ATAC Sequencing Protocol

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I. Reagents

1. 1X PBS
2. Nuclease-free H_2O
3. NP-40 10% (Sigma/Roche, catalog # 11332473001), store at 4ºC
4. Tween-20 10% (Sigma/Roche, catalog # 11332465001), store at 4ºC
5. Digitonin (Promega, catalog # G9441) - Dilute 1:1 with water to make 1% working stock, aliquot, store at -20ºC for up to 6 months, do not freeze/thaw more than 5 times
6. 1M Tris-HCl, pH 7.5 (ThermoFisher, catalog # 15567-027)
7. 5M NaCl
8. 1M $MgCl_2$ (Sigma, catalog # M1028)
9. Tagment DNA Enzyme 1 (TDE1) (Illumina, catalog # 15027865)
10. Tagment DNA Buffer (Illumina, catalog # 15027866)
11. MinElute Reaction Cleanup Kit (Qiagen, catalog # 28204)
12. Primers (see table at end of protocol)
13. NEBNext High-Fidelity 2X PCR Master Mix (NEB, catalog # M0541S)
14. SYBR Green I (ThermoFisher, catalog # S7563)
15. Agencourt AMPure XP magnetic beads (Beckman Coulter, catalog # A63880)
16. 80% Ethanol (made fresh)
17. Agilent High Sensitivity DNA Bioanalysis Kit (Agilent, catalog # 5067-4626)
18. Qubit dsDNA HS Assay Kit (ThermoFisher, catalog # Q32851)
19. Qubit fluorometer (ThermoFisher)
20. Agilent Bioanalyzer

Notes: This protocol is based on the Buenrostro et al. papers [1, 2], with the following modifications:

- ***Adjusted lysis step and transposition buffer based on Omni-ATAC protocol [3], which in our hands yielded larger number of peaks (~2x) and reduced mitochondrial reads by ~20%.***
- ***We found that column purification of the libraries does not remove primer-dimers (78 bp), and our libraries often contained large fragments 1,000-10,000 bp in length. Therefore, we use magnetic bead purification rather than column purification of the libraries. We recommend that you perform bioanalysis of your initial libraries to determine whether double-sided bead purification is needed.***
- ***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. We recommend that, if possible, you test different cell numbers and different transposase cell ratios. The Buenrostro et al. papers are based on using 50,000 cells. If we use 100,000 cells, then we double the volume of all reagents in the protocol.***

- ***We use live, freshly-isolated cells, not frozen. Reportedly, this revised protocol (Omni-ATAC) can be performed on snap-frozen cells or tissue [3]. Alternatively, you can slowly freeze cells using the protocol detailed in [4] (resuspend pellet in CryoStor buffer 50 ul / 50,000 cells and freeze at -80^{o}C in Mr. Frosty device). Pipet and centrifuge gently to avoid damaging/lysing cells prior to the cell lysis step.***

II. Resuspension Buffer

1. To use for Lysis Buffer and Wash Buffer, can be stored at room temperature long-term.

500 µl	1M Tris-HCl, pH 7.5 (final 10 mM)
100 µl	5 M NaCl (final 10 mM)
150 µl	1 M $MgCl_2$ (final 3 mM)
49.25 ml	nuclease-free H_2O
50 ml	

III. Cell Lysis

1. Centrifuge 100,000 cells to pellet [NOTE: The person who is sorting and aliquoting cells will tell you speed/time, to centrifuge usually this is 8,000 xg for 4 minutes], and then discard supernatant.

2. Add 100 µl cold Lysis Buffer, pipet up and down 3x gently to resuspend cells. [NOTE: Lysis buffer volume should be scaled to the number of cells; e.g. if you only have 50,000 cells, add 50ul cold Lysis Buffer]

97 µl	Resuspension Buffer
1 µl	10% NP-40 (final 0.1% v/v)
1 µl	10% Tween-20 (final 0.1% v/v)
1 µl	1% Digitonin (final 0.01% v/v)
100 µl	

3. Incubate on ice x3 minutes.

4. Add 1 ml Wash Buffer, invert tube 3 times gently.

990 µl	Resuspension Buffer
10 µl	10% Tween-20 (final 0.1% v/v)
1 ml	

5. Centrifuge at 500 xg for 10 minutes at 4^{o}C.

6. Discard supernatant (cytoplasm), keep pellet (nuclei).

IV. Transposition

1. While cells are centrifuging, make transposition reaction mix, using the Nextera DNA Library Prep Kit. [NOTE: Transposition reaction mix volume should be scaled to the number of cells; e.g. if you only have 50,000 cells, add 50ul transposition mix]

50 µl	2X TD Buffer (Tagment DNA Buffer)
33 µl	1X PBS

1 µl	10% Tween-20 (final 0.1% v/v)
1 µl	1% Digitonin (final 0.01% v/v)
5 µl	Tn5 Transposase (Tagment DNA Enzyme 1)
10 µl	nuclease-free H_2O
100	µl

2. Add transposition reaction mix to pellet, pipet up and down 6x gently to resuspend nuclei.
3. Incubate at 37°C for 30 minutes on thermomixer at 1,000 rpm.

V. DNA Purification

1. Isolate DNA using Qiagen MinElute Reaction Cleanup Kit.
2. Elute DNA in 10 µl EB (Elution Buffer).

Note:
OK to store DNA at -20°C at this point.

VI. PCR Amplification (Library Generation)

- Combine the following in a PCR tube for each sample:

10 µl	purified transposed DNA
10 µl	nuclease-free H_2O
2.5 µl	Ad1_noMX primer (25 µM)
2.5 µl	Ad2.* indexing primer (25 µM)
25 µl	NEBNext High-Fidelity 2X PCR Master Mix
50 µl	

- Amplify samples in PCR machine with following program:

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

- Remove tubes from PCR machine and use 5 µl of each partially-amplified library to perform qPCR to determine how many additional PCR cycles are needed. The goal is to stop amplification well prior to saturation to avoid variation among samples due to PCR bias.

5 µl	partially-amplified library
3.85 µl	nuclease-free H_2O
0.5 µl	Ad1_noMX primer (25 µM)
0.5 µl	Ad2.* indexing primer (25 µM)
0.15 µl	100X SYBR Green I
5 µl	NEBNext High-Fidelity 2X PCR Master Mix
15 µl	

- Perform qPCR using following program:

98°C	30 seconds	
98°C	10 seconds	] x20 cycles
63°C	30 seconds	
72°C	1 minute	

**qPCR Plate setup:

- Well type: unknown
- Collect fluorescence data for ROX and FAM
- Reference dye: ROX

**qPCR Thermal Profile setup:

- Pre-Melt/RT Segment: 1 plateau
- Amplification Segment: normal 3 skip
- Dissociation/Melt segment: uncheck this box

- Plot R vs Cycle Number. Calculate the number of additional PCR cycles needed for each sample, by determining the number of cycles needed to reach 1/3 of the maximum R.

- Continue PCR on remaining 45 µl of each partially-amplified library for the appropriate number (N) of cycles:

98°C	30 seconds	⎫
98°C	10 seconds	⎬ N cycles
63°C	30 seconds	⎬
72°C	1 minute	⎭

VII. Library Purification

1. Warm AMPure XP beads to room temperature, and vortex for 15 seconds to resuspend.
2. For double-sided bead purification (to remove primer dimers and large >1,000 bp fragments):
    a. Transfer each PCR sample to an epi tube, add 0.5X volume (22.5 µl) AMPure XP beads, pipet up and down 10x to mix thoroughly.
    b. Incubate at room temperature for 10 minutes.
    c. Place epi tubes in magnetic rack for 5 minutes.
    d. Transfer supernatant to new epi tube.
    e. Add 1.3X original volume (58.5 µl) AMPure XP beads, pipet up and down 10x to mix thoroughly. (This results in a final 1.8X bead buffer:sample ratio.)
    f. Incubate at room temperature for 10 minutes.
    g. Place epi tubes in magnetic rack for 5 minutes.
    h. Discard supernatant.
    i. Wash beads with 200 µl 80% EtOH (freshly made), pipet EtOH over beads 10x, then discard EtOH.
    j. Leave tube on magnetic rack with cap open for 10 minutes.
    k. Ensure all EtOH is removed.
    l. Resuspend beads in 20 µl nuclease-free H_2O, pipet up and down 10x to mix thoroughly.
    m. Incubate at room temperature for 2 minutes, then quickly spin epi tube down
    n. Place epi tube in magnetic rack for 1-5 minutes.
    o. Transfer supernatant to new epi tube.
3. Store purified libraries at -20°C.

VIII. Assessing Library Quality

1. Add 1 µl of each library to 3 µl nuclease-free H_2O (to make 1:4 dilution).

2. Run 1 µl of each diluted library on an Agilent High Sensitivity DNA Bioanalysis chip.
3. Use 1 µl of each diluted library to measure DNA concentration by QuBit.

IX. Sequencing

1. Use 50 bp paired-end (50PE) sequencing.
2. 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.

Table of PCR Primers (Illumina/Nextera i5 common adapter and i7 index adapters):

Ad1_noMX	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
Ad2.1_TAAGGCGA	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
Ad2.2_CGTACTAG	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
Ad2.3_AGGCAGAA	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
Ad2.4_TCCTGAGC	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
Ad2.5_GGACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
Ad2.6_TAGGCATG	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
Ad2.7_CTCTCTAC	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
Ad2.8_CAGAGAGG	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT
Ad2.9_GCTACGCT	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT
Ad2.10_CGAGGCTG	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT
Ad2.11_AAGAGGCA	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT
Ad2.12_GTAGAGGA	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT
Ad2.13_GTCGTGAT	CAAGCAGAAGACGGCATACGAGATATCACGACGTCTCGTGGGCTCGGAGATGT
Ad2.14_ACCACTGT	CAAGCAGAAGACGGCATACGAGATACAGTGGTGTCTCGTGGGCTCGGAGATGT
Ad2.15_TGGATCTG	CAAGCAGAAGACGGCATACGAGATCAGATCCAGTCTCGTGGGCTCGGAGATGT
Ad2.16_CCGTTTGT	CAAGCAGAAGACGGCATACGAGATACAAACGGGTCTCGTGGGCTCGGAGATGT
Ad2.17_TGCTGGGT	CAAGCAGAAGACGGCATACGAGATACCCAGCAGTCTCGTGGGCTCGGAGATGT
Ad2.18_GAGGGGTT	CAAGCAGAAGACGGCATACGAGATAACCCCTCGTCTCGTGGGCTCGGAGATGT
Ad2.19_AGGTTGGG	CAAGCAGAAGACGGCATACGAGATCCCAACCTGTCTCGTGGGCTCGGAGATGT
Ad2.20_GTGTGGTG	CAAGCAGAAGACGGCATACGAGATCACCACACGTCTCGTGGGCTCGGAGATGT
Ad2.21_TGGGTTTC	CAAGCAGAAGACGGCATACGAGATGAAACCCAGTCTCGTGGGCTCGGAGATGT
Ad2.22_TGGTCACA	CAAGCAGAAGACGGCATACGAGATTGTGACCAGTCTCGTGGGCTCGGAGATGT
Ad2.23_TTGACCCT	CAAGCAGAAGACGGCATACGAGATAGGGTCAAGTCTCGTGGGCTCGGAGATGT
Ad2.24_CCACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTGGGTCTCGTGGGCTCGGAGATGT

References

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