BD Rhapsody™ ATAC-Seq Assays

Technical Training Deck

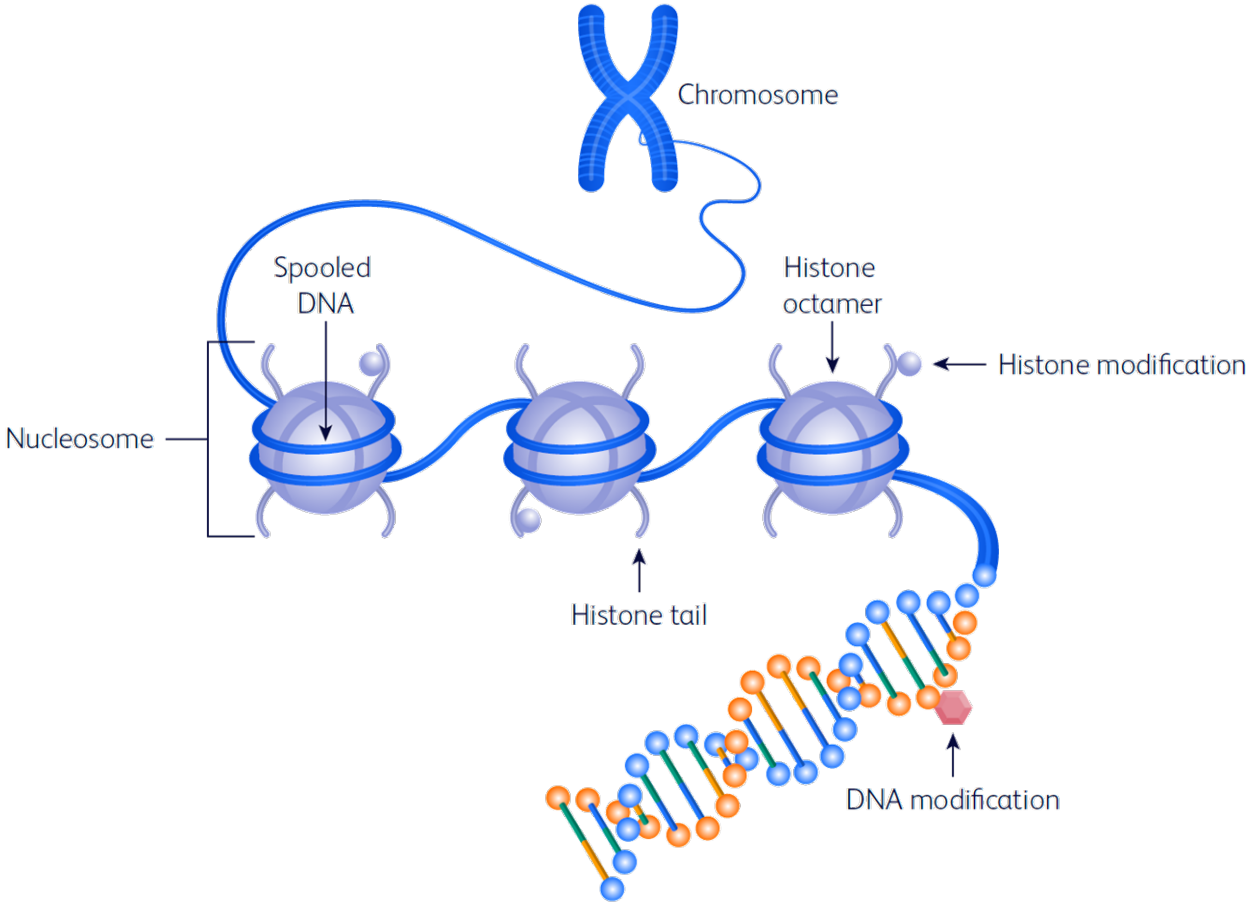
April 2024

BD

Introduction

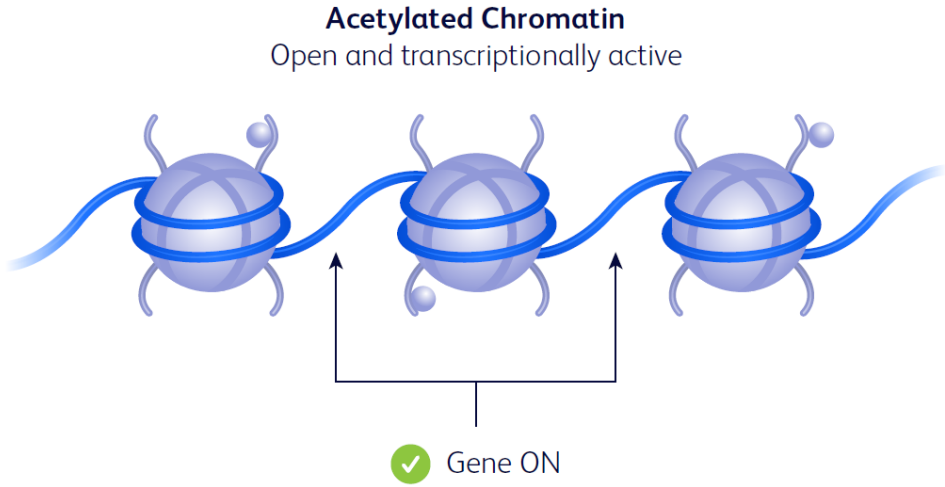
Introduction—chromatin structure

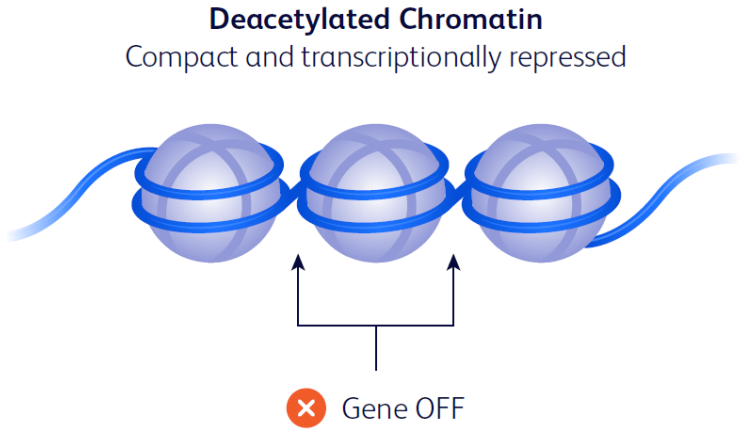
- DNA is packaged into a highly structured complex called chromatin.
- The structure of chromatin plays a crucial role in regulating gene expression through epigenetic processes.
- The basic unit of chromatin is the nucleosome, consisting of DNA wrapped around histone octamers.
- The histone tails protruding from the nucleosome are subject to various chemical modifications, including methylation and acetylation.
- These histone modifications can alter the degree of chromatin compaction and accessibility.
- Direct DNA modifications such as methylation can change the binding affinity of proteins to DNA and thereby influence gene expression.



