Omni ATAC-seq protocol

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Original protocol written 29-Jun-2018, **modified for use on 26/27-Jun-2019**

The Omni-ATAC protocol is an optimized version of the original ATAC-seq protocol. The Greenleaf and Chang lab's paper, "An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues", shows that the new protocol reduces the number of mitochondrial reads, increases the fraction of reads in peaks, and increases the fraction of reads surrounding transcription start sites. The amount of improvement depends heavily on cell type, but the overall effect is an improved signal to noise ratio that generates more peaks with less data. Amanda Ackermann's Omni ATAC protocol from the Kaestner lab says that compared to the original ATAC protocol, the optimized version "in our hands yielded larger number of peaks (almost double) and reduced mitochondrial reads by ~20%."

Reagents

Detergents

- Digitonin (Promega cat# G9441)
    - "Digitonin is supplied at 2% in DMSO. Dilute 1:1 with water to make a 1% (100x) stock solution. Avoid more than 5 freeze thaw cycles. Can be kept at -20°C for up to 6 months."[1]
- Tween20 (BioRad 1706531)
    - Supplied at 100%, dilute 1:9 in NF H2O to get to a 10% concentration solution.
    - Note: ackermann and greenleaf protocols use different brand (Sigma/Roche cat# 11332465001), already supplied at 10%.
- NP40 (Sigma/Roche cat# 11332473001)
    - "NP40 is supplied at 10%. Use at this concentration (100x stock). Store at 4°C."
    - Note, this is Nonidet P40, which is different from another commonly used NP40

Solutions

- 1M TrisHCl pH 7.5 (Invitrogen #15567027)
- 5M NaCl (Ambion/Thermo #AM9759)
- 1M MgCl2 (Ambion/Thermo #AM9530G)

Kits

- Nextera® DNA Sample Preparation Kit (Illumina #FC-121-1030)
- Zymo DNA Clean and Concentrator-5 Kit (Zymo #D4014)

PCR stuff

- NEBNext® HighFidelity 2X PCR Master Mix (NEB #M0541L)
- SYBR® Green Nucleic Acid Gel Stain (Invitrogen #S-7563)
- Greenleaf ATAC Primers (custom order these; see bottom of document)
- KAPA Library Quantification kit (KAPA #KK4854)

Other

- Nuclease free H2O (NF H2O)
- 80% Ethanol (made fresh)
- Agencourt AMPure XP magnetic beads (Beckman Coulter, catalog # A63880)

- Agilent High Sensitivity DNA Bioanalysis Kit (Agilent, catalog # 5067-4626)

Optional dead-cell cleanup reagents (needed only if % viable cells is less than 95%)

- DNase (Worthington cat# LS002007)
    - resuspended in Hanks balanced salt solution (HBSS) at a final concentration of 200 U/ml
- Ficoll (GE cat# 17144002)

Equipment needed: Thermoshaker, Thermocycler, qPCR machine, centrifuge, Agilent High Sensitivity Bioanalyzer + chip, Qubit dsDNA HS Assay Kit & fluorometer

Buffers/Mixes

1. Resuspension Buffer (can store at RT)
    a. 500uL 1M Tris-HCl, pH 7.5 (final 10mM)
    b. 100uL 5M NaCl (final 10mM)
    c. 150uL 1M MgCl2 (final 3mM)
    d. 49.25 ml NF H2O
2. Lysis Buffer, Wash Buffer, and Transposition Mix. For 12 samples, make:

Lysis buffer	vol (ul)
Resuspension buffer	640.2
NP-40	6.6
Tween-20	6.6
Digitonin	6.6

Wash buffer	vol (ul)
Resuspension buffer	13068
Tween-20	132

Transposition mix	vol (ul)
2x TD Buffer (from Illumina Nextera kit)	330
1X PBS	217.8
Tween-20	6.6
Digitonin	6.6
Tn5 Transposase	33
NF H2O	66

Procedure

Prepare Buffers

1. If not already available, dilute Digitonin and Tween-20 with NF H2O to make stock solutions.
2. Make resuspension buffer (if not already available).
3. For the number of samples to be prepared, calculate the amount of Lysis Buffer and Wash buffer needed.
4. Warm drug media, DPBS, and Trypsin (for other samples and for trypsin neutralization). Put 15ml falcon tubes (with water to 9ml), DPBS, digitonin, TD buffer, and Tn5 (TDE) on ice. Put Tween-20 and NP40 at RT on bench. Strap timer to clothing.
5. Make Lysis and Wash buffers. Put both buffers on ice.
6. Label the epi and lo-bind epi tubes to be used in step 2 of the “prepare cells” section.

Prepare cells

1. Perform experiment… end up with 10cm dishes of adherent cells (in this case, MCF-7 cells in different drug conditions)
2. Prepare single-cell suspension:
    a. Aspirate drug media (DMEM/F12 with 10% charcoal-stripped FBS, with drug)
    b. Wash each dish with 10mL DPBS
    c. Add 2.0mL 0.25% Trypsin to each plate, place in incubator for 7 min.
        i. While the cells trypsinize, get 2N 15ml tubes (N to spin cells in (A), N to transfer 2.5M cells to after counting (R)). Label 2N epi tubes + N Lobind epi tubes (3N total; N Lobind epi for 50k cells (A), N normal epi for Qiazol (R), N normal epi for counting (C)). Get Qiazol.
    d. Neutralize trypsin with 4mL hormone starvation media. “Wash” dish 3 times with the trypsin-drug media solution, then transfer to 15mL conical tube. Mix 6 times in conical tube (shear stress helps break apart cell clumps)
    e. Spin at 300 rcf for 3 min, aspirate media, resuspend in 1mL hormone starvation media. **Place samples on ice**.
3. Count cells. *(in parallel, first steps of miRNeasy protocol)*
    a. Biorad TC-20 machine method:
        i. Take 20ul cells from each (well-mixed) condition, mix with 20ul trypan blue. Pipet up and down 25 times to break apart cell clumps. Add 20ul to slide, count cells, calculate volume needed for 50k cells.
    b. Transfer amount of media necessary to move 50k cells into an epi tube. Place tubes on ice, waiting to start spinning until finishing the following miRNeasy steps:
        i. *[miRNeasy step]* Move up to 2.5 million cells to new 15mL conical tubes, centrifuge for 3 min at 300rcf.
        ii. *[miRNeasy step]* Aspirate all supernatant (leave as little as possible behind) from 15mL tubes. Then add 700ul Qiazol, immediately pipette up and down 4 times then transfer to an epi tube.

iii. *[miRNeasy step]* Vortex each microcentrifuge tube with Qiazol in it for 1 minute. Place tubes on ice, return to Omni-ATAC prep, then put in -80 as soon as possible. These lysates should be safe to store for "several months"

c. Centrifuge at 300 rcf at 4C for 4 min. **Place samples on ice**.

Transposition reaction

1. Aspirate media from epi tube → wash the 50k cells with 50uL cold 1x DPBS → centrifuge at 300 RCF at 4C for 3 min in a fixed angle centrifuge → discard supernatant.
2. Add 50uL cold Lysis Buffer, gently pipet up and down 3x to resuspend cells → incubate on ice for 3 minutes.
3. Add 1mL wash buffer, invert tube 3 times gently.
4. Centrifuge at 500rcf for 10 min at 4C. (Swinging bucket method: use open 15mL falcon tubes as sockets for the 1.5ml epi tubes)
    a. Make transposition mix while this is happening.
5. Aspirate supernatant, carefully avoiding cell pellet, using two pipetting steps (first aspirate down to 100uL with a p1000 then remove the final 100ul with a p200).
6. Resuspend cell pellet in 50ul transposition mixture by gently pipetting up and down 6 times.
7. Incubate reaction at 37C for 30 min in a thermomixer at 1000 RPM.
    a. [miRNeasy step] place Qiazol lysates in -80 now if there wasn't time to do it yet
    b. Take out Zymo kit, get ready to use
    c. Take out ATAC adapters, place on ice.

