

## ATAC-seq protocol (fta modified)

### (Reagents)

- (1) 1X PBS
- (2) Nuclease-free H<sub>2</sub>O
- (3) NP-40 10% (Sigma/Roche, catalog # 11332473001), store at 4 °C
- (4) Tween-20 10% (Sigma/Roche, catalog # 11332465001), store at 4 °C
- (5) 2% Digitonin in DMSO (Promega, catalog # G9441) - Dilute 1:1 with water to make 1% working stock, aliquot, store at -20 °C for up to 6 months, do not freeze/thaw more than 5 times
- (6) 1M Tris-HCl, pH 7.5 (Stowers Media Prep)
- (7) 5M NaCl (Stowers Media Prep)
- (8) 1M MgCl<sub>2</sub> (Sigma, catalog # M1028)
- (9) Nextera DNA Library Prep Kit (Illumina, catalog # FC-121-1030)
- (10) MinElute Reaction Cleanup Kit (Qiagen, catalog # 28204)
- (11) Primers (see table at end of protocol)
- (12) NEBNext High-Fidelity 2X PCR Master Mix (NEB, catalog # M0541S)
- (13) Agilent High Sensitivity DNA Bioanalysis Kit (Agilent, catalog # 5067-4626)
- (14) Qubit dsDNA HS Assay Kit & fluorometer (ThermoFisher, catalog # Q32851)

### (References)

- The primary protocol is based on the Buenrostro et al. papers  
Buenrostro, J.D., Wu, B., Chang, H.Y., and Greenleaf, W.J., 2015. ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. *Curr. Protoc. Mol. Biol.* 109:21.29.1-21.29.9. doi: 10.1002/0471142727.mb2129s109  
with the following modifications:

1. Adjusted lysis step and transposition buffer based on Omni-ATAC protocol  
An improved ATAC -seq protocol reduces background and enables interrogation of frozen tissues. *Nature Methods* 2017
2. Typically, 50,000-100,000 cells yield the best results. As few as 5,000 have been reported to work. The ratio of transposase to cell number is important. If possible, test different cell numbers and different transposase: cell ratios. Use live, freshly isolated cells, not frozen.
3. For all the centrifugation in the fixed-angle centrifuge, pay attention to the angle of the tube because the pellet will be located from the side to the bottom of the tube when spinning. Avoid disturbing the pellet as much as possible.

### Cell Lysis

1. Collect sorted blast cells (30k to 50k) or LSCs (5k-15k) in 1.5ml Eppendorf tube and spin down at 500g for 7 minutes in pre-chilled centrifuge.
2. Wash cells with 500µl cold RSB buffer (10 mM Tris-HCl pH 7.4, 10 mM NaCl, and 3 mM MgCl<sub>2</sub> in water), and centrifuge for 7 minutes to pellet.
3. After centrifugation, 400µl of supernatant was aspirated, which left 100µl of supernatant. This remaining 100µl of supernatant was carefully aspirated by pipetting with a P200 pipette tip to avoid the cell pellet.

5. Add 50µl cold Lysis Buffer (ATAC-seq RSB containing 0.1% NP40, 0.1% Tween-20, and 0.01% digitonin), and pipet up and down 3x gently to resuspend cells.
6. Incubate on ice for 3 minutes.
7. Add 500µl Wash Buffer (ATAC-seq RSB containing 0.1% Tween-20, without NP40 or digitonin), and invert tube 3 times gently.
8. Centrifuge at 500g for 10 minutes at 4°C.
9. Remove the supernatant (cytoplasm) with two pipetting steps, as described before, and keep pellet (nuclei).
10. While cells are centrifuging, make transposition reaction mix by using the Nextera DNA Library Prep Kit.  
※ Prepare for at least 2 samples for precise pipetting. For 5-15k LSCs, I still use 2.5µl Tn5 Transposase even it was designed for 50k cells.

### **25µl 2X TD Buffer**

- 16.5µl 1X PBS
- 0.5µl 10% Tween-20 (final 0.1% v/v)
- 0.5µl 1% Digitonin (final 0.01% v/v)
- 2.5µl Tn5 Transposase
- 5µl nuclease-free H<sub>2</sub>O

11. Add transposition reaction mix to pellet, pipet up and down 6 times gently to resuspend nuclei.
12. Incubate at 37°C for 30 minutes in water bath.

### **DNA Purification**

- Isolate DNA using Qiagen MinElute Reaction Cleanup Kit.
- Elute DNA in 10µl EB (Elution Buffer).
- \*\*OK to store DNA at -20°C at this point.**

### **PCR Amplification (Library Generation)**

Combine the following in a PCR tube for each sample:

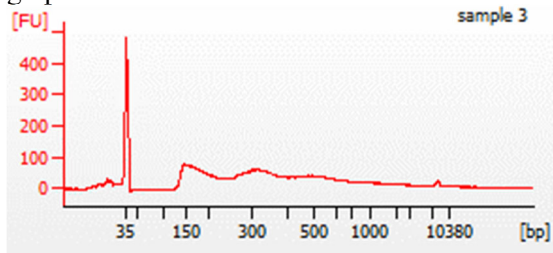
- 10µl purified transposed DNA
- 10µl nuclease-free H<sub>2</sub>O
- 2.5µl Ad1\_noMX primer (25µM)
- 2.5µl Ad2.\* indexing primer (25µM)
- 25µl NEBNext High-Fidelity 2X PCR Master Mix
- Total is 50µl

Amplify samples in PCR machine with following program:

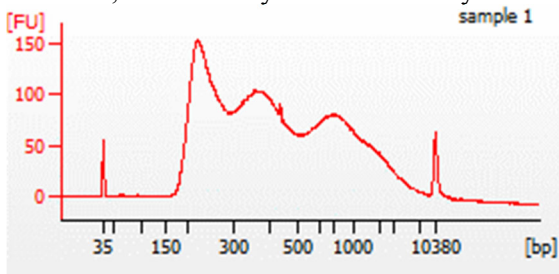
- 72°C 5 minutes
- 98°C 30 seconds
- 98°C 10 seconds
- 63°C 30 seconds x5 cycles
- 72°C 1 minute

### **Assessing Library Quality**

I skipped the qPCR step and amplify the purified transposed DNA for 14 cycles. I use Agilent High Sensitivity DNA Bioanalysis Kit to assess the quality of the library. The result usually looks like the graph below.



I use Pippin Prep 1.5% agarose, 250bp – 1.5kb for size selection of the amplified library. After size selection, the Bioanalyzer result usually looks like the graph below



Measure the library concentration with QuBit, fill the pooling sheet and send to MolBio core facility.

### Sequencing:

- Use 50 bp paired end (50PE) sequencing.
- Goal is to obtain >50 million genomic reads per sample minimum to assess open vs closed chromatin regions, and >200 million genomic reads per sample to detect transcription factor binding sites. Remember that many sequencing reads may map to contaminating mitochondrial DNA, the post-lysis wash step using Tween-20 further removes mitochondria and increases the complexity of the library,

### Primers

Ad1\_noMX

AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG;

Ad2.1\_TAAGGCGA

CAAGCAGAAGACGGCATAACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT;

Ad2.2\_CGTA TAG

CAAGCAGAAGACGGCATAACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT

Ad2.3\_AGGCAGAA

CAAGCAGAAGACGGCATAACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT

Ad2.4\_TCCTGAGC

CAAGCAGAAGACGGCATAACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT