



A Hologic Company

ATAC-seq Kit

Cat. No. C01080001 (8 rxns)

Cat. No. C01080002 (24 rxns)

ATAC-seq package for tissue

Cat. No. C01080005 (8 rxns)

Cat. No. C01080006 (24 rxns)



Please read this manual carefully
before starting your experiment

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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. 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 adapters 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.

Diagenode’s ATAC-seq kit is based on a highly validated protocol for cells and tissues, used for years in our Epigenomics Profiling Services and takes advantage of many successful Diagenode tools, such as loaded Tagmentase (Tn5 transposase), MicroChIP DiaPure Columns and Primer indexes for tagmented libraries kits. The ATAC-seq kit also contains a special qPCR step that has been optimized to estimate the amplification rate and to avoid an over-amplification of samples.

Depending on the type of sample (cell or tissue) the following products should be used for the complete ATAC-seq workflow:

A. Working with cells

1. ATAC-seq kit: 8 rxns - Cat. No. C01080001 or 24 rxns - Cat. No. C01080002
2. Primer indexes for tagmented libraries (Cat. No. C01011032 - C01011037)

B. Working with tissue

1. ATAC-seq package for tissue: 8 rxns - Cat. No. C01080005 or 24 rxns - Cat. No. C01080006.

The package is composed of:

Kit/format	ATAC-seq package for tissue - 8 rxns Cat. No. C01080005	ATAC-seq package for tissue - 24 rxns Cat. No. C01080006
Modules included	Tissue Nuclei Extraction for ATAC-seq 8 rxns (Cat. No. C01080003)	Tissue Nuclei Extraction for ATAC-seq 24 rxns (Cat. No. C01080004)
	ATAC-seq kit - 8 rxns (Cat. No. C01080001)	ATAC-seq kit - 24 rxns (Cat. No. C01080002)
	8 UDI for tagmented libraries (Cat. No. 01011035)	24 UDI for tagmented libraries - Set I (Cat. No. 01011034)

Kit Method Overview & Time Table

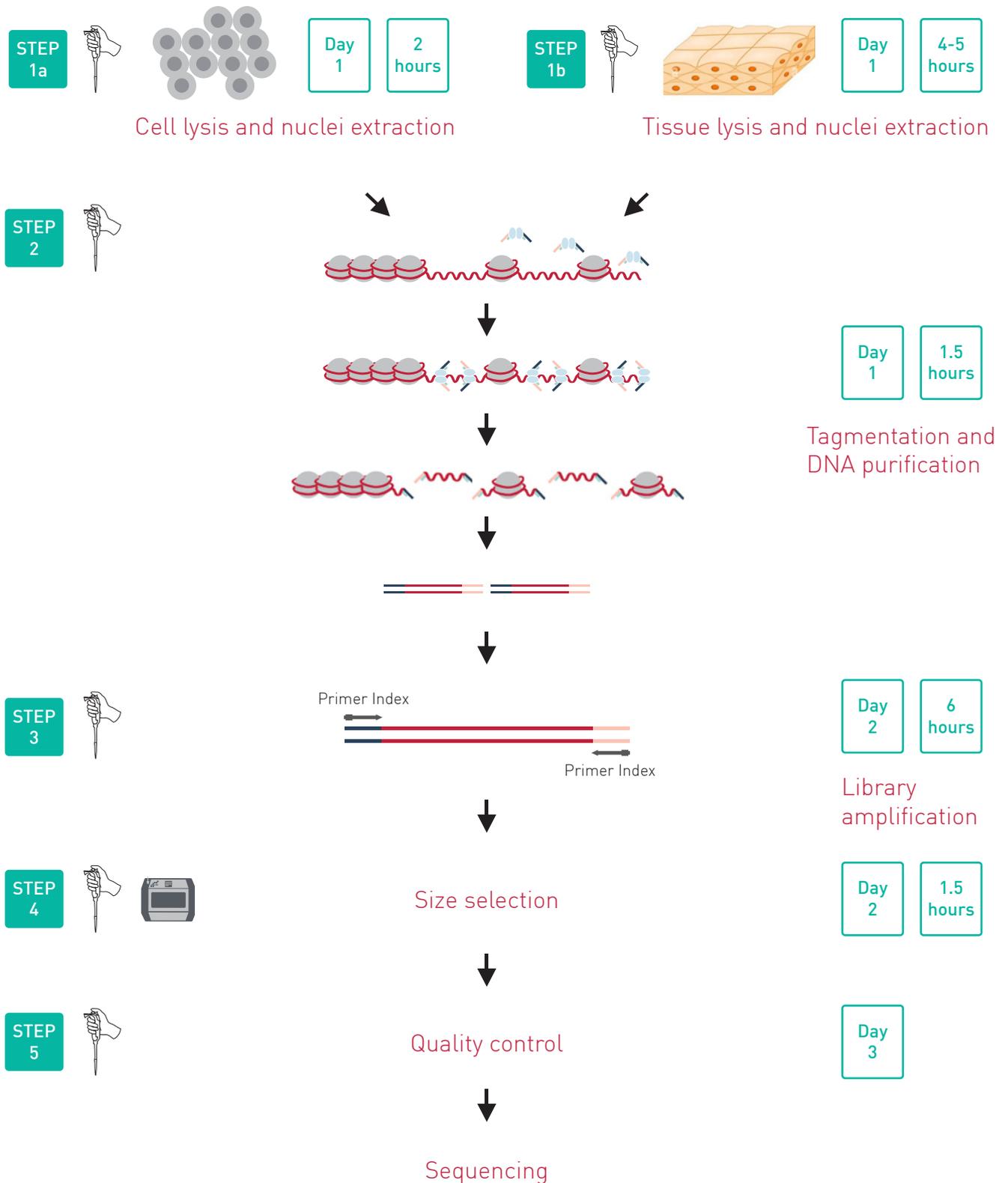


Figure 1. ATAC-seq workflow

Kit Materials

Depending on the type of sample (cell or tissue) the following products should be used for the complete ATAC-seq workflow:

A. Working with cells

1. ATAC-seq kit (8 rxns - Cat. No. C01080001 or 24 rxns - Cat. No. C01080002)
2. Primer indexes for tagmented libraries (Cat. No. C01011032 - C01011037)

B. Working with tissue

1. ATAC-seq package for tissue (8 rxns - Cat. No. C01080005 or 24 rxns - Cat. No. C01080006). The package is composed of:

Kit/format	ATAC-seq package for tissue - 8 rxns Cat. No. C01080005	ATAC-seq package for tissue - 24 rxns Cat. No. C01080006
Modules included	Tissue Nuclei Extraction for ATAC-seq 8 rxns (Cat. No. C01080003)	Tissue Nuclei Extraction for ATAC-seq 24 rxns (Cat. No. C01080004)
	ATAC-seq kit - 8 rxns (Cat. No. C01080001)	ATAC-seq kit - 24 rxns (Cat. No. C01080002)
	8 UDI for tagmented libraries (Cat. No. 01011035)	24 UDI for tagmented libraries - Set I (Cat. No. 01011034)

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 Cat. No. C01080001	Volume – 24 rxns Cat. No. C01080002	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

NOTE: * Volumes of both buffers contain excess to use 1 reaction in duplicate (for 8 rxns) or 3 reactions in duplicate (for 24 rxns) for optional protocol of nuclei isolation optimization for cells.

Table 2. Components stored at -20°C

Component	Cap color	Volume – 8 rxns Cat. No. C01080001	Volume – 24 rxns Cat. No. C01080002	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 Cat. No. C01080001	Volume – 24 rxns Cat. No. C01080002	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.

Components supplied with the Tissue nuclei extraction for ATAC-seq

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

Table 4. Components stored at +4°C

Component	Cap color	Volume – 8 rxns Cat. No. C01080003	Volume – 24 rxns Cat. No. C01080004	Storage
Lysis Buffer 1		3.6 ml	10.8 ml	+4°C
Lysis Buffer 2		5.4 ml	16.1 ml	+4°C
Nuclei Isolation Buffer 1		2.7 ml	8 ml	+4°C
Nuclei Isolation Buffer 2		4 ml	12 ml	+4°C
Nuclei Isolation Buffer 3		4 ml	12 ml	+4°C

Required Materials Not Provided

For ATAC-seq kit

- Gloves to wear at all steps
- Cold PBS
- Warm PBS (37°C)
- Cell medium
- Trypsin-EDTA to detach adherent cells
- 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
- Water bath
- Refrigerated 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)
- or 24 UDI for tagmented libraries - Set III (Cat. No. C01011037)

For Tissue Nuclei Extraction for ATAC-seq

- Dry ice
- Ice
- Scalpel and sterile blades
- Surgical tweezer
- Plastic cell culture dish
- Precise weighing machine (0.01g)
- Dounce homogenizer (2 or 5 ml) and corresponding pestles (loose and tight)
- Sterile 40 µm cell strainers
- Nuclease-free 2 ml DNA LoBind tubes
- Sterile syringe 1 or 2.5 ml + needle (22G)
- Refrigerated centrifuges with:
 - Swing-out rotor + adapters for 2 ml tubes
 - Fixed angle rotor for 2 ml tubes
- Protease Inhibitor Mix 200X (Diagenode, Cat. No. C12010011)

Remarks Before Starting

1. Starting material

1.1. Cells

The protocol of the ATAC-seq Kit has been optimized for the use of 50,000 cells per ATAC-seq reaction, and it is important to avoid larger cell numbers per reaction in order to achieve good tagmentation efficiency. The lowest recommended number per ATAC-seq reaction is 20,000 cells. However, we recommend starting from a larger number of cells (typically between 200,000 and 500,000 cells/sample) per cell lysis and nuclei extraction depending on the number of ATAC-seq reactions to be performed.

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

1.2. Tissues

For tissue samples we recommend using Diagenode's ATAC-seq package for tissue (8 rxns - Cat. No. C01080005 or 24 rxns - Cat. No. C01080006). The package is composed of:

8 rxns format: Tissue Nuclei Extraction for ATAC-seq, ATAC-seq kit and 8 UDI for Tagmented libraries.

24 rxns format: Tissue Nuclei Extraction for ATAC-seq, ATAC-seq kit and 24 UDI for Tagmented libraries - set I.

The Tissue nuclei extraction protocol has been optimized for 25 to 100 mg of fresh or frozen tissue per reaction to achieve a homogeneous tissue lysis and high number of pure isolated nuclei. If starting material is not limited, we recommend starting with 80-100 mg of tissue. The minimum starting amount that can be used with this protocol is 25 mg.

When weighing and cutting the tissue, always keep it in a plastic dish on dry ice to avoid degradation of samples. We recommend treating a maximum of 8 samples at one time to limit handling time and degradation of samples.

2. Lysis

2.1 Cells

The lysis time suggested in the protocol (3 min) works perfectly for a variety of cell types. However, some cells type might require an additional optimization for a proper lysis of the cell membranes, while keeping the nuclei integrity. We thus recommend testing several lysis times. The optimization protocol is described in the “Additional protocols” section. The cell lysis is optimal if :

- the viability is less than 5%
- the nuclei are intact and clearly visible under a microscope
- any visible double-membranes left.

2.2. Tissue lysis

We recommend preparing fresh buffers for tissue nuclei extraction for the number of desired reactions and to keep them at +4°C until use. These buffers cannot be stored and must be prepared daily.

During the homogenization and lysis step of tissue, we recommend using a glass dounce homogenizer with 2 types of pestles (loose and tight). If necessary, the dounce homogenizer can be used for several samples. In this case, we recommend washing it with 70% ethanol and ultra pure water between each sample to avoid contamination.

The Complete Nuclei Isolation Buffers 2 and 3 are very viscous. We recommend pipetting them slowly and smoothly to avoid the formation of air bubbles. We recommend using a syringe and needle to add them under the previous layer(s) to form a perfect three-layer gradient (see figure 2 in the Step 1b). Pay attention to not disturb the 3-layer gradient while manipulating tubes.

After centrifugation, pay attention to not disturb the 3-layer gradient and to gently pipette up the nuclei band only.

The type of rotor will impact the position of the cell pellet. For Tissue lysis and Nuclei extraction protocol, the 3-layers gradient will not be stable in a fixed angle rotor, it is why we recommend to use a swing-out rotor for this step.

3. Optimization of the number of amplification cycles

It is necessary to amplify libraries in order to have enough material for

sequencing. In order to ensure the best sequencing results, it is crucial to avoid an over-amplification to reduce PCR duplicates that will have to be discarded from the sequencing data.

The Diagenode's ATAC-seq library preparation step is divided into 3 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

When working with new type/amount of samples, we highly recommend performing the intermediate qPCR step that has been optimized to estimate the amplification rate and to avoid an over-amplification of samples. Moreover, it gives early indication if the experiment does not work (no qPCR amplification).

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 over-amplification of the samples. We therefore highly recommend following the protocol described at step 3, in order to obtain high quality libraries, with low numbers of PCR duplicates. For known sample type/amount or when the qPCR step has been optimized, this step can be skipped to make the protocol faster.

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 unique 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](https://www.diagenode.com/en/documents/primer-indexes-for-tagmented-libraries_manual)

5. Quantification

At the end of the ATAC-seq 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/adapters and a library itself.

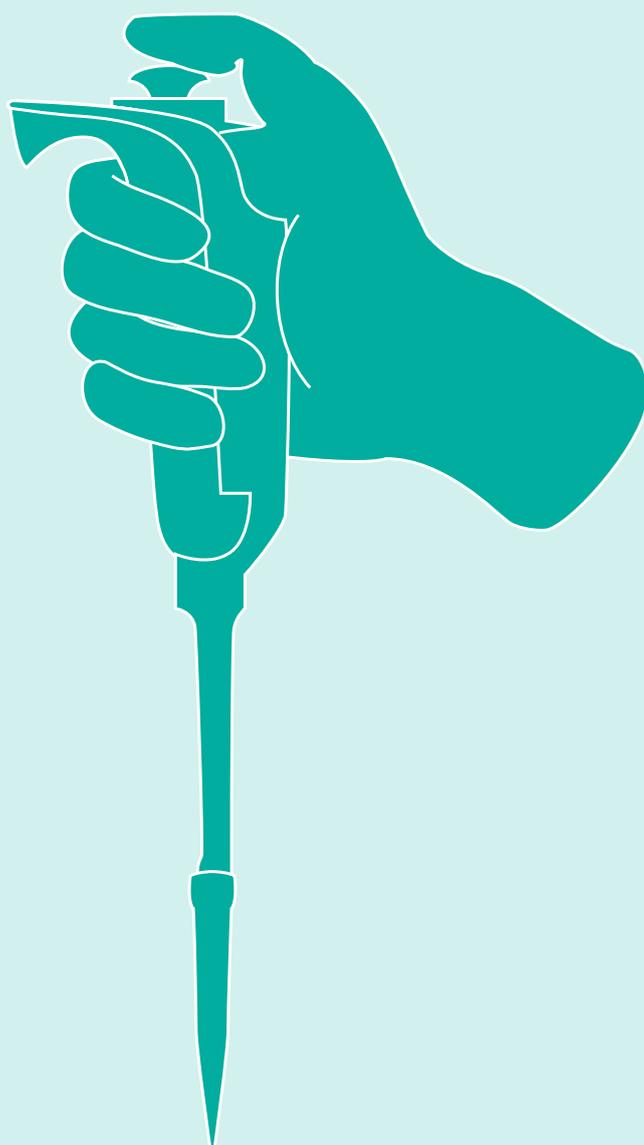
UV absorption/fluorescence detection-based methods (i.e., Nanodrop® (Thermo Scientific), Qubit®2.0 Fluorometer (Life Technologies), or QuantiT™ PicoGreen® dsDNA Assay Kit (Life Technologies) simply quantify total nucleic acid concentration. These methods do not discriminate adapter presence and offer 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.

6. Library profile

A good size selection of the library should get rid of PCR primers and adapters (peak around 150 bp) so as the final library profile shows a large distribution of fragment sizes starting from ~170 bp. A typical library size/profile is described in the section "Example of Results", Figure 5.

7. Sequencing recommendations

40M reads per sample are enough for open chromatin analysis, but 100M reads per sample are needed to study nucleosome positioning. We recommend single-end 100 bp or paired-end mode 50 bp for the sequencing.



PROTOCOL

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STEP 1a

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 1.4.

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 viable 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 rotor.
- 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 1b

Tissue Lysis and Nuclei Extraction



Buffer preparation

All volumes of buffers are given for one sample, when using several samples, prepare the buffers for desired number of reactions. Once freshly prepared, the buffers must be kept at +4°C until use.

NOTE: Additional Protease Inhibitor Mix (not provided) is required for the Tissue Lysis and Nuclei Extraction protocol.

