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A simple ATAC-seq protocol for population epigenetics

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bae6ibhe

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ABSTRACT

We describe here a step-by-step protocol for the generation of sequence-ready libraries for the Assay for Transposase Accessible Chromatin (ATAC-seq) that provides a positive display of accessible, presumably euchromatic regions. The protocol is straightforward and can be used with small individuals such as daphnia and schistosome worms, and probably many other biological samples of comparable size. This makes the method suitable for population epigenomics studies. This protocol is based on the protocol from Corces et al. (2016), Buenrostro et al. (2015) and Nextera DNA Library Prep Kit (2017) with some modifications. Controls must be done without organisms as input.

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KEYWORDS

Epigenetics, Epigenomics, ATAC-seq, Daphnia pulex, Schistosoma mansoni

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MATERIALS TEXT

Material
• Living Daphnia pulex, perfused S.mansoni worms or similar
• Phosphate buffered saline (PBS)
• 1% Molecular biology-grade IGEPAL CA-630
(Sigma-Aldrich, cat. no. I8896)
• 2xTD (Tagment DNA buffer from Nextera kit; Illumina, cat. no. FC-121-1030) ? NB: 2× tagmentation buffer can be prepared as follows: 20 mM Tris(hydroxymethyl)aminomethane; 10
mM MgCl2; 20% (vol/vol) dimethylformamide (Wang et al. 2013)
• TDE1 (Tagment DNA Enzyme from Nextera kit; Illumina, cat. no. FC-121-1030) ?NB: do not use the Nextera XT kit!
• Nuclease-free water (available from various molecular biology suppliers or use MilliQ water)
• QIAquick PCR Purification Kit (Cat No./ID: 28104)
• High fidelity DNA polymerase for PCR and corresponding buffers
• Universal Ad1_noMX primer (25 µM)
• Specific Index primer Ad2.*, different for each sample (25 µM)
• 10,000X SYBR I (Invitrogen, cat. no. S-7563)
diluted in water to 100X
• AMPure XP (Agencourt, cat. A63880) • Hard-Shell 96-well PCR plate, Microseal 'B' adhesive seals • Freshly prepared Ethanol 80%
• Refrigerated centrifuge
• 0.2 ml PCR tubes
• 1.5 ml Eppendorf tubes
Eppendorf ThermoMixer with agitation
• PCR thermal cycler
• qPCR instrument
• Magnetic rack
• 1 mL pipette
• 100 µL pipette
• 10 µL pipette
• Bioanalyzer High-Sensitivity DNA Analysis kit (Agilent 5067-4627)

1 Step 1: Daphnia/Schistosoma sampling
Set Eppendorf ThermoMixer with agitation to 37°C.
1. Cut a 1 mL pipette tip to create larger opening
2. Catch a single daphnia with pipette
3. Take a microphotograph
4. Remove all water by pipetting with 100 µL tip;
or
Perfuse S.mansoni worms and take single worm as dry as possible with forceps
5. Wash once with 50 µL of

cold 1x PBS buffer and
remove all supernatant by pipetting, being careful not to remove your sample;
6. Add to each sample

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25 µl 2× TD buffer
2.5 µl TDE1
0.5 µl 1% IGEPAL
22 µl Nuclease-free water
This gives 50 µL of transposase mixture for each sample.
Pipette up and down 10 times to disrupt cells. Proceed immediately to step 2.

2 Step 2: Chromatin Tagmentation
This step uses the Nextera Tn5 transposome to tagment
the chromatin, which is a process that fragments the chromatin and tags the DNA with adapter sequences in a single step. 1. Tagmentation reactions is incubated at 37°C for
30 min in an Eppendorf ThermoMixer with
agitation at 300 rpm.
2. Tagmented chromatin is immediately purified using a QIAGEN MinElute Reaction Cleanup kit (#28204) or a QIAquick PCR Purification Kit (#28106), and purified DNA is eluted into 20 µl of elution buffer (10 mM Tris-HCl, pH 8)

3 Step 3: Library amplification
This step amplifies the tagmented DNA using a limited cycle PCR program. PCR is carried out with a universal index Ad1 and an index (barcode) primer Ad2.*.

Option 1 (for Promega GoTag G2): Combine the following in a PCR tube for each sample

9.5 µl Nuclease-free MilliQ water
20 µl Purified transposed DNA
10 µl 5x GoTaq G2 buffer
4 µl MgCl2
2.5 µl Universal Ad1_noMX primer (25µM)
2.5 µl Specific Index primer Ad2.*,
different for each sample (25µM)
1 µl dNTPs (10 mM)
0.5 µl GoTaq G2

Or

Option 2 (for NEB mix, more convenient but more expensive):

20 µl Purified transposed DNA
2.5 µl Universal Ad1_noMX primer (25µM)
2.5 µl Specific Index primer Ad2.* ,
different for each sample (25µM)
25 µl NEBNext High-Fidelity 2X PCR Master Mix

In both options the final volume is 50 µl.

Pre-amplify samples in PCR machine with following program:

Step	Temp	Duration	Cycles
Pre-Warming	72°C	5 min	1
Initial denaturation	98°C	30 sec	1
Denaturation	98°C	10 sec	
Annealing	63°C	30 sec	5
Extension	72°C	1 min	
HOLD	12°C	∞	

In order to reduce GC and size bias in PCR, the PCR reaction is monitored using qPCR to stop amplification prior saturation.

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To run a qPCR side reaction, combine the following:

5 µl PCR product of the initial preamplification reaction (keep the remaining 45 µl at 4°C)
2.5 µl 5x GoTaq G2 buffer
0.1 µl GoTaq 2
3.14 µl Nuclease-free MilliQ water
0.25 µl Universal Ad1_noMX primer (25µM)
0.25 µl Ad2.* indexing primer (25µM)
1 µl MgCl2
0.25 µl dNTPs
0.1 µl 100X SYBR I

Total 12.5 µl

or (when using NEB mix)

5 µl PCR product of the initial preamplification reaction (keep the remaining 45 µl at 4°C)
4.41 µl Nuclease-free MilliQ water
0.25 µl Ad1_noMX primer (25 µM)
0.25 µl Ad2.* indexing primer (25 µM)
0.09 µl 100X SYBR I
5 µl NEBNext High-Fidelity 2X PCR Master Mix

Total 15 µl

Amplify samples in a qPCR machine with following program:

Step	Temp	Duration	Cycles
Initial denaturation	98°C	30 sec	1
Denaturation	98°C	10 sec	
Annealing	63°C	30 sec	20
Extension	72°C	1 min	
HOLD	12°C	∞	

To calculate the optimal additional number of cycles needed for the remaining 45 µl PCR, plot Relative fluorescence versus cycle number, and determine the cycle number that corresponds to one-third of the maximum fluorescent intensity (figure 1). In our experience, total of amplification cycles should not exceed 21.

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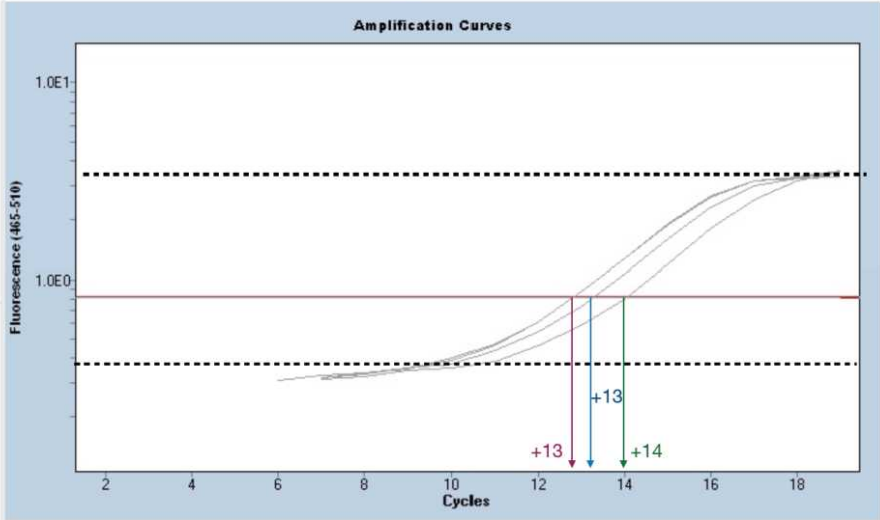

Fig. 1. Example amplification profile for four samples. (Xaxis) Number of PCR cycles. (Y-axis) Fluorescence intensity. An optimal number of additional cycles to perform for four ATAC-seq libraries are indicated.

