BD Rhapsody™ System

Single-Cell ATAC-Seq

Library Preparation Protocol

23-24473(01)
2024-03

Regulatory information

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

History

Revision	Date	Change made
23-24473(01)	2024-03	Initial release.

Contents

Introduction

This protocol provides instructions on generating a single-cell ATAC library using the BD Rhapsody™ Single-Cell Analysis System.

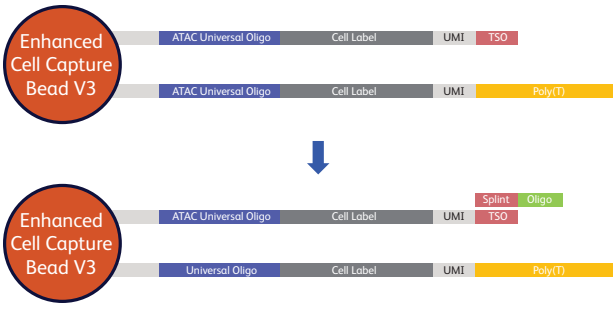
The library utilizes a specific variant of Tn5 transposase to fragment and simultaneously insert Next Generation Sequencing (NGS) primers into the accessible chromatin regions of individual cells or nuclei for sequencing on compatible Illumina® sequencers. It enables uncovering insights into the chromatin structure and factors that affect gene expression of cells at single-cell level.

For complete instrument procedures and safety information, see the *BD Rhapsody™ Single-Cell Analysis System Instrument User Guide*.

Workflow overview

The *BD Rhapsody™ System Single-Cell ATAC-Seq Library Preparation Protocol* enables profiling of the epigenomic landscape at a single nuclei level.

Splint bead: Starting from the BD Rhapsody™ Enhanced Bead V3 layout, add splint oligonucleotide to the beads to assist capturing of genomic DNA.

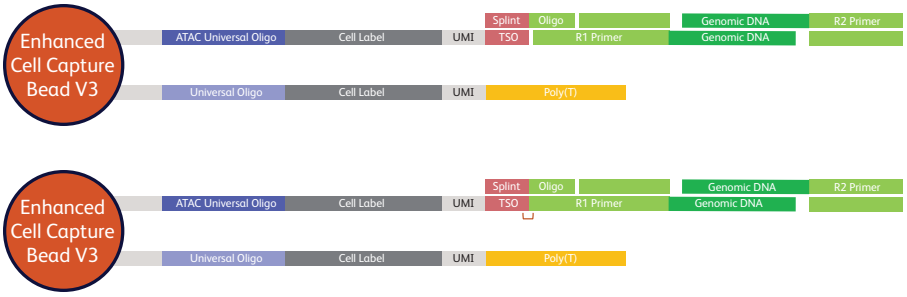


Nuclei isolation: Nuclei isolation protocol depends on the sample type. For details, see Nuclei isolation (page 13).

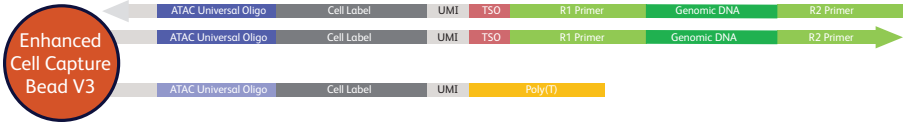
Tagmentation: During the bulk in-situ tagmentation process, expose the nuclei to a tagmentation mix, containing Tagmentase. This enzyme targets accessible genomic regions (open chromatin areas), cutting the DNA and simultaneously attaching preloaded adapter sequences to the ends of each DNA fragment. For substep details, see Tagmentation (page 13).

Single-cell capture: Perform cell lysis in a microwell. The genomic DNA sequences are captured by the splint-oligo-bonded TSO strands.

Ligation: Ligate BD Rhapsody™ bead oligo and tagmented DNA using DNA ligase.



Gap filling ATAC fragments: This process performs ATAC fragment gap filling and extension to beads oligo.

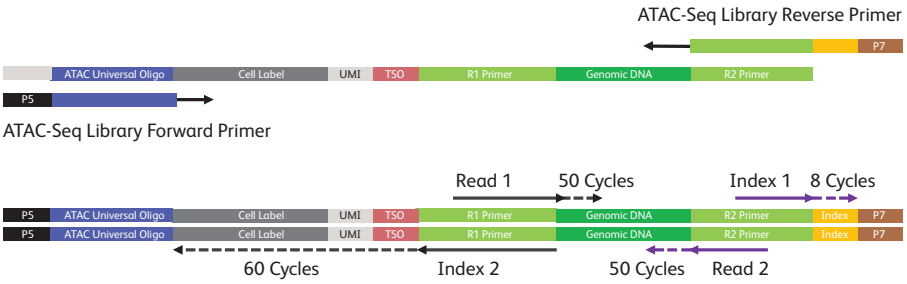


ATAC fragment denaturation and PCR amplification:

Supernatant: Denature the genomic DNA template off the bead. Illumina® adapters and indices are added during the ATAC product amplification.

Sequencing:

Read 1: 50 cycles
Read 2: 50 cycles
Index 1: 8 cycles
Index 2: 60 cycles



Required and recommended materials

Required reagents

Store the reagents at the storage temperature specified on the label.

Material	Supplier	Catalog No.
BD Rhapsody™ Tagmentation and Supplemental Reagents Kit	BD Biosciences	41926
BD Rhapsody™ ATAC-Seq Amplification Kit	BD Biosciences	41927
BD Rhapsody™ Enhanced Cartridge Reagent Kit V3[a]	BD Biosciences	667052
BD Rhapsody™ 8-Lane Cartridge	BD Biosciences	666262
Agencourt® AMPure® XP magnetic beads	Beckman Coulter	A63880
100% ethyl alcohol	Major supplier	–
Nuclease-free water	Major supplier	–
N,N-Dimethylformamide	MilliporeSigma	D4551-250ML
DyeCycle™ Green[b]	Thermo Fisher Scientific	V35004
Calcein AM[b]	Thermo Fisher Scientific	C1430
Draq7[b]	BD Pharmingen™	564904
Trypan Blue	Major supplier	–
70% ethyl alcohol or 70% isopropyl alcohol[c]	Major supplier	–

[a] The Enhanced Cartridge Reagent Kit V3 must be used before beginning this protocol.

[b] Protect DyeCycle™ Green, Calcein AM, and DRAQ7, from light. Avoid multiple freeze-thaw cycles of Calcein AM. See manufacturer's storage recommendations.

[c] To clean the BD Rhapsody™ Xpress System and the BD Rhapsody™ Scanner, see the *BD Rhapsody™ Single-Cell Analysis System Installation and Maintenance Guide*. Instead of 70% alcohol, 10% (w/v) bleach can be used.

