diagenode

Innovating Epigenetics Solutions

ATAC-seq Kit

Cat. No. C01080001 (8 rxns)

Cat. No. C01080002 (24 rxns)

USER GUIDE

V1 10_2021

Please read this manual carefully
before starting your experiment

Contents

Introduction

Gene expression is carefully regulated in the cells in order to manage a wide range of biological functions. The structure of chromatin is quite dynamic and contributes to this crucial regulatory process.

ATAC-seq, **A**ssay for **T**ransposase-**A**ccessible **C**hromatin, followed by next-generation sequencing, is a key technology to easily identify the "open" regions of the chromatin, which are usually associated with permissive gene expression. Indeed, the nuclei of the samples are incubated with a transposase, and only the genomic regions associated with open chromatin will be accessible to this transposase. During the process those regions will be cut and sequencing adaptors will be added, allowing their sequencing. High-throughput sequencing will then detect peaks, in open regions of the chromatin only., giving a map of the chromatin status in the whole genome of the sample.

The Diagenode's ATAC-seq kit is based on a highly validated protocol, used for years in our Epigenomics Profiling Services offer and takes advantage of many successful Diagenode's tools, such as the loaded Tagmentase (Tn5 transposase), the MicroChIP DiaPure Columns and the Primer indexes for tagmented libraries kits.

Kit Method Overview & Time Table

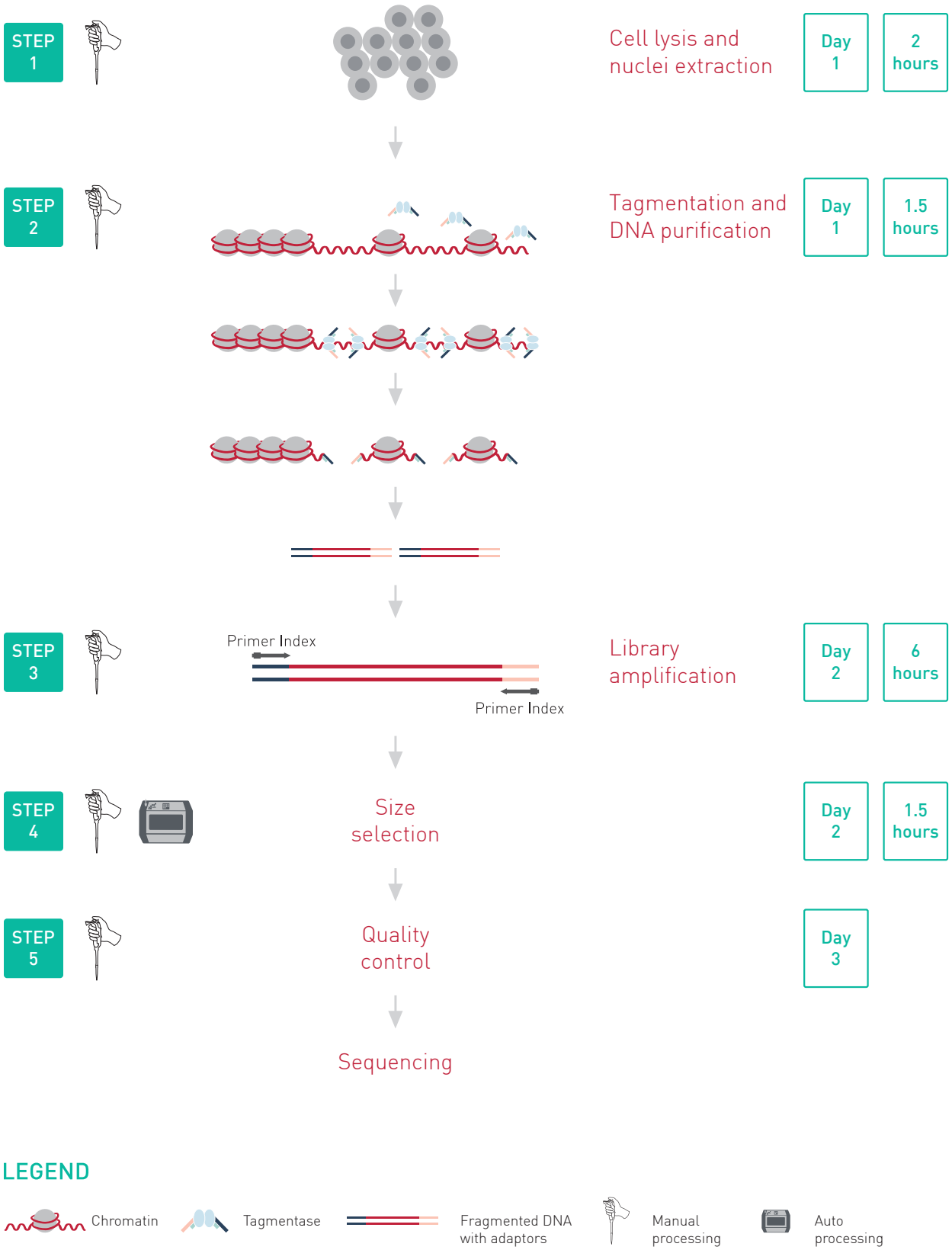


Figure 1. ATAC-seq workflow

Kit Materials

The ATAC-seq kit contains reagents necessary for all steps of the protocol. However, the primer indexes for multiplexing are not included in the kit and must be purchased separately (listed in the section "Required materials not provided"). The Diagenode's ATAC-seq kit is available in two formats, 8 or 24 reactions.

For IP-Star Compact users, two steps of the protocol are available as automated versions, size selection and clean-up. The reagents are supplied in quantities sufficient for up to 2 IP-Star runs (8 rxns kit) or up to 4 IP-Star runs (24 rxns kit).

Components supplied with the ATAC-seq Kit

***NOTE**: Upon receipt, store the components at the indicated temperatures.*

Table 1. Components stored at +4°C

Component	Cap color	Volume – 8 rxns	Volume – 24 rxns	Storage
ATAC Lysis Buffer 1	green	500 µl	1500 µl	+4°C
ATAC Lysis Buffer 2		10 ml	30 ml	+4°C
10% Tween20	red	10 µl	20 µl	+4°C
Nuclease-free water		650 µl	1900 µl	+4°C

Table 2. Components stored at -20°C

Component	Cap color	Volume – 8 rxns	Volume – 24 rxns	Storage
Protease inhibitor cocktail	black	23 µl	68 µl	-20°C
Tagmentase (loaded)	yellow	20 µl	60 µl	-20°C
2x High-Fidelity Mastermix	violet	240 µl	720 µl	-20°C
Tagmentation Buffer (2x)	blue	200 µl	600 µl	-20°C
100 x SYBR	none	3 µl	5 µl	-20°C

Table 3. Components stored at room temperature (RT)

Component	Cap color	Volume – 8 rxns	Volume – 24 rxns	Storage
ChIP DNA Binding Buffer		2 ml	6 ml	RT
DNA Wash Buffer*		700 µl	2.2 ml	RT
DNA Elution Buffer	white	560 µl	2.2 ml	RT
Spin columns		8	24	RT
Collection Tubes (2 ml)		8	24	RT

** Before first use, the DNA Wash Buffer must be completed by adding the 100% ethanol.*

ATAC-seq kit, format 8 rxns: add 2,8 ml of 100% ethanol to 700 µl of DNA Wash Buffer.

ATAC-seq kit, format 24 rxns: add 8,8 ml of 100% ethanol to 2,2 ml of DNA Wash Buffer.

Required Materials Not Provided

- Gloves to wear at all steps
- Cold PBS
- Warm PBS (37°C)
- 100% Ethanol, Molecular grade
- 80% Ethanol, Molecular grade
- AMPure XP beads (Beckman Coulter, Inc. #A63881)
- Trypan blue
- 2% Digitonin (Promega, G9441)
- Nuclease-free 0.2 ml tubes
- Nuclease-free 1.5 ml tubes
- 15 or 50 ml tubes
- Diagenode magnetic rack, DiaMag02 (Cat. No. B04000001) - if not using the IP-Star
- Waterbath
- Cold centrifuges
- Microscope
- Cell counter
- Thermocycler
- qPCR instrument and its associated tube strips/plates
- Primer indexes for tagmented libraries kit (Diagenode)
    - 8 SI for tagmented libraries (Cat. No. C01011033)
    - or 24 SI for tagmented libraries (Cat. No. C01011032)
    - or 8 UDI for tagmented libraries (Cat. No. C01011035)
    - or 24 UDI for tagmented libraries – Set I (Cat. No. C01011034)
    - or 24 UDI for tagmented libraries – Set II (Cat. No. C01011036)

Additionnal supplies (included and available separately)

Component	Cat. No.	Format
Tagmentase (loaded)	C01070012	10 µl and 30 µl
Tagmentation Buffer (2x)	C01019043	100 and 300 µl
MicroChIP DiaPure columns	C03040001	50 rxns

Remarks Before Starting

1. Starting material

This protocol has been optimized for the use of **50,000 cells** per ATAC-seq reaction, and it is important to avoid larger cell numbers in order to achieve good tagmentation efficiency. If starting material is limiting, do not go below 20,000 cells per reaction.

We recommend using fresh cells, but when needed, it is possible to cryopreserve cells and use them later for ATAC-seq, following these guidelines:

- Check that viability is higher than 90% before freezing
- Freeze the cells at a concentration of 1 million cells/ml
- Freeze cells with the standard reagents and procedure adapted to the cell type

2. Cell lysis

The lysis time must be carefully optimized in order to guarantee a proper lysis of the cell membranes, while keeping the nucleus integrity. We thus recommend to test several lysis times, when working for the first time with a new cell type. The optimization protocol is described in the "Additional protocols" section.

3. Optimization of the number of amplification cycles

It is necessary to amplify libraries in order to have enough material for sequencing. But as PCR can cause some amplification bias, it is really important to use as few cycles as possible, which also reduces the number of PCR duplicates that will have to be discarded from the sequencing data.

We recommend a qPCR step on an aliquot of the tagmented DNA. This qPCR uses the same settings as the final amplification and allows to monitor the efficiency of this amplification on each sample. This additional

step helps determine how many cycles are needed to produce a sufficient amount of library, while avoiding overamplification of the samples. We therefore highly recommend to follow the protocol described at step 3, in order to obtain high quality libraries, with low numbers of PCR duplicates.

4. Primer indexes

Diagenode primer indexes for tagmented libraries, compatible with the ATAC-seq kit, are available in several formats allowing a choice between single or dual-indexing and several numbers of different indexes. For more details about the differences between the kits and pooling guidelines for the indexes, please check the Primer index for tagmented libraries manual: https://www.diagenode.com/en/documents/primer-indexes-for-tagmented-libraries_manual

5. Quantification

At the end of the ATAC experiment, determine the concentration of the libraries with a highly sensitive method such as the dsDNA HS Assay Kit on the Qubit® system from ThermoFisher Scientific. PicoGreen® is also suitable but UV spectrophotometric methods such as the NanoDrop are usually not sufficiently sensitive.

There are several approaches available for library quantification including real-time PCR, UV absorption, fluorescence detection, or sizing and quantification using the Agilent Bioanalyzer or Fragment Analyzer (Advanced Analytical). It is important to understand the benefits and limitations of each approach.

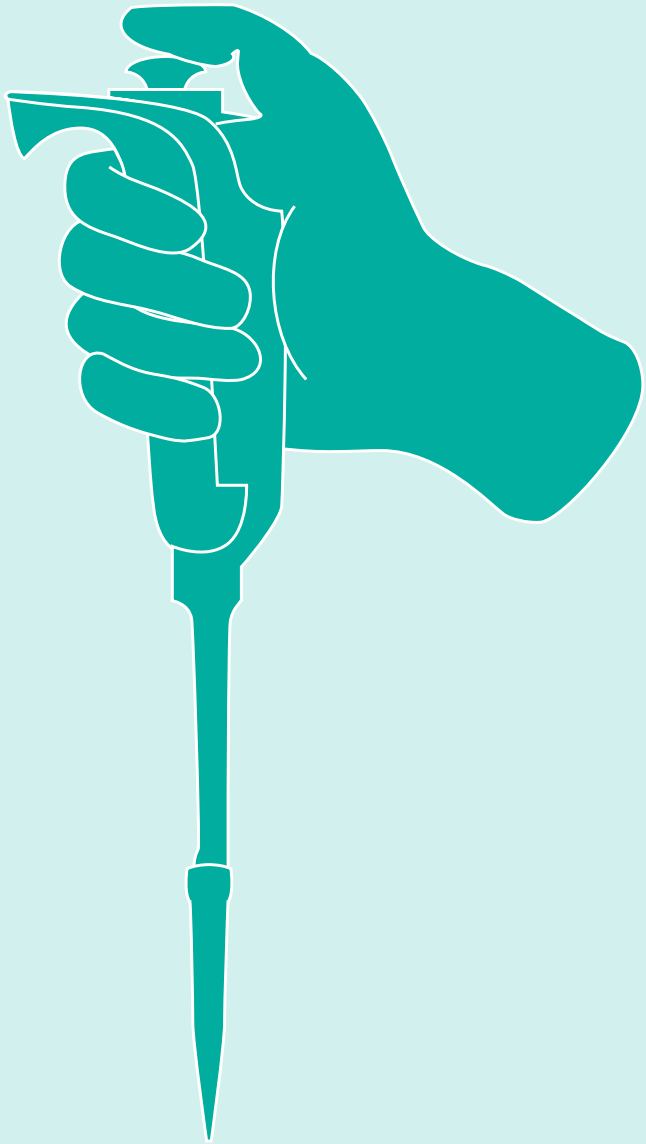
Real-time PCR-based approaches such as the KAPA Library Quantification Kit from Kapa Biosystems) quantify the library molecules that carry the Illumina adapter sequences on both ends and therefore reflect the quantity of the clustering competent library molecules. This approach assumes a relatively uniform size of sheared or fragmented starting gDNA

inserts used for library construction. Quantification by PCR can be done on unpurified libraries.

The Agilent Bioanalyzer system or Fragment Analyzer (Advanced Analytical) provide sizing and quantification information about the library analysed, but not about the clustering competency. Quantification can be done both on unpurified or purified samples. In a case of unpurified samples, a region corresponding to libraries should be limited in order to discriminate between primers/adaptors and a library itself.

UV absorption/fluorescence detection-based methods (i.e., Nanodrop® (Thermo Scientific), Qubit®2.0 Fluorometer (Life Technologies), or Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) simply quantify total nucleic acid concentration. These methods do not discriminate adapter presence and offer no information about the size of the library molecules. They can be used only on purified libraries. We recommend fluorescence-based assays over spectrophotometric measurements (e.g. NanoDrop) due to higher specificity and sensitivity.

PROTOCOL



STEP 1

Cell Lysis and Nuclei Extraction

1 *Day 1* *2 hours*

Buffer preparation

1.1 Add **2.5 µl of Protease Inhibitor Cocktail** (black cap) to **500 µl of cold PBS** and keep the tube on ice.

***NOTE**: This will be used to rinse the cells. When using several sample types, prepare 500 µl of PBS+PIC for each one.*

1.2 Define the number of ATAC reactions to be performed. For each reaction prepare **50 µl of ATAC Lysis Buffer 1** with **Digitonin** (e.g. add 0.5 µl of 2% Digitonin to 49.5 µl of ATAC Lysis Buffer 1 (green cap)). Keep it on ice.

***CAUTION**: Digitonin is toxic and care should be taken. Use full PPE including a mask, lab coat and gloves while handling Digitonin.*

Cell collection

For adherent cells

- Pre-warm PBS, culture medium and trypsin-EDTA at 37°C.
- Remove the medium and rinse a large number of cells (typically between **200,000** and **500,000 cells/sample**) with **pre-warmed PBS**. Gently shake the flask for 2 minutes.
- Remove the PBS and add sterile **trypsin-EDTA** to the culture flask to detach adherent cells from the bottom. Add enough trypsin-EDTA to completely cover the cells. Gently shake the culture flask for 1-2 minutes or until the cells start to detach. The time needed may depend on the cell type.

- Immediately add fresh culture medium to the cells when they are detached. For correct inactivation, the volume of medium must be 2x the volume of trypsin-EDTA. Transfer cell suspension to a 15 or 50 ml tube.
- Rinse the flask by adding **10 ml of warm PBS**. Add this volume to the tube containing the cells. Proceed immediately with step 1.3.

For suspension cells

Collect a large number of cells (typically between **200,000** and **500,000 cells/sample**) in a 15 or 50 ml tube and go directly to step 1.3.

For cryopreserved cells

- Thaw a large number of cells (typically between **200,000** and **500,000 cells/sample**) 2 minutes in a 37°C water bath.
- Transfer them to a 15 ml tube and go directly to step 1.3.

***NOTE**: If not all the cells are used, leftover cells can be frozen again. See "Remarks before starting" for more information regarding cryopreservation.*

1.3 Add **12 ml of warm PBS** to wash the cells.

1.4 Centrifuge cells at 500 x g for 10 minutes at 4°C in a swing out rotor.

1.5 Discard supernatant and resuspend the pellet with the **500 µl of cold PBS+PIC**.

1.6 Take an aliquot of **10 µl**, add **10 µl of Trypan blue** and count the cells.

1.7 Distribute **50,000 cells** in new 1.5 ml tubes, as determined by cell counting, using the total cell number.

Cell lysis

1.8 Centrifuge at 500 x g for 10 minutes at 4°C with a fixed angle.

1.9 Carefully take out all the supernatant and keep cell pellet on ice.

***NOTE**: Depending on the cell type, the cell pellet may be visible or not.*

1.10 Immediately add 50 µl of cold **ATAC Lysis Buffer 1 + Digitonin** to each tube. Pipette up and down 3 times and keep on ice for the optimized lysis time (generally 0 to 3 minutes).

***NOTE**: when doing the experiment on a new cell type for the first time we highly recommend to optimize the lysis time. See additional protocol: Nuclei isolation optimization for cells.*

1.11 Add **1 ml of cold ATAC Lysis Buffer 2** to each tube to stop the lysis and mix by inverting.

1.12 Centrifuge at 500 x g for 10 minutes at 4°C with a fixed angle.

1.13 Take out **1 ml** of supernatant and then centrifuge again at 500 x g for 5 minutes at 4°C.

***NOTE**: Depending on the cell type, the nuclei pellet may or may not be visible.*

1.14 Carefully discard all the leftover supernatant and keep the nuclei pellet on ice. Go directly to step 2.

STEP 2

Tagmentation and DNA Purification

1 *Day 1* *1.5 hour*

***NOTE**: Before first use, the **DNA Wash Buffer** must be completed by adding the **100% ethanol**.*

*ATAC-seq kit, **format 8 rxns**: add **2,8 ml of 100% ethanol** to **700 µl of DNA Wash Buffer**.*

*ATAC-seq kit, **format 24 rxns**: add **8,8 ml of 100% ethanol** to **2,2 ml of DNA Wash Buffer**.*

2.1 Prepare the **Tagmentation Mix** as described in the table below, for the number of desired reactions.

Reagent	Volume for 1 reaction
Tagmentation Buffer (2x) *(blue cap)*	25 µl
Tagmentase (loaded) *(yellow cap)*	2.5 µl
Digitonin 2%	0.25 µl
Tween20 10% *(red cap)*	0.5 µl
PBS	16.5 µl
Nuclease-free water	5.25 µl

***CAUTION**: Digitonin is toxic and care should be taken. Use full PPE including a mask, lab coat and gloves while handling Digitonin.*

***NOTE**: The Tagmentation Buffer (2x) is provided at -20°C, but it can also be stored at 4°C for up to 6 weeks.*

2.2 Add **50 µl of Tagmentation Mix** to each nuclei pellet and pipette up and down once.

2.3 Incubate the samples for exactly 30 minutes at 37°C.

2.4 Stop the reaction by adding **250 µl of Binding Buffer** to each sample as quickly as possible.

2.5 Transfer each sample to a provided Spin column in a Collection tube.

2.6 Centrifuge at ≥ 10,000 x g for 30 seconds. Discard the flow-through.

2.7 Add **200 µl of DNA Wash Buffer** to the column. Centrifuge at ≥ 10,000 x g for 30 seconds. Repeat wash step.

2.8 Add **12 µl of DNA Elution Buffer** (white cap) directly to the column matrix. Transfer the column to a new 1.5 ml microcentrifuge tube and centrifuge at ≥ 10,000 x g for 30 seconds to elute the DNA.

***NOTE**: Purified DNA can be stored several weeks at -20°C if needed.*

STEP 3

Library Amplification

2 *Day 2* *6 hours*

This step is divided into three parts:

a. A first PCR amplification for a few cycles

b. A qPCR to estimate the number of additional cycles needed

c. A second PCR amplification with a custom number of cycles for each sample

a. 1st PCR amplification

3.1 Prepare the **Amplification Mix** as described in the table below, for the number of desired reactions.

Reagent	Volume for 1 reaction
2x High-Fidelity Mastermix *(violet cap)*	25 µl
Nuclease-free water	14 µl

3.2 In a new 0.2 ml tube, mix **10 µl of tagmented DNA** from step 2.8 and **39 µl of Amplification Mix**.

3.3 Add **1 µl of primer pair** from a Primer index for tagmented libraries kit to each sample and mix.

***NOTE**: Primer pairs provided in the Primer index for tagmented libraries kits include indexes for the sequencing. Use different indexes for samples that you want to sequence in the same lane.*

3.4 Briefly spin the tubes and run the PCR program described in the table below.

Cycles	Temperature	Time
1	72°C	5 minutes
1	98°C	30 seconds
5	98°C	10 seconds
	63°C	30 seconds
	72°C	1 minute

***NOTE**: Samples can then be stored 4 hours on ice or several days at -20°C.*

b. qPCR to determine the number of additional cycles needed

3.5 Prepare the **qPCR Mix** as described in the table below, for the number of desired reactions.

Reagent	Volume for 1 reaction
2x High-Fidelity Mastermix *(violet cap)*	5 µl
Primer pair SI 1 or UDI 1	0.25 µl
100x SYBR	0.15 µl
Nuclease-free water	4.60 µl

3.6 Distribute **10 µl of qPCR Mix** in each well of a qPCR strip, add **5 µl of amplified DNA** from step 3.4 and mix.

3.7 Briefly spin the tubes and run the qPCR program described in the table below.

Cycles	Temperature	Time
1	98°C	30 seconds
20	98°C	10 seconds
	63°C	30 seconds
	72°C	1 minute

***NOTE**: Samples can then be stored 4 hours on ice or several days at -20°C.*

3.8 Determine the additional number of cycles needed, for each sample, as follows:

- Plot fluorescence versus Cycles.
- Record the maximum fluorescence intensity on the plot.
- Identify the Ct value corresponding to 1/3 of this maximum fluorescence. This value rounded up is the number of additional cycles needed.

***NOTES**: We recommend keeping the same number of cycles between technical replicates. If the maximum fluorescence intensity differs, take the lowest fluorescence intensity between replicates.*

*As general guidelines, **10** to **13 total cycles** (which means 5 cycles plus an additional 5 to 8 cycles) are typically applied to samples processed with **50,000 cells** per reaction.*

c. 2nd PCR amplification

3.9 Add amplification cycles to each pre-amplified library from step 3.4 with the PCR program described below and the X number of cycles determined at step 3.8:

Cycles	Temperature	Time
1	98°C	30 seconds
X (number of cycles = Ct at 1/3 fluorescence max)	98°C	10 seconds
	63°C	30 seconds
	72°C	1 minute

STEP 4

Size Selection – Manual Protocol

2 *Day 2* *1.5 hour*

***NOTE**: For IP-Star Compact users, the protocol for automated size selection is available in the section: Additional protocols.*

4.1 Carefully resuspend the room temperature AMPure XP beads by shaking and light vortexing until no pellet is visible at the bottom of the container.

4.2 Add **22.5 µl of AMPure XP beads** (corresponding to a 0.5x ratio). Mix by pipette 8 – 10 times until the mixture is homogeneous.

4.3 Incubate at room temperature for 10 minutes.

4.4 Place the tube on the DiaMag02 and wait until the beads are completely bound to the magnet (~2 minutes).

4.5 Without disturbing the pellet, carefully aspirate and transfer the supernatant to a new tube.

4.6 Add **58.5 µl of AMPure XP beads**. Mix by pipetting 8 – 10 times until the mixture is homogeneous.

4.7 Incubate at room temperature for 10 minutes.

4.8 Place the tube on the DiaMag02 and wait until the beads are completely bound to the magnet (~2 minutes).

4.9 Carefully aspirate by pipette and discard the supernatant without disturbing the pellet.

4.10 Wash the beads pellet 2 times as follows:

- With the tubes on the magnet, **add 100 µl** of freshly prepared **80% ethanol** without disturbing the bead pellet and wait for 5 seconds.
- Carefully aspirate by pipette and discard the supernatant without disturbing the pellet.

4.11 Leaving the tube open, let dry the beads on the DiaMag02 for 3 minutes.

4.12 Remove tubes from DiaMag02 and elute DNA by resuspending the beads in **20 µl of DNA Elution Buffer** (white cap).

4.13 Incubate for 10 minutes at room temperature.

4.14 Place the tube on the DiaMag02 and wait until the beads are completely bound to the magnet (~2 minutes).

4.15 Without disturbing the pellet, carefully aspirate and transfer the supernatant containing size selected libraries to a new tube.

STEP 5

Library QC

3 *Day 3*

5.1 Determine the concentrations of your samples by the use of a fluorescence-based assay such as the Qubit High Sensitivity assay (ThermoFisher Scientific).

5.2 Run a part of each library on a High Sensitivity chip for BioAnalyzer (Agilent) or on Fragment Analyzer (Advanced Analytical) according to the manufacturer's instructions.

***NOTE**: In some cases, for example when primer dimers are still present, an additional clean-up can be performed (see the protocol "Library clean-up" in the "Additional protocols" section). If all the samples have primer dimers to remove, at similar levels, it is possible to pool the samples first and to purify after.*

5.3 Your libraries are now ready for pooling and sequencing.

***NOTE**: Individual libraries, quantified and purified according to the above protocol, can be pooled at desired molar ratios to allow multiplex sequencing. Libraries that are being pooled must have been prepared with different indexes (for multiplexing and index pooling guidelines refer to the manual: Primer indexes for tagmented libraries. The minimal molar concentration needed to sequence the pool depends on the requirements of the sequencing platform. The total molarity is the sum of all the individual libraries' molarities in the final volume, e.g. if you add 5 µl of a 10 nM library to 5 µl of a 20 nM library, you have 10 µl of a 15 nM pool. If libraries are prepared from similar input amounts, they can be pooled by combining equal volume aliquots of each library.*

ATAC-seq Data Analysis Recommendations

ATAC-seq data analysis workflow

In the following chapter we will guide you through the basics of ATAC-seq data analysis. We will also provide some examples of software tools suitable for each step. We cite numerous analysis tools, including free and commercial softwares.

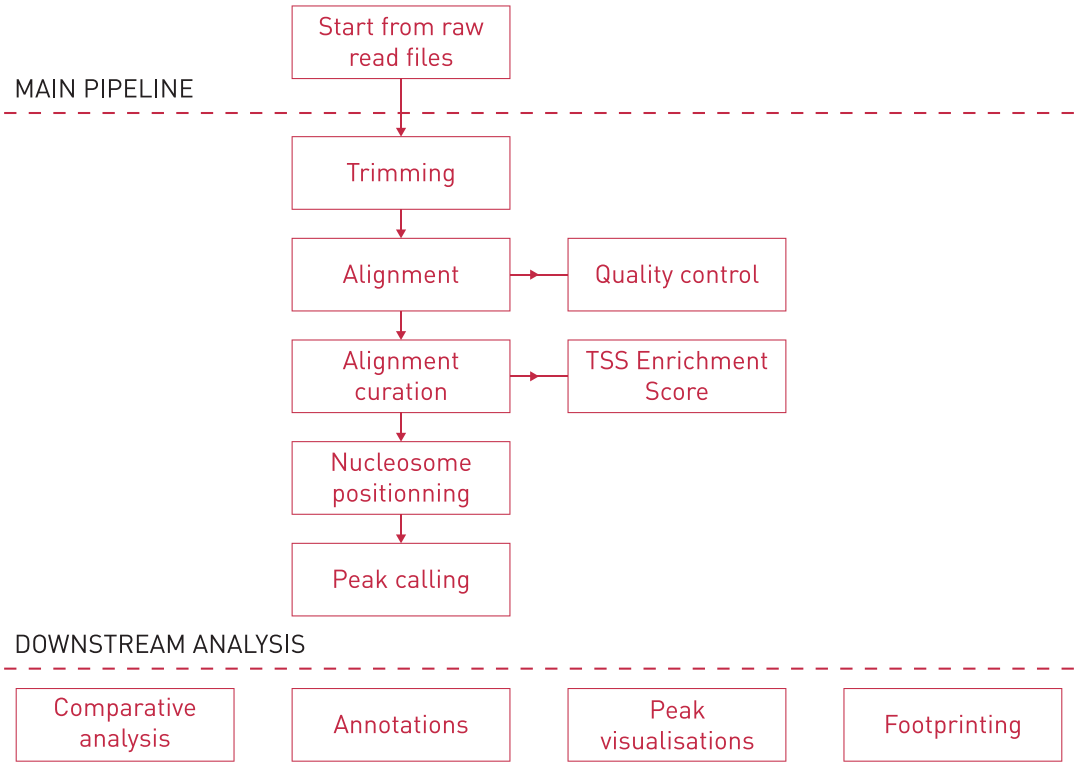


Figure 2. ATAC-seq data analysis workflow: general view

1. Trimming: use trimming to get rid of low-quality bases and artefacts in the readset, such as adapter contaminations.
   - **a.** Cutadapt
   - **b.** Trim Galore!
   - **c.** rimmomatic
   - **d.** NGmerge
2. **Alignment**: in this step you will map the reads against a known reference sequence. In order to assess the quality of your alignment, it can also be interesting to look at the fragment length distribution

(e.g. using qualimap) after alignment and assess if the distribution doesn't show any artifact. Such artifact could indicate some improper alignments caused by too short expected insert size parameters used for the alignment.

a. ELAND

b. Tmap

c. BWA

d. Bowtie2

3. (Optional step) **Quality control**: you can check the general quality of the sequencing and the alignment.

   a. FastQC

   b. Picard Tools

   c. samtools

4. **Alignment curation**: Removing bad alignments and technical artifacts. In order to get more reproducible results, we should remove several potential artifacts from our aligned reads:

   - Duplicated reads, often considered as PCR duplicates, should be remove as they are not coming from any biological signal.

     a. Samtools

     b. Picard

   - Bad mapping and improperly paired reads should also be removed

     a. Samtools

     b. Picard

   - Mitochondrial reads. As the mitochondrial genome is more accessible due to a lack of chromatin packaging it is also considered as noise in ATAC seq experiment and reads mapping on this chromosome should also be removed.

   - ENCODE blacklisted regions: those regions have been found to be regions where the probability of mapping reads was high and was thus shown to be highly unreliable to study enrichments. We usually also remove those regions before to process to peak calling.

     a. Bedtools

5. **Transcription Start Site (TSS) Enrichment Score**: should be computed as recommended by the ENCODE guidelines. Depending on the reference, a specific threshold is indicating the quality of the ATAC seq data analyzed (threshold table).
   a. ATACseqQC (R package)
6. **Switching reads for nucleosome positioning**: As a consequence of the Tn5 transposase used to generate ATAC seq profiles, we will need to shift the reads from +4 bp for the positive strand and -5 bp for the negative strand.
   a. Bedtools (+ custom scripts)
   b. ATACseqQC (R package)
7. **Peak calling**: during peak calling the software will detect sites of enrichment along the genome. Special care should be taken to ensure using proper parameters to call ATACseq peaks.
   a. MACS2
   b. GENRICH

After this general analysis, the peaks can be analyzed further to get answers to our biological questions. There are countless ways and tools for further analyses, the project goals determine which ones we should pick. Just as in the case of basic analysis, we recommend to thoroughly study the manual of the chosen software tool to understand its purpose and its function. Below we will list a few common analysis types to further process the peaks, along with example tools again.

8. **Footprinting**: ATAC seq data allows also to predict binding sites for several transcription factor by using the open chromatin information coupled to computational methods (e.g. hidden markov model). Several tools exist.
   a. CENTIPEDE
   b. ATACseqQC (R package)
   c. HINT-ATAC
   d. TOBIAS
9. **Visualization**: the peaks, the reads, and other data (e.g. gene positions) can be displayed in a suitable genome browser.
   a. IGV

b. IGB

c. USCS Genome Browser

10. **Descriptive statistics**: the peaks can be described in various useful ways, like how many reads fall into them, what is their number, mean size and significance etc.; these figures are also very useful when you compare datasets.

    a. Peak callers usually provide per peak and/or summary statistics after peak detection

    b. HOMER

    c. GREAT

    d. BEDTools

11. **Annotation, Gene Ontology, Pathway analysis**: after annotation/GO/Pathway analysis you will get a clear picture about which genomic features or pathways your peaks are associated to, providing an important information about disease mechanisms, the role of DNA binding proteins, or treatment effects.

    a. HOMER

    b. GREAT

    c. BEDTools

    d. ReactomePA

12. **Comparative analysis**: this type of analysis is the obvious choice when you have several datasets from comparable conditions (e.g. treated and untreated cells) or when you want to check the performance of your ChIP-seq by comparing it to a reference; there are many different ways to compare peaks, including checking the overlaps, the correlation of enrichment sizes and performing statistical tests on the peaksets

    a. HOMER

    b. BEDTools

    c. DiffBind

Example of Results

ATAC-Seq has been performed on 50,000 cryopreserved K562 cells, using the Diagenode's ATAC-seq kit and the 24 SI for Tagmented Libraries (Cat. No. C01011032). The quality control was then performed as described in step 5.

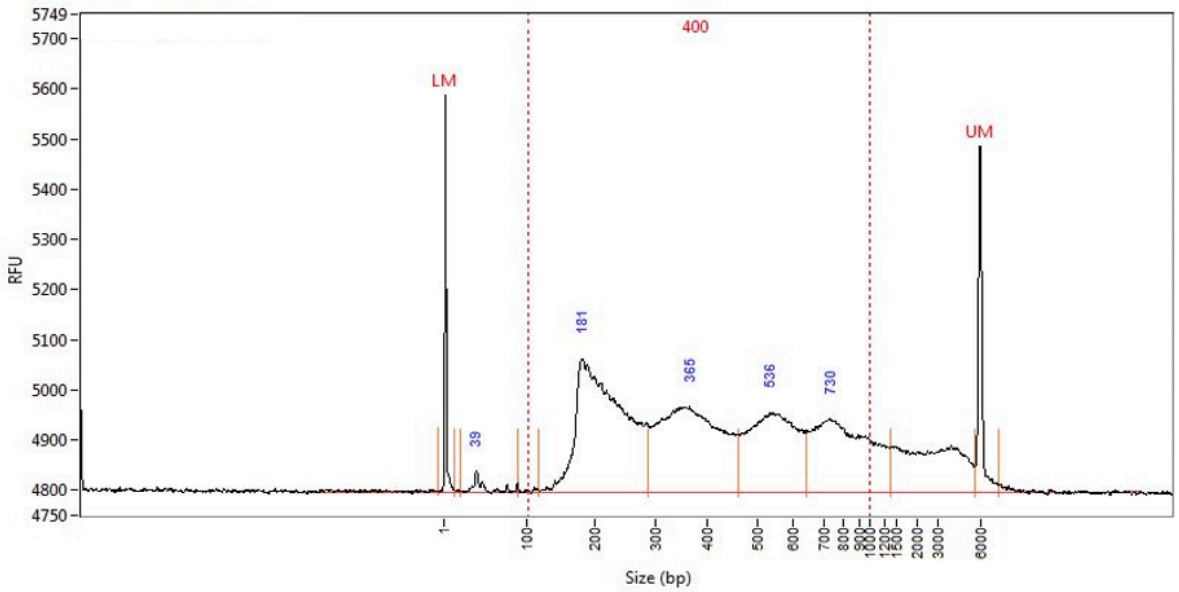


Figure 3. Library profile generated on Fragment Analyzer (Agilent)

The libraries were sequenced on Illumina's NovaSeq6000 in 2x50 bp mode, and the data analyzed as described in the section: ATAC-seq data analysis recommendations.

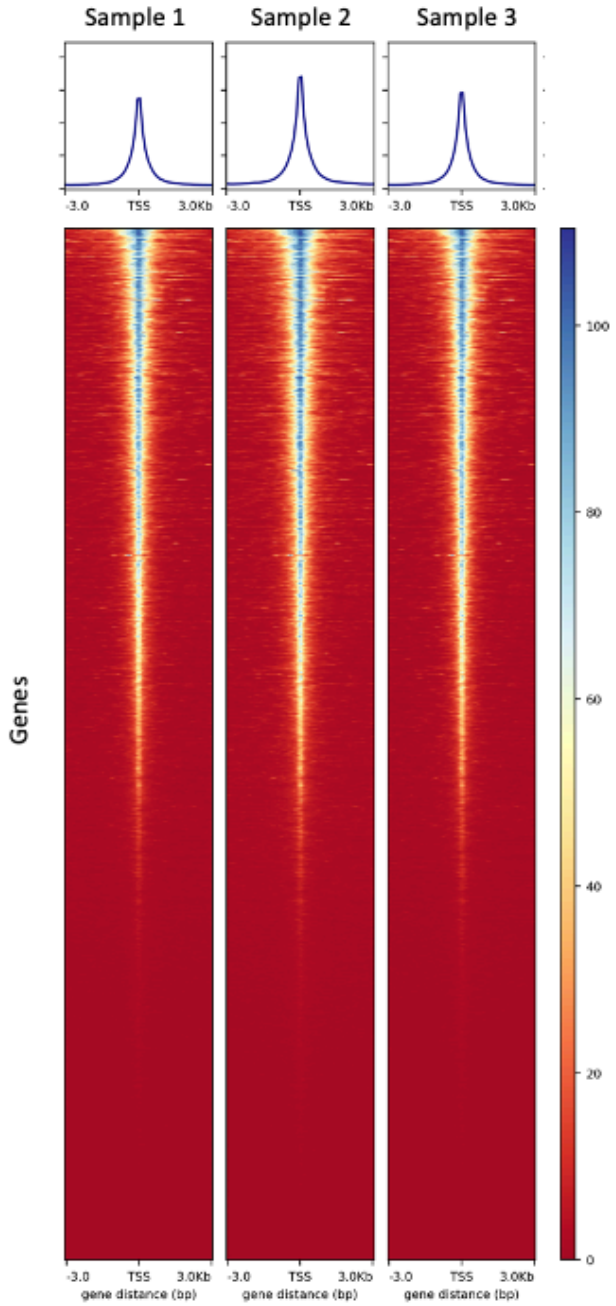


Figure 4. Enrichments at TSS of the ATAC-seq libraries

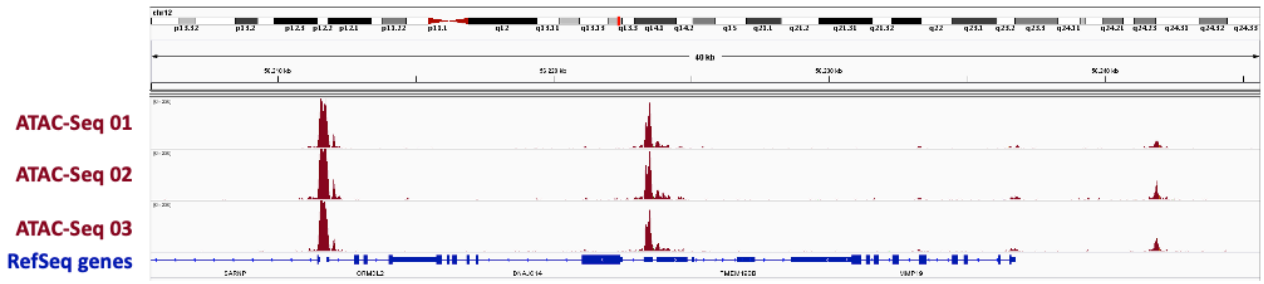


Figure 5. Sequencing profiles of the ATAC-seq libraries

ADDITIONAL PROTOCOLS

Nuclei Isolation Optimization For Cells

The aim of this protocol is to identify the optimal lysis conditions to extract a pure nuclei fraction for each new cell type.

Buffer preparation

1. Add **2.5 µl of Protease Inhibitor Cocktail** (black cap) to **500 µl of cold PBS** and keep the tube on ice.

   ***NOTE**: This will be use to rinse the cells. When using several sample types, prepare 500 µl of PBS+PIC for each one.*

2. Define the number of ATAC reactions to be performed. For each reaction prepare **50 µl of ATAC Lysis Buffer 1 with Digitonin** (e.g. add 0.5 µl of 2% Digitonin to 49.5 µl of ATAC Lysis Buffer 1 (green cap)). Keep it on ice.

   ***CAUTION**: Digitonin is toxic and care should be taken. Use full PPE including a mask, lab coat and gloves while handling Digitonin.*

Cell collection

For adherent cells

- Pre-warm PBS, culture medium and trypsin-EDTA at 37°C.
- Remove the medium and rinse a large number of cells (typically between **200,000** and **500,000 cells/sample**) with **pre-warmed PBS**. Gently shake the flask for 2 minutes.
- Remove the PBS and add sterile **trypsin-EDTA** to the culture flask to detach adherent cells from the bottom. Add enough trypsin-EDTA to completely cover the cells. Gently shake the culture flask for 1-2 minutes or until the cells start to detach. The time needed may depend on the cell type.

- Immediately add fresh culture medium to the cells when they are detached. For correct inactivation, the volume of medium must be 2x the volume of trypsin-EDTA. Transfer cell suspension to a 15 or 50 ml tube.
- Rinse the flask by adding **10 ml of warm PBS**. Add this volume to the tube containing the cells. Proceed immediately with step 3.

For suspension cells

Collect a large number of cells (typically between **200,000** and **500,000 cells/sample**) in a 15 or 50 ml tube and go directly to step 4.

For cryopreserved cells

- Thaw a large number of cells (typically between **200,000** and **500,000 cells/sample**) 2 minutes in a 37°C water bath.
- Transfer them to a 15 ml tube and go directly to step 3.

***NOTE**: If not all the cells are used, leftover cells can be frozen again. See "Remarks before starting" for more information regarding cryopreservation.*

3. Add **12 ml of warm PBS** to wash the cells.

4. Centrifuge cells at 500g for 10 minutes at 4°C in a swing out rotor.

5. Discard supernatant and resuspend the pellet with the **500 µl of cold PBS+PIC**.

6. Take an aliquot of **10 µl**, add **10 µl of Trypan blue**, and count the cells.

7. Distribute two times **50,000 cells** for each cell type in two new 1.5 ml tubes, as determined by cell counting, using the total cell number.

