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Enabling Epigenetics Research

High-Throughput ATAC-Seq Express Kit

Catalog No. 53158

(Version A2)

Revision	Date	Description of Change
A2	May, 2026	Updated storage temperature and quantity of ATAC Lysis Buffer

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 96 unique sequencing-ready Illumina®-compatible ATAC-Seq libraries from 50,000 - 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.
High-Throughput ATAC-Seq Express Kit	96 rxns	53158

Kit Components and Storage

The kit contains sufficient reagents to produce 96 unique next-gen sequencing-ready ATAC-Seq libraries. The reagents in this kit have multiple storage temperatures. The High-Throughput ATAC-Seq Express Kit is shipped at two temperatures, with two boxes on dry ice for components to be stored at -20°C, and a third 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
Pre-indexed Assembled Tn5 Transposomes	1 x 96 rxns	-20°C
P5 Primer (25 µM)	250 µL	-20°C
P7 Primer (25 µM)	250 µL	-20°C
ATAC Lysis Buffer	6 x 16 mL	RT or -20°C
2X Tagmentation Buffer	2 x 1.5 mL	-20°C
1% Digitonin	2 x 25 µL	-20°C
5X Q5 Reaction Buffer	960 µL	-20°C
dNTP Mix (10 mM each)	96 µL	-20°C
Q5 DNA Polymerase	48 µL	-20°C
SPRI Beads	5.76 mL	4°C
Silica Beads	288 µL	4°C
DNA Purification Binding Buffer*	4.8 mL	RT
DNA Purification Wash Buffer**	7.68 mL	RT
DNA Purification Elution Buffer	5.28 mL	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
- Thermomixer
- 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
- 10% Tween 20
- 10X PBS
- 100% Isopropanol

Note: The Pre-indexed Assembled Tn5 Transposomes plate is designed for user convenience. It can be used with as few as 8 wells (1 tear-away column) and up to 96 wells (12 tear-away columns). If only a portion of the plate is used, the remaining sections of the plate should be stored at -20°C for future use

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 50,000-100,000 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 the number of cells.

When working with primary cells 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 1X PBS from a 10X PBS stock and chill on ice. 5 mL per sample is required if processing tissue, 100 µL per sample is required for cells. If preparing for cells, make a minimum of 2 mL.

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 50,000 - 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.

    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. This protocol has been optimized for a range of cells, for best results we reccomend 50,000-100,00 cells per well. 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 50,000 - 100,000 cells into a fresh 0.2 mL PCR 8-tube strip tube or a 96-well plate on ice.
2. Centrifuge the cells at 500 x *g* for 5 minutes at 4˚C. If using PCR tubes, orient the tubes such that the pellet will be where you expect it to be. If there is no visible pellet, spin an addition 1000 x *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. Put two reservoirs, one for the 1X PBS and one for the **ATAC Lysis Buffer** on ice. Add 100 µL per sample of each buffer to its respective reservoir. If processing less than 20 samples, add 2 mL of buffer.
4. Once the cells are finished spinning, gently remove supernatant without disturbing or removing the pellet.

    To ensure the pellet is not accidentally removed, leave behind ~10 µL of liquid.

5. Using a multichannel pipette, add 100 µL of ice-cold PBS to each sample. Do not resuspend or disturb the pellet. Spin once more at 500 x *g* for 5 minutes at 4˚C.
6. Remove the supernatant very carefully, ensuring to not disturb the cell pellet.
7. Using a multichannel pipette, add 100 µL of ice-cold **ATAC Lysis Buffer** and gently resuspend the cell pellet.
8. Centrifuge at 500 x *g* for 10 minutes at 4˚C. During this time, prepare the Tagmentation Master Mix. The Tagmentation Master Mix can be aliquoted into PCR tubes for compatibility with multichannel pipettes.

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	7 µL
Total Volume	**21 µL**

1. 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.
2. Retrieve the **Pre-indexed Assembled Tn5 Transposomes** plate from -20°C storage. Tear off as many strips as required for the experiment. Return any unused wells to -20°C storage. Quick spin the wells to be used to collect all of the Tn5 at the bottom of the well.
3. Add 21 µL of Tagmentation Master Mix to each sample. Gently pipette mix or vortex the pellet nuclei to ensure the nuclei has been properly resuspended.
4. Carefully remove the caps from the **Pre-indexed Assembled Tn5 Transposomes** plate. When cold the lids are delicate and may snap. If this occurs, they can be replaced with standard 0.2 mL PCR tube caps. To decrease likelihood of the caps snapping, warm the top briefly by laying your palm on it or letting it sit on the bench top for 1-2 minutes.
5. With a multichannel, transfer the 21 µL of Tagmentation Master Mix and resuspended pellet to the **Pre-indexed Assembled Tn5 Transposomes** plate. Make sure to note the well that each sample was transferred to. Each well contains a unique index combination that will be used to identify the sample.
6. Reseal the **Pre-indexed Assembled Tn5 Transposomes** plate and incubate the tagmentation reaction at 37˚C for 30 minutes in a thermomixer (or similar device) set at 800 rpm.
7. Add 250 µL per sample of prepared **DNA Purification Binding Buffer** to a reservoir at room temperature. If processing 8 samples or less, add 2 mL.
8. After the incubation, add 125 µL (5 times the volume of your tagmentation reaction) of the prepared **DNA Purification Binding Buffer** to each sample.
9. Gently vortex the **Silica Beads**, ensuring they are well mixed and add 3 µL to each sample. Vortex or thoroughly pipette mix and let it sit for 5 minutes on ice.

Note: Residual **Silica Beads** may stick to the inside of pipette tips or tubes during transfer. This does not affect overall assay results

10. Put the tubes on a magnet and leave until the beads have completely pelleted (~2-5 minutes).
11. Add 500 µL of prepared **DNA Purification Wash Buffer** per sample to a reservoir. If processing less than 4 samples, add 2 mL.
12. Using a multichannel pipette, carefully remove and discard the supernatant without disturbing the bead pellet.
13. While still on the magnet, add 200 µL of **DNA Purification Wash Buffer** EtOH added. Remove the strip from the magnet and gently pipette up and down to mix.
14. 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.
15. 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.
16. 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.
17. Magnetize the reaction, allow the beads to completely pellet and transfer the supernatant into a new, labeled 0.2 mL PCR tube or 96 well plate. The samples can be stored at -20˚C or can move straight forward into PCR amplification.

PCR Amplification

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

A PCR Master Mix can be created prior to PCR amplification and stored at -20˚C to improve workflow.

PCR Master Mix

Reagent	1 sample	48 samples	96 samples
dNTP Mix,10 mM	1 µL	48 µL	96 µL
5X Q5 Reaction Buffer	10 µL	480 µL	960 µL
Q5 DNA Polymerase	0.5 µL	24 µL	48 µL
Total Volume	**11.5 µL**	**552 µL**	**1.104 mL**

Set up the PCR reactions by adding the components in the order shown below. The samples were already indexed during the tagmentation reaction and simply need to be amplified using a universal primer set. Refer to the plate map below for the index combination in each well.

Reagents	Volume
Tagmented DNA	33.5 µL
P7 Primer (25 µM)	2.5 µL
P5 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**

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 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.

Note: Prepare 400 µL of fresh 80% ethanol per sample.

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 freshly prepared 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.

Plate Map

Well Location		1	2	3	4	5	6	7	8	9	10	11	12
	Index	N505	N506	N507	N508	N510	N511	N513	N515	N516	N517	N518	N520
A	N701	N701, N505	N701, N506	N701, N507	N701, N508	N701, N510	N701, N511	N701, N513	N701, N515	N701, N516	N701, N517	N701, N518	N701, N520
B	N702	N702, N505	N702, N506	N702, N507	N702, N508	N702, N510	N702, N511	N702, N513	N702, N515	N702, N516	N702, N517	N702, N518	N702, N520
C	N703	N703, N505	N703, N506	N703, N507	N703, N508	N703, N510	N703, N511	N703, N513	N703, N515	N709*, N516	N703, N517	N703, N518	N703, N520
D	N704	N704, N505	N704, N506	N709*, N507	N704, N508	N704, N510	N704, N511	N704, N513	N704, N515	N704, N516	N704, N517	N704, N518	N704, N520
E	N705	N705, N505	N705, N506	N705, N507	N705, N508	N705, N510	N705, N511	N705, N513	N705, N515	N705, N516	N705, N517	N705, N518	N705, N520
F	N706	N706, N505	N706, N506	N706, N507	N706, N508	N706, N510	N706, N511	N706, N513	N706, N515	N706, N516	N706, N517	N706, N518	N706, N520
G	N707	N707, N505	N707, N506	N707, N507	N707, N508	N707, N510	N709*, N511	N707, N513	N707, N515	N707, N516	N707, N517	N709*, N518	N707, N520
H	N708	N708, N505	N708, N506	N708, N507	N708, N508	N708, N510	N708, N511	N708, N513	N708, N515	N708, N516	N708, N517	N708, N518	N708, N520

