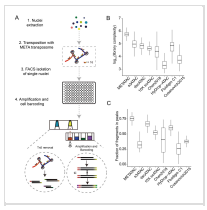

Version 2

Sep 30, 2022

METATAC V.1 V.2

Honggui Wu[1], Xiang Li[2,1,3], Fanchong Jian[2,1,4], Ayijiang Yisimayi[2,1,5], X. Sunney Xie[2,1,6]

[1]Biomedical Pioneering Innovation Center, Peking University, Beijing 100871, China;

[2]Beijing Innovation Center for Genomics, Peking University, Beijing 100871, China;

[3]Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China;

[4]College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, PRC;

[5]School of Life Sciences, Peking University, Beijing, 100871, China;

[6]School of Life Sciences, Peking University, Beijing 100871, China Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China

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Honggui Wu
Biomedical Pioneering Innovation Center, Peking University, ...

ABSTRACT

Abstract

Here we describe a protocol for multiplexed end-tagging amplification of transposase accessible chromatin (METATAC), a high-sensitivity single-cell ATAC-seq technique with the help of META chemistry and extensive biochemical modifications. We improved the protocol from three aspects, first, we used Omni-ATAC protocol to permeabilize cells, which greatly reduced mitochondrial reads to less than 0.5%. Second, we used sodium dodecyl sulfate (SDS) to release Tn5 from bound DNA, which enabled maximum DNA recovery. Third, we use META transposome instead Nextera transposome used in other protocols, which avoids half loss due to self-looping during amplification, thus further increasing library size. Specifically, META sequences also serve as a barcode to fragment decontamination, which provides high-sensitivity and precise single-cell chromatin accessibility analysis.

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scATAC-seq, chromatin accessibility, single-cell epigenomics

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REAGENT SETUP 1h 30m

1 Prepare METATAC Reagents 1h 30m

1.1 **5 mL ATAC resuspension buffer (ATAC-RSB)**

Mix **50 µL** 1 M Tris-HCl pH 7.5, **10 µL** 5 M NaCl, **15 µL** 1 M MgCl2, and bring the final volume to **5 mL** with nuclease-free H2O. Store the buffer at -20 °C for up to several months.

Citation: Honggui Wu, Xiang Li, Fanchong Jian, Ayijiang Yisimayi, X. Sunney Xie METATAC V.1
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A	B	C
Reagents	5 mL	Final conc.
1 M Tris-HCl pH 7.5	50 µL	10 mM
5 M NaCl	10 µL	10 mM
1 M MgCl2	15 µL	3 mM
Nuclease-free H2O	4925 µL	
Total	5 mL	

1.2 200 µL Omni-ATAC lysis buffer

50 µL for each reaction, mix **200 µL** ATAC-RSB with **2 µL** 10% IGEPAL CA-630 **Sigma** IGEPAL CA630 **Aldrich Catalog #I8896-50ML**, **2 µL** 10 Tween 20 Tween 20 **Bio-rad Laboratories Catalog #170-6606-MSDS**, Digitonin, **2 µL** 1% digitonin 40ul **Promega Catalog #G9441**. Freshly prepare before use.

A	B	C
Reagents	200 µL	Final conc.
ATAC RSB	200 µL	
10% IGEPAL CA630	2 µL	0.1%
10 % Tween 20	2 µL	0.1%
1% Digitonin	2 µL	0.01%

1.3 600 µL Omni-ATAC wash buffer

150 µL for each reaction, mix **600 µL** ATAC-RSB with **6 µL** 10% Tween 20. Freshly prepare before use.

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A	B	C
Reagents	600 µL	Final conc.
ATAC RSB	600 µL	
20% Tween 20	6 µL	0.1%

1.4 1 mL 2 x TD buffer

mix **20 µL** 1 M TAPS pH 8.5, **10 µL** 1 M MgCl2, **200 µL** DMF

NN-Dimethylformamide anhydrous **Sigma Aldrich Catalog #D4551-250ML**

, and bring the final volume to **1 mL** with nuclease-free H2O. Store the buffer at -20 °C for up to several months.

A	B	C
Reagents	1 mL	Final conc.
1 M TAPS pH 8.5	20 µL	20 mM
1 M MgCl2	10 µL	10 mM
DMF	200 µL	20%
Nuclease-free H2O	770 µL	

1.5 1 mL 2 x STOP buffer

mix **80 µL** 0.5 M EDTA, **10 µL** 1 M Tris-HCl pH 8.0, **10 µL** 0.1M spermidine, and bring the final volume to **1 mL** with nuclease-free H2O. Freshly prepare before use.

A	B	C
Reagents	1 mL	Final conc.
0.5 M EDTA	80 µL	40 mM
1 M Tris-HCl pH 8.0	10 µL	10 mM
0.1 M spermidine	10 µL	1 mM
Total	1 mL	

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1.6 50 µL Assemble META16 transposome

METATAC_Primer_v.1.xlsx

1) **Anneal META16 transposon**

A	B
Oligos	Sequence
META16-1	GGCACCGAAAA
META16-2	CTCGGCGATAAA
META16-3	GGTGGAGCATAA
META16-4	CGAGCGCATTAA
META16-5	AGCCCGGTTATA
META16-6	TCGGCACCAATA
META16-7	GCCTGTGGATTA
META16-8	GCGACCCTTTTA
META16-9	GCATGCGGTAAT
META16-10	GCGTTGCCATAT
META16-11	GGCCGCATTTAT
META16-12	ACCGCCTCTATT
META16-13	CCGTGCCAAAAT
META16-14	TCTCCGGGAATT
META16-15	CCGCGCTTATTT
META16-16	CTGAGCTCGTTTT
19 bp ME	5'-/phos/-CTGTCTCTTATACACATCT-3'
META Tranposon	5'-[META sequence]-AGATGTGTATAAGAGACAG-3'

A	B	C
Reagents	Per 50 µL	Final conc.
10 x Annealing Buffer	5 µL	1x
50 µM META16 Transposon	5 µL	5 µM
50 µM 19 bp ME	5 µL	5 µM
H2O	35 µL	

Mix thoroughly, then run the annealing program (95 °C, 1 min, gradual cooling, -0.1 °C /3s, 700 cycles to 25 °C, hold at 4 °C)

Recipe for 10x annealing buffer (500 mM NaCl, 100 mM Tris-HCl pH 8.0, 10 mM EDTA)

2) **Assemble METAT16 transposome**

Mix **25 µL** 5 µM Tn5 transposase and **25 µL** 5 µM annealed META16

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transposon, incubate at **21-24 °C** for 30 min, protected from light.

Assembled transposome can be stored at -80 °C for up to six months. Tn5 transposase was expressed in-house from the plasmid pTXB1-Tn5 or purchased from Vazyme (s111-01).

Assembled META16 transposome at a final concentration of 1.25 μM dimer.

Bulk Transposition 1h 30m

2 Harvest fresh culture in a conical centrifuge tube (15 ml or 50 ml) at room temperature, centrifuge at 500 x g for 5 min at room temperature, then wash twice with 1x PBS pH 7.4, count cell number, stain with Trypan blue, and ensure viability >90%. then aliquot 50, 000 cells to a 200 μL PCR tube. 20m

In order to have enough nuclei for FACS, we recommend preparing 2-3 replicates, which is enough for 50-60 96-well plates.

Our protocol also works well for cryopreserved samples and nuclei. For the cryopreserved sample, quickly thaw one tube of the cell at 37 °C water bath, then wash once with ice-cold PBS, count cell number, aliquot 50,000 each.

3 Pellet 50,000 viable cells at 500 x g at 4°C for 5 min in a swing bucket centrifuge, and remove supernatant carefully without disturbing the pellet. 6m

4 Add 50 μL ice-cold **Omni-ATAC lysis buffer** (step 1.2), pipette up and down 10 times, then incubate on ice for 3 min. 4m

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The incubation time depends on cell type, 3 min works well for most samples, but we found for the digestive system sample, incubation needs to extend to 5-10 min to get the periodic nucleosomal pattern.

5 Wash out lysis with 150 µL of ice-cold **Omni-ATAC wash buffer (step 1.3)** and invert the tube 3 times to mix. 2m

6 Pellet nuclei at 500 x g for 10 min at 4°C in a swing bucket centrifuge. 11m

7 Then wash one time with 50ul ice-cold Omni-ATAC wash buffer. Pellet nuclei at 500 x g at 4°C for 5min. 8m

8 **Transposition in Bulk** 50m

8.1 Prepare Transposition mix

A	B	C	D
Reagents	3 Rxn	Per Rxn	Final conc.
2 x TD buffer	37.5 µL	12.5 µL	
META 16 Transposome	6 µL	2 µL	100 nM
1 x PBS	28.5 µL	9.5 µL	
1% Digitonin	0.75 µL	0.25 µL	0.01%
10% Tween 20	0.75 µL	0.25 µL	0. 1%

8.2 Aspirate all supernatant, and avoid disrupting the visible pellet. Then resuspend the cell pellet in 25 µL of transposition mixture by pipetting up and down 10 times, then transfer to a 1.5 mL Lo-bind tube.

8.3 Incubate the reaction at 37°C for 30 minutes in a thermomixer with 1000 RPM mixing.

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8.4 Add 25 µL 2x Stop buffer to stop transposition and incubate on ice for 10 min.

FACS single nuclei 1h

9 Add 50 µL 0.5% BSA (by dissolving 0.25 g BSA in 50 mL 1x PBS pH 7.4), then add 5 µL 7-AAD to stain nuclei.

10 FACS sort single 7-AAD positive nuclei to a 96-well PCR plate, containing 1 µL nuclei lysis buffer (10 mM Tris-HCl pH 8.0, 20 mM NaCl, 1 mM EDTA pH 8.0, 15 mM DTT, 0.1% SDS, 60 µg/mL QIAGEN protease).

If not proceed immediately, seal the plate with Aluminum Sealing Film (Axygen, PCR-AS-600), and store at -80°C for several months without lysis.

All liquid transfer steps can be done with a multichannel pipette or with an automated liquid handler system (e.g., Beckman Biomek FXP liquid handler, Echo 525 acoustic liquid handler system). Here, we use Echo 525 to handle all the liquid transfer steps, which process 20 plates each time.

For cell lysis buffer, we use Echo 525 to aliquot with 384PP_AQ_BP calibration, 45 s/plate.

Amplification 3h 30m

11 Seal the plate with PCR sealing film (bio-rad, MSB1001), lysis was done by incubating at 65 °C 30m for 15 min.

12 After lysis, add 1 µL 3% Triton X-100 to quench SDS. Spin down in a plate centrifuge, vortex 20m to mix.

For 3% Triton X-100, we use Echo 525 to aliquot with 384PP_AQ_SPHigh calibration. 45

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s/plate.

13 Amplification

2h

13.1 Prepare preamplification mix

20m

A	B	C	D
Reagents	120 Rxn	Per rxn	Final conc.
2 x High fidelity Q5 master mix	360	3	1x
50 µM META16 primer mix	23.04	0.192	100 nM each
100 mM MgCl2	6	0.05	
Nuclease-free H2O	90.96	0.758	
Cell lysate	NA	2	NA

A	B
Oligos	Sequence
META 16 Primer	5'-[META sequence]-AGATGTGTATAAG-3'

META16 primer sequences see above .

13.2 Aliquot 4 µL above preamplification mix to each well, Spin down in a plate centrifuge, vortex to mix.

45m

For the preamplification mix, we use Echo 525 to aliquot with 384PP_AQ_BP calibration, 2 min/plate.

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13.3 Preamplification was incubated as
72°C, 5 min
98°C, 30 s
16 Cycles [98°C , 10 s; 62°C, 30 s; 72°C, 1 min]
72°C, 5 min
4°C hold.

50m

14 Cell barcoding

40m

Cell barcoding was realized using a 12 x 8 barcode combination. Premix 12 META16-ADP1 barcodes and 8 META16-ADP2 barcodes into 96 barcode combinations. Detailed sequences see below table.

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A	B
Oligos	Sequence
META16-ADP1	5'-CTTTCCCTACACGACGCTCTTCCGATCT-[CB1]-[META sequence]-AGATGTGTATAAG-3'
META16-ADP2	5'-GAGTTCAGACGTGTGCTCTTCCGATCT-[CB2]-[META sequence]-AGATGTGTATAAG-3'
CB1-1	GATATG
CB1-2	ATACG
CB1-3	CCGTCTG
CB1-4	TGCG
CB1-5	GAACTCG
CB1-6	ATGTAG
CB1-7	CCCG
CB1-8	TATGT
CB1-9	GAGTAAG
CB1-10	ATCG
CB1-11	CCTAG
CB1-12	TGACCG
CB2-1	ACTCTA
CB2-2	AGAGCAT
CB2-3	GGTATG
CB2-4	TCGATGC
CB2-5	CTACTAG
CB2-6	TATGCA
CB2-7	CACACGA
CB2-8	GTCGAT

Add 0.45 µL of one of 96 barcode mixes to each well.
Incubate as
98°C, 30 s,
5 cycles [98°C, 10 s, 62°C, 30 s, 72°C, 1 min]
72°C, 5 min

For the cell barcode, we use Echo 525 to aliquot with 384PP_AQ_BP calibration, 30 s/plate.

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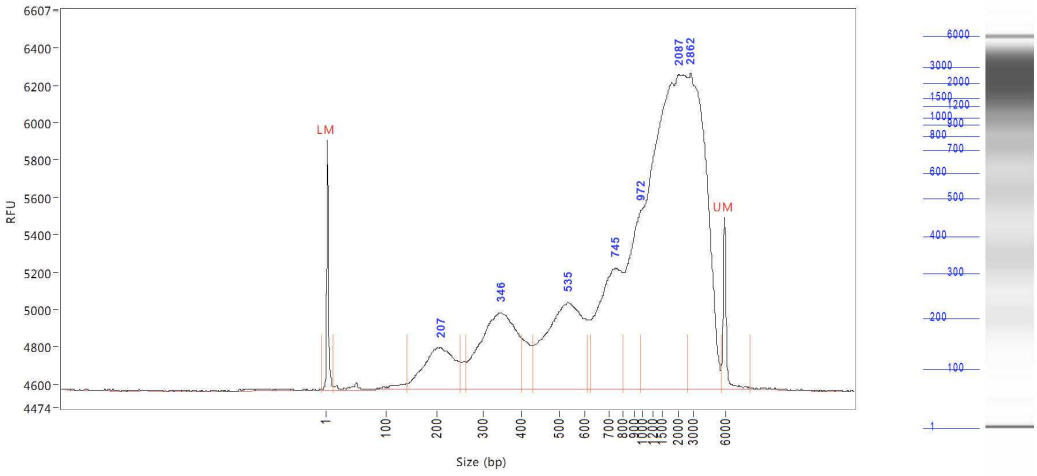
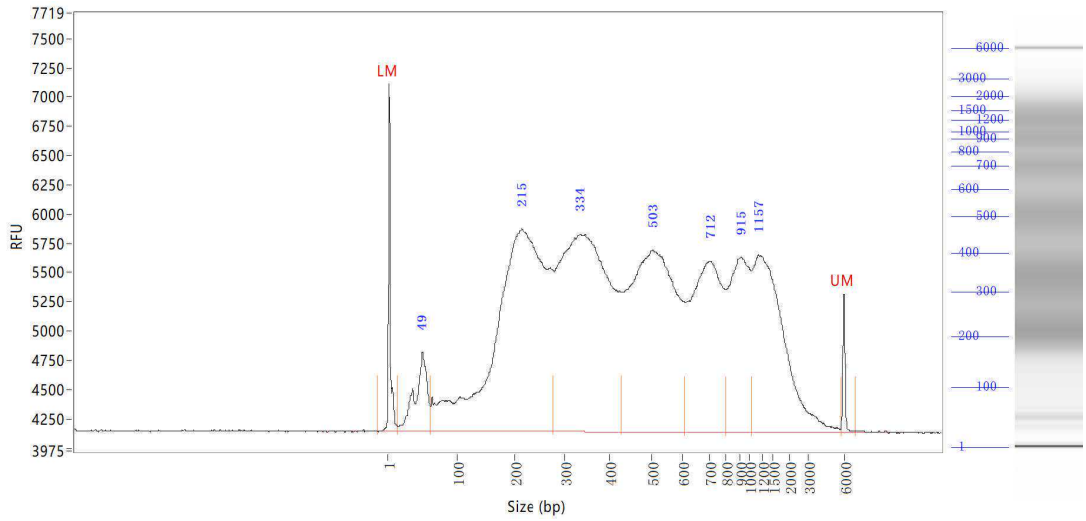
15 Pool a whole plate for purification, typical 200 µL/plate for purification. DNA was extracted with ZYMO DCC5.