Transposition reaction cleanup (Zymo DNA Clean and Concentrator-5 kit)

1. If the kit is new, prepare its buffers:
    a. Add 26 ml 95% EtOH to the 6 ml DNA Wash Buffer concentrate
    b. Add 104 ml 95% EtOH to the 24 ml DNA Wash Buffer concentrate
2. Add 250 ul DNA Binding Buffer to the 50ul ATAC reactions. Vortex briefly.
3. Transfer the mixture to a Zymo-Spin column in a collection tube.
4. Centrifuge 30 seconds at 12,000 rcf. Discard the flow-through.
5. Add 200ul DNA Wash Buffer to each column. Centrifuge for 30 seconds at 12,000 rcf. Discard flow-through. Repeat this step once.
6. Add 23.2ul elution buffer to the column and let incubate for one minute. Transfer to a new 1.5ml microcentrifuge tube, centrifuge for 30 seconds to elute the DNA. Use all ~20 ul of product in the following PCR (note: at this step, **samples are safe to freeze**).
7. Elute DNA in 21 ul elution buffer, place on ice. This elution typically results in ~20 ul of product. Use all 20 ul of product in the following PCR. (note: safe to store at -20C at this point)

Pre-amplification

1. Set up PCR tubes. In each tube, add:
    a. 20ul purified transposed DNA
    b. 25ul NEBNext master mix
    c. 2.5ul Ad1_NoMX primer (25uM in NF H2O)
    d. 2.5ul Ad2_<sample-dependent index> (25uM in NF H2O)
2. Amplify for 5 cycles, using the following program
    a. 72C, 5 min
    b. 98C, 30 seconds
    c. [: 98C, 10 seconds
    d. 63C 30 seconds
    e. 72C 1 minute :] (x5 cycles in the brackets)
3. Store samples on ice, proceed immediately to next step

qPCR to determine additional number of cycles

1. Use 10ul from each pre-amplified library to make a qPCR plate with the following:
    a. 10uL pre-amplified library
    b. 1.0 ul Ad1_NoMX primer
    c. 1.0 ul Ad2_<sample-dependent index> primer
    d. 0.30ul 100x SYBR Green I
    e. 10ul NEBNext master mix
    f. 7.70 ul NF H2O
2. Perform qPCR with the following program:
    a. 98C, 30 seconds
    b. [:98C, 10 seconds
    c. 63C, 30 seconds
    d. 72C, 1 minute :] (x20 cycles)
3. 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 ⅓ of maximum R. (Note, some libraries may be “over-amplified” from the pre-amplification and that is okay)
4. With the remaining 40 ul of each pre-amplified library (don’t need to add any more reagents), perform the needed number of additional cycles (N) determined by the previous step. Use the following program:
    a. 98C 30 seconds
    b. [: 98C 10 seconds
    c. 63C 30 seconds
    d. 72C 1 minute :] (xN cycles)

Library purification (using bead-based Ackermann method from Kaestner lab)

