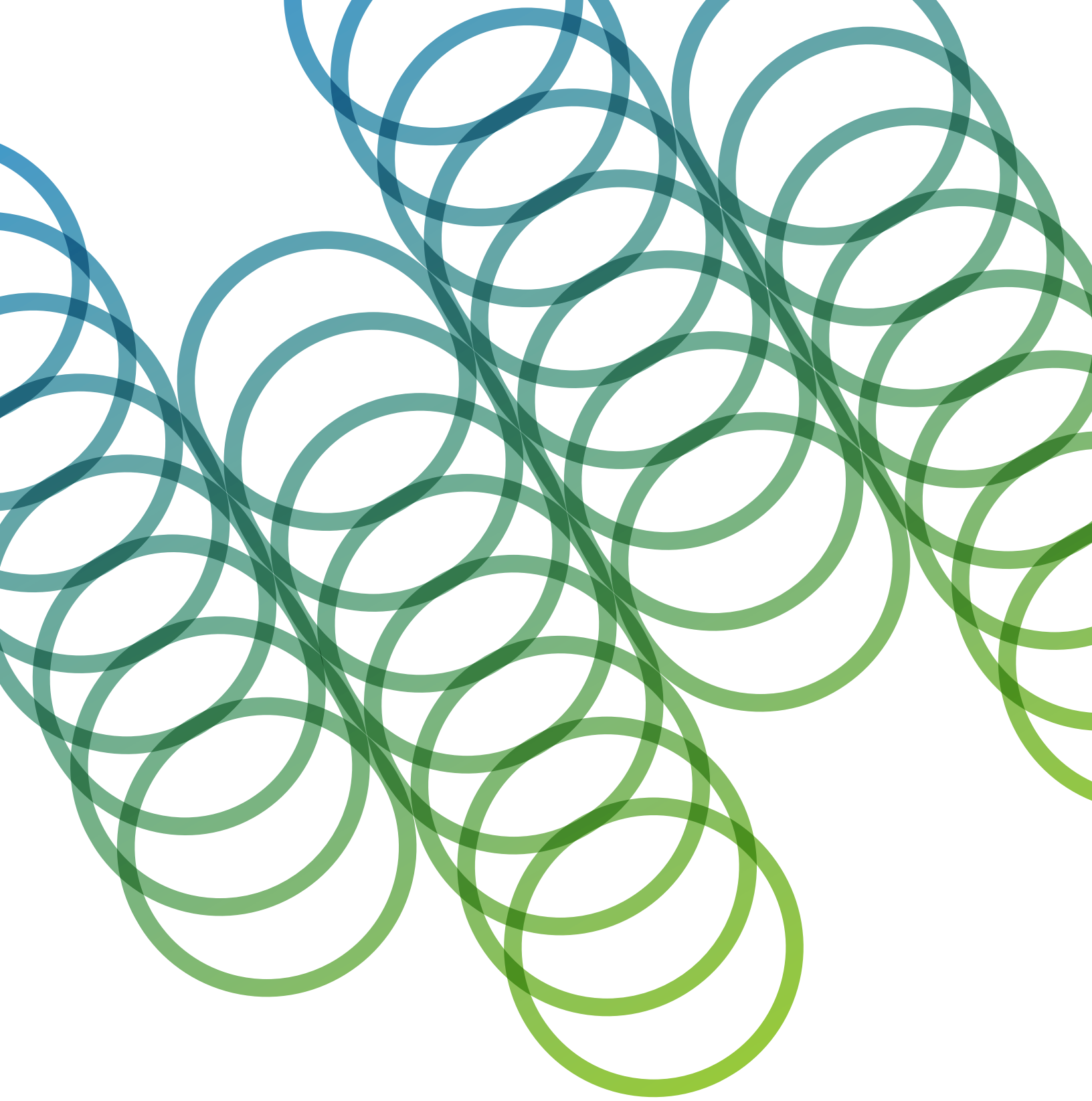

EpiCypher®
Bringing Epigenetics to Life

CUTANA™ ATAC-seq Kit Version 1

User Manual Version 1.0

EpiCypher®

CUTANA™

ATAC-seq Kit

Use with CUTANA™ Nextera-Compatible CDI Primers

Kit Version 1

Catalog No. 14-1121-24rxn: 24 Reaction Kit

Catalog No. 14-1190-24rxn: 24 Reaction CDI Primers

Upon receipt, store indicated components at 4°C, -20°C, -80°C, and room temperature (RT)

See p. 7 for storage instructions.

EpiCypher, Inc. PO Box 14453, Durham, NC 27713
Ph: 1-855-374-2461 www.epicypher.com Email: info@epicypher.com
Tech Support: support.epicypher.com techsupport@epicypher.com

24 Hour Emergency Phone Number:
US & Canada: 1-800-535-5053 | International: 1-352-323-3500

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See EpiCypher's Tech Support Center at support.epicypher.com for additional ATAC-seq FAQs and troubleshooting guidance.

Introduction

Assay for **T**ransposase-**A**ccessible **C**hromatin using **seq**uencing (ATAC-seq) is a widely adopted epigenomic assay for profiling chromatin accessibility[1]. The assay leverages hyperactive Tn5, a prokaryotic transposase, to cleave and tag the genomic DNA at open chromatin regions with sequencing adapters. Tagmented fragments are then amplified via PCR, yielding next-generation sequencing-ready libraries.

Open chromatin regions are enriched for functional elements such as promoters, enhancers, and transcription factor binding sites, providing multilayered insights into gene regulation mechanisms. ATAC-seq can therefore be used to compare regulatory landscapes across cell types, developmental states, disease conditions, or experimental treatments.

The CUTANA™ ATAC-seq Kit, when combined with Nextera-Compatible CDI Primers (EpiCypher 14-1190-24rxn), contains key reagents required to generate 24 sequencing-ready libraries with high consistency and sensitivity. Key features of CUTANA™ ATAC-seq include:

- **A streamlined and rapid workflow.** Adapted from the Omni-ATAC-seq protocol[2], the CUTANA ATAC-seq workflow incorporates the use of 8-strip tubes and bead-based DNA purification, and can be completed in just a few hours.
- **High-quality and reproducible data.** Consistently generates robust open chromatin profiles (Figure 1).
- **Diverse sample compatibility.** Validated for cells and nuclei, including cryopreserved nuclei extracted from tissue samples. Sample prep guidelines are available at support.epicypher.com.
- **Low input requirements.** Although it is recommended to target 50,000 nuclei per standard reaction, comparably high-quality data can be generated down to 1,000 nuclei (Figure 2), making this kit well-suited for rare sample types and low-input applications.
- **Comprehensive customer support.** Support.epicypher.com provides detailed information about ATAC-seq assay optimization, sample prep, sequencing analysis, and troubleshooting.