1h 30m



It's a typical amplicon of GM12878 cells, but the fragment size distribution varies according to different cell types.

16 For fragment analysis, we use Agilent Fragment analyzer DNF474 kit, only samples with clear periodic nucleosome patterns are used for sequencing.



Wxamplified library showed good periodic nucleosome pattern.

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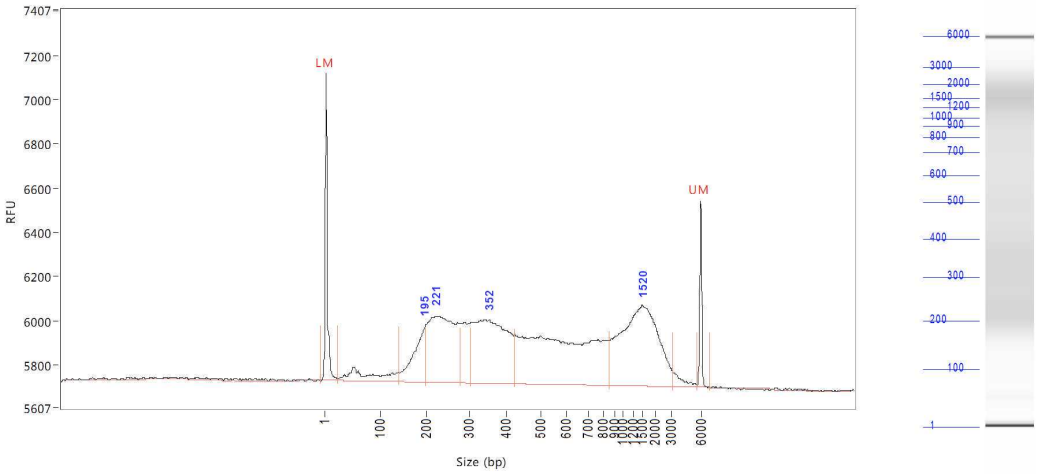

Examplified library showed poor periodic nucleosome pattern.

Library preparation

17 Library Preparation

1h 30m

17.1 Prepare Library prep mix

A	B	C	D
Reagents	40 Rxn	Per rxn	Final conc.
2x Q5 master mix	600	15	1x
NEB universal primer(10 µM)	20	2	0.67 µM
Neb i7 Index primer(10 µM)		2	0.67 µM
100 mM MgCl2	1	0.1	
Template		10.9	
Total		30	

17.2 Library preparation was done by incubating as

98°C, 30 s
2 cycle [98°C, 10 s, 68°C, 30 s, 72°C, 1 min]
72°C, 5 min

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2 cycles of amplification are critical to avoid residual cell barcode primers in purified amplicons causing cell-to-cell contamination.

17.3 Purify with ZYMO DCC5, then purify with 1.1 x SPRI beads to remove primer dimers.

18

METATAC Library schematic diagram

Sequencing

19 For sequencing, we sequenced our sample on Illumina Hiseq 4000 or NovaSeq sequencer with 9 Gb/plate.

20 **Raw read processing.**
Raw Read Preprocessing. For both read 1 and read 2, the first 4 to 7 bases and the following 11 to 13 bases are paired cell barcodes and META sequence, respectively (step 1.6 attachment). We used a custom Python script to parse barcodes and split reads into individual fastq files for each cell, allowing up to one mismatch. Meanwhile, META sequences were annotated to read the name, allowing up to two mismatches. Then we used cutadapt to trim adapter sequences from both ends according to the 19-bp mosaic end (ME) sequence, with parameters -e 0.22 -a CTGTCTCTTATACACATCT and -e 0.22 -g AGATGTGTATAAGAGACAG for both read 1 and read 2. Processed reads were mapped to reference genome with bowtie2 -X 2000 –local –mm –no-discordant –no-mixed. hg38 (GRCh38, v26) reference genome was used for human cells, and mm10 (GRCm38, vM19) reference genome was used for mouse cells. Reads with mapping quality of less than 30 were filtered out from the further analysis. PCR duplicates were identified and removed with a custom script, according to their positions on the genome and META tags. Paired-end reads were converted to fragments with Tn5 insertion centering correction (R1 start +4 and R2 end 5). Finally, for each cell, contaminated fragments from other cells were removed based on the aligned coordinates, META sequences, and read frequency.

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