ACTIVE MOTIF®

Enabling Epigenetics Research

ATAC-Seq Express Kit

Catalog No. 53157

(Version A1)

Information in this manual is subject to change without notice and does not constitute a commitment on the part of Active Motif, Inc. It is supplied on an “as is” basis without any warranty of any kind, either explicit or implied. Information may be changed or updated in this manual at any time.

The manufacturer of this documentation is Active Motif, Inc.

For Research Use Only. Not for use in diagnostic procedures.

Illumina® is a registered trademark of Illumina, Inc.

Contents

Overview

The Assay for Transposase-Accessible Chromatin via Sequencing (ATAC-Seq) method was first introduced in 2013[1]. ATAC-Seq is a rapid assay of the epigenetic state by enabling identification of open chromatin regions. In the assay, intact nuclei are treated with a hyperactive Tn5 transposase mutant which is able to simultaneously tag the target DNA with sequencing adapters and fragment the DNA in a process termed "tagmentation"[2,3].

Because of the assay's speed, simplicity, sensitivity, and applicability to a wide range of sample types, ATAC-Seq has become a commonly used epigenetic assay, and can serve as a gateway to further, more detailed, epigenetic analyses. The ATAC-Seq Express Kit provides the reagents necessary to produce 16 unique sequencing-ready Illumina®-compatible ATAC-Seq libraries from 20 - 30 mg tissue or 1,000 - 100,000 cells per reaction.

ATAC-Seq Express Advantages

- Assess the epigenetic profile of open chromatin regions
- Yields next-gen sequencing-ready processed samples in hours
- Simple and rapid three-step protocol
- Silica beads for fast post-amplification sample purification

Product	Format	Catalog No.
ATAC-Seq Express Kit	16 rxns	53157

Kit Components and Storage

The kit contains sufficient reagents to produce 16 unique next-gen sequencing-ready ATAC-Seq libraries. The reagents in this kit have multiple storage temperatures. The ATAC-Seq Kit is shipped at two temperatures, with one box on dry ice for components to be stored at -20°C, and a second box at room temperature for components to be stored at 4°C. Please store components according to the storage conditions below. All reagents are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Storage
ATAC Lysis Buffer	17 mL	RT
Assembled Transposomes	170 µL	-20°C
10X PBS	500 µL	RT
2X Tagmentation Buffer	425 µL	-20°C
10% Tween 20	10 µL	RT
1.0% Digitonin	10 µL	-20°C
Q5 High-Fidelity DNA Polymerase (2U/µL)	10 µL	-20°C
5X Q5 Buffer	2 × 130 µL	-20°C
10 mM dNTPs	40 µL	-20°C
i7 Indexed Primer 1	10 µL	-20°C
i7 Indexed Primer 2	10 µL	-20°C
i7 Indexed Primer 3	10 µL	-20°C
i7 Indexed Primer 4	10 µL	-20°C
i5 Indexed Primer 1	10 µL	-20°C
i5 Indexed Primer 2	10 µL	-20°C
i5 Indexed Primer 3	10 µL	-20°C
i5 Indexed Primer 4	10 µL	-20°C
SPRI Beads	1 mL	4°C
Silica Beads	52 µL	4°C
DNA Purification Binding Buffer*	6 mL	RT
DNA Purification Wash Buffer**	10 mL	RT
DNA Purification Elution Buffer	672 µL	RT

*DNA Purification Binding Buffer must be reconstituted to a final concentration of 60% isopropyl alcohol prior to use. Add 9 mL of 100% isopropanol to the DNA Purification Binding Buffer bottle prior to use.

**DNA Purification Wash Buffer must be reconstituted to a final concentration of 80% ethanol prior to use. Add 40 mL of 100% ethanol to the DNA Purification Wash Buffer prior to use.

Additional Materials Required

- 25 mL buffer reservoir
- Thermal Shake Touch, with PCR plate block
- Microcentrifuge
- Rotator
- 0.2 mL PCR 8-tube strips
- Single-channel pipettes and appropriate tips
- Multi-channel pipettes and appropriate tips
- Magnetic plate for PCR tubes
- TapeStation and associated reagents
- 100% Ethanol
- Vortex
- Trypan Blue
- Deionized Water
- 40 µm filter (for tissue samples)
- Razor blade (for tissue samples)
- 5 cm petri dish (for tissue samples)
- 15 mL conical tubes (for tissue samples)
- Dounce homogenizer (for tissue samples)
- Thermocycler with heated lid
- 1x PBS
- 100% Isopropanol

Experimental Protocol

Experimental Design Considerations

ATAC-Seq is highly sensitive to differences in chromatin state, cell type, and sample quality. 2-3 biological replicates per condition is strongly recommended. Replicates improve the reliability, reproducibility, and statistical power of your results.

The kit has been optimized for 1,000-100,000 cells for most sample types; however, variation between sample types is possible. The assembled transposome (Tn5)-to-cell ratio has the greatest impact on the degree of tagmentation and the resulting shape of the amplified library (see FAQ for more details). Therefore, when working with a new sample type, it is recommended to perform an initial trial by titrating either the number of cells or the amount of Tn5 added to the Tagmentation Master Mix.

Example:

Keeping Assembled Transposomes amount (10 µL) the same:

Sample 1: 50k cells
Sample 2: 100k cells
Sample 3: 200k cells

Keeping cell number (50k cells) the same:

Sample 1: 10 µL
Sample 2: 5 µL
Sample 3: 2 µL

Note: Add additional H_2O to keep total Tagmentation Master Mix volume 25 µL

When working with primary cells or tissue, it is recommended to include an easy-to-use immortalized cell line alongside your experimental sample as a procedural control. One or two reactions are sufficient. This control helps account for any sample-type-specific variation that may occur. While sequencing is not required for the control, the amplified library should be visualized on a DNA fragment analyzer alongside your experimental samples. If the control yields a library, but the experimental samples do not, this indicates that the protocol was executed properly, and that the sample preparation likely requires optimization.

Prepare Buffers

Prepare DNA Purification **Binding Buffer**: add 9 mL 100% isopropanol to the DNA Purification **Binding Buffer** bottle to a final concentration of 60% isopropanol.

Prepare DNA Purification **Wash Buffer**: add 40 mL of 100% ethanol to the DNA Purification **Wash Buffer** bottle, the final concentration of ethanol is 80%

Tissue Sample Preparation

This protocol is designed for 20 to 30 mg of tissue per reaction. Fresh tissue can be used or tissue that has been flash frozen at -80°C.

If working with frozen tissue samples that are fibrous an alternate protocol for nuclei isolation is provided in **Appendix Section C**.

Take SPRI beads out of 4°C and warm to room temperature by simply placing the vial of SPRI beads on the bench top.

1. For each sample, label a 5 cm petri dish and place on wet ice, along with a labeled 15 mL conical tube containing 5 mL ice-cold PBS.
2. Transfer each sample to its corresponding dish, mince with a razor blade, and transfer to the corresponding 15 mL conical tube containing ice-cold PBS using a 1 mL pipette tip (the tip can be cut to widen the bore in order to avoid clogging the tip).
3. Centrifuge the 15 mL conical tubes at 500 × *g* for 5 minutes at 4°C.
4. Aspirate PBS from centrifuged tube, and add 1 mL ATAC Lysis Buffer.
5. Using a 1 mL pipette tip with a widened bore, transfer each sample to a 1 mL dounce homogenizer and dounce slowly for 30 strokes using a tight-fitting pestle (for example, small-clearance, type B).
6. Filter each homogenized sample through a 40 µm mesh strainer and collect in a fresh 1.5 mL microcentrifuge tube. Immediately after filtration, take a 10 µL aliquot for cell counting.
7. Count the nuclei in each aliquot using Trypan Blue cell-viability staining (stain in a 1:1 ratio of sample volume:0.4% Trypan Blue). Only nuclei stained blue by Trypan Blue should be counted.
8. Invert samples gently to mix, then aliquot 1,000 - 100,000 nuclei into a new tube.
9. Centrifuge the new nuclei aliquots at 500 × *g* at 4°C for 5 minutes. During this time, prepare the Tagmentation Master Mix, see table in **Tagmentation Reaction and Purification**.
10. Aspirate supernatant and proceed immediately to the Tagmentation Reaction and Purification steps.
11. After the spin, remove the supernatant very carefully, ensuring the cell pellet is not disturbed.

    This is a critical step; take extra care to remove the ATAC Lysis Buffer but not lose nuclei

    Leave behind 5-10 µL of the volume to ensure the pellet does not get accidentally become aspirated. Immediately continue to the Tagmentation Reaction and Purification steps.

Cell Sample Preparation

1,000 to 100,000 fresh cells or cryopreserved frozen cells can be used. Cells must be of high quality to preserve viability. Cryopreserved cells should be cryopreserved in a controlled rate freeze with media formulated to protect against the ice crystal formation and subsequent cell damage.

If working with adherent cells that are difficult to detach or detachment methods that decrease cell viability substantially an alternate protocol for in-well lysis is provided in **Appendix Section B**.

Take SPRI and Silica Beads out of 4°C and warm to room temperature by simply placing the vial of beads on the bench top.

1. Count cells and aliquot 1,000 to 100,000 cells into a fresh 0.2 mL PCR 8-tube strip tube on ice.

Note: If using ATAC-Seq Spike-In Control Nuclei (Catalog No. 53154), add the recommended number to each sample in this step.

2. Centrifuge the cells at 500 × *g* for 5 minutes at 4°C. If there is no visible pellet, spin an addition 1000 × *g* for 5 minutes at 4°C. For stability when centrifuging, place the PCR tubes into a 96-well plate and spin the plate in a swing bucket centrifuge.
3. If processing 4 or more samples at once, put two reservoirs, one for the 1x PBS and one for the ATAC Lysis Buffer on ice. Add 2 mL of each buffer to its respective reservoir. When adding buffer to samples, use a multichannel pipette.
4. Once the cells are finished spinning, gently remove supernatant without disturbing or removing the pellet. For best results, use a single channel pipette to remove supernatant and a multichannel to add buffers. To ensure the pellet is not accidentally removed, leave behind ~10 µL of liquid.
5. Add 100 µL of ice-cold PBS. Do not resuspend or disturb the pellet. Spin once more at 500 × *g* for 5 minutes at 4°C.
6. Remove the supernatant very carefully, ensuring to not disturb the cell pellet. For best results, use a single channel pipette to remove supernatant and a multichannel to add buffers.
7. Add 100 µL of ice-cold ATAC Lysis buffer and gently resuspend the cell pellet.

Note: Lysis should occur rapidly for most cell types. To confirm successful lysis, stain an aliquot with Trypan Blue and observe the sample under a microscope. If lysis is complete, all cells should appear blue, indicating membrane permeability.

8. Centrifuge at 500 × *g* for 10 minutes at 4°C. During this time, prepare the Tagmentation Master Mix, see table in **Tagmentation Reaction and Purification**.
9. Aspirate supernatant and proceed immediately to the Tagmentation Reaction and Purification steps.

10. After the spin, remove the supernatant very carefully, ensuring the cell pellet is not disturbed.

    ⚠ This is a critical step; take extra care to remove the ATAC Lysis Buffer but not lose nuclei

    Leave behind 5-10 µL of the volume to ensure the pellet does not get accidentally become aspirated. Immediately continue to the Tagmentation Reaction and Purification steps.

Tagmentation Reaction and Purification

Tagmentation Master Mix

Reagents	Volume
2X Tagmentation Buffer	12.5 µL
10X PBS	1 µL
1.0% Digitonin	0.25 µL
10% Tween 20	0.25 µL
H_2O	1 µL
Assembled Transposomes	10 µL
Total Volume	**25 µL**

Note: If optimizing Assembled Transposomes volume, adjust the volume of H_2O so the total volume remains 25 µL.

1. Add 25 µL of Tagmentation Master Mix to each sample, sample does not have to be on ice. Gently pipette to mix or vortex to resuspend nuclei in the Tagmentation Master Mix.
2. Incubate the tagmentation reaction at 37°C for 30 minutes in a thermomixer (or similar device) set at 800 rpm.
3. If processing 4 or more samples at once, add 2.2 mL of prepared DNA Purification **Binding Buffer** to a reservoir at room temperature.
4. After the incubation, add 125 µL (5 times the volume of your tagmentation reaction) of the prepared DNA Purification **Binding Buffer** to each sample.
5. Gently vortex the silica beads and briefly spin down to collect them at the bottom of the tube. Add 3 µL of beads to each reaction, wiping away any excess from the outside of the pipette tip. Vortex each reaction briefly to mix, then incubate on ice for 5 minutes.
6. Put the tubes on a magnet and leave until the beads have completely pelleted (~2-5 minutes).
7. If processing 4 or more samples, add 500 µL of prepared DNA Purification **Wash Buffer** per sample to a reservoir.

8. Using a multichannel pipette, carefully remove and discard the supernatant without disturbing the bead pellet.
9. While still on the magnet, add 200 µL of DNA Purification **Wash Buffer with EtOH added**. Remove the strip from the magnet and gently pipette up and down to mix.
10. Place back on the magnet and incubate for 30 seconds. After 30 seconds remove the supernatant and repeat wash for a total of two washes.
11. With the tube still on the magnet, let the residual wash buffer evaporate for about 2 minutes or until the beads are no longer shiny. If there is excess wash buffer remaining, quickly spin down the tubes, re-magnetize and remove any of the residual wash buffer. Over drying the beads can cause them to crack, reducing the overall yield.
12. Resuspend the beads in 35 µL of DNA Purification **Elution Buffer**. Gently pipette up and down, mixing well, and allow the beads to sit at RT for 5 minutes.
13. Magnetize the reaction, allow the beads to completely pellet and transfer the supernatant into a new, labeled 0.2 mL PCR tube. If needed, the samples can be temporarily stored at -20°C but it is recommended to proceed with PCR amplification.

Measuring DNA concentration before PCR amplification does not provide a reliable QC for assay success. This measurement only reflects the total amount of genomic DNA recovered, not whether tagmentation was successful. If chromatin has been properly tagmented with Nextera adapters, PCR amplification is required to generate detectable library fragments and confirm assay performance.

PCR Amplification

If a kit such as the KAPA Real-Time Library Amplification Kit is used, an initial 72°C extension step is essential.

1. Set up the PCR reactions by adding the components in the order shown below. If libraries are to be multiplexed for sequencing on the same flow cell, ensure that a unique i5 and/or i7 index combination is used for each.

   Each sample will require a combination of one i7 Indexed Primer and one i5 Indexed Primer in the PCR Amplification. There are 4 × 4 = 16 unique combinations of i7/i5 primers for a total of 16 samples that can be multiplexed. These Indexed Primers are based on Illumina's Nextera adapters.

i7 Indexed Primer	i5 Indexed Primer
i7 Indexed Primer 1 = i7 N701	i5 Indexed Primer 1 = i5 N501
i7 Indexed Primer 2 = i7 N702	i5 Indexed Primer 2 = i5 N502
i7 Indexed Primer 3 = i7 N703	i5 Indexed Primer 3 = i5 N503
i7 Indexed Primer 4 = i7 N704	i5 Indexed Primer 4 = i5 N504

It is important that a unique index primer combination is used for each sample for sample identification after demultiplexing.

Example combinations:

	N501	N502	N503	N504
N701	N701 + N501	N701 + N502	N701 + N503	N701 + N504
N702	N702 + N501	N702 + N502	N702 + N503	N702 + N504
N703	N703 + N501	N703 + N502	N703 + N503	N703 + N504
N704	N704 + N501	N704 + N502	N704 + N503	N704 + N504

Reagents	Volume
Tagmented DNA	33.5 µL
i7 Indexed Primer (25 µM)	2.5 µL
i5 Indexed Primer (25 µM)	2.5 µL
dNTPs (10 mM)	1 µL
5X Q5 Reaction Buffer	10 µL
Q5 Polymerase (2 U/µL)	0.5 µL
Total	**50 µL**

If multiple samples are being processed simultaneously, a master mix containing dNTPs, 5X Q5 Reaction Buffer, and Q5 Polymerase can be made.

2. Perform PCR using the following program on a thermal cycler (with a heated lid):

72°C for 5 minutes	
98°C for 30 seconds	
98°C for 10 seconds 63°C for 30 seconds 72°C for 1 minute	**10 cycles**
Hold for 10°C	

Bead Clean Up

1. Add 60 µL (1.2x) well-mixed, room temperature SPRI Beads to each sample.
2. Vortex briefly to mix and incubate for 5 minutes at room temperature to allow beads to bind.
3. Apply magnet to collect beads.
4. Once the solution is clear, aspirate the supernatant.
5. With the magnet still applied to the sample, add 180 µL 80% ethanol to each sample without mixing.
6. Incubate for 30 seconds at room temperature.
7. Aspirate the supernatant.
8. Repeat **steps 5-7** for a second ethanol wash.
9. Allow tubes to sit at room temperature so that residual ethanol can evaporate. Once the beads transition from shiny to matte (2-5 minutes), proceed to the next step.
10. With the tubes separated from the magnet, add 20 µL DNA Purification **Elution Buffer**.
11. Cap tubes and vortex to mix.
12. Incubate samples for 5 minutes at room temperature.
13. Apply magnet to collect beads.
14. Once the solution is clear, transfer each supernatant containing the eluted DNA to a fresh tube.

