ATAC SEQUENCING

I. Reagents

- 1X PBS
- Nuclease-free H_2O
- NP-40 10%
- Nextera DNA Library Prep Kit (Illumina, catalog # FC-121-1030)
- MinElute Reaction Cleanup Kit (Qiagen, catalog # 28204)
- Primers (see table at end of protocol)
- NEBNext High-Fidelity 2X PCR Master Mix (NEB, catalog # M0541S)
- SYBR Green I (ThermoFisher, catalog # S7563)
- Agencourt AMPure XP magnetic beads (Beckman Coulter, catalog # A63880)
- 80% Ethanol (made fresh)
- Agilent High Sensitivity DNA Bioanalysis Kit (Agilent, catalog # 5067-4626)
- Qubit dsDNA HS Assay Kit & fluorometer (ThermoFisher, catalog # Q32851)

II. Notes

1. The primary protocol is based on the Buenrostro et al. papers, with the following modification:
    - 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.
2. Cells must be alive, fresh, not frozen.

III. Cell Lysis

1. Wash 50,000 cells with 50 µl cold 1X PBS in epi tube, centrifuge to pellet, discard supernatant.
2. Add 50 µl cold lysis buffer, pipet up and down to resuspend cells.
    - 10 µl 1M Tris·Cl, pH 7.4 (final 10 mM)
    - 2 µl 5 M NaCl (final 10 mM)
    - 3 µl 1 M $MgCl_2$ (final 3 mM)
    - 10 µl 10% NP-40 (final 0.1% v/v)
    - 975 µl nuclease-free H_2O
    - 1,000 µl **Total**
3. Centrifuge at 500 xg for 10 minutes at 4°C.
4. Discard supernatant (cytoplasm), keep pellet (nuclei).

IV. Transposition

1. While cells are centrifuging, make transposition reaction mix, using the Nextera DNA Library Prep Kit. Volumes per sample of 50,000 cells:

- 25 µl 2X TD Buffer
- 2.5 µl Tn5 Transposase
- 22.5 µl nuclease-free H_2O
- 50 µl **Total**

2. Add transposition reaction mix to pellet, pipet up and down to resuspend nuclei.
3. Incubate at 37ºC for 30 minutes.

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 -20ºC at this point.

VI. PCR Amplification (Library Generation)

1. 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
2. Amplify samples in PCR machine with following program:
   - 72ºC 5 minutes
   - 98ºC 30 seconds
   - 98ºC 10 seconds
   - 63ºC 30 seconds **x5 cycles**
   - 72ºC 1 minute
3. 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
   - 4.41 µl nuclease-free H_2O
   - 0.25 µl Ad1_noMX primer (25 µM)
   - 0.25 µl Ad2.* indexing primer (25 µM)
   - 0.09 µl 100X SYBR Green I
   - 5 µl NEBNext High-Fidelity 2X PCR Master Mix
   - 15 µl **Total**
4. Perform qPCR using following program:
   - 98ºC 30 seconds
   - 98ºC 10 seconds
   - 63ºC 30 seconds **x20 cycles**
   - 72ºC 1 minute
5. Plot R vs Cycle Number.
6. 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.

7. 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
   - 63°C 30 seconds
   - 72°C 1 minute

   ] **N cycles**

VII. Library Purification

1. Warm AMPure XP beads to room temperature, and vortex to resuspend.
2. For single left-sided bead purification (to remove primer dimers):
   - Transfer each PCR sample to an epi tube, add 1.8X volume (81 µl) AMPure XP beads, pipet up and down 10x to mix thoroughly.
   - Incubate at room temperature for 10 minutes.
   - Place epi tubes in magnetic rack for 5 minutes.
   - Discard supernatant.
   - Wash beads with 200 µl 80% EtOH (freshly made), pipet EtOH over beads 10x, then discard EtOH.
   - Leave tube on magnetic rack with cap open for 10 minutes.
   - Ensure all EtOH is removed.
   - Resuspend beads in 20 µl nuclease-free H_2O, pipet up and down 10x to mix thoroughly.
   - Place epi tube in magnetic rack for 1-5 minutes.
   - Transfer supernatant to new epi tube.

3. For double-sided bead purification (to remove primer dimers and large >1,000 bp fragments):
   - 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.
   - Incubate at room temperature for 10 minutes.
   - Place epi tubes in magnetic rack for 5 minutes.
   - Transfer supernatant to new epi tube.
   - 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.)
   - Incubate at room temperature for 10 minutes.
   - Place epi tubes in magnetic rack for 5 minutes.
   - Discard supernatant.
   - Wash beads with 200 µl 80% EtOH (freshly made), pipet EtOH over beads 10x, then discard EtOH.
   - Leave tube on magnetic rack with cap open for 10 minutes.
   - Ensure all EtOH is removed.
   - Resuspend beads in 20 µl nuclease-free H_2O, pipet up and down 10x to mix thoroughly.
   - Place epi tube in magnetic rack for 1-5 minutes.
   - Transfer supernatant to new epi tube.
4. 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

Use 50 bp paired-end (50PE) sequencing to obtain >50 million reads per sample minimum to assess open vs closed chromatin regions, and >200 million reads per sample to detect transcription factor binding sites.

X. Table of PCR Primers (based on TruSeq indices)

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

XI. References

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