8. Centrifuge at 500 g for 10 minutes at 4°C with a fixed angle.

9. Take out all the supernatant and keep cell pellet on ice

***NOTE**: Depending on the cell type, the cell pellet may be visible or not.*

10. Immediately add **50 µl of cold ATAC Lysis Buffer 1 + Digitonin** to each tube. Pipette up and down 3 times and keep on ice. For each cell type incubate one replicate for 0 minutes and a second one for 3 minutes.

11. Add 1 ml of cold ATAC Lysis Buffer 2 to each tube to stop the lysis, either immediately or after 3 minutes according on the replicate.

12. Centrifuge at 500 x g for 10 minutes at 4°C with a fixed angle.

13. Take out **1 ml of supernatant** and then centrifuge again at 500 x g for 5 minutes at 4°C.

    ***NOTE**: Depending on the cell type, the nuclei pellet may or may not be visible.*

14. Discard all the leftover supernatant and resuspend the nuclei pellet in 30 µl of PBS.

15. Take an aliquot of **10 µl**, add **10 µl of Trypan blue**, and count the nuclei. Measure the viability of the sample. After lysis the viability should be very low (less than **5%**).

16. Analyze the solution under a microscope to check nuclei purity. Choose the condition for which nuclei are clearly visible, intact and without any double-membranes left.

Library Clean-Up – Manual Protocol

1. Carefully resuspend the room temperature AMPure XP beads by shaking and light vortexing until no pellet is visible at the bottom of the container.

2. Estimate the library volume and add nuclease-free water to reach **50 µl**.

3. Add **90 µl of AMPure XP beads**. Mix by pipetting 8 – 10 times until the mixture is homogeneous.

4. Incubate at room temperature for 10 minutes.

5. Place the tube on the DiaMag02 and wait until the beads are completely bound to the magnet (~2 minutes).

6. Carefully aspirate by pipette and discard the supernatant without disturbing the pellet.

7. Wash the beads pellet 2 times as follows:

   - With the tubes on the magnet, add **100 µl** of freshly prepared **80% ethanol** without disturbing the bead pellet and wait for 5 seconds.
   - Carefully aspirate by pipette and discard the supernatant without disturbing the pellet.

8. Leaving the tube open, let the beads dry on the DiaMag02 for 5 minutes.

9. Remove tubes from DiaMag02 and elute DNA by resuspending the beads in **20 µl of DNA Elution Buffer** (white cap).

10. Incubate for 10 minutes at room temperature.

11. Place the tube on the DiaMag02 and wait until the beads are completely bound to the magnet (~2 minutes).

12. Without disturbing the pellet, carefully aspirate and transfer the supernatant containing purified libraries to a new tube.

Size Selection – Automated Protocol

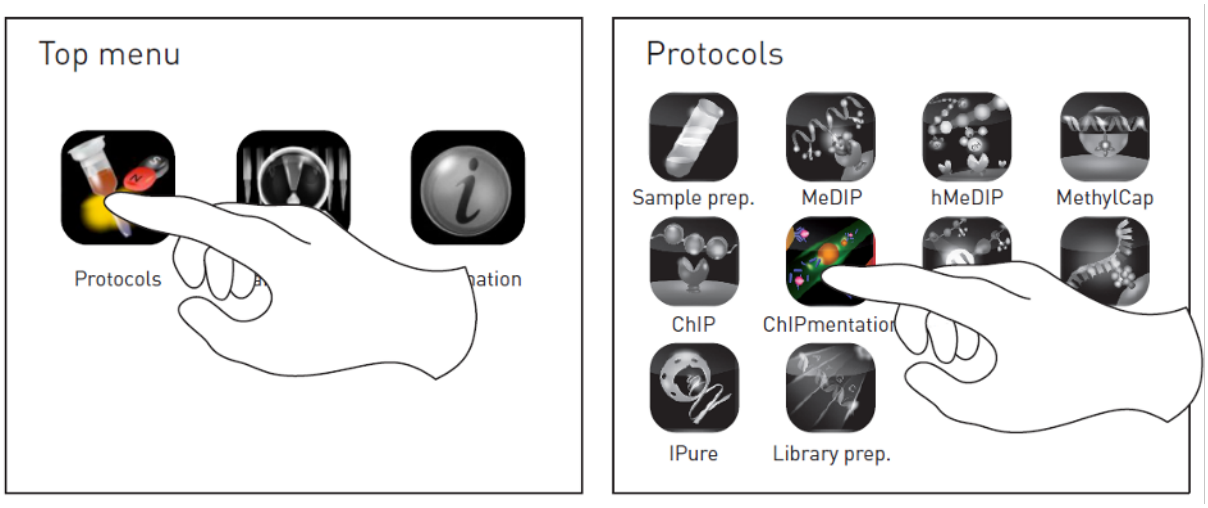
30 min hands on time

1h10 min run for each series of 8 samples

This section describes the protocol for size selection using the IP-Star Compact Automated System.

> ***NOTE**: Use the IP-Star Compact and room temperature AMPure XP beads for the size selection.*

1. Switch on the IP-Star and select "**Protocols**" icon and then "**ChIPmentation**".



2. Select "**ATAC_size_selection_8**".

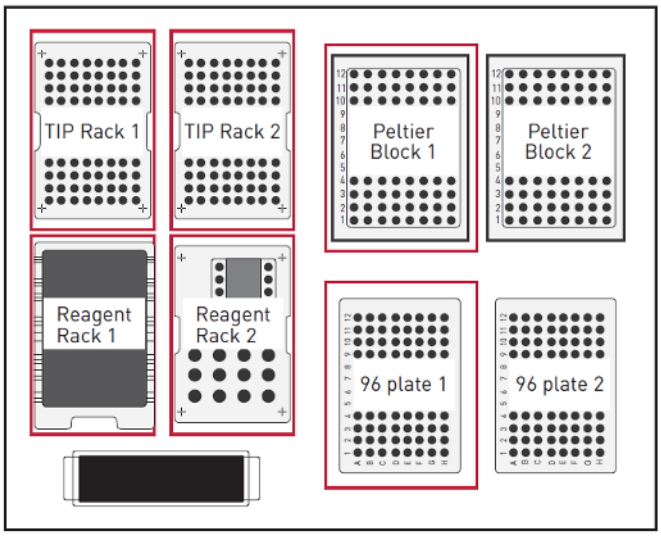
3. Set up the exact number of samples that you want to process by pressing the black box.

> ***NOTE**: The Peltier Block 1 is now cooling down to 4°C to keep your samples cold.*

4. Set up all the plastics on the platform according to the screen layout.

   - Fill **TIP Rack 1** with tips according to the screen.
   - Fill **Reagent Rack 1 & 2** with reagent containers according to the screen.

- Fill **96 plate 1** with a 96 well microplate.
- Fill **Peltier Block 1** with 200 µl tube strips according to the screen.



5. Fill the robot with all reagents.

   - Add **5 µl of nuclease-free water** to each sample to have a final volume of **50 µl**. Put your samples in lane 1 of the **Peltier Block**.
   - Distribute **90 µl** of room temperature **AMPure XP beads** in row 1 of the **96-well Microplate**.