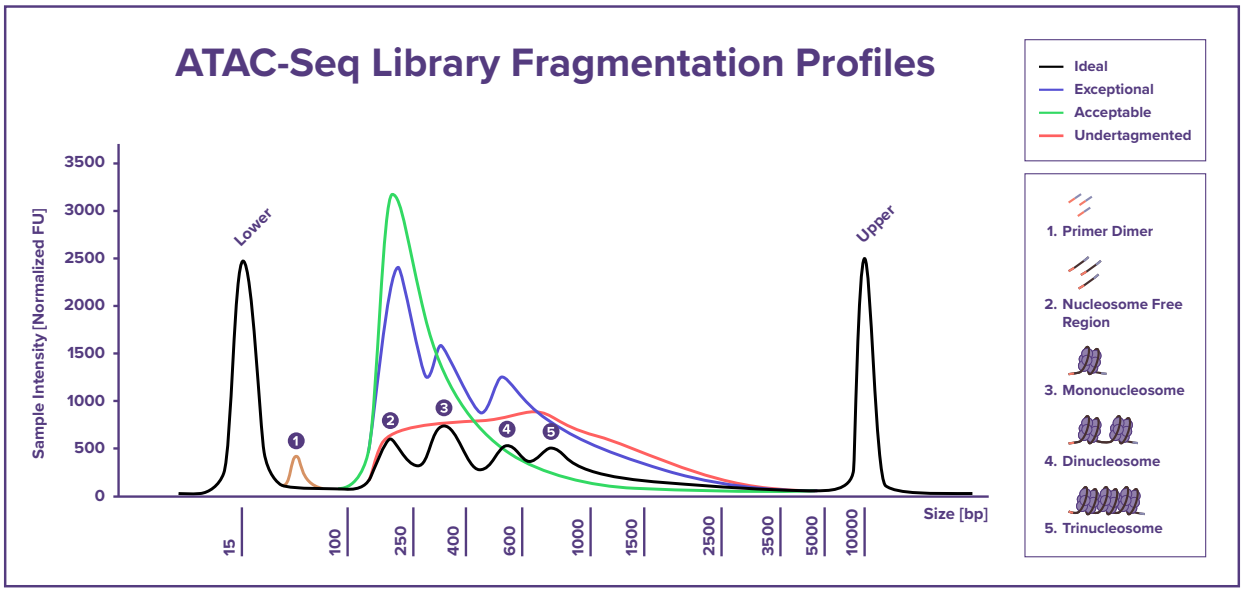
At this stage, libraries are ready for quantification and sequencing. Use a library quantification kit for next-generation sequencing to quantify the library (*e.g.* Kapa Biosystems, Catalog No. KR0405). PCR amplified libraries can also be analyzed to assess size distribution with a Bioanalyzer, TapeStation, or similar instrument to assess size distribution.

Appendix

Section A. Library Quality Control

After library amplification and SPRI bead clean-up it is recommended to measure the library concentration via Qubit or KAPA qPCR. It is also recommended to assess DNA fragment size distribution with a Bioanalyzer, TapeStation, or similar instrument using High Sensitivity D1000/D5000 or similar tape. A final concentration between 1-30 ng/µL is expected.

Please see the example image below to assess your libraries fragmentation pattern:



Tagmentation is the process by which Tn5 transposase fragments DNA and inserts adapters at regions of open chromatin. The extent of tagmentation directly influences fragment size distribution and overall sequencing quality. The two most critical factors impacting tagmentation efficiency are the Tn5-to-cell ratio and the quality of the input cells.

A high-quality library typically shows 1-3 peaks between 200-600 bp, with fragment sizes tapering off above 1,000 bp. This profile is generally suitable for sequencing.

- If excessive primer dimers (>5%) are present, they should be removed with a second SPRI bead cleanup.
- If there is an abundance of fragments >1,000 bp, it may indicate under-tagmentation or PCR artifacts.
- If sufficient DNA is present within the desired range, the library can still be sequenced, as larger fragments typically do not cluster efficiently on the flow cell. However, an overabundance of large fragments can skew the pool and reduce the proportion of usable fragments.