Recommended consumables

Supplies	Supplier	Catalog No.
Gilson™ PIPETMAN™ DIAMOND Tipack™ filter tips, 100–1200 µL for BD Rhapsody™ P8xP1200 µL pipette (or BD Rhapsody™ P1200 µL pipette) (Recommended) Or ZAP™ SLIK 1000 µL low-retention aerosol filter pipet tips for BD Rhapsody™ P8xP1200 µL pipette (or BD Rhapsody™ P1200 µL pipette) (Alternative)	Thermo Fisher Scientific Labcon	F171803G 1177-965-008-9
Low retention, filtered pipette tips (20 µL, 200 µL, 1000 µL)	Major supplier	–
Falcon® tube with cell strainer cap	Corning	352235
INCYTO disposable hemocytometer	INCYTO	CN DHC-N01-5
60-mL reagent reservoir self-standing[a]	BD Biosciences	666626
Corning® 96-well polypropylene cluster tube, 8-tube strip format, sterile[b]	Corning	4413
0.2-mL PCR 8-strip tubes	Major supplier	–
15-mL conical tube	Major supplier	–
50-mL conical tube	Major supplier	–
DNA LoBind® tubes, 1.5 mL	Eppendorf	022431021
DNA LoBind® tubes, 2.0 mL	Eppendorf	022431048
DNA LoBind® tubes, 5.0 mL	Eppendorf	0030108310
Reagent reservoir (sterile, non-pyrogenic, RNase/DNase free), 10 mL	VistaLab	3054-1012 3054-1013
Reagent reservoir (sterile, non-pyrogenic, RNase/DNase free), 25 mL	VistaLab	3054-1002 3054-1003
Deep 96-well 2-mL polypropylene plate	Major supplier	–
Lint-free cloth (Kim-Wipes)	Major supplier	–
Qubit™ assay tubes	Thermo Fisher Scientific	Q32856
Qubit™ dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32851
Agilent High Sensitivity DNA Kit	Agilent	5067-4626

[a] Waste collection container for the BD Rhapsody™ HT Xpress System.

[b] These are the bead retrieval tubes to be used with the BD Rhapsody™ HT Xpress System.

Required equipment

Equipment	Supplier	Catalog No.
Single-channel pipettes (P20, P200, P1000)	Major supplier	–
BD Rhapsody™ HT Xpress Package	BD Biosciences	666730
BD Rhapsody™ Scanner	BD Biosciences	633701
Hemocytometer adapter[a]	BD Biosciences	633703
BD Rhapsody™ P8xP1200 µL pipette-HTX[b]	BD Biosciences	666718
BD Rhapsody™ P1200 µL Pipette – HTXc	BD Biosciences	666719
Temperature-controlled centrifuge	Major supplier	–
Eppendorf ThermoMixer® C	Eppendorf	5382000023
Microcentrifuge for 1.5–2.0-mL tubes	Major supplier	–
Microcentrifuge for 0.2-mL tubes	Major supplier	–
Qubit™ 3.0 Fluorometer	Thermo Fisher Scientific	Q33216
Agilent® 2100 Bioanalyzer	Agilent Technologies	G2940CAG
Invitrogen™ DynaMag™-2 magnet	Thermo Fisher Scientific	12321D
Low-profile magnetic separation stand for 0.2-mL, 8-strip tubes	V&P Scientific, Inc.	VP772F4-1
Ice bucket	Major supplier	–
Vortexer	Major supplier	–
Digital timer	Major supplier	–

[a]Included with the BD Rhapsody™ Scanner.

[b] Part of the BD Rhapsody™ Xpresss Package. Items can be ordered separately.

[c] Only required if not using the BD Rhapsody™ P8xP1200µL Pipette – HTX.

Before you begin

- Ensure that you have the correct kits for this protocol. Matching cap colors indicate that you have the correct kit.

BD Rhapsody™ Enhanced Cartridge Reagent Kit V3

Cap Color	Name	Quantity
	BD Rhapsody™ HT Enhanced Cell Capture Beads V3	4
	Sample Buffer	1
	Cartridge Wash Buffer 1	1
	Cartridge Wash Buffer 2	1
	Lysis Buffer	4
	Bead Wash Buffer	1
	Waste Collection Container	4
	1M DTT	1

ATAC-Seq Amplification Kit

Cap Color	Reagent Name	Quantity
	Ligation Buffer	1
	Ligase	1
	Nuclease Free Water	2
	Gap-Filling Enzyme	1
	Gap-Filling Buffer	1
	ATAC-Seq Library Forward Primer	1
	ATAC-Seq Library Reverse Primer 1	1
	ATAC-Seq Library Reverse Primer 2	1
	ATAC-Seq Library Reverse Primer 3	1
	ATAC-Seq Library Reverse Primer 4	1
	ATAC-Seq Library Reverse Primer 5	1
	ATAC-Seq Library Reverse Primer 6	1
	ATAC-Seq Library Reverse Primer 7	1
	ATAC-Seq Library Reverse Primer 8	1
	dNTP	1
	Gap-Filling Enhancer	1
	Elution Buffer	1
	Bead Resuspension Buffer	1
	PCR MasterMix	1

ATAC-Seq Tagmentation and Supplemental Reagents Kit

Cap Color	Name	Quantity
Purple	Tagmentase	1
Purple	Tagmentation Buffer	1
Purple	10X PBS	1
Purple	Digitonin 2%	1
Purple	Tween 20 10%	1
White	Nuclease Free Water	1
Blue	Universal ATAC-Seq Splint Oligo	1
Blue	Splint Oligo Annealing Buffer	1
Blue	Splint-Bead Wash Buffer	1
Green	Nuclei Buffer	2
White	Proteinase K, Molecular Biology Grade	1

- Thaw reagents (not enzymes) in the BD Rhapsody™ Tagmentation and Supplemental Reagents Kit (Cat. No. 41926) and BD Rhapsody™ ATAC-Seq Amplification Kit (Cat. No. 41927) at room temperature (15–25 °C), and then place on ice. Keep enzymes at –25 °C to –15 °C.

  Note: Only thaw the reagents needed for the day.
- Dilute 2% Digitonin to 1% Digitonin with nuclease-free water.
- Prepare tagmentation buffer with dimethylformamide (DMF): Thaw and transfer 200 µL of tagmentation buffer into a new 1.5-mL LoBind® tube, add 50 µL of 100% DMF into the tube, and mix by vortexing. Tagmentation buffer with DMF can be stored at –20 °C for later use.
- Place on ice the following components of the BD Rhapsody™ Enhanced Cartridge Reagent Kit V3 (Cat. No. 667052):
  - Sample buffer
  - 1M DTT
  - Bead wash buffer
  - BD Rhapsody™ Enhanced Cell Capture Beads V3
- Visually inspect the lysis buffer for any precipitation. If precipitation is not present, leave the lysis buffer at room temperature (15–25 °C) until ready to use. If precipitation is present, incubate the lysis buffer at room temperature for 1 hour. Invert to mix, but do not vortex. Once the solution is clear, continue leaving the lysis buffer at room temperature until ready to use.
- Open the tube while holding the DTT tube vertically. The solution is overlain with an inert/non-oxygen-containing gas. A non-vertical tube will allow the inert gas to pour off. After opening the DTT tube once, seal and store the tube at –20 °C.
- Thaw Calcein AM. Once at room temperature (15–25 °C), resuspend Calcein AM (1 mg; Thermo Fisher Scientific Cat. No. C1430) in 503.0 µL dimethyl sulfoxide (DMSO) for a final stock concentration of 2 mM. Follow the manufacturer's instructions and protect it from light.