   ***NOTE**: Resuspend the beads with pipetting up and down several times before dispensing them.*

   - Fill the container of the **Reagent Rack 1** with freshly prepared **80% Ethanol** according to the screen.
   - Fill the container of **Reagent Rack 2** with **DNA Elution Buffer** (white cap) (**=Resuspension Buffer**) according to the screen.
   - Check the proper insertion of the racks and the consumables.

6. Close the door and press "**Run**" to start.

7. After the run, recover your samples on the upper row of the **Left Peltier Block**. The final volume is **20 µl** for each sample.

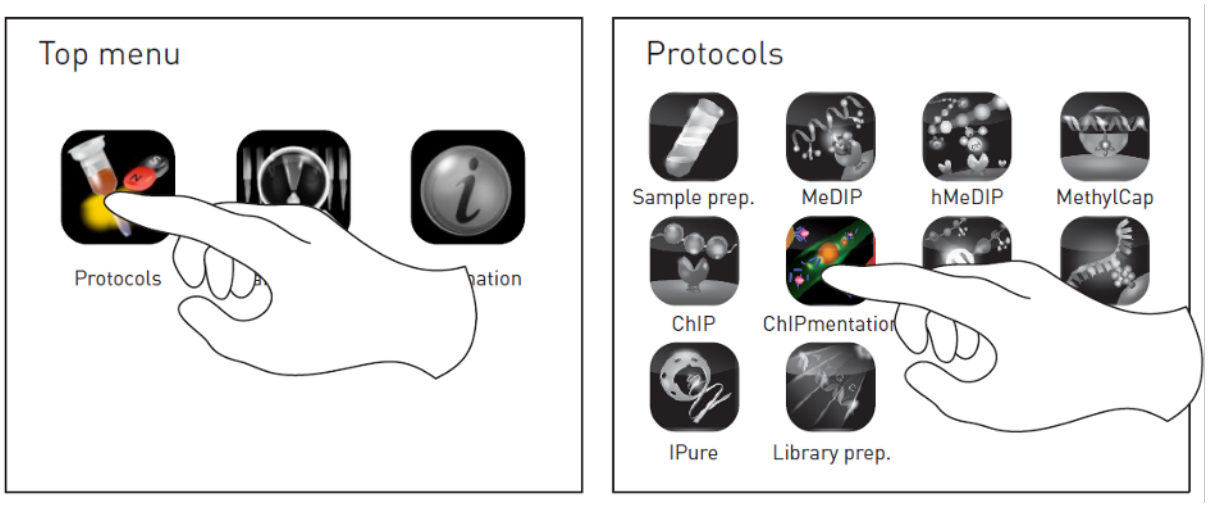
8. Press "OK" and "Back" until the homepage appears on the screen. Press "Shutdown" and wait until the screen is black before switching off the IP-Star.

   ***NOTE**: Remove all the plastics from the platform, empty the waste shuttle and clean the inner side of the IP-Star with 70% ethanol.*

Libraries Clean-Up – Automated Protocol

***NOTE**: Use the IP-Star Compact and room temperature AMPure XP beads for the clean-up.*

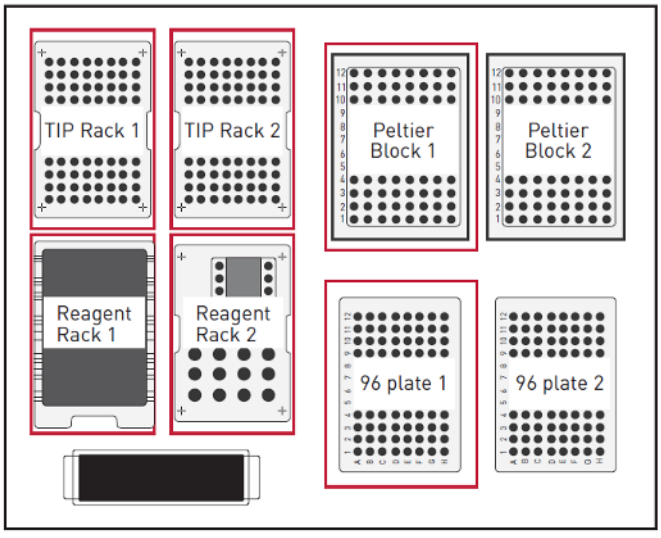
1. Select "**Protocols**" icon and then "**ChIPmentation**".



2. Select "**ChIPmentation_Purification_08**" if you plan to run between **1** and **8** samples, or "**ChIPmentation_Purification_16**" if you plan to run between **9** and **16** samples.

3. Set up the exact number of samples that you want to process by pressing the black box.

***NOTE**: The Peltier Block is now cooling down to 4°C to keep your samples cold.*



4. Set up all the plastics on the platform according to the screen layout.

   - Fill **TIP Rack 1** (and **2** if processing more than 8 samples) with tips according to the screen.
   - Fill **Reagent Rack 1 & 2** with reagent containers according to the screen.
   - Fill **96 plate 1** with a 96 well microplate.
   - Fill **Peltier Block 1** with 200 µl tube strips according to the screen.

5. Estimate the library volume and add nuclease-free water to reach **50 µl**.

6. Fill the robot with all reagents.

   - Put your samples in lane 1 (and 2 if processing more than 8 samples) of the **Peltier Block 1**.
   - Distribute **90 µl** of room temperature **AMPure XP Beads** in row 1 (and 5 if processing more than 8 samples) of the **96-well Microplate**.

   ***NOTE**: Resuspend the beads with pipetting up and down several times before dispensing them.*

   - Fill the container of the **Reagent Rack 1** with freshly prepared **80% Ethanol** according to the screen.
   - Fill the container of **Reagent Rack 2** with **DNA Elution Buffer** (white cap) (**Resuspension Buffer**) according to the screen.
   - Check the proper insertion of the racks and the consumables.

7. Close the door and press "Run" to start.

8. After the run, recover your samples on the upper row of the **Peltier Block 1**. The final volume is **20 µl** per each sample.

9. Press "OK" and "Back" until the homepage appears on the screen. Press "Shutdown" and wait until the screen is black before switching off the IP-Star.

   ***NOTE**: Remove all the plastics from the IP-Star platform, empty the waste shuttle, and clean the inner side of the IP-Star with 70% ethanol.*

FAQs

My centrifuge does not have the same kind of rotor as specified in the manual (swing-out rotor or fixed angle) so can I still use it?

The type of rotor will impact the position of the cell pellet. In the manual, the position which is more suitable for the step of the experiment is described, but if not available it can be replaced by another rotor.

What is the composition of buffers included in the kit?

The composition of the buffers is proprietary.

How many reads should I target when sequencing ATAC-seq libraries?

40M reads per sample are enough for open chromatin analysis, but 100M reads per sample are needed to study nucleosome positioning.

Regarding the questions related to the IP-Star® Compact, please refer to the troubleshooting guide and the list of error codes pages 29-31 of the SX-8G IP-Star® Compact manual.

Related Products

Product	Reference
Tagmentase (Tn5 transposase) loaded	C01070012
Tagmentation Buffer (2x)	C01019043
8 SI for Tagmented libraries	C01011033
24 SI for tagmented libraries	C01011032
8 UDI for tagmented libraries	C01011035
24 UDI for tagmented libraries - Set I	C01011034
24 UDI for tagmented libraries - Set II	C01011036
MicroChIP DiaPure columns	C03040001
DiaMag 0.2ml magnetic rack	B04000001
IP-Star® Compact Automated System	B03000002

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