Introduction—open vs closed chromatin

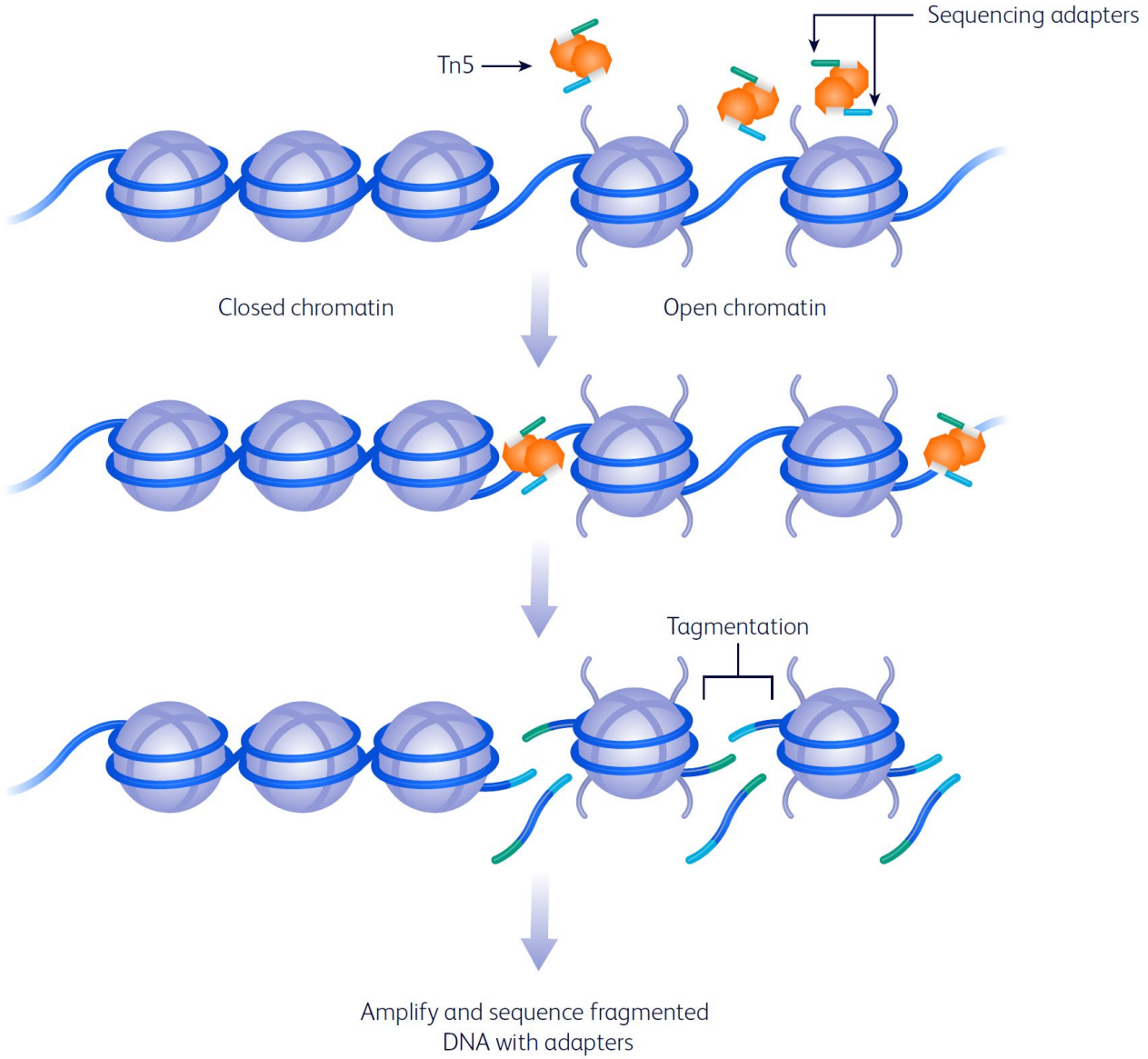
- As was mentioned earlier, histone modifications can change the degree to which chromatin is accessible. For instance:
- Acetylation of histone proteins is associated with an open, transcriptionally active state of chromatin.
- A relaxed chromatin structure is more accessible to transcriptional machinery, allowing genes in these regions to be expressed or “turned on.”
- In contrast, deacetylated chromatin is more condensed and has a compact structure.
- This condensed chromatin structure is generally associated with gene repression or “turning genes off,” as the tightly packed DNA is inaccessible to the machinery required for gene expression.