Homogenization Buffer: Prepare **450 µl per sample** by mixing:

Reagents	Volume for 1 sample
Lysis Buffer 1	450 µl
Protease Inhibitor Mix 200X	2.25 µl

Tissue Lysis Buffer: Prepare **800 µl per sample** by mixing:

Reagents	Volume for 1 sample
Homogenization Buffer	133 µl
Lysis Buffer 2	667 µl
Protease Inhibitor Mix 200X	4 µl

Complete Nuclei Isolation Buffer 1: Prepare **400 µl per sample** by mixing:

Reagents	Volume for 1 sample
Homogenization Buffer	66.7 µl
Nuclei Isolation Buffer 1	333.3 µl

Complete Nuclei Isolation Buffer 2: Prepare **600 µl per sample** by mixing:

Reagents	Volume for 1 sample
Homogenization Buffer	100 µl
Nuclei Isolation Buffer 2	500 µl

Complete Nuclei Isolation Buffer 3: Prepare **600 µl per sample** by mixing:

Reagents	Volume for 1 sample
Homogenization Buffer	100 µl
Nuclei Isolation Buffer 3	500 µl

Tissue lysis

- 1.1 Weigh and cut on dry ice the tissue (fresh or frozen). Take **25-100 mg of tissue** per ATAC-seq reaction.
- 1.2 Using a scalpel blade, chop the tissue into small pieces on a plastic cell culture dish that is kept on dry ice.
- 1.3 Transfer the tissue pieces in a dounce homogenizer containing **800 µl of Tissue Lysis Buffer** and allow it to thaw for a **few minutes** on ice.
- 1.4 Disrupt the tissue with loose pestle first for at least 20 strokes and then the same with tight pestle to form a homogeneous suspension.
- 1.5 Filter by gravity the tissue lysis suspension two times through a 40 µm cell strainer (use a new filter each time) placed on top of a 50 ml tube. Recover the cell suspension inside the 50 ml tube but also in the bottom of the filter (filtered part) with a pipette.

NOTE: The volume of recovered suspension after two rounds of correct filtering should be around 450-500 µl. This cell suspension must be kept on ice.

Nuclei extraction

- 1.6 Take **400 µl** of the recovered suspension and put them in a 2 ml DNA LoBind tube.

- 1.7 Add **400 µl of Complete Nuclei Isolation Buffer 1** and pipette up and down 3 times.
- 1.8 Take **600 µl of Complete Nuclei Isolation Buffer 2** using a 1 ml syringe and 22G needle.
- 1.9 Slowly add it under the previous mixture. Pay attention to not mix the Complete Nuclei Isolation Buffer 2 with the previous mixture and to create a perfect second layer.
- 1.10 Take **600 µl of Complete Nuclei Isolation Buffer 3** using a new 1 ml syringe and 22G needle.
- 1.11 Slowly add it under the previous mixture. Pay attention to not mix the Complete Nuclei Isolation Buffer 3 with the previous layer of Complete Nuclei Isolation Buffer 2 and to create a perfect third layer.

NOTE: We recommend adding the Complete Isolation Buffers 2 and 3 very slowly and smoothly avoiding the formation of air bubbles under the previous layer(s) using a syringe and a needle such as described in the picture below.

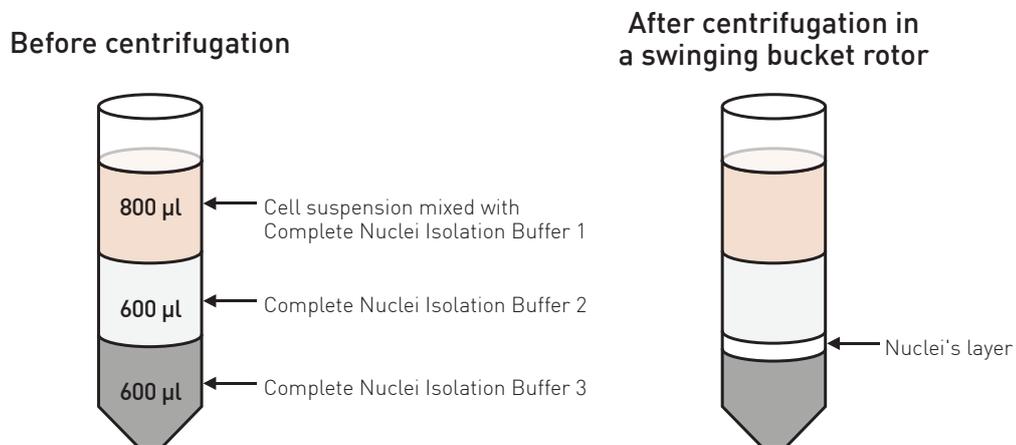


Figure 2. Schema of 3 layers in a 2 ml tube before and after centrifugation.

- 1.12 Gently put the 2 ml tubes into the cold centrifuge with a swing-out rotor.
- 1.13 Centrifuge at 3500 x g for **20 minutes** at 4°C.

NOTE: Pay attention to not disturb the gradient and to use a swing-out rotor (using adapters for 2 ml tubes) to allow a better gradients separation and the acceleration sets up at 5 and the brake at 0.

1.14 A thin “whitish” band should appear between layers 2 and 3 (from the top). Using a P200 pipette, collect this band carefully (200-400 μ l) and transfer it to a new 1.5 ml tube.

NOTE: Pay attention to not disturb the layers during the pipetting and to not take any solution excepted the white band of nuclei.

