH = 5.2). Purify using MinElute PCR Purification Kit (28004, Qiagen) and elute in 20 µl EB.
16. Size selection with Ampure XP beads (A63880, Beckman Coulter). Add 180 µl EB and 110 µl beads (0.55 x sample volume) and mix 10 times by pipetting.
17. Incubate 5 min at room temperature. Separate on magnetic stand.
18. Transfer supernatant (300 µl) to a new tube.
19. Add 190 µl beads (1.5 x sample volume).
20. Incubate 5 min at room temperature. Separate on magnetic stand.
21. Wash beads 2 times with 200 µl EtOH (70 %).
22. After the second wash resuspend beads in 20 µl EB by pipetting 10 times.
23. Separate on magnetic stand and transfer 17 µl of final library to a new tube.

Quantification of library and quality check

24. Quantify final libraries using Qubit (1 µl/sample, Qubit dsDNA HS Assay Kit (Q32851), Thermo Fisher Scientific) and check for library size distribution using 4200 TapeStation (High Sensitivity D1000 ScreenTape (5067-5584) and Reagents (5067-5583), Agilent Technologies)**.**
25. Optional: Quantify final library using the KAPA DNA Quantification Kit (KK4953, KAPA Biosystems)
26. Samples are now ready for sequencing on Illumina platform e.g. HiSeq4000 (50 bp PE, Nextera v2 libraries).

Appendix

A. PCR Primer

PCR primers were ordered from IDT. Primer sequences were the same as in the Nextera Index XT Kit v2 (FC-131-2001, Illumina).

i5-Primer

Name	Index sequence	Full sequence
S502	CTCTCTAT	AATGATACGGCGACCACCGAGATCTACACCTCTCTATTCGTCGGCAGCGTC
S503	TATCCTCT	AATGATACGGCGACCACCGAGATCTACACTATCCTCTTCGTCGGCAGCGTC
S505	GTAAGGAG	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGTCGTCGGCAGCGTC
S506	ACTGCATA	AATGATACGGCGACCACCGAGATCTACACACTGCATATCGTCGGCAGCGTC
S507	AAGGAGTA	AATGATACGGCGACCACCGAGATCTACACAAGGAGTATCGTCGGCAGCGTC
S508	CTAAGCCT	AATGATACGGCGACCACCGAGATCTACACCTAAGCCTTCGTCGGCAGCGTC
S510	CGTCTAAT	AATGATACGGCGACCACCGAGATCTACACCGTCTAATTCGTCGGCAGCGTC
S511	TCTCTCCG	AATGATACGGCGACCACCGAGATCTACACTCTCTCCGTCGTCGGCAGCGTC

i7-Primer

Name	Index sequence	Full sequence
N701	TCGCCTTA	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGG
N702	CTAGTACG	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGG
N703	TTCTGCCT	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGG
N704	GCTCAGGA	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGG
N705	AGGAGTCC	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGG
N706	CATGCCTA	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGG
N707	GTAGAGAG	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGG
N710	CAGCCTCG	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGG
N711	TGCCTCTT	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGG
N712	TCCTCTAC	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGG