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## **A simple ATAC-seq protocol for population epigenetics**

[version 1; peer review: 1 approved with reservations]

Ronaldo de Carvalho Augusto 1,2, Aki Minoda<sup>3</sup>, Christoph Grunau 1

1 Univ. Perpignan Via Domitia, IHPE UMR 5244, CNRS, IFREMER, Univ. Montpellier, Perpignan, 66860, France

2 LBMC, Laboratoire de Biologie et Modélisation de la Cellule Univ Lyon, ENS de Lyon, Université Claude Bernard Lyon 1, CNRS, UMR 5239, INSERM, U1210, Lyon, 69007, France

3 RIKEN Center for Integrative Medical Sciences, Epigenome Technology Exploration Unit, Tsurumi, Kanagawa, 230-0045, Japan

Corresponding author : Grunau C ([christoph.grunau@univ-perp.fr](mailto:christoph.grunau@univ-perp.fr))

### **Abstract**

We describe here a protocol for the generation of sequence-ready libraries for population epigenomics studies. The protocol is a streamlined version of the Assay for transposase accessible chromatin with high-throughput sequencing (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, and it requires little molecular biology handling expertise.

### **Keywords**

epigenetics, epigenomics, ATAC-seq, *Daphnia pulex*, *Schistosoma mansoni*

## Introduction

Understanding the dynamic cross-talk between epigenetic mechanisms and environmental cues of animal populations is of fundamental importance for ecologists and evolutionary biologists (Shi *et al.*, 2019). The *dynamics* of *chromatin* has long been of interest as a source of phenotypic variance within and among animal populations (Hu & Barrett, 2017; Zhang *et al.*, 2018) and can affect their ecological performance (Augusto *et al.*, 2019; Hawes *et al.*, 2018). In eukaryotic cells, chromatin is a dynamic structure that provides epigenetic information to regulate cell function and gene expression (Chen & Dent, 2014). The physical organization of the chromatin landscape modulates accessibility of genomic regions and dynamically response to both external and internal stimuli. In general, accessible genomic regions are enriched in regulatory elements during gene activity while inaccessible regions restrict binding of transcriptional regulators resulting in gene silencing (Stergachis *et al.*, 2013). Assay for transposase accessible chromatin with high throughput sequencing (ATAC-Seq) is a technique used to assess genome-wide chromatin accessibility. ATAC-seq uses the Tn5 transposase, which fragments DNA and adds adapters simultaneously in open chromatin regions (that are accessible to Tn5 transposase). Deep sequencing of the PCR amplified Tn5 accessible regions provides a high-resolution map of accessible chromatin regions in the genome. We reasoned that this technique can not only be used to establish functional links between chromatin structure and gene function, but also to quantify epigenetic diversity in populations. This would require generation of ATAC-seq chromatin maps in single individuals. In addition, the technique should be sufficiently robust to be used by scientists who are experts in the field of population (epi)genetics and ecology, but having potentially received little training in molecular biology.

Here, we describe a streamlined and robust method for ATAC-Seq of individuals of the crustacea *Daphnia pulex* and also for the trematode *Schistosoma mansoni*. Our procedure is based on the protocol from Buenrostro *et al.* (2015); Corces *et al.* (2016) and Nextera DNA Library Preparation Kit (2017). Besides their ecological and epidemiological importance, both abovementioned organisms show high phenotypic plasticity in response to environmental cues (e.g. the presence of predator for daphnia) or during their development (schistosoma). This phenotypic plasticity is in line with epigenetic plasticity and makes them suitable models for monitoring chromatin accessibility changes. To validate the robustness of the method, we ask 13 experimenters with different levels of expertise in molecular biology to run the experimental procedure independently using *D. pulex*. This validation was performed during the summer school 'Epigénétique en Ecologie et Evolution' organized by the RTP3E in June 2018. ATAC-Seq on *S. mansoni* was done in our laboratory. The procedure provides robust results with individual *D. pulex* and adult worms of *S. mansoni* specimens, but other organisms of similar cell number can probably be used. Controls must be done without organisms as input.

## Materials and methods

### ***Animal sampling and transposase mixture***

Fresh adults *S. mansoni* worms were collected from 4 female 4-week-old Swiss OF1 mice (weight mean 18g) supplied by Charles River, L'arbresle, France. Mice had been infected by peritoneal injection with 150 mixed sexes cercaria. Hepatic perfusions were performed with lethal injection of 1mg per kg body weight of sodium pertobartial solution (Dolehal, Vetoquinol, Lure, France) after 65 days post infection. Water and food were given *ad libitum*, 12h light/dark cycle, 25°C. Housing, feeding and animal care followed the national ethical standards established in the writ of 1 February 2013 (NOR: AGRG1238753A) setting the conditions for approval, planning and operation of establishments, breeders and suppliers of animals used for scientific purposes and controls. The French *Ministère de l'Agriculture et de la Pêche* and French *Ministère de l'Éducation Nationale de la Recherche et de la Technologie* provided permit A66040 to our laboratory for experiments on animals

and certificate for animal experimentation (authorization 007083, decree 87–848) for the experimenters.

Living *Daphnia pulex* were sampled by pipetting through a 1 mL automatic pipette with enlarged openings of the pipetting tips. To avoid experimenter bias, 13 different persons sampled at least one individual. Finally, each specimen was then individually transferred to a 1.5 mL Eppendorf tube and was immediately processed for ATAC-seq library preparation as follows.

The necessary material is listed in Table 1 and Table 2 and must be prepared in advance. In addition, nuclease-free water, high fidelity DNA polymerase for PCR and corresponding buffers, freshly prepared 80% ethanol, refrigerated centrifuge, 0.2 ml PCR tubes, 1.5 ml tubes, ThermoMixer with agitation, PCR thermal cycler, qPCR instrument, magnetic rack, 1 mL pipette, 100  $\mu$ L pipette, and 10  $\mu$ L pipette are needed.

An Eppendorf ThermoMixer was then set with agitation to 37°C and the following steps performed.

- For *D. pulex* remove all water by pipetting with 100  $\mu$ L tip;

or

Perfuse *S. mansoni* worms and take single worm as dry as possible with forceps

- Wash once with 50  $\mu$ L of cold 1x PBS buffer and remove all supernatant by pipetting, being careful not to remove your sample;

- Add to each sample

- 25  $\mu$ L 2x TD buffer (Wang *et al.*, 2013)

- 2.5  $\mu$ L TDE1

- 0.5  $\mu$ L 1% IGEPAL

- 22  $\mu$ L Nuclease-free water

This gives 50  $\mu$ L of transposase mixture for each sample. The samples are pipetted up and down 10 times to disrupt cells.

### **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.

- Tagmentation reactions are incubated at 37°C for 30 min in an Eppendorf ThermoMixer with agitation at 300 rpm.

- Tagmented chromatin is immediately purified using a QIAGEN MinElute Reaction Cleanup kit or a QIAquick PCR Purification Kit, and purified DNA is eluted into 20  $\mu$ L of elution buffer (10 mM Tris-HCl, pH 8).

### **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, as described in Table 3 (Buenrostro *et al.*, 2015). Two library amplification methods were tested and validated in our hands as follows:

**Option 1** (for Promega GoTag G2). Combine the following in a PCR tube for each sample: 9.5  $\mu$ L Nuclease-free MilliQ water;

20 µl Purified transposed DNA; 10 µl 5x GoTaq G2 buffer; 4 µl MgCl<sub>2</sub>; 2.5 µl Universal Ad1\_noMX primer (25 µM); 2.5 µl Specific Index primer Ad2.\*, different for each sample (25 µM); 1 µl dNTPs (10 mM); 0.5µl GoTaq G2.

Or

**Option 2** (for NEB mix, more convenient but more expensive). Combine the following in a PCR tube for each sample: 20 µl purified transposed DNA; 2.5µl Universal Ad1\_noMX primer (25µM); 2.5µl Specific Index primer Ad2.\*, different for each sample (25µM); 25µl NEBNext High-Fidelity 2X PCR Master Mix

In both options the final volume is 50 µl. The samples are preamplified using a PCR machine with the program described in Table 4.

In order to reduce GC and size bias in PCR, the PCR reaction is monitored using qPCR to stop amplification prior to saturation. To run a qPCR side reaction, we combined the following depending on the option that had been chosen previously:

**Option 1:** 5µl PCR product of the initial pre-amplification 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 (25 µM); 0.25 µl Ad2.\* indexing primer (25 µM); 1µl MgCl<sub>2</sub>; 0.25 µl dNTPs; 0.1 µl 100X SYBR I

or

**Option 2:** 5 µl PCR product of the initial pre-amplification reaction;(keep the remaining 45 µl at 4°C); 4.41 µl Nuclease-free MilliQ water; 0.25 µl Ad1\_noMX primer (25 µM); 0.25µl Ad2.\* indexing primer (25 µM); 0.09µl 100X SYBR I; 5µl NEBNext High-Fidelity 2X PCR MasterMix

The samples are amplified in a qPCR machine with the program set out in Table 5.

To calculate the optimal additional number of cycles needed for the remaining 45 µl PCR, relative fluorescence is plotted against cycle number and the cycle number that corresponds to one-third of the maximum fluorescent intensity is determined (Figure 1). In our experience, the total number of amplification cycles should not exceed 21 (Augusto *et al.*, 2019).

The remaining 45 µl PCR reaction is run with the additional number of cycles and purified with a QIAGEN MinElute Reaction Cleanup kit or a QIAquick PCR Purification Kit or similar, and eluted with 20 µl of elution buffer (10 mM Tris-HCl, pH 8).

Fragments are separated by electrophoresis through a 1.5% agarose gel or on a Bioanalyzer chip. A ladder that corresponds to the nucleosome-free region and multiple nucleosome-size fragments should be seen (one nucleosome = about 150 bp). A single band at around 150 bp indicates sample degradation or overfragmentation. Ideally, five bands should be obtained, three bands are acceptable (Figure 2).

#### **AMPure XP beads double-side purification**

This step enriches for the nucleosome-free (~300 bp). Removing small fragments (primer dimers) is important for optimal sequencing. First 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. Incubate at room temperature for 10 minutes and place tubes in magnetic rack for 5 minutes. Transfer supernatant to new tube and add 58.5 µl (1.3X original volume, to remove small fragments) AMPure XP beads, pipet up and down 10 times to mix thoroughly. Incubate at room temperature for 10 minutes, place tubes in magnetic rack for 5 minutes and discard supernatant. Wash beads with 200 µl 80% ethanol (freshly made), pipet ethanol over beads 10 times, then discard ethanol. Ensure all ethanol is removed. 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. Resuspend beads in 20 µl nuclease-free water, pipet up and down 10 times to mix thoroughly, place tube in magnetic rack for 1–5 minutes and transfer supernatant to new tube. This step can be replaced by Diagenode IP-Star, size selection 320 bp.

We have not systematically investigated if different purification procedures influence on the result. Purified libraries should be stored at -20°C.

#### **Libraries check**

Size profiling can be performed using an Agilent Bioanalyzer High Sensitivity DNA Assay. Expected profiles are shown in Figure 2. Bioanalyzer profiles or KAPA library quantification kit are used to quantify libraries and proceed to sequencing.

A step-by-step protocol is available from Protocols.io at <https://doi.org/10.17504/protocols.io.bae6ibhe>.

## **Results**

### ***The method can be used by scientists with low expert level in molecular biology***

The protocol described in the methods section was tested by 13 volunteers with molecular biology expertise level ranging from untrained, some with many years of experience and others who had retired from active wet-bench work several years ago. Each volunteer processed one experiment using *Daphnia*. In only two cases ATAC-seq library production did not succeed.

### ***The method can be used with small organisms***

After having had firmly established that the method delivers robust results for *Daphnia*, we used it on individual adult worms of *Schistosoma mansoni* and the protocol delivered expected results in terms of fragmentation, number of PCR cycles and library insert size distribution (Figure 3). Data underlying these results are available at Zenodo (Augusto et al., 2020).

## **Discussion**

Phenotypically, plasticity plays an important role in development and evolution. The relative contribution of genetic and epigenetic components to heritable plasticity is a matter of lively scientific debate (Hu & Barrett, 2017; Roquis et al., 2018). One of the caveats of analyzing epigenetic information is that it is stored in several, very different bearers of information (e.g. DNA methylation, modification of histones, non-coding RNA and topological position in the interphase nucleus). Nevertheless, these types of information converge towards a change in chromatin structure which can be approximated by DNA accessibility. We reasoned that a straightforward ATAC-seq method to map the chromatin accessibility status in populations with high phenotypic plasticity would facilitate further investigations of the role of epigenetics in plasticity. This study field is also of particular importance to field ecologists. We therefore set-out to establish a robust, easy to use protocol that can be used with little molecular biology training. Our protocol was successfully used in the framework of a summer school 'Epigénétique en Ecologie et Evolution' by participants with different levels of expertise in molecular biology using *D. pulex*. We also used single adult *S. mansoni* worms as biological material. We believe that our protocol is suitable for fast epigenotyping of other organisms as well. From our experience, the only parameter that it might be necessary to optimize is tagmentation reaction time if over- or under-fragmentation occurs. A potential issue is contamination with microorganisms whose DNA might be present in the libraries

## **Data availability**

Zenodo: Supporting data for “A simple ATAC-seq protocol for population epigenetics”.  
<http://doi.org/10.5281/zenodo.3828600> (Augusto et al., 2020).

This project contains the following underlying data:

- Agarose picture (TIF). (Example of electrophoresis fragment separation.)
- Agarose profile (PNG) (Example of fragment separation on a BioAnalyser chip.)
- BioAnalyser, BioAnalyser 2–4 (PDF). (BioAnalyser profiles generated in this study.)
- qPCR cycles (XLSX). (Quantification of qPCR cycles for each indicated organism.)
- qPCR plot (JPG). (qPCR amplification cycles plot.)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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We are grateful to the 13 volunteers who tested the procedure

### **Competing interests:**

No competing interests were disclosed.

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**Table 1. Externally sourced materials.**

Item name	Vendor	Catalogue ID
1% Molecular biology-grade IGEPAL CA-630	Sigma-Aldrich	I8896
2xTD (Tagment DNA buffer from Nextera kit)	Illumina	FC-121-1030
TDE1 (Tagment DNA Enzyme from Nextera kit)	Illumina	FC-121-1030
QIAquick PCR Purification Kit	Qiagen	28104
AMPure XP beads	Agencourt	A63880
Bioanalyzer High-Sensitivity DNA Analysis kit	Agilent	5067-4627
10,000X SYBR I	Invitrogen	S-7563

**Table 2.** Reagents produced in the laboratory.

Phosphate buffered saline (PBS)	137mM NaCl; 27mM KCl; 100mM Na <sub>2</sub> HPO <sub>4</sub> ; 18mM KH <sub>2</sub> PO <sub>4</sub>
Tagmentation buffer (TD buffer) ( <a href="#">Wang et al., 2013</a> )	20 mM Tris(hydroxymethyl)aminomethane; 10 mM MgCl <sub>2</sub> ; 20% (vol/vol) dimethylformamide