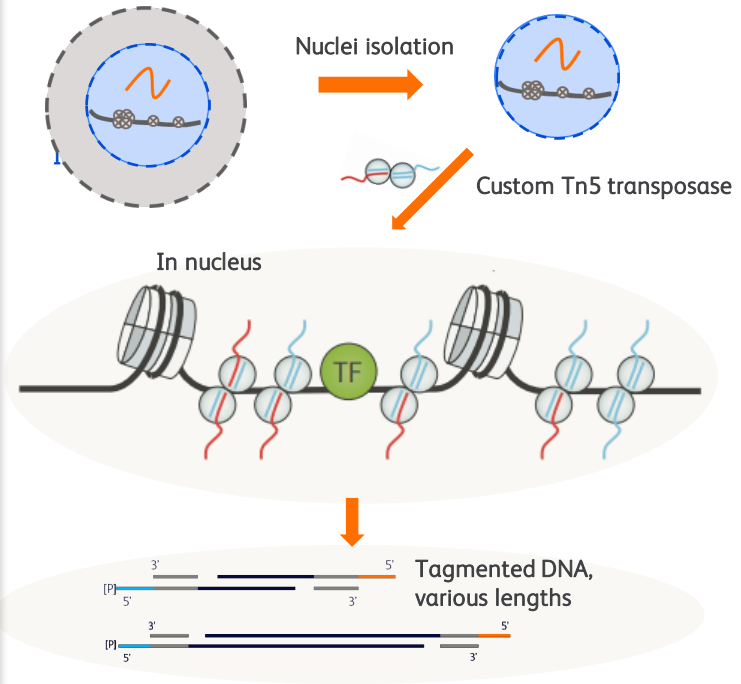
Introduction—ATAC-seq

- ATAC-seq utilizes a prokaryotic transposase enzyme called Tn5, which has been loaded with sequencing adapters.
- These Tn5 complexes can insert the adapters into regions of open, accessible chromatin, but not into closed, condensed chromatin.
- This process of simultaneously fragmenting and tagging the DNA is called tagmentation.
- The tagmented DNA fragments from open chromatin regions are then amplified and prepared for high-throughput sequencing.
- By sequencing these fragments and mapping them back to a reference genome, researchers can identify regions associated with gene regulatory elements like promoters and enhancers.



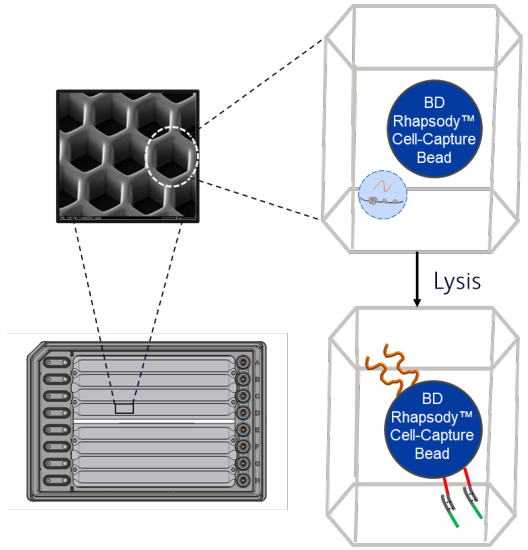
Introduction—BD Rhapsody™ ATAC-Seq Assay workflow

1. Sample preparation and in situ bulk tagmentation



- Tagmentation: 37 °C 30 min, with detergents

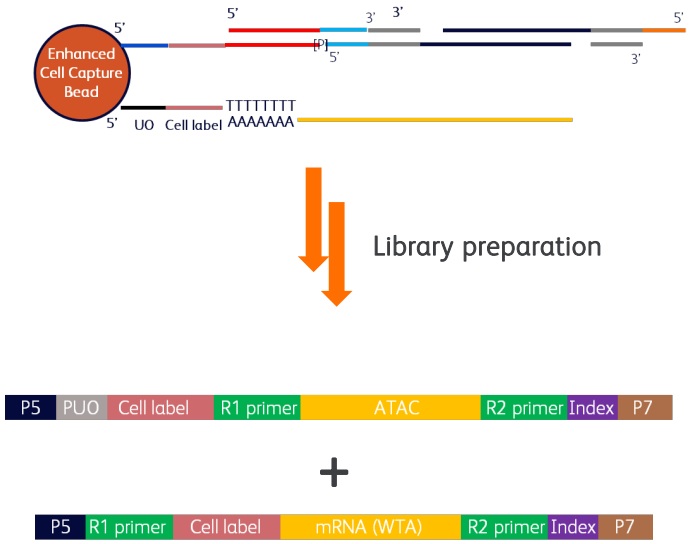
2. BD Rhapsody™ System single-cell partitioning, lysis and target capture



Both ATAC fragments and mRNA can be captured on beads

- Lysis: 37 °C 10 min; after adding lysis buffer, physically move cartridge to incubator (37 °C)

3. Library preparation and sequencing



- Standalone ATAC-Seq: Ligation → gap filling → index PCR and sequencing
- Multiomic ATAC-Seq: Ligation → RT (gap filling) → index PCR and sequencing