Figure: Pre-indexed Assembled Tn5 Transposomes Indexed Primer Plate Map

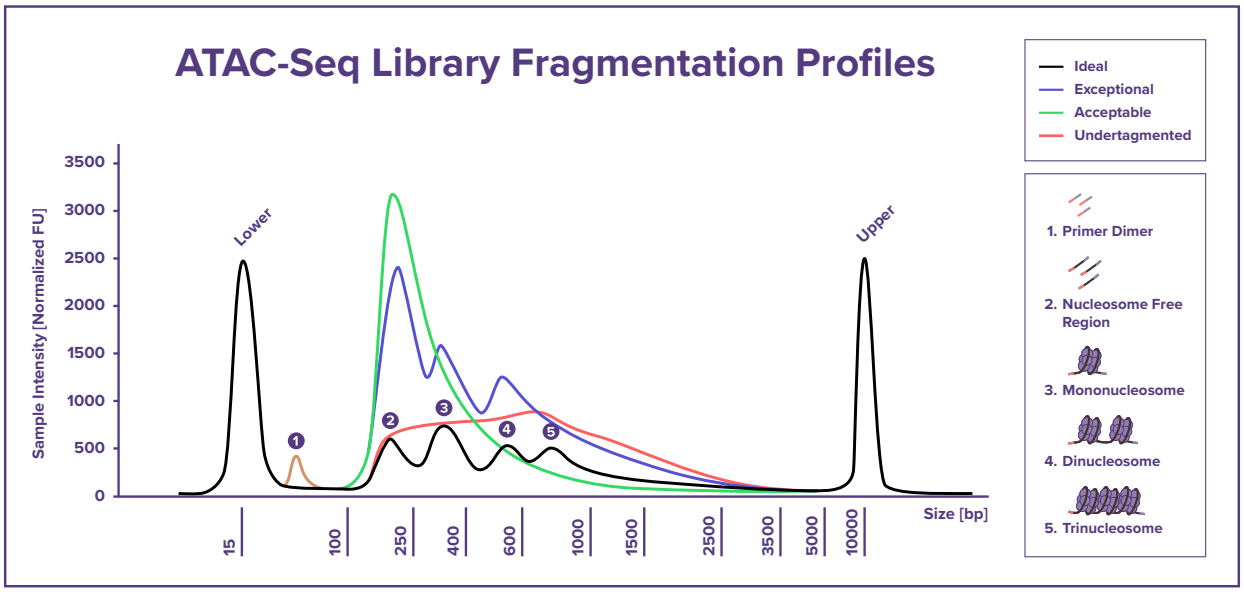
Each well contains 4 µL of 8 µM Pre-indexed Assembled Tn5 Transposomes. This concentration is a combination of the indexed primer pairs and Tn5 (8 µM Tn5 + 4 µM oligo A + 4 µM oligo B). The Pre-indexed Assembled Tn5 Transposomes have been validated in ATAC-Seq using 50,000 cells. All i5 and i7 index combinations were tested to ensure efficiency in a biologically relevant tagmentation reaction. i5 and i7 index combinations that were identified to produce less sequencing reads were replaced with index N709 and normal sequencing reads resulted after re-testing to confirm they yield optimal results. Wells where N709 replaced the index in use for other wells in that row are highlighted with red N709* in the plate map above to easily see the changes in the logical layout of the plate.

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 21 µL of prepared Tagmentation Master Mix to each well. Gently pipette to mix, and then transfer to **Pre-indexed Assembled Tn5 Transposomes** plate.
6. Proceed to **Step 6** of **Tagmentation Reaction and Purification** on **page 7**.

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

P7 Primer

5'- CAA GCA GAA GAC GGC ATA CGA -3'

P5 Primer

5'- AAT GAT ACG GCG ACC ACC GA -3'

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.

Frequently Asked Questions

Question	Answer
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.
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. 53153) which contains lysis buffer, tagmentation buffer, and digitonin.
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.

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