ATAC-Seq Kit

Quick Guide

Catalog No. 53150
vA1

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Tissue Sample Preparation

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 tubes at 500 x *g* for 5 minutes at 4ºC. Remove supernatant.
4. Add 1 mL ATAC Lysis Buffer and using a 1 mL wide bore pipette tip, transfer each sample to a 1 mL dounce homogenizer. Slowly dounce for 30 strokes.
5. Filter each sample through a 40 µm mesh strainer and collect in a fresh 1.5 mL microcentrifuge tube.
6. Immediately after filtration, take a 10 µL aliquot for cell counting using Trypan Blue cell-viability staining. Aliquot 50,000-100,000 cells into a new tube.
7. Centrifuge tubes at 500 x *g* at 4ºC for 5 minutes. During this time prepare **Tagmentation Master Mix**, see table below. After centrifuge, remove supernatant and proceed to the Tagmentation Reaction and purification steps.

Cell Sample Preparation

1. Count cells and aliquot 50,000 to 100,000 cells into a fresh 1.5 mL centrifuge tube for each sample.
2. Centrifuge cells at 500 x *g* for 5 minutes at 4ºC. Remove supernatant. If there is no pellet, do additional centrifuge at 1000 x *g* for 5 minutes at 4ºC.
3. Add 100 uL cold PBS. Centrifuge at 500 x *g* at 4ºC. Remove supernatant.
4. Resuspend pellet in 100 uL ice-cold ATAC Lysis Buffer. Centrifuge at 500 x *g* at 4ºC for 10 minutes.
5. Transfer sample to a PCR tube on ice. Centrifuge at 500 x *g* for 10 minutes at 4ºC. During this time prepare **Tagmentation Master Mix**. After centrifuge, remove supernatant and move directly to tagmentation.

Tagmentation Master Mix (Per sample)

Reagents	Volume
2X Tagmentation Buffer	25 µL
10X PBS	2 µL
1.0% Digitonin	0.5 µL
10% Tween 20	0.5 µL
H_2O	12 µL
Assembled Transposomes	10 µL

Tagmentation Reaction and Purification

1. Add 50 uL of tagmentation master mix to each sample, pipetter to resuspend. Incubate at 37ºC for 30 minutes in a thermomixer at 800 rpm.
2. Immediately following the tagmentation reaction, transfer each sample to a clean 1.5
3. Add 250 µL DNA Purification Binding Buffer and 5 µL 3 M sodium acetate to each sample.
4. If the color of the sample is anything other than bright yellow, add additional 3 M sodium acetate in 5 µL increments until the proper color is achieved.

5. Mix each sample and transfer to a labeled DNA purification column. Centrifuge columns at 17,000 x *g* for 1 minute.
6. Discard the flow-through, return the collection tube to the column, then add 750 uL of wash buffer. Centrifuge at 17,000 x *g* for 1 minute.

Note: 100% ethanol must be added to the Wash Buffer before the first use, to a final concentration of 80%. (40 mL of 100% Ethanol to the DNA Purification Wash Buffer bottle)

7. Discard the flow-through. With the column cap open, centrifuge at 17,000 x *g* for 2 minutes to remove residual Wash Buffer from the column.
8. Add 35 uL of DNA purification elution buffer to the center of the column matrix. Incubate at RT for 1 minute. Centrifuge column at 17,000 x *g* for 1 minute.
9. Purified DNA can be stored at -20°C, or you may proceed directly with the PCR Amplification of Tagmented DNA steps that follow.

PCR Amplification of Tagmented DNA

PCR Reaction Master Mix (Per sample)

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

1. Set up the PCR reactions by adding the components in the order shown above. 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.
2. Perform PCR using the following program on a thermal cycler (with a heated lid):

   72°C 5 minutes
   98°C for 30 seconds
   10 cycles of: 98°C for 10 seconds, 63°C for 30 seconds, 72°C for 1 minute
   Hold at 10°C.

3. Perform SPRI clean-up with 60 µL SPRI bead solution (1.2X the sample volume), eluting in 20 µL DNA Purification Elution Buffer. Have 400 µL of freshly-prepared 80% ethanol ready per sample:

   A. Add 60 µL well-mixed, RT SPRI Beads to each sample.
   B. Vortex briefly to mix and incubate for 5 minutes at RT to allow beads to bind. Apply magnet to collect beads.
   C. Once the solution is clear, aspirate the supernatant.
   D. Perform two washes, each with 180 uL of 80% ethanol, leaving the beads on the magnet for both washes.
   E. Allow tubes to sit at RT so that residual ethanol can evaporate. Once the beads transition from shiny to matte (2-5 minutes), proceed to the next step.
   F. Once beads are dry, add 20 uL of DNA purification Elution Buffer and vortex. Leave at RT for 5 minutes. Apply magnet to collect beads.
   G. Once the solution is clear, transfer each supernatant containing the eluted DNA to a fresh tube.

4. 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.