**Table 3. PCR Primer ID and sequence.**

Index ID	Sequence
Ad1_noMX:	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATG TG
Ad2.1_TAAGGCG A	CAAGCAGAAGACGGCATAACGAGATTTCGCCTTAGTCTCGTGGGCTCGGA GATGT
Ad2.2_CGTACTA G	CAAGCAGAAGACGGCATAACGAGATCTAGTACGGTCTCGTGGGCTCGG AGATGT
Ad2.3_AGGCAGA A	CAAGCAGAAGACGGCATAACGAGATTTCTGCCTGTCTCGTGGGCTCGGA GATGT
Ad2.4_TCCTGAG C	CAAGCAGAAGACGGCATAACGAGATGCTCAGGAGTCTCGTGGGCTCGG AGATGT
Ad2.5_GGACTCC T	CAAGCAGAAGACGGCATAACGAGATAGGAGTCCGTCTCGTGGGCTCGG AGATGT
Ad2.6_TAGGCAT G	CAAGCAGAAGACGGCATAACGAGATCATGCCTAGTCTCGTGGGCTCGG AGATGT
Ad2.7_CTCTCTAC	CAAGCAGAAGACGGCATAACGAGATGTAGAGAGGTCTCGTGGGCTCGG AGATGT
Ad2.8_CAGAGAG G	CAAGCAGAAGACGGCATAACGAGATCCTCTCTGGTCTCGTGGGCTCGGA GATGT
Ad2.9_GCTACGC T	CAAGCAGAAGACGGCATAACGAGATAGCGTAGCGTCTCGTGGGCTCGG AGATGT
Ad2.10_CGAGGC TG	CAAGCAGAAGACGGCATAACGAGATCAGCCTCGGTCTCGTGGGCTCGG AGATGT
Ad2.11_AAGAGG CA	CAAGCAGAAGACGGCATAACGAGATTGCCTCTTGTCTCGTGGGCTCGGA GATGT
Ad2.12_GTAGAG GA	CAAGCAGAAGACGGCATAACGAGATTCCTCTACGTCTCGTGGGCTCGGA GATGT
Ad2.13_GTCGTG AT	CAAGCAGAAGACGGCATAACGAGATATCACGACGTCTCGTGGGCTCGG AGATGT
Ad2.14_ACCACT GT	CAAGCAGAAGACGGCATAACGAGATACAGTGGTGTCTCGTGGGCTCGG AGATGT
Ad2.15_TGGATC TG	CAAGCAGAAGACGGCATAACGAGATCAGATCCAGTCTCGTGGGCTCGG AGATGT
Ad2.16_CCGTTT GT	CAAGCAGAAGACGGCATAACGAGATACAAACGGGTCTCGTGGGCTCGG AGATGT
Ad2.17_TGCTGG GT	CAAGCAGAAGACGGCATAACGAGATACCCAGCAGTCTCGTGGGCTCGG AGATGT
Ad2.18_GAGGG GTT	CAAGCAGAAGACGGCATAACGAGATAACCCCTCGTCTCGTGGGCTCGG AGATGT
Ad2.19_AGTTG GG	CAAGCAGAAGACGGCATAACGAGATCCCAACCTGTCTCGTGGGCTCGG AGATGT

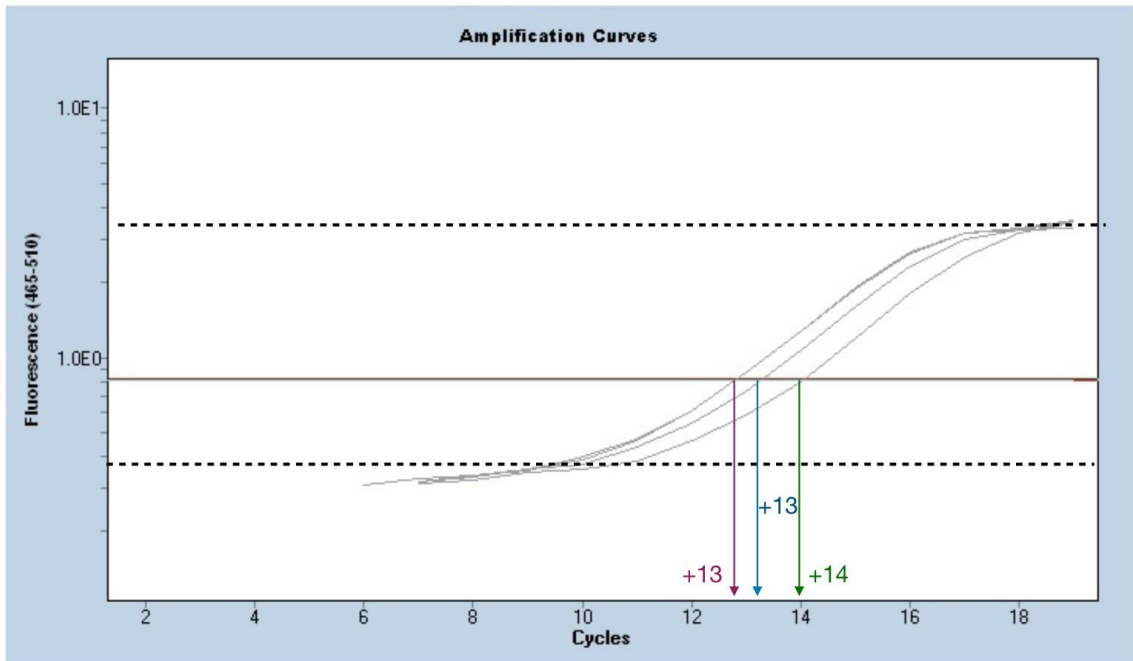
Ad2.20_GTGTGG TG	CAAGCAGAAGACGGCATAACGAGATCACCACACGTCTCGTGGGCTCGG AGATGT
Ad2.21_TGGGTT TC	CAAGCAGAAGACGGCATAACGAGATGAAACCCAGTCTCGTGGGCTCGG AGATGT
Ad2.22_TGGTCA CA	CAAGCAGAAGACGGCATAACGAGATTGTGACCAGTCTCGTGGGCTCGG AGATGT
Ad2.23_TTGACC CT	CAAGCAGAAGACGGCATAACGAGATAGGGTCAAGTCTCGTGGGCTCGG AGATGT
Ad2.24_CCACTC CT	CAAGCAGAAGACGGCATAACGAGATAGGAGTGGGTCTCGTGGGCTCGG AGATGT

