Improved - Assay for Transposase-Accessible Chromatin with high-throughput sequencing (OMNI-ATAC-seq)

This protocol mainly uses either the Illumina Nextera kit or the Vazyme Trueprep kit to prepare ATAC-seq libraries for the Illumina platform. Please look at the manufacturer's instructions and product information for more details.

The Omni-ATAC protocol is an improved protocol of the original ATAC-seq[1,2] protocol. The following adjustment on the lysis buffer and tagmentation buffer increase the signal to noise ratio significantly and reduced mitochondrial reads by ~20%.

Typically, the protocol yields best results with 50,000-100,000 cells but the protocol can be scale to work with different numbers of cell by optimizing the cell: transposons ratio.

Cells can be freshly harvested or snap-frozen cells[3]. For tissues, isolation of nuclei, please refer to Corces *et al*., 2017 for more details.

Notes:

- Make sure the cells are viable prior to tagmentation. A viability above 90% is recommended and preferably around 95%. If too many dead cells are present, they should be removed before lysis to avoid affecting the results[3].
- The use of LoBind tube is recommended.
- Some steps may need to be optimized depending on cell type

The following kit is required:

i) Illumina FC-121-1030 Nextera DNA Library Prep Kit (24 samples) @ Vazyme TD501TruePrep DNA Library Prep Kit V2 for Illumina®
ii) Illumina FC-121-101 Nextera Index Kit (24 indexes, 96 samples) @ Vazyme TD202/3 TruePrep Index Kit V2/V3 for Illumina (96reactions/384reactions)
iii) QIAGEN MinElute PCR purification kit (QIAGEN, cat# 28004)
iv) AMPure XP beads (Beckman, cat# A63881)
v) KAPA HiFi HotStart ReadyMix (ROCHE, cat# KK2601)
vi) KAPA Library Quantification Kit (ROCHE, cat# KK4854)
vii) Fragment Analyzer kit DNF-474-0500
viii) Qubit™ dsDNA HS (ThermoFisher, cat# Q32854)

I. Preparation of buffer:

1) Prepare the ATAC-Resuspension buffer (RSB) and keep it on ice for later use (~1.1ml per sample).

ATAC-Resuspension buffer (RSB)		
Reagent	**Final concentration**	**2ml**
1M Tris HCl (pH 7.4)	10mM	20µl
5M NaCl	10mM	4µl
1M $MgCl_2$	3mM	6µl
25X ROCHE Complete Protease Inhibitor Cocktail	1X	80µl
ddH_2O		1890µl

II. Cell lysis

2) Spin down 50,000 viable cells (>90%) at 500 x g at 4°C for 5 minutes.
3) Remove all supernatant carefully without disturbing the cells.

4) Add 50 µl of cold ATAC-RSB with detergent to the cell pellet and pipette gently to mix.

ATAC-RSB with detergent		
Reagent	**Final concentration**	**100µl**
ATAC-RSB		97.8
10% Igepal CA-630	0.1%	1µl
10% Tween-20	0.1%	1µl
5% Digitonin (Invitrogen™ BN2006)	0.01%	0.2µl

5) Incubate the cells on ice for 3 minutes.
6) Dilute out the detergent by adding 1 ml of cold ATAC-RSB containing 0.1% Tween-20 only.

ATAC-RSB with 0.1% Tween-20		
Reagent	**Final concentration**	**1ml**
ATAC-RSB		990µl
10% Tween-20	0.1%	10µl

7) Spin down the nuclei at 500 x g at 4°C for 10 minutes.

III. Tagmentation

8) Carefully remove the supernatant and resuspend the nuclei in the transposase mix as follow:

Illumina Transposase mix	
Reagent	**50µl**
PBS	16.9µl
2x TD Buffer	25µl
Tn5 (TDE1)	2.5µl
5% digitonin	0.1µl
10% Tween-20	0.5µl
ddH_2O	5µl

Vazyme Transposase mix	
Reagent	**50µl**
PBS	31.9µl
5x TTBL	10µl
Tn5 (TTE Mix V50)	2.5µl
5% digitonin	0.1µl
10% Tween-20	0.5µl
ddH_2O	5µl

9) Incubate the tubes at 37°C for 30 minutes in either a thermocycler or Thermomixer(1000rpm).