If needed, a right-side size selection can be performed to remove large fragments. Use caution, as this step may also lead to the loss of some desired material. A 0.5x SPRI bead ratio is recommended for right-side selection when moving forward.

Dead or dying cells can significantly compromise ATAC-Seq data quality by disrupting chromatin structure and nuclear integrity. These cells often allow non-specific access to Tn5 transposase, leading to over-tagmentation, high background noise, and an excess of short DNA fragments. In addition, damaged or lysed cells release mitochondrial DNA, which can dominate sequencing reads and reduce usable data. Chromatin decondensation in dying cells may also generate false signals of accessibility. To minimize these effects, it is critical to start with a high-viability cell population, avoid harsh handling, and consider dead cell removal prior to the assay.

ATAC Library Quick Tips:

- Aim for 1-3 peaks between 200-600bp. Fragments should be trailing off after 800bp.
- A final library concentration of 20-30 ng/µL is achievable; 1 ng/µL or greater is acceptable.
- Excess primers dimers (>5%) should be removed using a second SPRI bead clean-up
- Large fragments (>600bp) in undertagmented libraries can be removed using a right-side SPRI bead selection if sufficient library is present in the 200-600bp range.
- After cell quality, the Tn5:cell number ratio has the largest impact on the fragmentation pattern. This may need to be optimized for your sample type.
- Learn more about tagmentation based library QC at: activemotif.com/blog-library-qc

If you would like assistance assesing your library, please send you fragmentation profile report to tech_service@activemotif.com

Section B. Alternate Protocol for In-Well Lysis

This protocol is ideal for use with adherent cells that are sensitive to detachment methods, which may negatively impact cell viability. It can also be adapted as a high-throughput approach for processing multiple samples in parallel.

Adherent Cell Protocol (96-well Format)

1. Grow cells to confluence in a 96-well tissue culture plate.

Approximately 40,000–50,000 cells per well are expected, but this is cell type dependent.

2. Aspirate the media, then wash each well with 200 µL of PBS.
3. Add 100 µL of ice-cold ATAC Lysis Buffer to each well.

Do not disrupt the cells by pipetting.

Incubate for 10 minutes on ice.

4. Carefully discard the supernatant.
5. Add 25 µL of prepared Tagmentation Master Mix to each well. Gently pipette to mix, and then transfer to a PCR strip tube or PCR plate.
6. Proceed to **Step 11** of the main ATAC-Seq protocol.

Note: The tagmentation reaction can be performed in the original 96-well TC plate if using a thermomixer with a 96-well flat bottom plate adapter.

Section C. Alternate Protocol for Nuclei Isolation from Frozen Tissue

This nuclei isolation method is useful when working with frozen tissue samples that are fibrous, such as bone, muscle, or insect exoskeleton. In these cases, the douncing step is not necessary.

Nuclei Isolation from Frozen Tissue (Mortar and Pestle)

1. Pre-chill a mortar and pestle in a bed of dry ice. Add liquid nitrogen as needed to keep the tissue frozen throughout the process.
2. Place the frozen tissue in the mortar and grind it into a fine powder, removing any large chunks.

Caution: Sample loss can occur during the transfer of powdered tissue. To minimize loss, you may start with a conical tube with a wide opening, then transfer to a pre-chilled 1.5 mL tube after the 10-minute incubation in lysis buffer.

Transfer the powdered tissue into a pre-chilled 1.5 mL tube using a cold spatula.

Tip: Work quickly to prevent thawing.

3. Add 1 mL of ATAC Lysis Buffer, resuspend thoroughly, and incubate on ice for 10 minutes.
4. Pass the suspension through a 40 µm cell strainer to remove debris.
5. Centrifuge at 500 × *g* for 5 minutes at 4°C.
6. Carefully discard the supernatant.
7. Resuspend the pellet in 500 µL of cold PBS to wash.
8. Take a small aliquot and count nuclei using AOPI or Trypan Blue.
9. Use 50,000-100,000 nuclei per reaction.
10. Proceed to the tagmentation step (refer to **step 9** above).