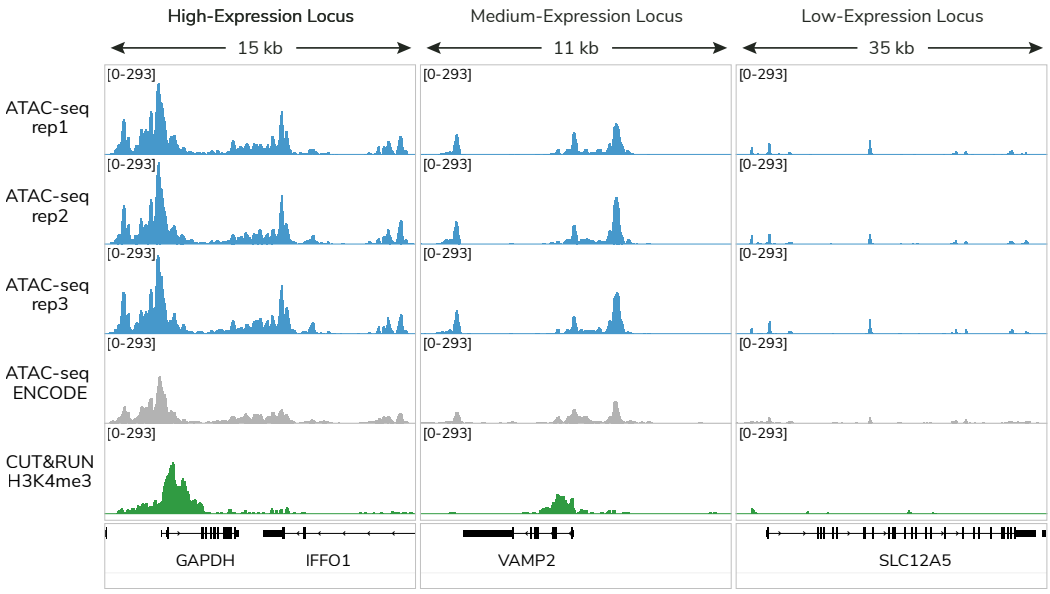


FIGURE 1

The CUTANA ATAC-seq Kit demonstrates reproducible performance across experiments by multiple scientists on multiple days. Representative genome browser tracks show results using 50,000 K562 nuclei at locations of genes known to have high, medium, and low expression by RNA-seq (SRR4235541, SRR4235542). Clear peaks with expected open chromatin profiles are observed using 20–50 million unique sequencing reads per reaction. The ENCODE ATAC-seq track (GSE170214) and a representative CUT&RUN H3K4me3 track are shown at the same locations for reference.

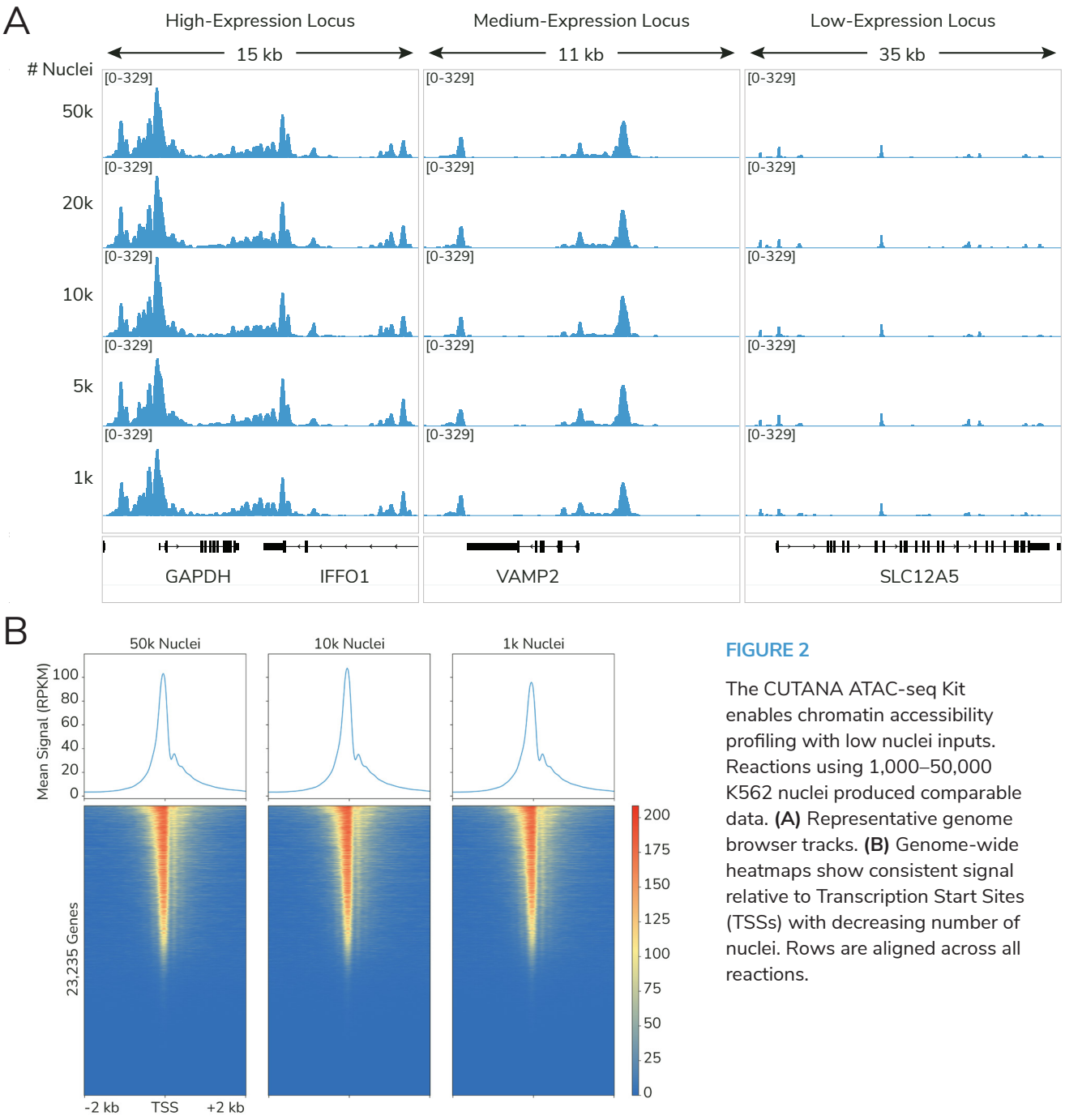


FIGURE 2

The CUTANA ATAC-seq Kit enables chromatin accessibility profiling with low nuclei inputs. Reactions using 1,000–50,000 K562 nuclei produced comparable data. **(A)** Representative genome browser tracks. **(B)** Genome-wide heatmaps show consistent signal relative to Transcription Start Sites (TSSs) with decreasing number of nuclei. Rows are aligned across all reactions.