- Thaw DyeCycle™ Green at room temperature (15–25 °C). Follow the manufacturer's instructions and protect it from light.
- Aliquot 100% ethyl alcohol and cartridge reagent buffers in 10-mL or 25-mL reagent reservoirs based on the number of lanes used, as instructed by the following table. Do not aliquot for single lane.

Component	For 1 lane (mL)	For 2 lanes (mL)	For 3 lanes (mL)	For 4 lanes (mL)	For 5 lanes (mL)	For 6 lanes (mL)	For 7 lanes (mL)	For 8 lanes (mL)
100% Ethyl Alcohol	0.05	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cartridge Wash Buffer 1	0.76	3.50	5.25	7.00	8.75	10.50	12.25	14.00
Cartridge Wash Buffer 2	0.38	2.00	3.00	4.00	5.00	6.00	7.00	8.00

- For a single cartridge workflow, adhere to the specified buffer volume as recommended in the BD Rhapsody™ protocol for Single Cell Capture and cDNA Synthesis.
- When conducting the experiment in accordance with the *BD Rhapsody™ HT Xpress System Instrument User Guide* for a scanner-free workflow, it is essential to utilize a thermomixer for the **Bead Agitation** step. It is important to keep the cartridge leveled.

Best practices

- Use low-retention filtered pipette tips.
- Use wide-bore tips when handling nuclei.
- When working with BD Rhapsody™ Enhanced Cell Capture Beads, use low-retention filtered tips and LoBind® tubes. Never vortex the beads. Pipet-mix only.
- Bring AMPure XP magnetic beads to room temperature before use.
- Remove supernatants without disturbing AMPure XP magnetic beads.
- It is recommended to use a swinging-bucket centrifuge for pelleting cells.
- For a complete list of materials for the BD Rhapsody™ system, see the *BD Rhapsody™ Single-Cell Analysis System Instrument User Guide* (Doc ID 23-24257).

Important: The BD Rhapsody™ Enhanced Cartridge Reagent Kit V3 (Cat. No. 667052) must be used for this protocol. The BD Rhapsody™ Tagmentation and Supplemental Reagents Kit (Cat. No. 41926) is not compatible with the BD Rhapsody™ Enhanced Cartridge Reagent Kit (Cat. No. 664887).

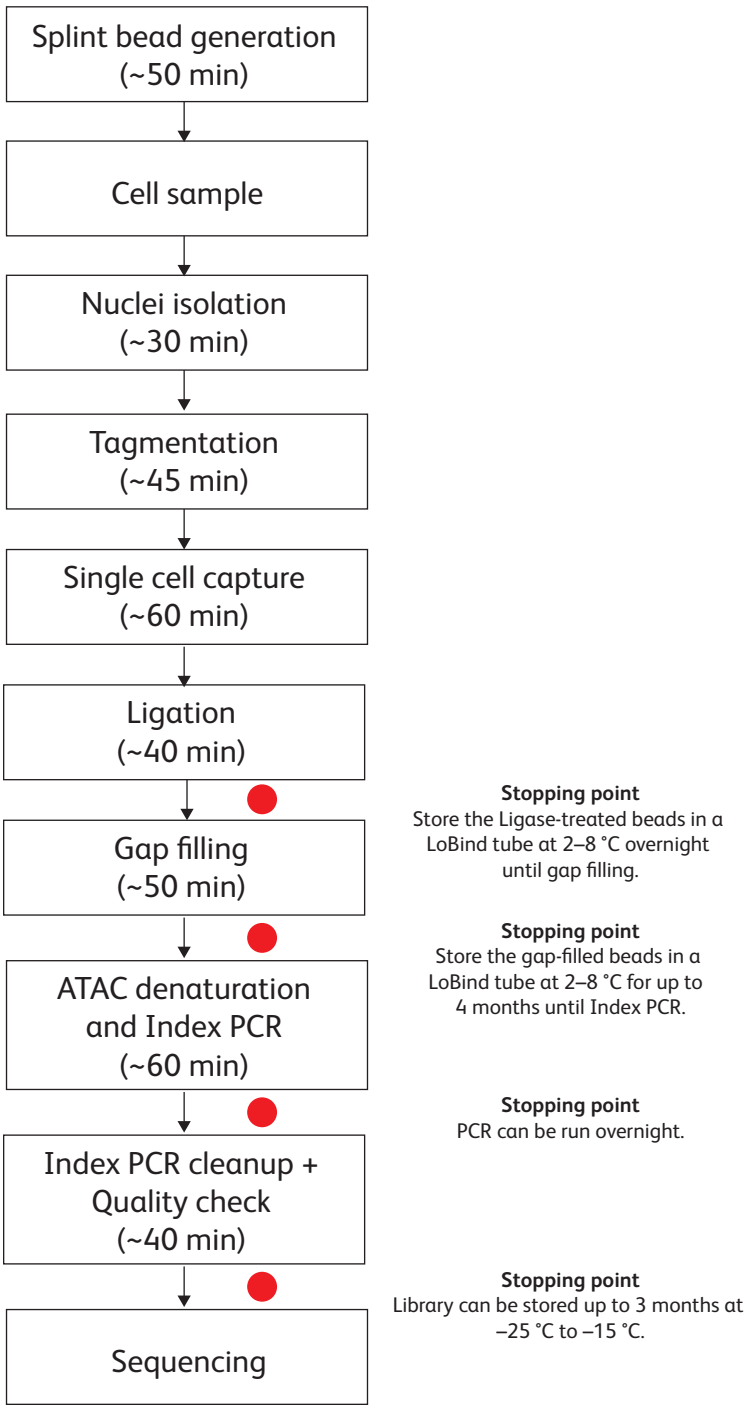
Additional documentation

- *BD Rhapsody™ Preparing Single-Cell Suspensions Protocol* (Doc ID: 23-24126)
- *BD Rhapsody™ HT Single-Cell Capture and Analysis System Single-Cell Capture and cDNA Synthesis Protocol* (Doc ID: 23-24252)
- *BD® Single-Cell Multiomics Bioinformatics Handbook* (Doc ID: 54169)

Safety information

For safety information, see the *BD Rhapsody™ Single-Cell Analysis System Instrument User Guide* (Doc ID: 23-24257).