1.15 Take an aliquot of **10 μ l**, add **10 μ l of Trypan blue** and evaluate for nuclei purity and counting.

NOTE: Measure the viability of the sample. After lysis the viability should be less than 5%. Analyze the solution under a microscope to check nuclei purity. The nuclei should be clearly visible, intact and without any double-membranes left.

1.16 Distribute **50,000 nuclei** in a new 1.5 ml tube on ice, as determined by nuclei counting, using the total nuclei number.

1.17 Add **1 ml of cold ATAC Lysis Buffer 2** to each tube and mix by inverting.

1.18 Centrifuge at 1000 x g for **10 minutes** at 4°C with a fixed angle rotor.

1.19 Take out 1 ml of supernatant and then centrifuge again at 1000 x g for **5 minutes** at 4°C.

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

1.20 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 up to 10 times.

- 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 $\geq 10,000 \times g$ for 30 seconds. Discard the flow-through.
- 2.7 Add 200 µl of DNA Wash Buffer to the column. Centrifuge at $\geq 10,000 \times g$ 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 $\geq 10,000 \times g$ 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

NOTE: The Primer indexes for tagmented libraries kit (single indexes or UDIs) are required to be used in this section. If you are using the ATAC-seq package for tissue, the Primer indexes are included (UDI for tagmented libraries); If you are using the ATAC-seq kit, the Primer indexes must be purchased separately.

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

***NOTE:** When working with new type/amount of samples, we highly recommend performing the intermediate qPCR step that has been optimized to estimate the amplification rate and to avoid and over-amplification of samples. For known sample type/amount or when the qPCR step has been optimized, this step can be skipped to make the protocol faster.

a. First 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 μ l
Nuclease-free water	14 μ l

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 indexes 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 (<i>violet cap</i>)	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.

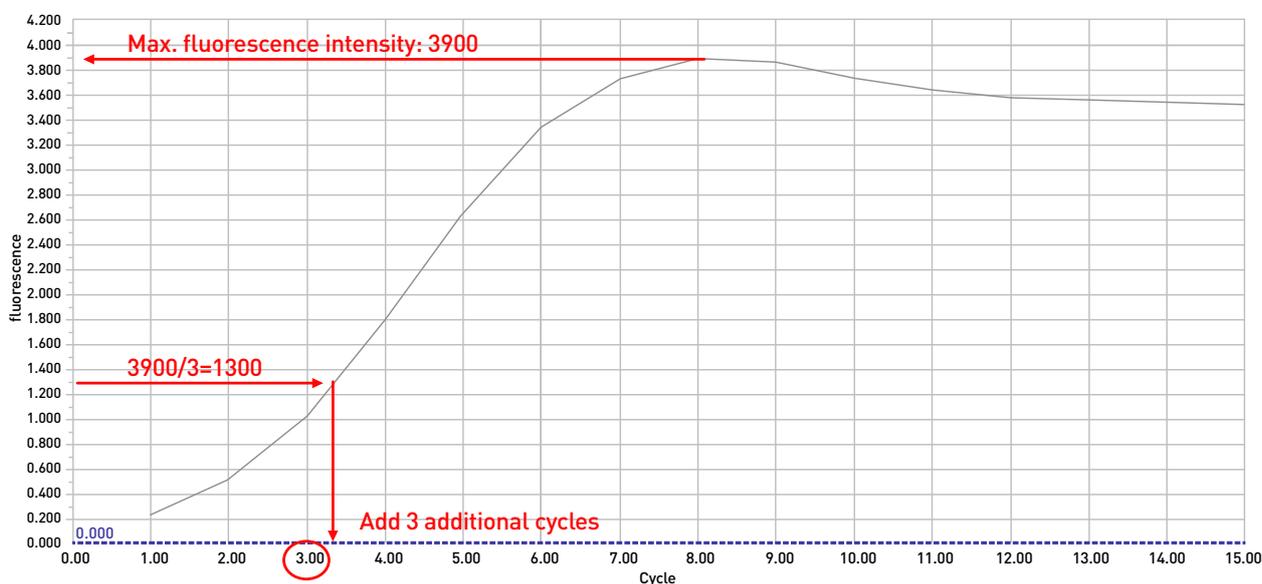


Figure 3. Example of estimation of the additional number of cycles.

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/nuclei** per reaction.

c. Second 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 μ l** 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 μ l 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



-
- 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 μ l of a 10 nM library to 5 μ l of a 20 nM library, you have 10 μ l 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.

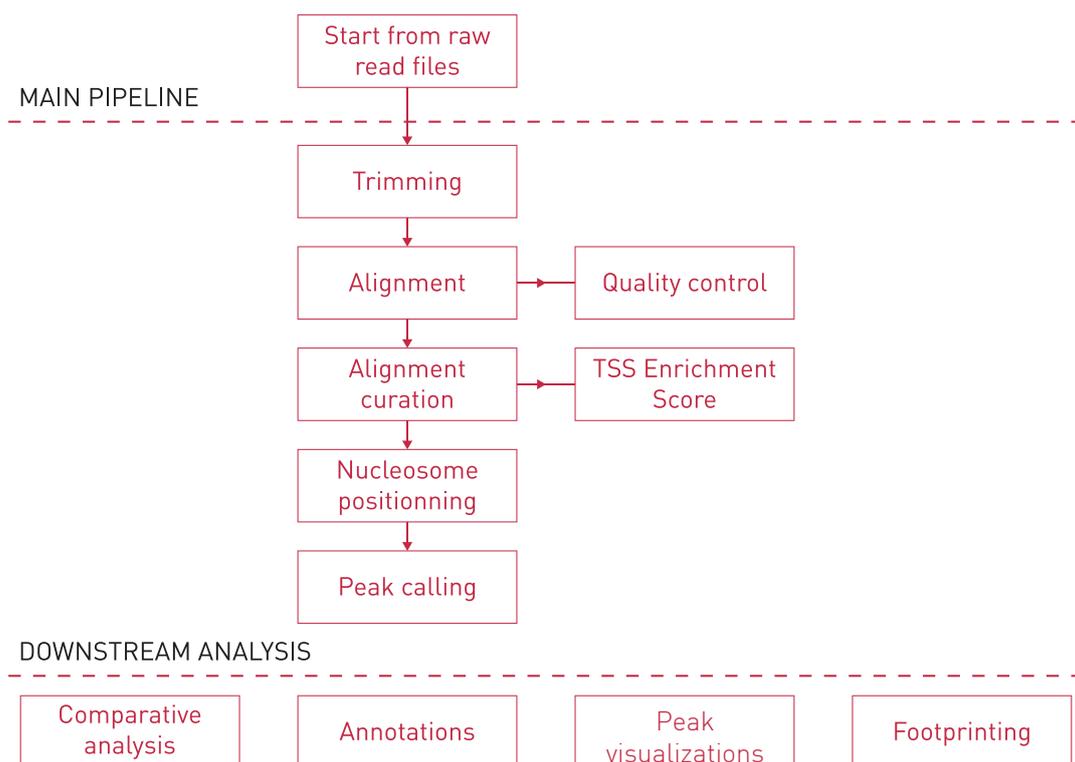


Figure 4. ATAC-seq data analysis workflow: general view

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. Trimmomatic
 - 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 removed 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 ATAC-seq 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. UCSC 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 using Fragment Analyzer (Agilent) for the size assessment.

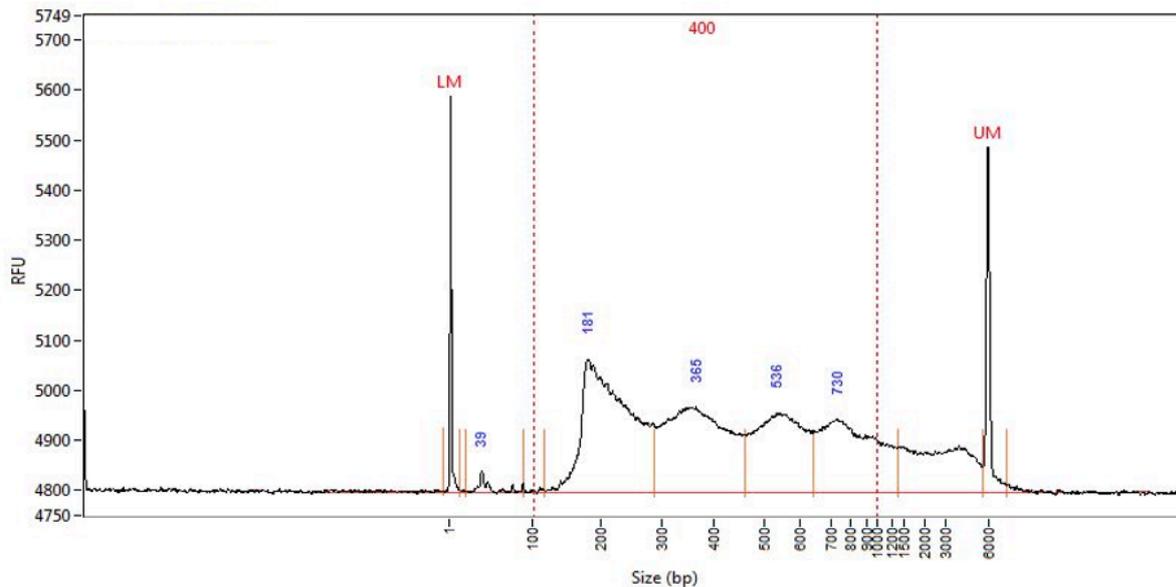


Figure 5. Typical ATAC-seq library profile.

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.

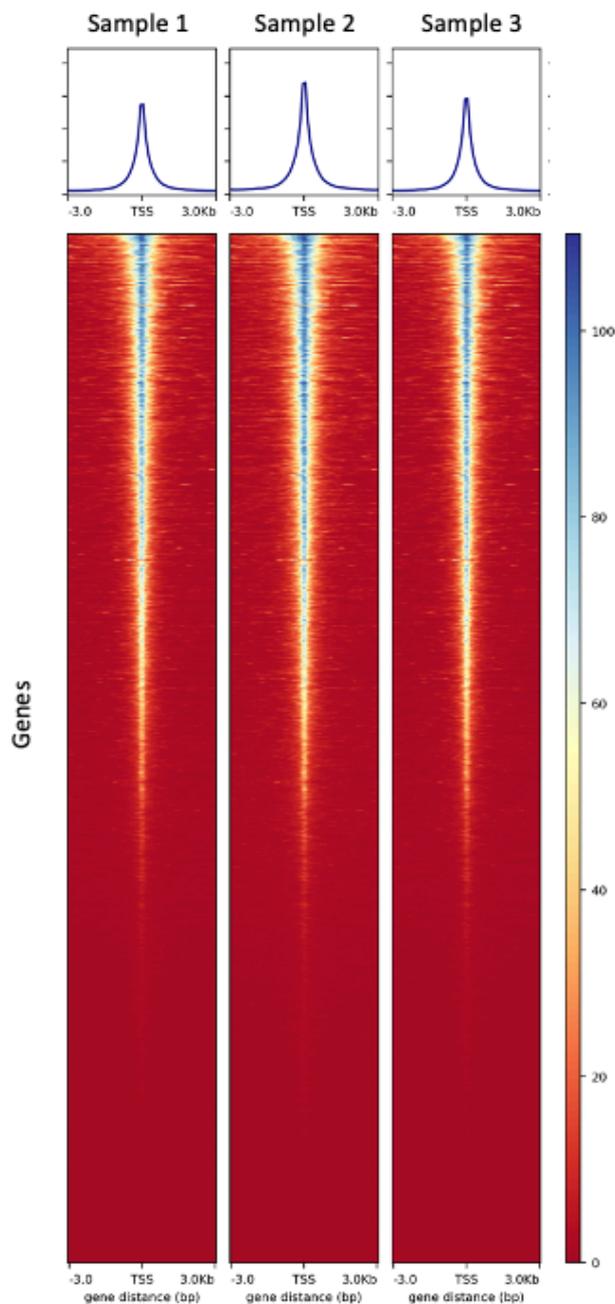


Figure 6. Enrichments at TSS of the ATAC-seq libraries

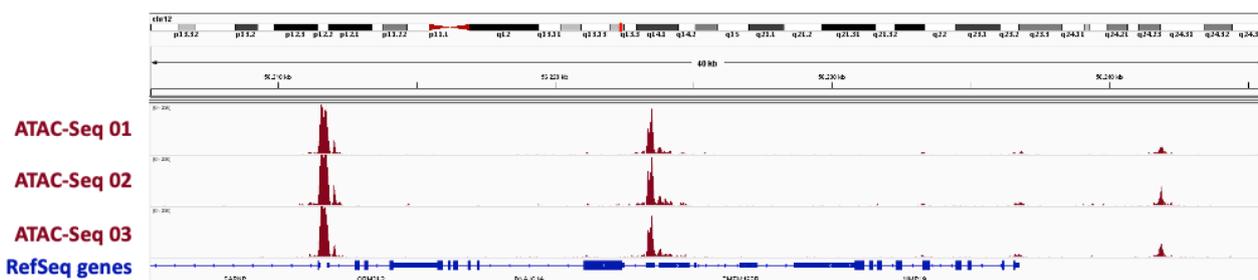


Figure 7. Sequencing profiles of the ATAC-seq libraries



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 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 μ l**.
3. Add **90 μ l 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 μ l** 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 μ l 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

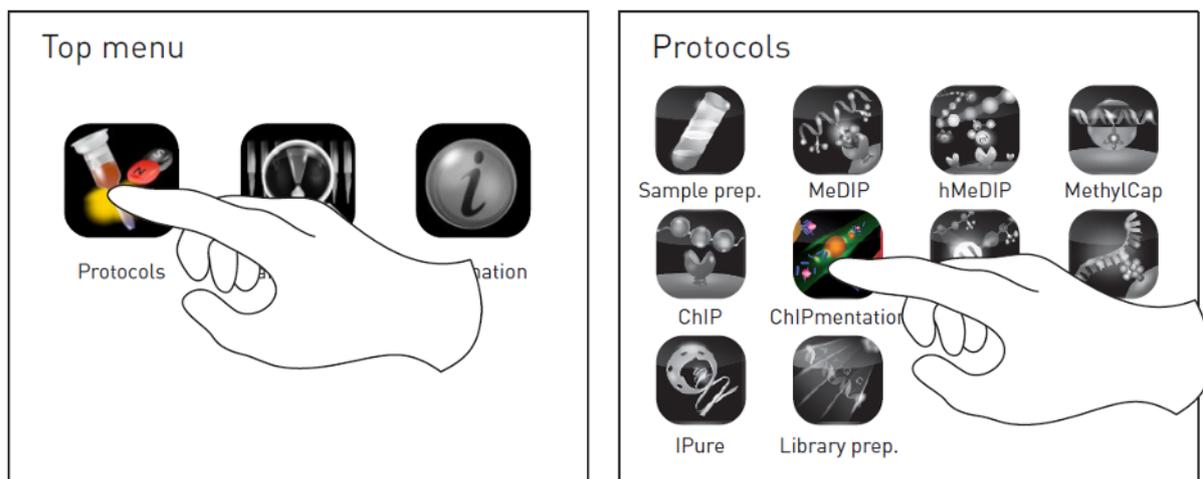
 30 min hands on time

 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**”.

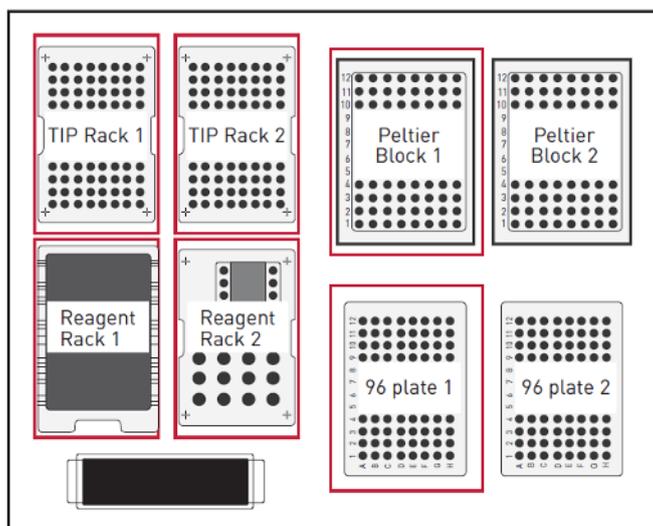


2. Select “**ATAC_size_selection_8**”.
3. Set up the exact number of samples that you want to process by pressing the black box.

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 μ l tube strips according to the screen.



5. Fill the robot with all reagents.

- Add **5 μ l of nuclease-free water** to each sample to have a final volume of **50 μ l**. Put your samples in lane 1 of the **Peltier Block**.
- Distribute **90 μ l** 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 μ l** for each sample.

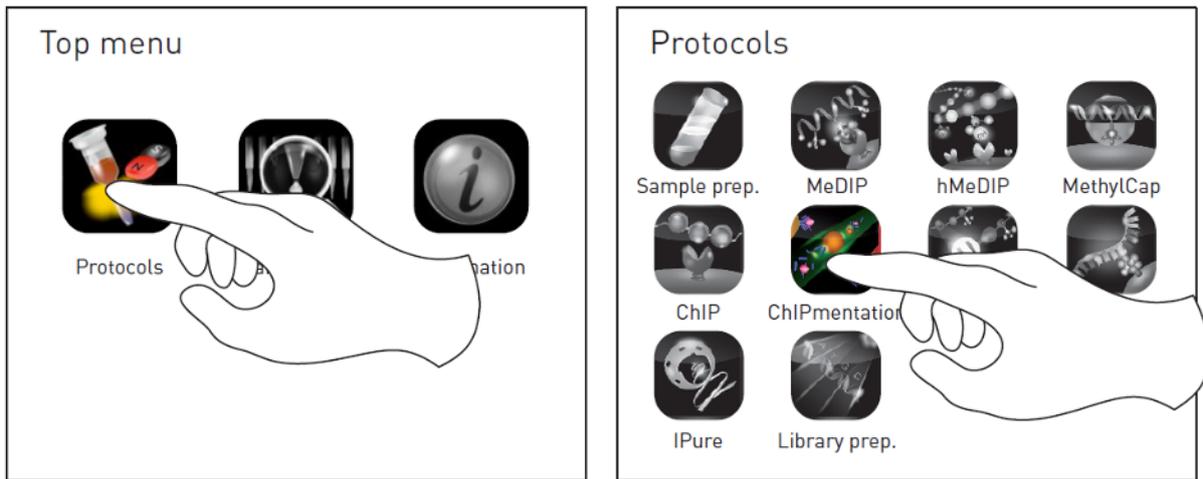
8. Press “OK” and “Back” until the homepage appears on the screen. Press “Shutdown” and wait until the screen is black before switching off the IP-Star.

***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

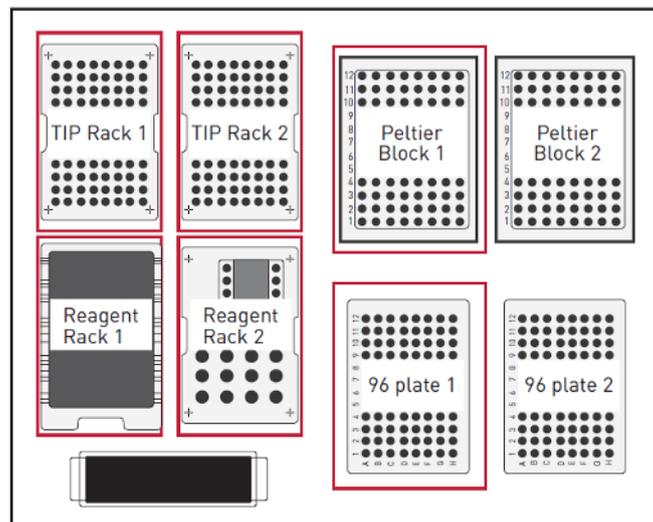
NOTE: Use the IP-Star Compact and room temperature AMPure XP beads for the clean-up.

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 μ l tube strips according to the screen.
5. Estimate the library volume and add nuclease-free water to reach **50 μ l**.
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 μ l** 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 μ l** 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.

For Tissue lysis and Nuclei extraction protocol, the 3-layer gradient will not be stable in a fixed angle rotor, it is why we recommend using a swing-out rotor for this step.

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.

What is the difference between single and dual-indexing? What is the best for ATAC-seq libraries?

With single-indexing, during the PCR amplification, only one primer will contain a barcode, whereas the second primer is universal. This means that only one side of the library will bare a barcode. This is enough to demultiplex the pool of samples after sequencing.

Nevertheless with some sequencers, (e.g. the NovaSeq6000), a phenomenon called index hopping has been observed. This index hopping introduces mistakes in the reading of the index and can lead to a misattribution of some reads, to the wrong sample. To identify those mistakes you can use Unique Dual-Indexing. In this case, the two PCR primers are adding unique barcodes to each sample, on each side of the insert. This way, if one index is wrongly associated to one sample, the second index will allow to see it. Therefore when sequencing on NovaSeq, Unique Dual-Indexing is recommended, but not mandatory.

Related Products

Product	Reference
ATAC-seq kit (8 rxns)	C01080001
ATAC-seq kit (24 rxns)	C01080002
ATAC-seq package for tissue (8 rxns)	C01080005
ATAC-seq package for tissue (24 rxns)	C01080006
Tissue Nuclei Extraction for ATAC-seq (8 rxns)	C01080003
Tissue Nuclei Extraction for ATAC-seq (24 rxns)	C01080004
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
24 UDI for tagmented libraries - Set III	C01011037
Tagmentase (Tn5 transposase) loaded	C01070012
Tagmentation Buffer (2x)	C01019043
MicroChIP DiaPure columns	C03040001
Protease Inhibitor Mix 200X	C12010011
DiaMag 0.2ml magnetic rack	B04000001
IP-Star® Compact Automated System	B03000002

Revision history

Version	Date of modification	Description of modifications
V2 11_2024	November 2024	Quick correction typo page 16
V2 01_2022	January 2022	Modification of the manual by addition of the protocol for ATAC-seq on tissue. Following it, all sections of the manual have been completed by the information related to tissue processing: Introduction, Workflow, Kit materials, Required materials not provided, Remarks before starting.

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