Outline of Workflow

Here, we review the main steps of the CUTANA™ ATAC-seq workflow (Figure 3):

- **Intact nuclei isolation**
  Nuclei are extracted from bulk cell populations. High-quality sample prep is essential for successful ATAC-seq reactions. Detailed quality check steps are included to confirm the quality and accurate counting of the input sample.
- **Tn5-mediated tagmentation and DNA cleanup**
  Adapter-loaded Tn5 tagments accessible DNA in the genome and appends sequencing adapters on both ends. Tagmented DNA is purified with a user-friendly approach using CUTANA DNA Purification Beads.
- **Indexing PCR and library cleanup**
  Tagmented DNA is amplified by PCR with CUTANA Nextera-Compatible Primers (sold separately; see EpiCypher 14-1190-24rxn), and cleaned up with CUTANA DNA Purification Beads. These kits generate up to 24 uniquely barcoded libraries that can be multiplexed for sequencing (See Appendix).
- **Library quality analysis and Illumina® next-generation sequencing**
  Purified libraries are analyzed on Agilent Bioanalyzer® or TapeStation® to evaluate fragment size distribution and determine library concentrations. See Protocol Section V for example TapeStation traces of ATAC-seq libraries. High-quality libraries are diluted and pooled for Illumina paired-end sequencing (2 x 50 bp).

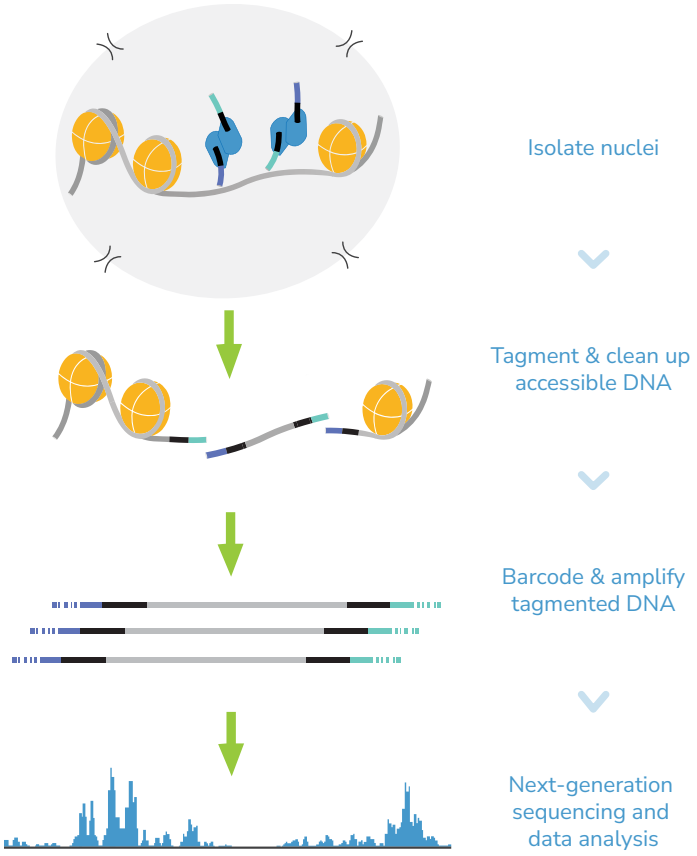


FIGURE 3
Overview of the CUTANA™ ATAC-seq workflow.

Included in the Kit

Kit components are stable for 6 months upon date of receipt. Store as outlined below.

Store at room temperature (RT) upon receipt:

Item	Catalog No.	Notes before use
8-strip Tubes	10-0009-12	Enables use of multi-channel pipettors.
10X PBS	21-1035-12	Use to prepare 1X PBS, free of calcium and magnesium.
Stop Solution	21-1036-12	Use to quench tagmentation.
DNA Purification Beads	21-1407-12	**DO NOT FREEZE.** Slightly viscous: thoroughly mix before use and pipette carefully to ensure correct volume.
0.1X TE Buffer	21-1025-12	Use to elute tagmented DNA and ATAC-seq libraries.

Store at 4°C upon receipt:

Item	Catalog No.	Notes before use
10X Wash Buffer	21-1037-12	Use to prepare Wash Buffer 1.
10% Tween-20	21-1038-12	Use to prepare Wash Buffer 2.
Lysis Buffer	21-1039-12	Use to prepare ATAC-seq Nuclei Extraction Buffer.

Store at -20°C upon receipt:

Item	Catalog No.	Notes before use
2X Tagmentation Buffer	21-1040-12	Use to prepare Tagmentation Master Mix.
Enzyme Diluent	21-1041-12	**GLYCEROL CONTAINING BUFFER: quick spin before use.** Use to dilute Tn5 Transposome during the assay if necessary.
Non-Hot Start 2X PCR Master Mix	15-1018-12	Use for PCR amplification and indexing of ATAC-seq libraries.
5% Digitonin	21-1004-12	Thaw at RT. Use to prepare ATAC-seq Nuclei Extraction Buffer and Tagmentation Master Mix.

Store at -80°C upon receipt:

Item	Catalog No.	Notes before use
Tn5 Transposome	15-1036-12	**GLYCEROL CONTAINING BUFFER: thaw and quick spin before use.** Tn5 loaded with barcoded adapters, use with Nextera-Compatible Primers to amplify ATAC-seq libraries.

Materials Required but Not Supplied

NEXTERA-COMPATIBLE PRIMERS:

- CUTANA™ Nextera-Compatible CDI Primer Set 1 (EpiCypher 14-1190-24rxn)

REAGENTS:

- Molecular biology grade water, DNase and RNase free
- 100% Ethanol (200 proof), any vendor
- 0.4% Trypan Blue (e.g., Invitrogen T10282)

EQUIPMENT:

- 1.5 mL microcentrifuge tubes
- 15 mL and 50 mL conical tubes
- Low-retention filter pipette tips
- Magnetic separation rack for 8-strip tubes (EpiCypher 10-0008)
- 8-channel multi-pipettor (e.g., VWR 76169-250) and multi-channel reagent reservoirs (e.g., Thermo Fisher Scientific 14-387-072)
- Automated cell counter or brightfield/phase contrast microscope
- Cell counting slides or a hemocytometer
- Vortex (e.g., Vortex-Genie® 2, Scientific Industries SI-0236)
- Benchtop centrifuge/mini-centrifuge with an 8-strip tube adapter (e.g., from Fisher Scientific, Benchmark Scientific)
- Refrigerated microcentrifuge
- Thermocycler with heated lid (e.g., from BioRad, Applied Biosystems, Eppendorf)
- Thermomixer (Optional)

  * See application note at Step 24

- Capillary electrophoresis machine and required reagents, e.g., Agilent TapeStation with D1000 ScreenTape (5067-5582) and D1000 reagents (5067-5583) or Agilent Bioanalyzer with High Sensitivity DNA Kit (5067-4626)

SUCCESS METRICS AND OPTIMIZATION OVERVIEW

- The CUTANA ATAC-seq Kit provides multiple quality control checks (Figure 4), including established ATAC-seq success metrics, to support reliable ATAC-seq workflows[5].
- To assess ATAC-seq library quality based on enrichment of accessibility signals around Transcription Start Sites (TSSs), calculate the TSS enrichment score according to ENCODE recommendations as a functional signal-to-background measure.
- Sample prep and efficient tagmentation are key to ATAC-seq success. Ensure samples and tagmentation reagents are handled properly.
- For reactions within the validated input range (1,000–50,000 nuclei), use 5 Units of Tn5 Transposome per reaction as the standard condition. If over-tagmentation is observed, dilute the Tn5 Transposome as needed.
- Visit the ATAC-seq Tech Support Center or contact techsupport@epicypher.com for our most up-to-date tips and troubleshooting guidelines.