Time considerations



Procedure

The range of intended total nuclei load on a cartridge is between 1,000 to 50,000 nuclei for this protocol. Nuclei load below or above this recommended range may not be suitable for current protocol configuration. Follow the procedures listed in this section.

Important: The BD Rhapsody™ Enhanced Cartridge Reagent Kit V3 (Cat. No. 667052) must be used for this protocol.

Splint bead generation

1. Set the thermomixers to 25 °C and 70 °C.
2. Obtain BD Rhapsody™ Enhanced Cell Capture Beads V3.
3. Place the tube on a magnet rack to magnetize beads down until the solution is clear.
4. Remove and discard the supernatant.
5. Remove the tube from the magnet rack and pipet 1 mL of splint-bead wash buffer to the tube.
6. Resuspend beads by slowly pipetting up and down 10 times.
7. Transfer the resuspended beads into a new 1.5-mL LoBind® tube.
8. Place the tube on a magnet rack to magnetize beads down until the solution is clear.
9. Remove and discard the supernatant.
10. Remove the tube from the magnet rack and add the following into the tube.

Color	ATAC-Seq Tagmentation and Supplemental Kit Component	Volume (µL)
●	Splint-bead wash buffer	160
●	Splint oligo annealing buffer	20
●	Universal ATAC-Seq splint oligo	20

11. Briefly centrifuge, then resuspend the beads by slowly pipetting up and down 10 times.
12. Place the tube in the thermomixer at 70 °C and incubate for 5 minutes with 1200 rpm mixing.
13. Transfer the tube to the thermomixer at 25 °C and incubate for 30 minutes with 1200 rpm mixing.
14. Remove the tube from the thermomixer, briefly centrifuge, and place the tube on a magnet rack to magnetize the beads down until the solution is clear.
15. Remove and discard the supernatant.
16. Remove the tube from the magnet rack and add 1 mL of splint-bead wash buffer.
17. Resuspend the beads by slowly pipetting up and down 10 times.
18. Place the tube on the magnet rack to magnetize the beads down until the solution is clear.
19. Remove and discard the supernatant.
20. Repeat wash with 1 mL of splint-bead wash buffer 2 more times for a total of three washes.
21. After the final wash, resuspend the beads in 380 µL of cold sample buffer by slowly pipetting up and down 10 times.
22. Store on ice if proceeding on the same day, or at 2–8 °C for up to 1 week.

Nuclei isolation

Note: Reagents required for nuclei isolation are not included in the kits.

For optimal results with your specific sample type, refer to established nuclei isolation protocols and identify the method best suited for your cells or tissues of interest.

Prepare nuclei suspension for tagmentation

Based on the expected nuclei recovery, resuspend the isolated nuclei pellet in Nuclei Buffer with a wide bore tip by gently pipet-mixing 10 times, targeting for 10,000 nuclei/µL and keep on ice.

1. Dilute an aliquot of nuclei 20-fold in cold sample buffer for nuclei counting.
   a. Pipet 95 µL of cold sample buffer into a new 1.5-mL LoBind® tube.
   b. Ensure the nuclei are well suspended by gently pipet-mixing.
   c. Pipet 5 µL of the nuclei suspension into the tube with 95 µL cold sample buffer. Keep the remaining nuclei on ice.
   d. Pipet 0.5 µL of 5 mM DyeCycle Green into the tube.
   e. Gently pipet-mix with a wide-bore tip 10 times and incubate on ice for 5 minutes to stain the nuclei, protected from light.
2. Count the stained nuclei immediately using the BD Rhapsody™ Scanner.

   IMPORTANT: If the expected total recovery is less than 50,000 nuclei, skip the counting step and use all in the tagmentation reaction.
   a. Ensure the stained nuclei are well suspended by gently pipet-mixing.
   b. Pipet 10 µL into INCYTO disposable hemocytometer and count using the scanner.
   c. Multiply the reading by 20 to calculate the concentration of unstained nuclei.
      - If unstained nuclei concentration is >10,000 nuclei/µL, dilute the nuclei to 10,000 nuclei/µL with modified nuclei buffer and keep on ice.
      - If the unstained nuclei concentration is <10,000 nuclei/µL, keep on ice. Adjust the nuclei volume and nuclease-free water in the tagmentation reaction.

Tagmentation

1. Set a thermomixer to 37 °C.
2. In a new 1.5-mL LoBind® tube, add the following reagents in order. Pipet-mix the buffer and Tn5 tagmentase 10 times before adding nuclei. Add 50,000 nuclei and gently pipet-mix 5–10 times with a wide-bore tip.

Tagmentation mix

Color	Kit Component	Volume (µL)
●	Tagmentation buffer with DMF	25
○	Nuclease-free water	13[a]
●	10X PBS	2
●	Digitonin 1%[b]	0.5
●	Tween20, 10%	0.5
●	Tagmentase	4
	Nuclei	5[a]
	[a] If the nuclei concentration is less than 10,000 nuclei/µL, adjust the volume of nuclei and nuclease-free water. [b] 1% Digitonin is diluted from 2% stock with nuclease-free water.	

Note: Successful tagmentation has been performed with 50,000 nuclei in 50 µL reaction. When using less than 50,000 nuclei, proportionally scale down the reaction.

3. Incubate the reaction at 37 °C for 30 minutes in a thermomixer without shaking.

   Note: During the incubation time, start priming the cartridge by following step 1 in section BD Rhapsody™ cartridge workflow (no scan other than indicated) (page 15).

4. After incubation, add 400 µL of cold sample buffer into the tagmentation mix.
5. Gently pipet-mix 5 times and keep on ice.
6. Wet the cell strainer with 50 µL of sample buffer. Filter the tagmented nuclei through a Falcon tube with cell strainer cap. Place the tube on ice.
7. Stain an aliquot of tagmented nuclei and count the stained nuclei immediately using the BD Rhapsody™ Scanner.

   Note: DO NOT STAIN THE ENTIRE SAMPLE.

   Nuclei staining dye can impact ATAC-seq data. Consequently, no cartridge scanner metrics will be collected.

