

**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<sup>1,2</sup> 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<sup>3</sup>. 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<sup>3</sup>.
- ❖ 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 <sub>2</sub>	3mM	6µl
25X ROCHE Complete Protease Inhibitor Cocktail	1X	80µl
ddH <sub>2</sub> O		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  $\mu$ l of cold ATAC-RSB with detergent to the cell pellet and pipette gently to mix.

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

- 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		990 $\mu$ l
10% Tween-20	0.1%	10 $\mu$ l

- 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:

2x TD Buffer		
Reagent	Final Concentration	100ml
1M Tris-HCl pH 7.6	20 mM	2ml
1M MgCl <sub>2</sub>	10 mM	1ml
Dimethyl Formamide	20%	20ml
ddH <sub>2</sub> O		77ml

Transposase mix	
Reagent	50 $\mu$ l
PBS	16.9 $\mu$ l
2x TD Buffer	25 $\mu$ l
Tn5	2.5 $\mu$ l*
5% digitonin	0.1 $\mu$ l
10% Tween-20	0.5 $\mu$ l
ddH <sub>2</sub> O	5 $\mu$ l

\*For homemade Tn5, experimental optimization is necessary as there is batch to batch difference in enzymatic efficiency.

- 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  $\mu$ l of PB buffer to the tube and mix well.  
12) Transfer the mixture to the column and wash with 750  $\mu$ l PE buffer.  
13) Elute the DNA using 20  $\mu$ l 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 tagged fragments with indexed primers with the following PCR mix and cycles

PCR Mix	
Reagent	Volume ( $\mu$ L)
2X KAPA HiFi Hotstart Readymix	25
Primer 1 (N7XX)	2.5
Primer 2 (N5XX)	2.5
Sample	20
<b>TOTAL</b>	<b>50</b>

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	$\infty$	

15) Transfer 1  $\mu$ l 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  $\mu$ l 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	$\infty$	

\*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  $\mu$ l of PB buffer to the tube and mix well.

21) Transfer the mixture to the column and wash with 750 $\mu$ l PE buffer.

22) Elute the DNA using 20  $\mu$ l of EB.

**VII. Size selection**

23) Check the library size using Fragment Analyzer, AATI or Bioanalyzer, Agilent.

24) Take 10  $\mu$ l 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 190 $\mu$ l of ddH<sub>2</sub>O or 10 mM Tris-Cl(pH8.0) to 200 $\mu$ l.

- 27) Add 110 $\mu$ l (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 190 $\mu$ l (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  $\mu$ l of ddH<sub>2</sub>O 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:**

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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