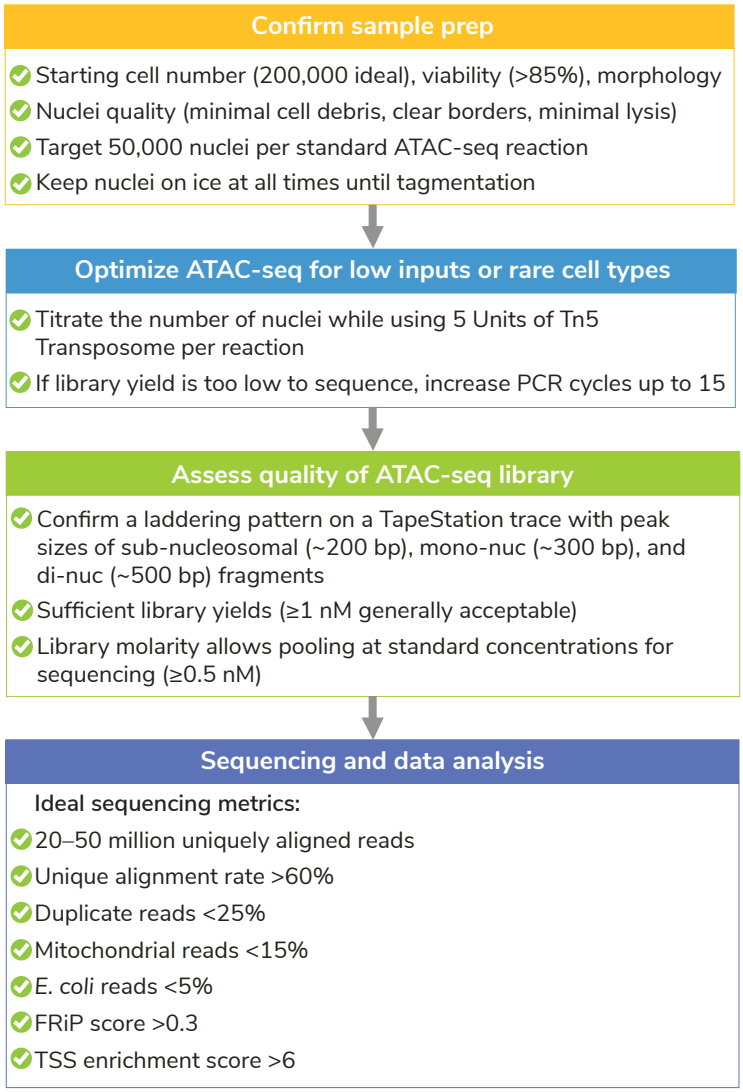


FIGURE 4

Quality control metrics and optimization tips to ensure successful open chromatin profiling using the CUTANA™ ATAC-seq Kit.

SECTION I: BUFFER PREP (~30 MIN)

IMPORTANT NOTES ON BUFFER PREP

* Buffers are prepared **FRESH** on the day of every ATAC-seq experiment.
* Volumes in Table 1 are per ATAC-seq reaction and include 20% excess to account for pipetting errors. You do **NOT** need to add additional volume.

1. Gather kit reagents stored at RT, 4°C, -20°C, and -80°C needed for ATAC-seq: **10X PBS, 10X Wash Buffer, Tween-20, Lysis Buffer, 2X Tagmentation Buffer, Tn5 Transposome, Non-Hot Start 2X PCR Master Mix**, and **Digitonin**. Place **Digitonin** at RT and all other reagents on ice to thaw or equilibrate.
2. Prepare **1X PBS**, **Wash Buffer 1**, and **ATAC-seq Nuclei Extraction Buffer** as outlined in Table 1. Place on ice.
3. To a new tube labeled **Wash Buffer 2**, add Wash Buffer 1 and 10% Tween-20 as outlined in Table 1. Place on ice.
4. To a new tube labeled **Tagmentation Master Mix**, add 2X Tagmentation Buffer, 1X PBS, 5% Digitonin, and 10% Tween-20 as outlined in Table 1. Place on ice.
5. Dispense 37.5 µL Tagmentation Master Mix per reaction to labeled 8-strip tubes. Place on ice.

Buffer Sample Scaling Calculations

COMPONENT	[Final]	1rxn	8rxn	12rxn
1X PBS - keep on ice for same day use, store remainder at RT				
10X PBS	1X	30 µL	240 µL	360 µL
Molecular Grade H_2O	-	270 µL	2.16 mL	3.24 mL
Wash Buffer 1 - keep on ice for same day use				
10X Wash Buffer	1X	240 µL	1.92 mL	2.88 mL
Molecular Grade H_2O	-	2.16 mL	17.3 mL	25.9 mL
ATAC-seq Nuclei Extraction Buffer - keep on ice for same day use				
Lysis Buffer	-	60 µL	480 µL	720 µL
5% Digitonin*	0.01%	0.12 µL	0.96 µL	1.44 µL
Wash Buffer 2 - keep on ice for same day use				
Wash Buffer 1	-	1.2 mL	9.6 mL	14.4 mL
10% Tween-20	0.1%	12 µL	96 µL	144 µL
Tagmentation Master Mix - keep on ice for same day use				
2X Tagmentation Buffer	-	30 µL	240 µL	360 µL
1X PBS	2 mM	14.3 µL	114.4 µL	171.6 µL
5% Digitonin*	0.01%	0.12 µL	0.96 µL	1.44 µL
10% Tween-20	0.1%	0.6 µL	4.8 µL	7.2 µL

**For fewer than 8 reactions, dilute Digitonin stock 10-fold in molecular grade water before use.*

TABLE 1

Combine reagents as instructed in the table to prepare ATAC-seq Buffers. Calculations for 8 and 12 reactions are provided. **All buffers include 20% extra volume to account for pipetting error – no additional overage is needed.**

SECTION II: NUCLEI EXTRACTION (~1 HR)

ATAC-SEQ SAMPLE PREP NOTES

* **High-quality sample prep is essential to ATAC-seq.** The quality control steps in this protocol support robust nuclei extraction for successful ATAC-seq.
* This protocol uses 50,000 fresh K562 nuclei per ATAC-seq reaction as an example. Ideal cell viability may vary by sample type, treatments, or processing conditions. The goal is to harvest cells with good integrity and minimal lysis.

6. Count starting cells (in tissue culture flask, tube, cryopreserved cells, etc.) and confirm cell integrity as follows:
    a. Transfer 10 µL cells to a fresh tube.
    b. Add 10 µL 0.4% Trypan Blue. Pipette 3 times to mix.
    c. Quickly transfer 10 µL of the cell-Trypan Blue mixture to a cell counting slide. Obtain cell counts, determine viability, and confirm expected cellular morphology using a brightfield/phase microscope or cell counter. See Figure 5A (p. 13).
    d. Perform 2 counts and calculate average.
7. Harvest at least 200,000 cells per reaction by centrifugation at 500 x g for 5 min at 4°C.