Section D. Index Primers and Sample Sheet Information

Index 1 (i7) Primers

CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG

Index 2 (i5) Primers

AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC

The sample sheet's index sequences are used for demultiplexing and correctly identifying pooled samples. Note that some sequencing platforms require the reverse complement of the index for demultiplexing. Please refer to the table below:

i7 Index	i7 Sequence	Sample Sheet
N701	TCGCCTTA	TAAGGCGA
N702	CTAGTACG	CGTACTAG
N703	TTCTGCCT	AGGCAGAA
N704	GCTCAGGA	TCCTGAGC

i5 Index	i5 Sequence	Sample Sheet (NovaSeq v1.0 Reagent Kits, MiSeq, HiSeq 2000/2500)
N501	TAGATCGC	TAGATCGC
N502	CTCTCTAT	CTCTCTAT
N503	TATCCTCT	TATCCTCT
N504	AGAGTAGA	AGAGTAGA

i5 Index	i5 Sequence	Sample Sheet (NovaSeq v1.5 Reagent Kits, iSeq, MiniSeq, NextSeq, HiSeq 3000/4000)
N501	TAGATCGC	GCGATCTA
N502	CTCTCTAT	ATAGAGAG
N503	TATCCTCT	AGAGGATA
N504	AGAGTAGA	TCTACTCT

Sequence for Read 1 and Read 2 adapter trimming: CTGTCTCTTATACACATCT.

Frequently Asked Questions

Question	Answer
What is the lowest number of cells that I can use?	For optimal data quality, at least 50,000 cells are recommended. However, we have successfully generated usable data from as few as 1,000 cells. The minimum number of cells required will depend on the sample type. When using low input amounts, expect to see a higher duplication rate and fewer detectable peaks.
I don't have a thermomixer. How can I perform the tagmentation reaction?	A PCR machine set to 37°C with the lid set to lowest temperature can be used.
Can I increase the PCR cycle number to increase my library yield?	We have not found that increasing PCR cycles improves data quality. It is more important to optimize the tagmentation reaction conditions and sample quality.
What is the expected percentages of mitochondrial reads?	Mitochondrial reads typically account for ~20% of total reads in most ATAC-Seq experiments. High levels of mitochondrial reads can consume a large portion of sequencing depth and reduce data quality. If mitochondrial reads exceed 50%, it may indicate poor sample quality or cell membrane integrity issues. Cells with high energy demand (*e.g.*, liver, muscle, or cancer cells) naturally contain more mitochondria and may yield 30-40% mitochondrial reads. Digitonin is included in the buffers to help reduce mitochondrial contamination by more selectively permeabilizing the plasma membrane.
I want to pool more than 16 samples. Does Active Motif offer more indexing primers?	Yes, please find additional primers here: activemotif.com/catalog/1357/nextera-compatible-multiplex-primers
What length do you recommend sequencing and how many reads?	PE38 minimum. PE42 or PE50 are also commonly used. **Example PE38:** Read 1: 38 Index 1: i7 Index: 8 Index 2: i5 Index: 8 Read 2: 38 30 million reads are recommended. 20 million reads is the minimum for meaningful data in most cases. For sophisticated analysis like foot printing or differential analysis more reads are necessary.
What is the recommended pooling concentration?	Pooling is typically done at an equimolar concentration of 0.5-4 nM. The exact concentration may vary depending on the sequencing platform and protocol. Always follow the manufacturer's guidelines for accurate loading.
Can I purchase more lysis buffer separately for optimization?	Yes. We offer the ATAC-Seq Buffer set (Catalog No. 53154) which contains lysis buffer, tagmentation buffer, and digitonin.

Question	Answer
Can Active Motif sequence my library?	Yes, we can! Please e-mail sales@activemotif.com and you will be connected with our services team.
Can Active Motif analyze my data?	Yes, we can! Please e-mail sales@activemotif.com and you will be connected with our services team.

References

1. Buenrostro, J. D., *et al.* (2013) *Nat. Methods* 10: 1213-1218.
2. Adley, A., *et al.* (2010) *Genome Biol.* 11.
3. Corces, M. R., *et al.* (2017) *Nat. Methods* 14: 959-962.

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

North America	Toll free:	877 222 9543
	Direct:	760 431 1263
	Fax:	760 431 1351
	E-mail:	tech_service@activemotif.com
Europe	Direct:	+32 (0)2 653 0001
	Fax:	+32 (0)2 653 0050
	E-mail:	eurotech@activemotif.com
Japan	Direct:	+81 (0)3 5225 3638
	Fax:	+81 (0)3 5261 8733
	E-mail:	japantech@activemotif.com
China	Direct:	(86)-21-20926090
	Cell Phone:	18521362870
	E-mail:	techchina@activemotif.com