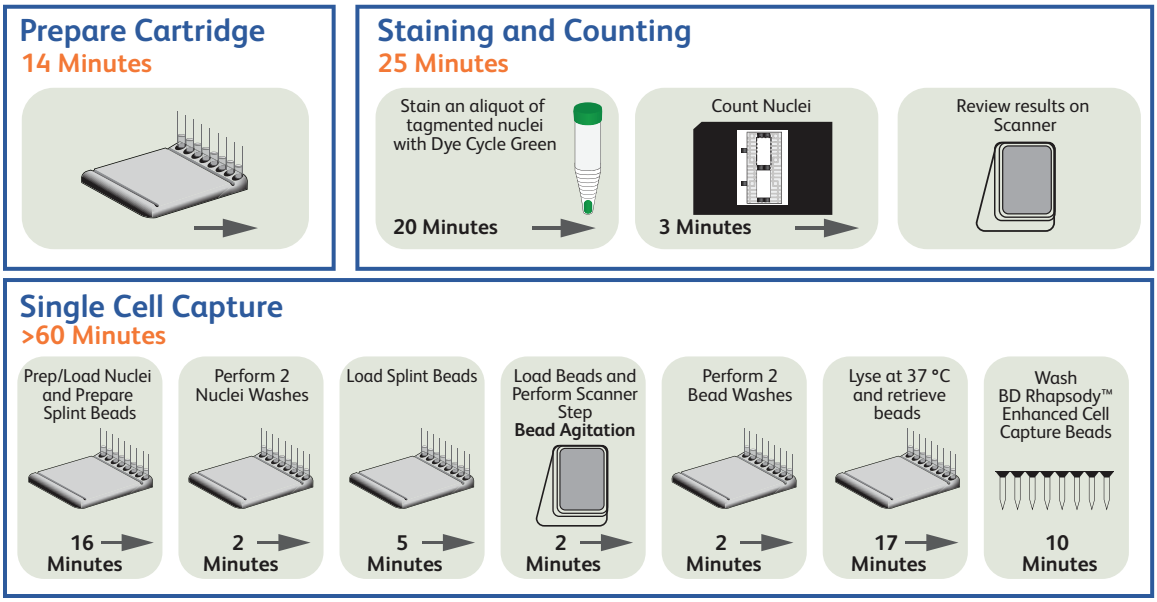
   a. Ensure the nuclei are well suspended by gently pipet-mixing.
   b. Pipet 50 µL of the nuclei suspension into a new 1.5-mL LoBind® tube. Keep the remaining nuclei suspension on ice.
   c. Pipet 1.25 µL of 5-times diluted DyeCycle™ Green* (1 mM) into the tube containing the 50 µL nuclei aliquot.

      Note: *Dilute 5 mM DyeCycle™ Green 5 fold to 1 mM with DMSO.

   d. Pipet-mix 5 times with wide-bore tip and incubate on ice for 5 minutes to stain the nuclei, protected from light.

8. Count the stained nuclei immediately using the BD Rhapsody™ Scanner.
   a. Ensure the stained nuclei are well suspended by gently pipet-mixing.
   b. Pipet 10 µL into INCYTO disposable hemocytometer and count using the scanner.
   c. Viability information is not applicable. Use the concentration to calculate loading dilution.

BD Rhapsody™ cartridge workflow (no scan other than indicated)



1. Prime the cartridge.
   a. Prior to priming the cartridge, scan at least one lane of the empty cartridge for Cell Load scan. For detailed instructions, see *BD Rhapsody™ HT Single-Cell Capture and Analysis System Single-Cell Capture and cDNA Synthesis Protocol* (Doc ID: 23-24252).
   b. Place waste collection container and cluster tube in the BD Rhapsody™ HT Xpress System.
   c. Carefully peel off the seal on the cartridge inlet of the lanes to be used.
   d. Prime the cartridge using the following table steps with BD Rhapsody™ P8xP1200µL pipette:

Step number	Material to load	Volume (µL/lane)	Pipette mode	Incubation at room temperature
1	100% ethyl alcohol	50	EtOH Prime	N/A
2	Air	380	Prime/Wash	N/A
3	Room temp. Cartridge Wash Buffer 1	380	Prime/Wash	1 min
4	Air	380	Prime/Wash	N/A
5	Room temp. Cartridge Wash Buffer 1	380	Prime/Wash	3 min
6	Air	380	Prime/Wash	N/A
7	Room temp. Cartridge Wash Buffer 2	380	Prime/Wash	≤4 hr

2. Prepare single-nuclei suspension for cartridge loading:

   Use the BD Rhapsody™ Scanner to calculate the number of nuclei for cartridge loading.

a. Use the Samples Calculator on the scanner to calculate the volumes of tagmented nuclei and sample buffer needed to prepare a nuclei suspension of 380 µL (this volume is for one lane).

b. Select the correct cartridge type. For the BD Rhapsody™ 8-Lane Cartridge, use 0120.

c. Prepare 380 µL nuclei suspension for cartridge loading by mixing unstained tagmented nuclei with cold sample buffer according to the displayed volumes on the scanner. Ensure the stock solution of each sample is well suspended by gently pipet-mixing with a wide-bore tip before pooling. Keep the nuclei suspension on ice.

d. If working with multiple samples, transfer each tube of prepared nuclei suspension into a 96-deep-well plate for multiple lane loading. Keep the 96-deep-well plate on ice.

3. Load tagmented nuclei in the cartridge:

a. Load the cartridge with materials listed in the following table using the BD Rhapsody™ P8xP1200µL pipette:

Material to load	Volume (µL) 1 lane	Pipette mode
Air	380	Prime/Wash
• Gently pipet mix with a multi-channel pipette to completely resuspend the nuclei. • Set the BD Rhapsody™ P8xP1200µL pipette (or BD Rhapsody™ P1200µL pipette) to **Load** mode. • Immediately load.		
Nuclei suspension	320	Load

Note: Air bubbles that might appear at the inlet or outlet of the cartridge do not affect cartridge performance.

b. Incubate at room temperature (15–25 °C) for 8 minutes.

4. Wash the loaded nuclei with cold sample buffer:

Note: Do not omit this step. It is necessary to obtain good ATAC data.

a. Place the cartridge on the BD Rhapsody™ HT Xpress System.

b. Set the BD Rhapsody™ P8x1200 µL pipette to **Prime/Wash** mode.

c. Load the cartridge with materials listed in the following table using the BD Rhapsody™ P8x1200 µL pipette:

Material to load	Volume (µL) 1 lane	Pipette mode
Air	380	Prime/Wash
Cold sample buffer	380	Prime/Wash
Air	380	Prime/Wash
Cold sample buffer	380	Prime/Wash

5. Load and wash Cell Capture Beads:

a. Place the cartridge on the BD Rhapsody™ HT Xpress System.

b. Set the BD Rhapsody™ P8xP1200 µL pipette to **Prime/Wash** mode.

c. Bring the splint beads generated from the Splint bead generation (page 12) steps.

d. Load the cartridge with materials listed below using the BD Rhapsody™ P8xP1200 µL pipette:

Material to load	Volume (µL) 1 lane	Pipette mode
Air	380	Prime/Wash
• Gently pipet mix with a multi-channel pipette to completely resuspend the beads. • Set the BD Rhapsody™ P8xP1200µL pipette (or BD Rhapsody™ P1200µL pipette) to Load mode. • With a new set of pipette tips, immediately load the beads. Check the pipette tips to make sure that there are no air bubbles inside the tips before loading. Otherwise, dispense the beads into the 96-deep well plate and aspirate with a new set of pipette tips.		
Splint Beads	320	Load

e. Incubate the cartridge at room temperature (15–25 °C) for 3 minutes.

f. Perform scanner step: **Bead Agitation**.

g. After bead agitation is complete, tap **OK**, then **Eject**. Remove the cartridge from the scanner.

h. Place the cartridge on the BD Rhapsody™ HT Xpress System.

i. Set the BD Rhapsody™ P8xP1200µL pipette to Prime/Wash mode.

j. Load the cartridge with materials listed below using the BD Rhapsody™ P8xP1200µL pipette:

Material to load	Volume (µL) 1 lane	Pipette mode
Air	380	Prime/Wash
Cold sample buffer	380	Prime/Wash
Air	380	Prime/Wash
Cold sample buffer	380	Prime/Wash

6. Lyse nuclei:

a. Add 75.0 µL of 1 M DTT to one room-temperature 15-mL lysis buffer bottle and briefly vortex mix. Use the lysis buffer with DTT within 24 hours, and then discard.

b. Pipette 0.5 mL of lysis buffer with DTT into a new 1.5-mL LoBind® tube. Add 25 µL of Proteinase K to the tube immediately before the lysis step, and gently pipet-mix 5 times.

Note: 0.5 mL is enough for one lane. Scale up proportionally if multiple lanes are used.

c. Set the BD Rhapsody™ P8xP1200µL pipette to Lysis mode.

d. Load the cartridge with materials listed using the BD Rhapsody™ P8xP1200µL pipette:

Material to load	Volume (µL) 1 lane	Pipette mode
Lysis buffer with DTT and Proteinase K	280	Lysis

e. Carefully remove the cartridge from the BD Rhapsody™ HT Xpress System. Slowly transfer the cartridge into an incubator at 37 °C and incubate for 10 minutes. Maintain the recommended lysis time for best performance.

Note: It is important to keep the cartridge leveled.

7. Retrieve cell-capture beads:
   a. Place the cluster tube 8-tube strip into the BD Rhapsody™ HT Xpress System drawer. Label the tubes appropriately.
   b. Ensure that the BD Rhapsody™ P8xP1200µL pipette is set to **Retrieval** mode.
   c. Move the front slider to BEADS on the BD Rhapsody™ HT Xpress System.
   d. Carefully bring the cartridge from the 37 °C incubator to the BD Rhapsody™ HT Xpress System and allow the cartridge to cool down for 5 minutes.
   e. Gently pull the top RETRIEVAL slider toward and on top of the cartridge.
   f. Leave the retrieval magnet in the down position for 1 minute.
   g. Aspirate 1,000 µL lysis buffer with DTT using the BD Rhapsody™ P8xP1200µL pipette.
   h. Press down on the BD Rhapsody™ P8xP1200µL pipette to seal against the gasket.
   i. Push back the top RETRIEVAL magnet, and immediately load 1,000 µL lysis buffer with DTT.
   j. Remove the pipette from the gasket and purge the tips.
   k. Move the front slider to OPEN and remove the cluster tube with the bottom adapter to a flat, secure surface.
   l. Remove the cluster tube from the bottom adapter. Gently pipet-mix the beads and transfer into a new 1.5-mL LoBind® tube. Keep on ice.
   m. If beads are still left in the cluster tube, add 100 µL of lysis buffer with DTT, rinse the cluster tube, and transfer into the 1.5-mL LoBind® tube from the previous substep.
   n. Immediately proceed to the next step (Wash cell capture beads).
8. Wash cell capture beads:
   a. Place the tube on a magnet rack for 2 minutes.
   b. Remove and discard the supernatant. Avoid leaving lysis buffer or bubbles in the tube.
   c. Remove the tube from the magnet, and pipet 1.0 mL cold bead wash buffer into the tube. Pipet-mix.
   d. Place the tube on the magnet rack for 2 minutes. Remove and discard the supernatant.
   e. Remove the tube from magnet, and pipet 1.0 mL cold bead wash buffer into the tube. Pipet-mix, and place on ice.

      Note: Start ligation ≤30 minutes after washing retrieved cell capture beads with bead wash buffer.

Ligation

Before you begin, thaw reagents (except for the enzymes) in the BD Rhapsody™ ATAC-Seq Amplification Kit to room temperature. Keep enzymes at –25 °C to –15 °C.

1. Set a thermomixer to 25 °C.
2. In a new 1.5-mL or 2.0-mL LoBind® tube, add the following components:

   Ligation mix

Color	Kit component	For 1 library (µL)	For 1 library with 10% overage (µL)	For 4 libraries with 10% overage (µL)	For 8 libraries with 10% overage (µL)
●	Ligation buffer	20	22	88	176
●	Ligase	10	11	44	88
○	Nuclease-free water	170	187	748	1496
	Total	**200**	**220**	**880**	**1760**

3. Gently vortex ligation mix, briefly centrifuge and place on ice.
4. Place the tube of washed cell capture beads on a magnet rack for 2 minutes. Remove and discard the supernatant.
5. Remove the tube from the magnet and pipet 200 µL of ligation mix into the tube.
6. Resuspend the beads by pipet-mixing 10 times.
7. Transfer the whole reaction into a new 1.5-mL LoBind® tube.
8. Incubate the tube in the thermomixer at 25 °C for 30 minutes with 1,200 rpm mixing.
9. Remove the tube from the thermomixer and place on the magnet for 2 minutes. Remove and discard the supernatant.
10. Resuspend the beads in 200 µL of bead resuspension buffer.

    STOPPING POINT: Ligase-treated beads can be stored at 2–8 °C overnight.

Gap Filling

1. Program the thermomixer.
   a. 1,200 rpm and at 25 °C for 10 minutes.
   b. 1,200 rpm and at 37 °C for 15 minutes.
   c. 1,200 rpm and at 45 °C for 10 minutes.
   d. 1,200 rpm and at 55 °C for 10 minutes.

   IMPORTANT: Confirm “Time Mode” is set to **Time Control** before the program begins.

2. In a new 1.5-mL or 2.0-mL LoBind® tube, add the following components and mix.

   Gap Filling mix

Color	Kit component	For 1 library (µL)	For 1 library with 10% overage (µL)	For 4 libraries with 10% overage (µL)	For 8 libraries with 10% overage (µL)
○	Nuclease-free Water	138	151.8	607.2	1214.4
●	dNTP	20	22	88	176
●	Gap-filling enhancer	12	13.2	52.8	105.6
●	Gap-filling buffer	20	22	88	176
●	Gap-filling enzyme	10	11	44	88
	Total	**200**	**220**	**880**	**1760**

3. Place the tube with ligase-treated beads on a magnet rack for 2 minutes. Remove and discard the supernatant.
4. Resuspend the beads in 200 µL of gap filling mix.
5. Place the tube in the thermomixer. Start the program set in step 1.
6. After incubation, place the tube on the magnet rack for 2 minutes. Remove and discard the supernatant.
7. Resuspend the beads in 200 µL of bead resuspension buffer.

   STOPPING POINT: Gap filling-treated beads can be stored at 2–8 °C for up to 4 months.

   IMPORTANT: If necessary, subsample to have desired number of gap filling-treated beads.

Performing single-cell ATAC Library Index PCR

1. Set a thermomixer to 95 °C.
2. Choose between using the entire sample or a sub-sample of the gap filling-treated beads. If using the entire sample, skip to step 4. If using a subsample, proceed to step 3.
3. (Optional) Subsample the gap filling-treated beads:
   - Determine the volume of beads to subsample for sequencing, based on the expected number of nuclei captured on beads in the final bead-resuspension volume.
   - Completely resuspend the beads by pipet-mixing, then pipet the calculated volume of bead suspension into a new 1.5-mL LoBind® tube. If needed, bring the total volume up to 200 µL with bead resuspension buffer.

   Note: The remaining beads can be stored in bead resuspension buffer at 4 °C for up to 4 months.
4. Place the tube with gap filling-treated beads on a magnet rack for 2 minutes. Remove and discard the supernatant.
5. Pipet 40 µL of elution buffer to the beads. Pipet-mix.
6. Incubate the tube in the thermomixer at 95 °C for 5 minutes (no shaking).
7. Remove the tube from the thermomixer and immediately place the tube on ice for 5 minutes.
8. Remove the tube from ice, quick spin, and then place the tube on a magnet rack until the solution is clear.
9. Transfer the entire supernatant to a new 0.2-mL PCR tube. Keep on ice.
10. Pipet 40 µL of elution buffer to the beads. Pipet-mix.
11. Incubate the tube in the thermomixer at 95 °C for 5 minutes (no shaking).
12. Remove the tube from the thermomixer and immediately place the tube on ice for 5 minutes.
13. Remove the tube from ice, quick spin and then place the tube on the magnet rack until the solution is clear.
14. Transfer the entire supernatant into the PCR tube with the previously collected 40 µL eluted supernatant. Total 80 µL of ATAC products.
15. Resuspend the beads with 200 µL bead resuspension buffer. Store the beads at 2–8 °C.
16. In a new 1.5-mL tube, add the following components. Gently vortex mix, briefly centrifuge, then place on ice.

ATAC index PCR mix

Color	Kit component	For 1 library (µL)	For 1 library with 10% overage (µL)	For 4 libraries with 10% overage (µL)	For 8 libraries with 10% overage (µL)
◯	PCR master mix	30	33	132	264
◯	ATAC-Seq Library Forward Primer	6	6.6	26.4	52.8
🔴	ATAC-Seq Library Reverse Primer(1–8)[a]	6	6.6	–	–
	Total	**42**	**46.2**	**158.4**	**316.8**

a. For more than one ATAC library, use a different ATAC-Seq Library Reverse Primer for each library.

17. Combine the ATAC index PCR mix with ATAC products as follows:
    - For one sample, the ATAC index PCR mix includes an ATAC-Seq Library Reverse Primer. Combine 42 µL of the mix with 80 µL of ATAC product. Pipet-mix 10 times, and then split the reaction volume (122 µL) into two 0.2-mL PCR tubes.
    - If working with multiple samples, the ATAC index PCR mix does not include ATAC-Seq Library Reverse Primer because the reverse primer must be sample-specific. In separate tubes for each sample, combine 36 µL of the ATAC index PCR mix with 80 µL of ATAC product and 6 µL of the ATAC-Seq Library Reverse Primer that is specifically assigned to the sample. Pipet-mix 10 times, and then split the reaction volume (122 µL) into two 0.2-mL PCR tubes.
18. Gently vortex mix and briefly centrifuge.
19. In post-amplification workspace. Run the following PCR program. (Volume = 60 µL)

Step	Cycles	Temperature	Time
Hot start	1	98 °C	45 s
Denaturation	12–16 cycles[a]	98 °C	10 s
Annealing		66 °C	30 s
Extension		72 °C	30 s
Final extension	1	72 °C	1 min
Hold	1	10 °C	∞

a. Suggested PCR cycles might need to be optimized for different cell types and number of cells.

STOPPING POINT: The PCR can run overnight.

Recommended number of PCR cycles

Number of cells in ATAC PCR	Suggested number of PCR cycles
≥10,000	12
10,000–5,000	13
4,999–1,000	14
<1,000	16

Purifying ATAC Index PCR product

1. Perform the purification in post-amplification workspace.
2. Bring AMPure XP beads to room temperature (15–25 °C) and vortex at high speed for 1 minute until beads are fully resuspended.
3. In a new 5.0-mL LoBind® tube, prepare 2 mL (per sample) fresh 80% (v/v) ethyl alcohol by combining 1.6 mL absolute ethyl alcohol, molecular biology grade, with 0.4 mL of nuclease-free water. Vortex the tube for 10 seconds to mix.

   Note: Make fresh 80% ethyl alcohol (1 mL/sample) and use it within 24 hours.
4. When the ATAC Index PCR is complete, briefly centrifuge to collect the contents at the bottom of the tubes.
5. In a new 1.5-mL LoBind® tube, combine the two reactions of each sample for a total volume of 120 µL.

6. Pipet 144 µL of AMPure XP beads into the tube containing 120 µL of ATAC Index PCR products. Pipet-mix 10 times.
7. Incubate at room temperature (15–25 °C) for 5 minutes.
8. Place the tube on the 1.5-mL tube magnet for 3 minutes. Remove and discard the supernatant.
9. Keeping the tube on the magnet, gently add 500 µL of fresh 80% ethyl alcohol into the tube and incubate for 30 seconds. Remove and discard the supernatant.
10. Repeat step 9 once for a total of two washes.
11. Keeping the tube on the magnet, use a small-volume pipette to remove and discard any residual supernatant from the tube.
12. Air-dry the beads at room temperature (15–25 °C) for 5 minutes.
13. Remove the tube from the magnet and resuspend the beads in 40 µL of Elution Buffer. Pipet-mix until the beads are fully resuspended.
14. Incubate at room temperature (15–25 °C) for 2 minutes.
15. Briefly centrifuge and place the tube on the magnet until the solution is clear, usually ≤30 seconds.
16. Transfer the supernatant (~40 µL) containing the purified ATAC library into a new 1.5-mL LoBind® tube.

    STOPPING POINT: Store at 2–8 °C if proceeding on the same day, or at –25 °C to –15 °C for up to 3 months.

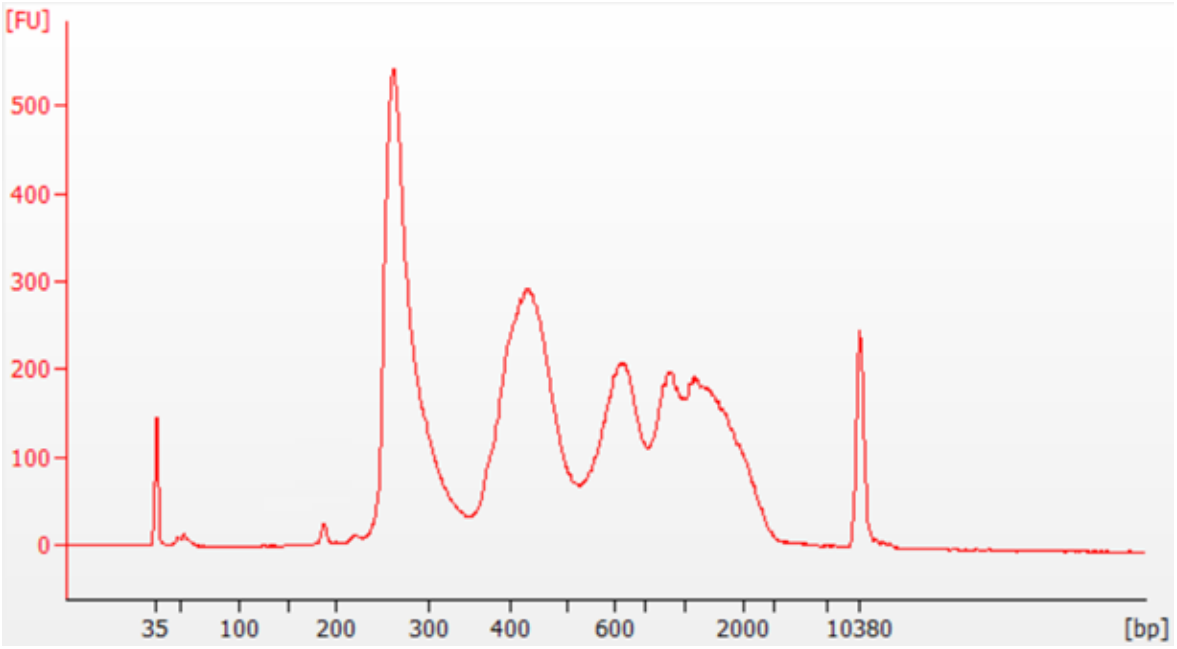
Performing quality control on the final sequencing libraries

1. Measure the concentration of each ATAC library by quantifying 2 µL of the final sequencing library with a Qubit Fluorometer and Qubit dsDNA HS assay, and perform quality control of the ATAC library using either of the following systems:
   a. Agilent 2100 Bioanalyzer using the Agilent High Sensitivity DNA Kit
   b. Agilent 4200 TapeStation system using the Agilent High Sensitivity D1000 or D5000 ScreenTape Assay
2. If needed, dilute the library to the quantitative range of the Agilent 2100 Bioanalyzer. Measure the fragment size of the library following the manufacturer's instructions.
   a. If the concentration is >5 ng/µL, dilute the library to ≤5 ng/µL with elution buffer.
   b. Measure the average fragment size of the ATAC libraries within the size range of 200–1,000 bp by using the Agilent Bioanalyzer with the Agilent High Sensitivity DNA Kit (Agilent Cat. No. 5067-4626). Follow the manufacturer's instructions.

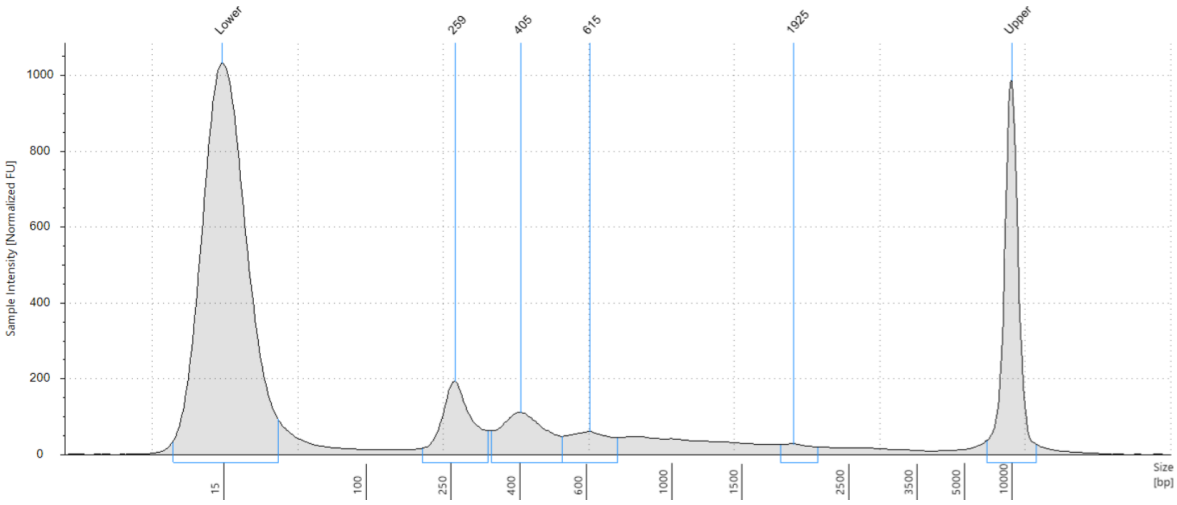
      The following diagram shows a representative ATAC library trace from the Bioanalyzer and TapeStation, where the majority of the fragments are distributed between ~200–2000 bp.

Figure 1 BD Rhapsody™ ATAC Library

A. Bioanalyzer High Sensitivity DNA trace



B. TapeStation high-sensitivity D5000 trace



Sequencing

ATAC library requirements

- Recommended sequencing depth: 50,000 read pairs per cell.
- Required parameters:

Parameter	Requirement
Platform[a]	Illumina®[a]
Paired-end reads	Recommended: Read 1: 50 cycles; Read 2: 50 cycles Index 1: 8 cycles; Index 2: 60 cycles
PhiX	1% recommended
Analysis	See the *BD® Single-Cell Multiomics Bioinformatics Handbook* (Doc ID: 54169)
a. To review the Illumina® Index 1 (i7) sequences, see the following table.	

Illumina® Index (i7) sequences

Library Reverse Primer	Sequences
1	TAAGGCGA
2	CGTACTAG
3	AGGCAGAA
4	TCCTGAGC
5	GGACTCCT
6	TAGGCATG
7	CTCTCTAC
8	CAGAGAGG

ATAC library sequencing recommendations

- For a NextSeq High or Mid Output run and MiniSeq High or Mid Output run, load the flow cell at a concentration in the range of 1.5–1.8 pM with 1% PhiX spike-in.

Single-cell ATAC-library sequencing analysis pipeline

Contact your local Field Application Specialist (FAS) or scomix@bdscomix.bd.com for access to the latest BD Rhapsody™ sequence analysis pipeline.

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