Run the remaining 45 µl PCR reaction with the additional number of cycles and purify with a QIAGEN MinElute Reaction Cleanup kit (#28204) or a QIAquick PCR Purification Kit (#28106) or similar, and elute with 20 µl of elution buffer (10 mM Tris-HCl, pH 8). Run it on a 1.5% agarose gel or a Bioanalyzer chip. You should see a ladder that corresponds to the nucleosomefree region and multiple nucleosome-size fragments (one nucleosome = about 150 bp). A single band at around 150bp indicates sample degradation or over-fragmentation.
Ideally, five bands should be obtained, three bands are acceptable (figure 2).

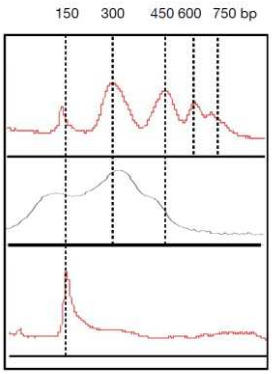

Fig. 2. Examples of fragmentation profiles. (Xaxis) Base pairs. (Y-axis) Fluorescence intensity.
Peaks correspond to nucleosome-free region, mono- to tetra-nucleosome fractions.
Bottom lane: too strong fragmentation, thus Tn5 incubation time needs to be decreased.

4 Step 4: AMPure XP beads double side purification This step enriches for the nucleosome-free (~300 bp).

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Removing small fragments (primer dimers) is important for optimal sequencing. We have not systematically investigated if different purification procedures influence on the result.

1. Transfer 45 µl to an Eppendorf tube (or use PCR tube directly). Add 22.5 µl (0.5X original volume, to remove large fragments) AMPure XP beads, pipet up and down 10 times to mix thoroughly.
2. Incubate at room temperature for 10 minutes.
3. Place tubes in magnetic rack for 5 minutes.
4. Transfer supernatant to new tube.
5. Add 58.5 µl (1.3X original volume, to remove small fragments) AMPure XP beads, pipet up and down 10 times to mix thoroughly
6. Incubate at room temperature for 10 minutes.
7. Place tubes in magnetic rack for 5 minutes.
8. Discard supernatant.
9. Wash beads with 200 µl 80% ethanol (freshly made), pipet ethanol over beads 10 times, then discard ethanol. Ensure all ethanol is removed.
10. Leave tube on magnetic rack with cap open for 3 to maximum 10 minutes depending on ambient humidity. The beads should be 'glowing' but not wet. Be careful not to over-dry them, which will decrease elution efficiency.
11. Resuspend beads in 20 µl nuclease-free water, pipet up and down 10 times to mix thoroughly.
12. Place tube in magnetic rack for 1-5 minutes.
13. Transfer supernatant to new tube.

OR: use Diagenode IP-Star, size selection 320 bp.

Store purified libraries at -20˚C.

5 Step 5: Libraries check

Perform size profiling on Agilent Bioanalyzer High Sensitivity DNA Assay. Expected profiles are shown in figure 3. Use Bioanalyzer profiles or KAPA library quantification kit to quantify libraries and proceed to sequencing.

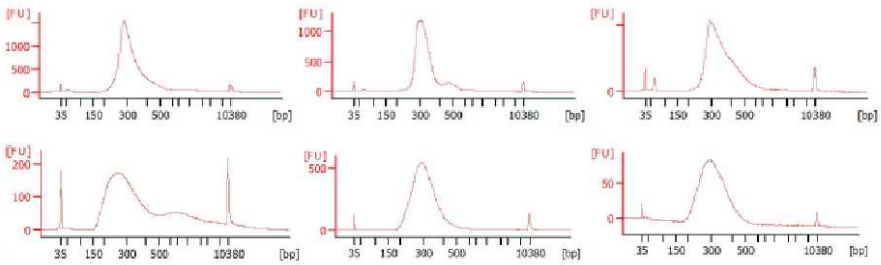

Fig. 3. Examples of BioAnalyser profiles of ATAC-seq libraries after size selection. X-axis in base-pairs, y-axis fluorescence intensity. Fragment size should be between 150 and 800 bp.

End matter

6 Notes

It would certainly be good to have always the same number of fragments after Tn5 incubation. It could be

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envisaged to incubate aliquots of each sample for 10, 20 and 30 min, and then use the sample from the incubation time that provides a fixed, e.g. 3-nucleosome peaks for all samples.

TD buffer can be prepared as follows: 20 mM Tris(hydroxymethyl)aminomethane; 10 mM MgCl2; 20% (vol/vol) dimethylformamide (Wang et al. 2013)

Table of PCR Primers (based on TrueSeq indices):
www.nature.com/nmeth/journal/v13/n11/extref/nmeth.3999-S5.xlsx

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

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