* A starting input of 200,000 cells is recommended to recover up to 50,000 nuclei per reaction. Actual nuclei recovery may vary by sample type and preparation conditions. If starting material is limited, empirically determine the input cell number needed to recover sufficient nuclei for tagmentation.
* To minimize sample loss during centrifugation, batch process 200,000 to 500,000 cells for nuclei extraction in a single 1.5 mL Eppendorf tube. Isolated nuclei will be aliquoted into 8-strip tubes in subsequent steps for individual tagmentation reactions.

8. Carefully remove media without disturbing cell pellet. Resuspend in 100 µL per reaction of cold **1X PBS**, gently pipetting 5–10 times. Keep cells and nuclei on ice from this point forward.
9. Perform another cell count with Trypan Blue as previously described.
10. Transfer up to 500,000 cold PBS-resuspended cells into each 1.5 mL tube for batch-processed nuclei extraction. Keep cells on ice.
11. Spin 500 x g for 5 min at 4°C. Pipette to remove supernatant without disturbing the pellet.
12. Gently resuspend cells in 1 mL cold **Wash Buffer 1** per 1.5 mL tube.
13. Spin 500 x g for 5 min at 4°C. Carefully and slowly remove **entire** supernatant without disturbing the pellet. Keep cell pellet on ice.

* It is important to remove as much of the supernatant as possible to avoid diluting the subsequent lysis step: First use a P1000 pipette, then a P200.

NOTE

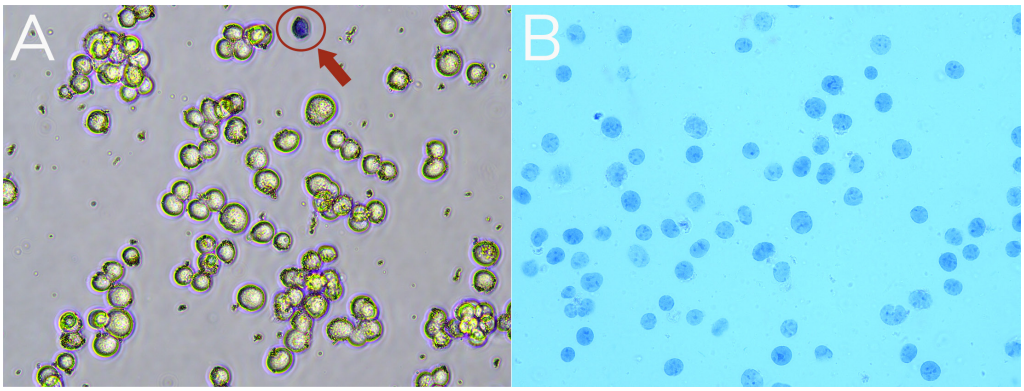
* **Nuclei extraction is time sensitive.** Perform Steps 14–15 in batches of 4 tubes or fewer to avoid over-lysis. Use a timer to closely monitor the lysis duration and work quickly.

14. Resuspend cell pellet in 50 µL cold **ATAC-seq Nuclei Extraction Buffer** per tube (with ≤500,000 cells) by gentle pipetting. Place on ice and incubate for 3 minutes.
15. When 3-minute lysis is complete, quench by adding 1 mL of cold **Wash Buffer 2** per tube. Cap tube and invert a few times to mix before returning to ice.
16. When all samples are lysed and quenched, pellet nuclei by spinning 500 x g for 5 min at 4°C.
17. Carefully remove tubes from the centrifuge and gently place on ice. One tube at a time, gently remove all supernatant, without touching the far side walls or bottom of tube with nuclei. Work quickly as nuclei pellets are fragile and may loosen over time.

* During centrifugation, make sure all tubes face the same orientation, or mark the side of the tube towards the outer perimeter of the rotor. Pellets may or may not be visible, and it should be assumed that nuclei can pellet against the entire side of the tube facing outward of the rotor.
* Remove as much supernatant as possible after centrifugation: mitochondria are in the supernatant, and carryover will result in higher mitochondrial reads in sequencing.

18. Resuspend nuclei in 12 µL cold 1X PBS per reaction (e.g., if starting with cells batch processed for two reactions in an Eppendorf tube, use 24 µL cold 1X PBS). Gently rinse entire side wall of the Eppendorf tube while resuspending. If multiple Eppendorf tubes were processed with the same sample type, they should be pooled for counting. **Keep nuclei on ice**.
19. Count extracted nuclei and confirm integrity as follows (note that accurate nuclei counting is essential to ensure ATAC-seq quality and reproducibility):
    a. Transfer 2 µL nuclei to a fresh tube and add 8 µL 1X PBS.
    b. Add 10 µL 0.4% Trypan Blue. Pipette 3 times to mix.
    c. Transfer 10 µL of the nuclei-Trypan Blue mixture to a cell counting slide. Obtain nuclei counts, determine lysis completeness, and confirm expected morphology using a brightfield/phase microscope or cell counter. See Figure 5B.
    d. Calculate average from 2 counts. Multiply by 5 to determine actual nuclei concentration.
20. If the average nuclei concentration for a sample type is ≥5 x 10^6 / mL, bring the concentration down to 5 x 10^6 / mL by adding cold 1X PBS. This will result in a resuspension that contains 50,000 nuclei per 10 µL for each reaction.
21. If average nuclei for a sample type is <5 x 10^6 / mL, calculate actual number of nuclei per 10 µL.

* The CUTANA ATAC-seq Kit is validated for inputs ranging from 1,000 to 50,000 human nuclei using 5 Units of Tn5 Transposome per reaction. For reactions using fewer than 50,000 nuclei, use cold 1X PBS to adjust the concentration so that the resuspension contains the desired number of nuclei per 10 µL. See this Tech Support Center article for additional guidance on low-input samples.



Sample	Success Metrics	Troubleshooting Tips
Starting cells Figure 5A	Cells should be bright white (Trypan Blue excluded), round, and unclumped. Fresh cells ideally show >90% viability. Excess dead cells may reduce DNA yields and impact data quality.	Optimize cell culture conditions; use fresh media, evaluate potential contamination issues.
Extracted nuclei Figure 5B	Debris visible but mostly not attached to nuclei. Most nuclei should be intact.	Closely monitor cells during nuclei extraction by Trypan Blue staining to optimize incubation time.

FIGURE 5

Representative images of K562 cell and nuclei samples for ATAC-seq. Samples were stained with Trypan Blue and visualized under brightfield microscope. **(A)** Starting cells are mostly excluding Trypan Blue. A dead cell (blue; Trypan positive) is circled in red, whereas the remaining live cells are bright white and round. **(B)** Successful nuclei extraction shows Trypan Blue stained nuclei.

SECTION III: TAGMENTATION AND CLEANUP (~1.5 HRS)

NOTE

* This protocol recommends using 5 Units of CUTANA Tn5 Transposome per ATAC-seq reaction with 1,000–50,000 human nuclei. If working with a novel sample type, an organism with a genome size that differs significantly from human, or if over-tagmentation was observed in a previous ATAC-seq reaction (see Figure 7, p. 18, for example TapeStation traces), we recommend performing a Tn5 titration to establish the optimal Tn5:nuclei ratio. See this Tech Support Center article for more recommendations on Tn5 Transposome optimization.
* For reactions that require lower amounts of Tn5 Transposome, dilute Tn5 Transposome with Enzyme Diluent proportionally to keep the volume per reaction consistent (e.g., for a reaction with 0.5 Units of Tn5 Transposome, perform a 1:10 dilution of stock Tn5 Transposome in Enzyme Diluent, then add 2.5 µL of the dilution to the reaction). Use diluted Tn5 Transposome FRESH on the day of experiment and discard after use.

22. To 8-strip reaction tubes with 37.5 µL **Tagmentation Master Mix** (prepared in Step 5), add 2.5 µL **Tn5 Transposome** per reaction. While on ice, gently mix by pipetting.
23. While on ice, add 10 µL nuclei prepared according to Step 20 or 21. Gently mix by pipetting and quick spin to collect liquid. Total volume per tube at this point is 50 µL.
24. Perform tagmentation by incubating tubes at 37°C for 30 minutes at 1,000 rpm in a thermomixer that is compatible with 8-strip tubes.
    * *If an 8-strip tube-compatible thermomixer is unavailable, perform tagmentation in a static thermocycler, removing samples for brief vortexing every 10 min throughout the 30 min reaction.*
25. After tagmentation is complete, quick spin tubes to collect liquid and add 5 µL **Stop Solution** per reaction. Mix by gentle vortexing for 5 seconds and quick spin.
26. Transfer samples to a thermocycler pre-heated to 68°C with a heated lid at 105°C.
27. Incubate for 10 minutes at 68°C.
28. Remove tubes from thermocycler, quick spin, and allow to cool to RT.
29. Prepare 85% Ethanol (EtOH) fresh using 100% EtOH and molecular biology grade water. Make 500 µL per reaction: 425 µL 100% EtOH + 75 µL water. Note that these calculations include extra volume to account for pipetting error.
30. Vortex **DNA Purification Beads** to fully resuspend. Slowly add 99 µL DNA Purification Beads (1.8X reaction volume) to each reaction. Ensure pipette tip is free of extra droplets when dispensing to reactions.
31. Mix well by vortexing for 5 seconds to an even resuspension (critical for DNA Purification Bead binding). Quick spin tubes and incubate 5 min at RT.
32. Place tubes on a magnet for 2–5 min at RT, until solution clears (Figure 6A). Pipette to remove supernatant without disturbing beads.

33. Keeping tubes on the magnet, add 180 µL 85% EtOH directly onto beads. Pipette to remove supernatant.
34. Repeat the previous step one time.
35. Remove tubes from magnet. Quick spin to collect liquid, with caps facing in, to avoid dislodging beads. Return to magnet and pipette to remove residual EtOH.
36. Remove tubes from magnet and air-dry, caps open, for 2–5 min at RT. Beads should appear damp matte brown (Figure 6B). If beads are crackly and/or light brown, they are too dry and DNA yield will be reduced.
37. Add 20 µL **0.1X TE Buffer** to each reaction to elute tagmented DNA. Pipette and/or vortex to fully resuspend beads and quick spin. Incubate for 2 min at RT.
38. Place tubes on magnet for 2 min at RT.
39. Transfer 17.5 µL of eluate to new 8-strip tubes. This is the tagmented DNA.

    Safe pause point. Tagmented DNA can be stored at -20°C.

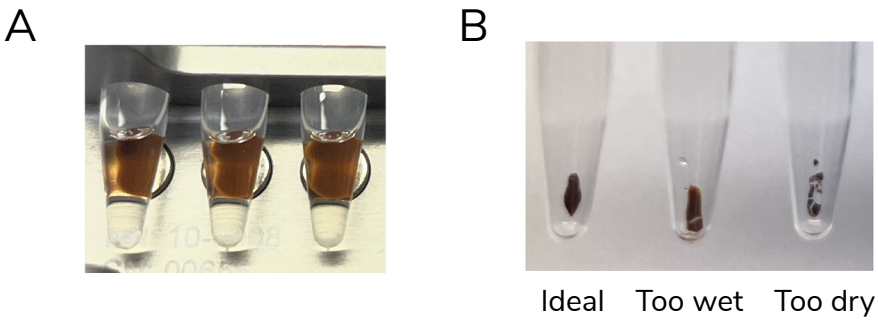


FIGURE 6

Expected bead appearances during tagmentation product cleanup. **(A)** Beads after mixing with tagmentation reactions and magnetic separation. **(B)** Air-dried beads after EtOH washes.

SECTION IV: PCR AMPLIFICATION AND CLEANUP (~1.5 HRS)

40. Assign a unique pair of i5 and i7 Primers from **CUTANA Nextera-Compatible CDI Primer Set 1 (EpiCypher 14-1190-24rxn)** to each ATAC-seq reaction as instructed in Appendix.

* **Illumina sequencing runs containing only ATAC libraries should include at least two i5 barcodes (sequential odd and even; e.g., i501 and i502) to ensure proper color balancing on the sequencer.**

41. Retrieve the 10 µM **i5** and **i7 Primers**, thaw on ice, and quick spin before each use. If necessary, thaw frozen tagmented DNA on ice.

42. For indexing PCR, add primers and PCR Master Mix directly to reactions in 8-strip tubes containing 17.5 µL tagmented DNA on ice. To each reaction add: 6.25 µL **i5 Primer**, 6.25 µL **i7 Primer**, and 30 µL **Non-Hot Start 2X PCR Master Mix**. Gently vortex to mix and quick spin. Change tips between each addition to prevent cross-contamination.

43. Place tubes in a thermocycler with a heated lid set to 105°C. Perform PCR using the parameters below to amplify tagmented DNA.

Step #	Temperature	Time	Cycles
1	72°C	5 min	1
2	98°C	30 sec	1
3	98°C	10 sec	10*
4	63°C	30 sec	
5	72°C	1 min	
6	4–12°C	∞	1

* 10 PCR cycles is the standard condition and generates robust open chromatin profiles from 10,000-50,000 K562 nuclei. Optimize as needed for lower nuclei inputs, less-characterized samples, or if troubleshooting low yields. For ultra-low input (e.g., 1,000 nuclei), use 15 cycles to increase yield for sequencing.

Increasing PCR cycles may result in higher duplication rates and lower unique alignment rates.

44. Remove tubes from thermocycler, quick spin, and allow to return to RT.

45. Prepare 85% Ethanol (EtOH) fresh using 100% EtOH and molecular biology grade water. Make 500 µL per reaction: 425 µL 100% EtOH + 75 µL water. Note that these calculations include extra volume to account for pipetting error.

46. Vortex DNA Purification Beads to fully resuspend. Slowly add 108 µL DNA Purification Beads (1.8X reaction volume) to each reaction. Ensure pipette tip is free of extra droplets when dispensing to reactions.

47. Mix well by pipetting and/or vortexing to an even resuspension (critical for DNA Purification Bead binding). Quick spin tubes and incubate 5 min at RT.

48. Place tubes on a magnet for 2–5 min at RT, until solution clears. Pipette to remove supernatant without disturbing beads.
49. Keeping tubes on the magnet, add 180 µL 85% EtOH directly onto beads. Pipette to remove supernatant.
50. Repeat the previous step one time.
51. Remove tubes from magnet. Quick spin to collect liquid, with caps facing in, to avoid dislodging beads. Return to magnet and pipette to remove residual EtOH.
52. Remove tubes from magnet and air-dry, caps open, for 2–5 min at RT. Beads should appear damp matte brown (Figure 6B, see p. 15). If beads are crackly and/or light brown, they are too dry and DNA yields will be reduced.
53. Add 20 µL 0.1X TE Buffer to each reaction to elute tagmented DNA. Pipette and/or vortex to fully resuspend beads and quick spin. Incubate for 2 min at RT.
54. Place tubes on magnet for 2 min at RT.
55. Transfer 17.5 µL of eluate to new 8-strip tubes. This is the ATAC-seq library.
    Safe pause point. Library can be stored at -20°C.

SECTION V: ANALYSIS OF LIBRARY FRAGMENT SIZE (~1 HR)

56. Use 1 µL ATAC-seq library for analysis on the Agilent Bioanalyzer (High Sensitivity DNA Kit) or TapeStation (D1000 ScreenTape System). Set region to 150–700 bp for quantitation. Typically, libraries made from 50,000 K562 nuclei input can be expected to be >20 nM. Lower library yield is expected with lower-input or difficult samples, and is generally acceptable if ≥1 nM. See **Figure 7** for more expected TapeStation results.

NOTE

* An ideal ATAC-seq library shows a laddering pattern between 150–1,000 bp, with peaks corresponding to the size of subnucleosomal (~200 bp), mononucleosomal (~300 bp), dinucleosomal (~500 bp) fragments including adapters. Trinucleosomal peaks may also be visible.
* Over-tagmentation in ATAC-seq is shown by only 1 visible peak (~200 bp) in a TapeStation trace. Under-tagmented ATAC-seq fragments often show 2-3 shallow discernable peaks (>300 bp) with high molecular weight species (>1,000 bp). These libraries may still produce good-quality sequencing data. However, over-tagmentation may result in lower signal-to-noise and lower FRiP scores, and under-tagmentation may result in inaccurate quantitation and problems balancing pooled libraries.
* See this Tech Support Center article for help troubleshooting fragment distribution traces prior to sequencing.

SECTION VI: ILLUMINA® SEQUENCING AND DATA ANALYSIS

57. Select the appropriate Illumina sequencing platform based on the number of ATAC-seq libraries and desired sequencing depth. Paired-end sequencing is recommended (2 x 50 bp cycles). For sufficient coverage, sequence each library to contain ideally 20–50 million unique reads (after removing multimapping, duplicate, and DAC exclusion list reads).
58. Dilute and pool libraries using molarity calculations and load onto Illumina sequencer. General steps:
    a. Dilute each library to the same nM concentration, depending on final yields. For NextSeq 2000 and NextSeq 500/550, dilute to 1-4 nM.
    b. Pool equimolar libraries into one tube as instructed in this Tech Support Center article.
    c. Dilute pooled libraries following Illumina guidelines (support.illumina.com).
    d. When setting up a multiplexed sequencing run, ensure that each library contains a unique i5 and i7 Primer pair and that dual indexes are correctly assigned. For a full list of index sequences, download the CUTANA Nextera-Compatible Combinatorial Dual Indexing Primers spreadsheet at epicypher.com/14-1190 under Resources.

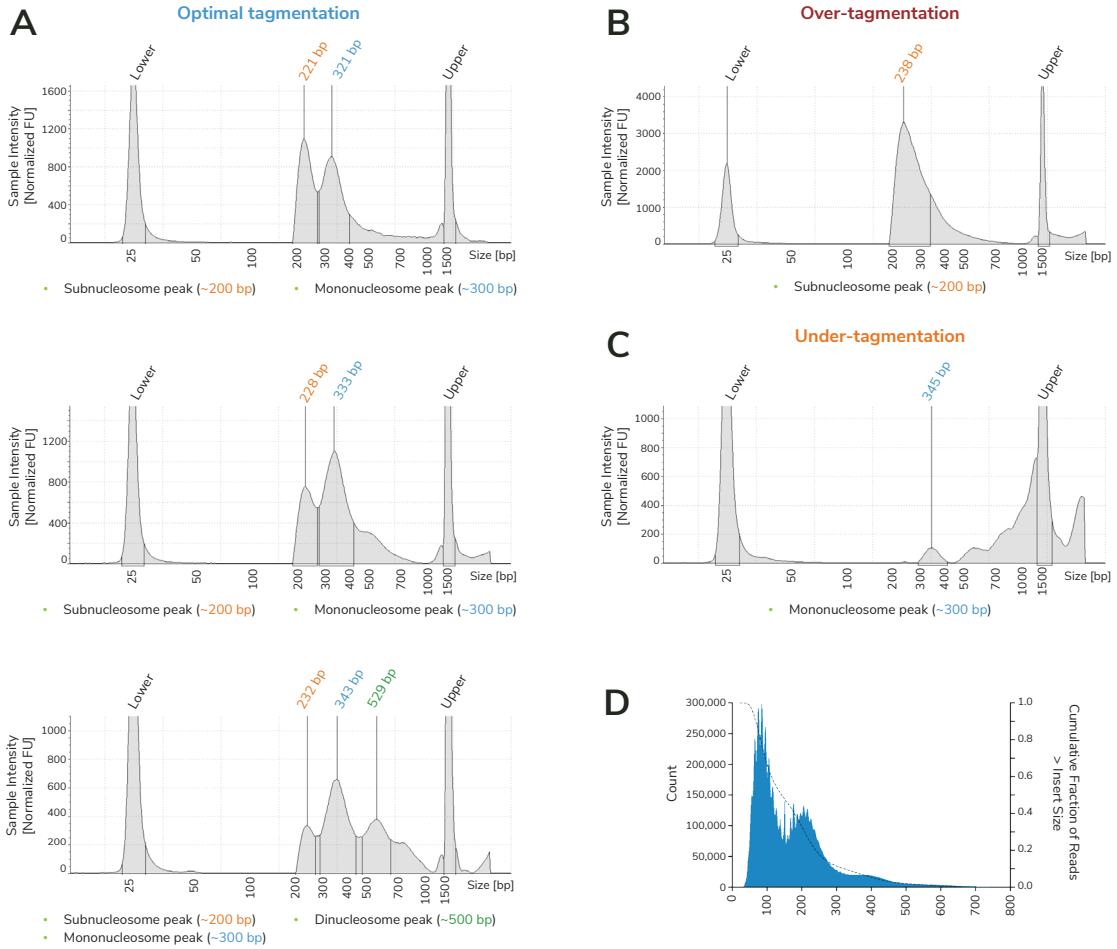


FIGURE 7

Commonly seen fragment size distributions from ATAC-seq libraries. Expected TapeStation traces for **(A)** optimally tagmented libraries, **(B)** over-tagmented libraries, and **(C)** under-tagmented libraries. **(D)** Typical insert size distribution histogram from sequenced ATAC-seq libraries using the CUTANA ATAC-seq Kit.

Frequently Asked Questions

See EpiCypher's Tech Support Center at support.epicypher.com for additional FAQs, troubleshooting guidance, and development updates on ATAC-seq.

1. How many cells are needed for ATAC-seq?

ATAC-seq can be successful with as few as 1,000 nuclei with robust cell types. However, when establishing a new cell line or if input material is plentiful, we recommend starting with 200,000 human cells to achieve 50,000 nuclei per standard reaction.

2. Should I dilute my Tn5 Transposome for low input reactions?

We recommend keeping Tn5 concentration at 5 Units per reaction for inputs ranging from 1,000 to 50,000 nuclei to ensure efficient tagmentation. See this Tech Support Center article for more tips on approaching low input ATAC-seq reactions.

3. Can I use cells or nuclei that have been cryopreserved?

Yes, properly cryopreserved cells and nuclei can be used. For quality control purposes, check cell permeability with Trypan Blue staining immediately after thawing. Properly cryopreserved cells show >85% viability (Trypan Blue excluding) and can yield good ATAC-seq libraries. If using cryopreserved nuclei, skip Steps 6–15. See this Tech Support Center article for more on sample types compatible with the CUTANA ATAC-seq Kit and sample prep notes.

4. What is the expected ATAC-seq library yield? What should I do if my yield is too low?

The yields for ATAC-seq libraries may vary based on sample types and input amount. Standard 50,000 K562 nuclei input consistently generates libraries above 20 nM. In general, lower library yield is expected with lower-input or difficult samples and is generally acceptable if ≥1 nM. See this Tech Support Center article for more optimization tips to improve yield and sample quality for low input ATAC-seq reactions.

5. What is the expected fragment size distribution from ATAC-seq libraries?

An ideal ATAC-seq library shows a laddering pattern between 150–1,000 bp, with peaks corresponding to the sizes of subnucleosomal (~200 bp), mono-nuc (~300 bp), di-nuc (~500 bp) fragments including adapters. Trinucleosomal peaks may also be visible.

6. What is the recommended read length and sequencing depth for ATAC-seq libraries?

We recommend performing 2 x 50 bp paired-end sequencing for standard ATAC-seq libraries.

20–50 million uniquely aligned reads typically provides reliable ATAC-seq data. The exact number of reads required is based on the specific research needs. Refer to this Tech Support Center article to learn more about the impact of read depths on data quality.

7. What is the expected percentage of duplicate reads and mitochondrial reads?

A high-quality ATAC-seq reaction is expected to contain <25% duplicate reads and <15% mitochondrial reads[3]. However, these metrics can vary with sample type, input number, sample prep quality, and PCR cycle number. See this Tech Support Center article for troubleshooting guidance.

8. Can I use CUTANA Cloud to analyze my ATAC-seq data?

The CUTANA Cloud ATAC-seq data analysis pipeline is coming soon! Check back on our website for updates, or reach out to our tech support team at techsupport@epicypher.com to get notified when it's live.

Appendix: Illumina Sequencing and Primer Selection Guide

ILLUMINA SEQUENCING PLATFORMS

* The CUTANA ATAC-seq Kit is compatible with Illumina high-throughput sequencing platforms (e.g., NextSeq 1000/2000).
* Paired-end sequencing (2 x 50 bp) is recommended for ATAC-seq.
* Libraries should be sequenced to a depth of 20–50 million uniquely aligned reads.
* The sequencing depth required for an ATAC-seq library may exceed the capacity of MiSeq systems. We suggest pooling with other labs on a higher-throughput sequencing platform.

PRIMER SELECTION GUIDE

* **Index sequences are available in an easy-to-copy spreadsheet at epicypher.com/14-1190 under Resources.**
* **Primers are not included in this kit. For best compatibility, we recommend using CUTANA™ Nextera-Compatible CDI Primer Set 1 (EpiCypher 14-1190-24rxn).**

We recommend pairing this kit with **CUTANA™ Nextera-Compatible CDI Primer Set 1 (EpiCypher 14-1190-24rxn)** to enable a combinatorial dual indexing strategy. Specifically, each ATAC-seq library is prepared with a distinct combination of two 8 bp barcodes, or indexes: one at the 5' end (i5 index), and the second at the 3' end (i7 index). The CDI Primer Set 1 comes with four i5 Primers and six i7 Primers, which can be used to generate 24 uniquely indexed libraries for multiplexed sequencing.

Figure 8 illustrates proper primer organization to facilitate successful i5 and i7 Primer pair selection and pipetting. Illumina sequencing runs containing only ATAC libraries should include at least two i5 barcodes (sequential odd and even; e.g., i501 and i502) to ensure proper color balancing on the sequencer. Do **NOT** repeat pairs of i5 and i7 Primers in a sequencing run. If an experiment will be combined with others on a single lane or flow cell, ensure that there is no overlap of primer pairs.

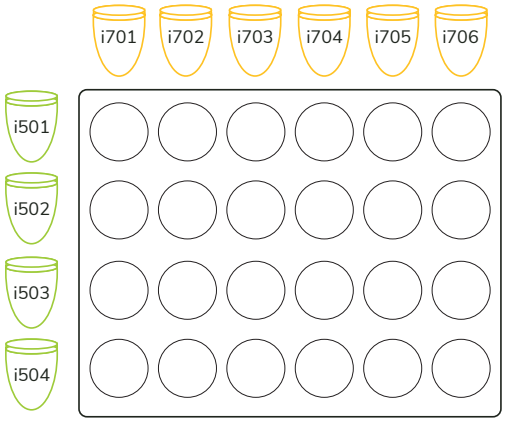


FIGURE 8

i5 and i7 Primers provided in **CUTANA™ Nextera-Compatible CDI Primer Set 1 (EpiCypher 14-1190-24rxn)** are organized to guide primer pair selection for successful multiplexed ATAC-seq sequencing. Orange tubes indicate i7 Primers i701–i706. Green tubes indicate i5 Primers i501–i504.

References

1. Buenrostro, Jason D et al. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. **Nat *Methods*** 10,12, 1213–8 (2013).
2. Corces, M Ryan et al. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. **Nat *Methods*** 14,10, 959–962 (2017).
3. Grandi, F.C., Modi, H., Kampman, L. et al. Chromatin accessibility profiling by ATAC-seq. **Nat *Protoc*** 17, 1518–1552 (2022).