1. Warm AMPure XP beads to room temperature, vortex to resuspend them
2. Perform either step 3 or step 4 below: (perform step 4)
3. Single-sided bead purification (remove primer dimers; see Ackermann protocol for alternative method to remove primer dimers and fragments > 1000kb)
    a. Transfer each PCR sample to an epi tube → add 1.8X volume (72ul) AMPure XP beads, pipet up and down 10x to mix thoroughly
    b. Incubate at RT for 10 minutes
    c. Prepare 80% ethanol:
        i. mix 1.58 mL NF H2O with 8.42 mL 95% EtOH
    d. Place epi tubes in magnetic rack for 5 min
    e. Discard supernatant (supernatant should have the small fragments of DNA; ratio of original sample to amount of AMPure XP added determines how small of a DNA fragment size will stick to the beads)
    f. Wash beads with 80% EtOH (freshly made). Pipet EtOH over beads 10x, then discard EtOH.
    g. Leave tube on magnetic rack with cap open for 10 min
    h. Ensure all EtOH is removed
    i. Resuspend beads in 20uL NF H2O, pipet up and down 10x to mix thoroughly.
    j. Place epi tube in magnetic rack for 1-5 min
    k. Transfer supernatant to new epi tube.
4. **Double-sided bead purification (to remove primer dimers and large >1,000 bp fragments):**
    a. Transfer 38ul of each PCR sample to an epi tube, add 0.5X volume (19 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 (49.4 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 H2O, pipet up and down 10x to mix thoroughly.
    m. Place epi tube in magnetic rack for 1-5 minutes.
    n. Transfer supernatant to new epi tube.
5. Store purified libraries at -20C

Assessing library quality

1. Add 1uL each library to 3uL NF H2O. (makes 1:4 dilution)
2. Run 1uL of each diluted library on an Agilent High Sensitivity DNA Bioanalysis chip
    a. Use the protocol here: (Agilent High Sensitivity Quick Start Guide) https://www.agilent.com/cs/library/usermanuals/public/G2938-90322_HighSensitivityDNAKit_QSG.pdf
3. Use 1uL of each diluted library to measure DNA concentration by QuBit

Greenleaf ATAC Primers		
Primer name	**INDEX**	**Primer Sequence to order**
ATAC_Ad1_noMX:	N/A	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
ATAC_Ad2.1_TAAGGCGA	TAAGGCGA	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.2_CGTACTAG	CGTACTAG	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.3_AGGCAGAA	AGGCAGAA	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.4_TCCTGAGC	TCCTGAGC	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.5_GGACTCCT	GGACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.6_TAGGCATG	TAGGCATG	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.7_CTCTCTAC	CTCTCTAC	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.8_CAGAGAGG	CAGAGAGG	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.9_GCTACGCT	GCTACGCT	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.10CGAGGCTG	CGAGGCTG	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.11AAGAGGCA	AAGAGGCA	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.12GTAGAGGA	GTAGAGGA	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.13GTCGTGAT	GTCGTGAT	CAAGCAGAAGACGGCATACGAGATATCACGACGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.14ACCACTGT	ACCACTGT	CAAGCAGAAGACGGCATACGAGATACAGTGGTGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.15TGGATCTG	TGGATCTG	CAAGCAGAAGACGGCATACGAGATCAGATCCAGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.16CCGTTTGT	CCGTTTGT	CAAGCAGAAGACGGCATACGAGATACAAACGGGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.17TGCTGGGT	TGCTGGGT	CAAGCAGAAGACGGCATACGAGATACCCAGCAGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.18GAGGGGTT	GAGGGGTT	CAAGCAGAAGACGGCATACGAGATAACCCCTCGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.19AGGTTGGG	AGGTTGGG	CAAGCAGAAGACGGCATACGAGATCCCAACCTGTCTCGTGGGCTCGGAGATGT

ATAC_Ad2.20GTGTGGTG	GTGTGGTG	CAAGCAGAAGACGGCATACGAGATCACCACACGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.21TGGGTTTC	TGGGTTTC	CAAGCAGAAGACGGCATACGAGATGAAACCCAGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.22TGGTCACA	TGGTCACA	CAAGCAGAAGACGGCATACGAGATTGTGACCAGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.23TTGACCCT	TTGACCCT	CAAGCAGAAGACGGCATACGAGATAGGGTCAAGTCTCGTGGGCTCGGAGATGT
ATAC_Ad2.24CCACTCCT	CCACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTGGGTCTCGTGGGCTCGGAGATGT