**Table 4.** PCR program for library preamplification.

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

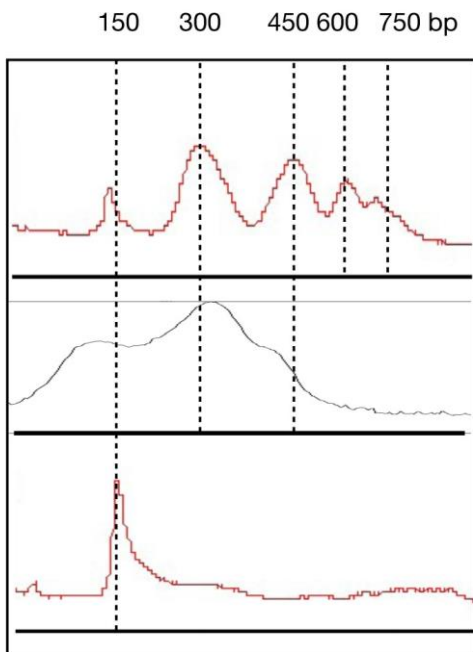
**Table 5.** PCR program for library amplification.

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



**Figure 1. Example amplification profile for four samples.**

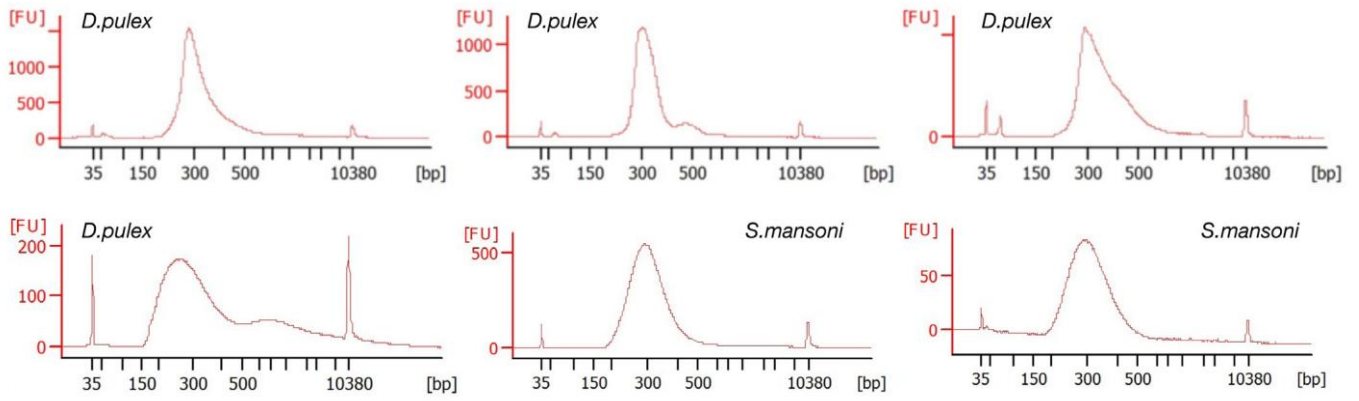
(X-axis) Number of PCR cycles. (Y-axis) Fluorescence intensity. An optimal number of additional cycles to perform for four ATAC-seq libraries are indicated.



**Figure 2. Examples of fragmentation profiles.**

(X-axis) 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.





**Figure 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.