IV. DNA Purification – Post tagmentation

10) Purify DNA immediately using MinElute kit.
11) Add 100 µl of PB buffer to the tube and mix well.
12) Transfer the mixture to the column and wash with 750 µl PE buffer.
13) Elute the DNA using 20 µl of EB.
    The chromatin/DNA is now tagmented. If amplification of the tagmented fragments is not performed immediately, store DNA in -20°C.

V. Library amplification

14) Amplify the tagmented fragments with indexed primers with the following PCR mix and cycles

PCR Mix	
Reagent	**Volume (µL)**
2X KAPA HiFi Hotstart Readymix	25
Primer 1 (N7XX)	2.5
Primer 2 (N5XX)	2.5
Sample	20
TOTAL	**50**

Thermocycler conditions			
Stage 1	72	05:00	
Stage 2	98	00:30	
Stage 3	98	00:10	5 cycles
	63	00:30	
	72	01:00	
Stage 4	4	∞	

15) Transfer 1 µl of amplified product to a new tube and store the remained at 4°C. (Proceed immediately to quantify the concentration of the amplified product.)
16) Dilute the aliquoted 1 µl into 1:1,000 and perform a library quantification with KAPA library quantification kit.
17) Calculate additional cycles needed to achieve 10 nM taking the dilution factor into consideration.
18) Run the rest of the stored product for additional cycles.

Thermocycler conditions			
Stage 1	98	00:30	
Stage 2	98	00:10	*Calculated # of cycles
	63	00:30	
	72	01:00	
Stage 3	4	∞	

*Determined by the library quantification qPCR in step 18.

VI. DNA Purification – Post Amplification

19) Purify amplified DNA fragments using MinElute kit.
20) Add 250 µl of PB buffer to the tube and mix well.
21) Transfer the mixture to the column and wash with 750µl PE buffer.
22) Elute the DNA using 20 µl of EB.

VII. Size selection

23) Check the library size using Fragment Analyzer, AATI or Bioanalyzer, Agilent.
24) Take 10 µl of the samples and perform a size selection using the AMPure XP beads to select for fragments of 150bp to 800bp (0.55X and then 1.5X, this should be optimized based on initial library size).
25) Remove AMPure XP beads from 4°C and equilibrate to room temperature
26) Adjust the sample volume by adding 190µl of ddH_2O or 10 mM Tris-Cl(pH8.0) to 200µl.
27) Add 110µl (0.55X) of beads to the tube and mix properly
28) Incubate for 5 minutes and place the tube on a magnetic stand for 2 minutes or until the mixture becomes clear.
29) Transfer the supernatant to a fresh tube and add 190µl (1.5X) of beads to the supernatant.
30) Mix properly and incubate for 5 minutes.
31) Place the tube on a magnetic rack for 2 minutes or until the mixture becomes clear.
32) Wash the beads twice with 70% EtOH for 30 seconds without disturbing the beads on the magnetic rack.
33) Remove all the residual ethanol and air-dry the pellet for 2-5 minutes.

34) Elute the DNA using 20 µl of ddH_2O or 10 mM Tris-HCl, pH8.0. Over dried pellet will result in poor resuspension and elution.

VIII. Library quality check, quantification and sequencing

35) Quantify the samples and run the library on a Fragment Analyzer, AATI or Bioanalyzer, Agilent to check the size distribution.
36) Quantify the final library with library quantification kit.
37) Dilute and pool multiple libraries if required to desired concentration base on the sequencing platform requirement.
38) Run the sequencing.

References:

1. Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ (2013) Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nature methods 10: 1213-1218
2. Buenrostro JD, Wu B, Chang HY, Greenleaf WJ (2015) ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. Current protocols in molecular biology / edited by Frederick M Ausubel [et al] 109: 21.29.21-21.29.29
3. Corces MR, Trevino AE, Hamilton EG, et al. (2017) An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. Nature methods 14: 959
4. Milani P, Escalante-Chong R, Shelley BC, et al. (2016) Cell freezing protocol suitable for ATAC-Seq on motor neurons derived from human induced pluripotent stem cells. Scientific Reports 6: 25474
5. Ackermann AM, Wang Z, Schug J, Naji A, Kaestner KH (2016) Integration of ATAC-seq and RNA-seq identifies human alpha cell and beta cell signature genes. Molecular Metabolism 5: 233-244