

The following document is a protocol for **ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing)** on frozen cell pellets using the **Illumina Tagmentation kit (TDE/Tn5 transposase)** requires a critical initial step for nuclei isolation from the frozen starting material. This version incorporates key modifications, such as the **Omni-ATAC** lysis buffer components, known to improve results with non-fresh/frozen cells and reduce mitochondrial reads.

## ATAC-seq Protocol: Frozen Cell Pellets (Omni-ATAC Variation)

This protocol is adapted from the Omni-ATAC method, focusing on isolating **high-quality nuclei** from a cryopreserved pellet for subsequent tagmentation.

### 1. Thawing and Nuclei Isolation

Step	Action	Notes
1.1	Prepare <b>cold 1x PBS</b> and <b>ATAC-seq Lysis Buffer</b> (Omni-ATAC formula: e.g., RSB buffer with 0.1% NP40, 0.1% Tween-20, and 0.01% Digitonin). Keep all reagents and centrifuge at <b>4°C</b> .	Working with cold reagents is crucial to maintain nuclear integrity.
1.2	Retrieve cell pellet from -80°C or liquid nitrogen. <b>Immediately</b> thaw quickly by gently rubbing the vial in your hand for about 10-20 seconds or placing in a 37°C water bath for about 1 minute.	Do <b>not</b> thaw completely; keep it mostly frozen and on ice after initial thaw.
1.3	Add <b>1 mL cold 1x PBS</b> to the pellet and gently resuspend by slow pipetting (P1000) or gentle flicking. Transfer to a 1.5 mL microcentrifuge tube.	Rapid dilution of cryoprotectant (e.g., DMSO) minimizes cell stress.
1.4	Centrifuge at <b>500xg for 5 minutes at 4°C</b> .	
1.5	<b>Carefully</b> aspirate and discard the supernatant, avoiding the pellet.	
1.6	Resuspend the pellet in <b>500 µL</b> of cold <b>1x PBS</b> . Centrifuge again at <b>500xg for 5 minutes at 4°C</b> .	This second wash helps remove residual cryoprotectant.

1.7	<b>Carefully</b> aspirate and discard the supernatant, leaving the cell pellet.	The nucleus pellet can be fragile or nearly invisible.
1.8	Add <b>50 µL cold ATAC-seq Lysis Buffer</b> . Gently pipette up and down <b>3-5</b> times with a P200 tip to <b>resuspend</b> and lyse the cells.	<b>The number of pipetting strokes is critical for optimal lysis.</b> Check for about 50,000 nuclei/sample.
1.9	Incubate the suspension <b>on ice</b> for exactly <b>3 minutes</b> .	<b>Timing is essential for consistent lysis.</b>
1.10	Add <b>1 mL cold Wash Buffer</b> (RSB buffer with 0.1% Tween-20, no NP40 or Digitonin) and gently invert <b>3</b> times to mix.	This dilutes the detergents and stops the lysis reaction.
1.11	Centrifuge at <b>500xg for 10 minutes at 4°C</b> to pellet the nuclei.	
1.12	<b>Very carefully</b> aspirate and discard the supernatant.	The nucleus pellet can be fragile or nearly invisible.
1.13	Resuspend the nuclei pellet in <b>50 µL cold 1x Tagment DNA (TD) Buffer</b> (from the Illumina kit).	This is the final resuspension volume for tagmentation.
1.14	<b>Optional QC:</b> Take a 1-5 µL aliquot, stain with Trypan Blue or DAPI, and check for <b>intact nuclei</b> using a hemocytometer or automated cell counter.	Intact, non-clumped nuclei are vital for ATAC-seq success. Adjust volume with TD buffer to normalize to the required nuclei count (e.g., 50,000).

## 2. Tagmentation (Using Illumina TDE1)

Step	Action	Reagents/Details
2.1	Prepare the <b>Tagmentation Master Mix</b> on ice (scale for 1 reaction plus 1 extra):	
	<b>25 µL 2x TD Buffer</b>	From Illumina kit (Cat #15027866).

	<b>2.5 <math>\mu</math>L TDE1</b> (Tn5 Transposase)	From Illumina kit (Cat #15027865).
	<b>22.5 <math>\mu</math>L</b> Nuclease-Free <b>H2O</b>	
	<b>Total 50 <math>\mu</math>L Master Mix</b>	
<b>2.2</b>	Add <b>50 <math>\mu</math>L</b> of the Tagmentation Master Mix to the 50 $\mu$ L nuclei suspension (from step 1.13/1.14). <b>Total reaction volume is 100 <math>\mu</math>L.</b>	
<b>2.3</b>	Gently pipette up and down <b>6</b> times to mix the nuclei with the transposase. Transfer the mixture to a <b>0.2 mL PCR tube</b> .	<b>Do not vortex</b> or mix vigorously, as this can shear the chromatin.
<b>2.4</b>	Incubate in a thermal cycler at <b>37°C for 30 minutes</b> with the <b>lid heated</b> .	Tagmentation step.
<b>2.5</b>	<b>Purify</b> the tagmented DNA using a commercial PCR clean-up kit (e.g., Qiagen MinElute or Zymo DNA Clean & Concentrator) according to the manufacturer's instructions.	This step removes the Tn5 enzyme.
<b>2.6</b>	Elute the tagmented <b>DNA</b> in <b>10 <math>\mu</math>L</b> Elution Buffer (EB) or Nuclease-Free <b>Water</b> .	Proceed immediately to PCR amplification or store at -20°C.

### 3. PCR Amplification and Final Library Cleanup

This is the final step to add the unique indexing primers and amplify the library.

<b>Step</b>	<b>Action</b>	<b>Reagents/Details</b>
<b>3.1</b>	Set up the initial <b>5 cycles PCR</b> reaction using the <b>10 <math>\mu</math>L</b> of purified tagmented DNA:	
	<b>10 <math>\mu</math>L</b> Tagmented DNA	
	<b>25 <math>\mu</math>L</b> NEBNext High-Fidelity <b>2x</b> PCR Master Mix	
	<b>2.5 <math>\mu</math>L</b> of 25 $\mu$ M Nextera Primer <b>Ad1</b>	

	<b>2.5 µL</b> of 25 µM Indexed Primer <b>Ad2.x</b>	Use the appropriate unique i7 index.
	<b>10 µL</b> Nuclease-Free <b>H2O</b>	
	<b>Total 50 µL reaction</b>	Run <b>5</b> cycles: <b>72°C</b> for 5 minutes; <b>98°C</b> for 30 seconds; 5 cycles of (98°C for 10 seconds, 63°C for 30 seconds, 72°C for 1 minute).
<b>3.2</b>	Determine <b>Additional</b> PCR Cycles.	Remove a <b>5 µL</b> aliquot for a qPCR side reaction to avoid over-amplification.
<b>3.3</b>	Run the remaining <b>45 µL</b> for the <b>additional cycles</b> determined in Step 3.2.	
<b>3.4</b>	<b>Clean up</b> the final PCR product using a <b>1.8x</b> AMPure XP bead selection for fragment size selection.	This removes short adapter/primer-dimers (about 80 bp) and large genomic fragments (greater than 1,000 bp).
<b>3.5</b>	Elute the final ATAC-seq library in <b>20 µL</b> Nuclease-Free <b>Water</b> .	
<b>3.6</b>	<b>Final QC:</b> Quantify the library using a Qubit dsDNA HS assay and analyze fragment distribution on an Agilent Bioanalyzer (High Sensitivity DNA chip).	Look for the characteristic <b>nucleosome ladder pattern</b> (about 150 bp for nucleosome-free, about 300 bp for mono-nucleosome, etc.).