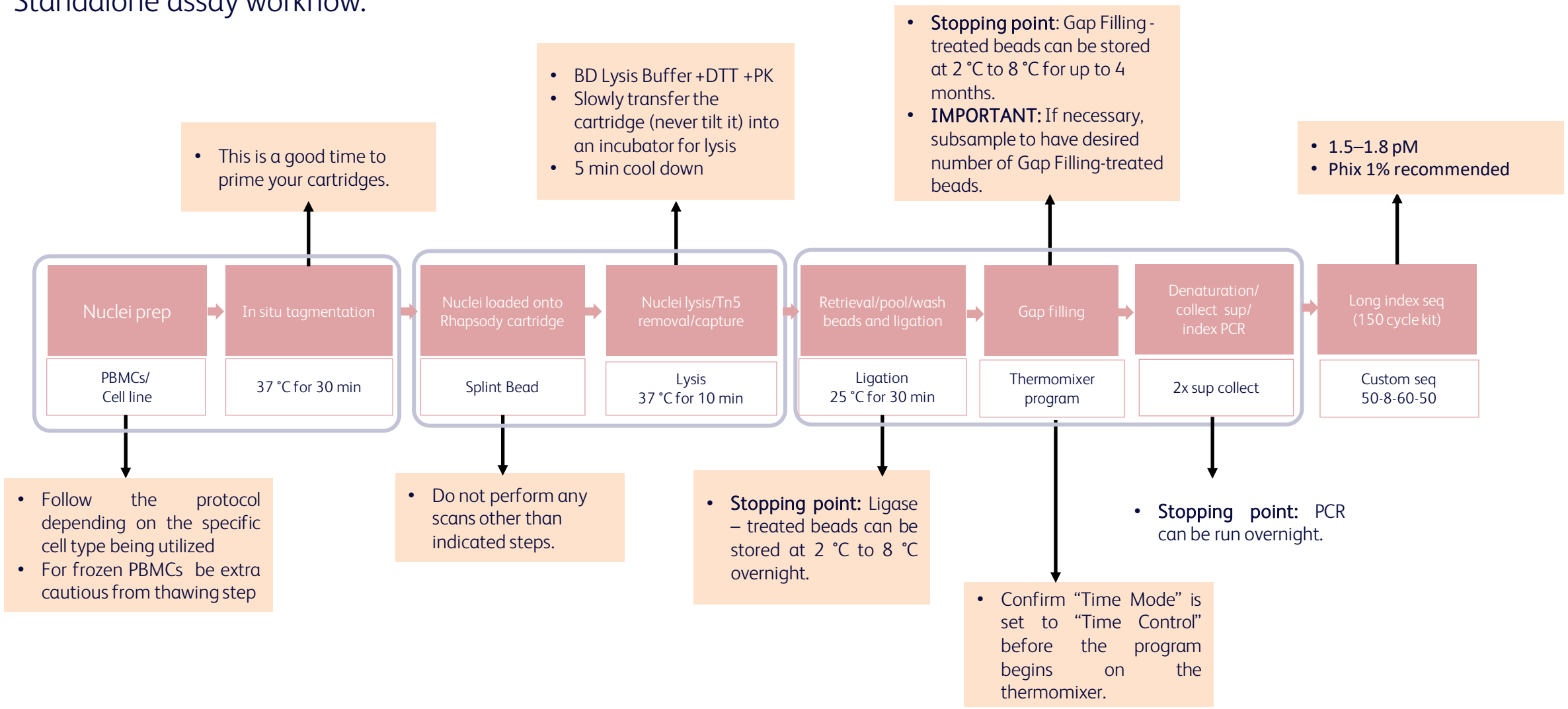
BD Rhapsody™ ATAC-Seq Assay Workflow

BD Rhapsody™ ATAC-Seq vs BD Rhapsody™ Whole Transcriptome Analysis (WTA) Assays

- Major differences between the two workflows on the BD Rhapsody™ HT Xpress System:
  1. Working on isolated nuclei
  2. Using Splint oligo loaded beads
  3. Tagmentation
  4. No scanning during the cartridge run except the bead agitation step (prior to priming the cartridge, scan at least one lane of the empty cartridge for cell load scan).
  5. Lysis time is 10 min at 37 °C in the incubator with 5 min cooling down
  6. Nuclei lysis: adding Proteinase K to the lysis buffer along with DTT
  7. Ligation for 30 min at room temperature
  8. Gap filling for standalone [no ExoI step] and Gap S(RT) and Exo I step for multiomics
  9. Single-Cell ATAC Library Index PCR, purifying and QC
  10. Sequencing strategy for ATAC library same i7 index for library indexing and long i5 index for cell label (50X50X60)

BD Rhapsody™ ATAC-Seq Assay

- Standalone assay workflow:



Library preparation steps (Single Cell ATAC-Seq)

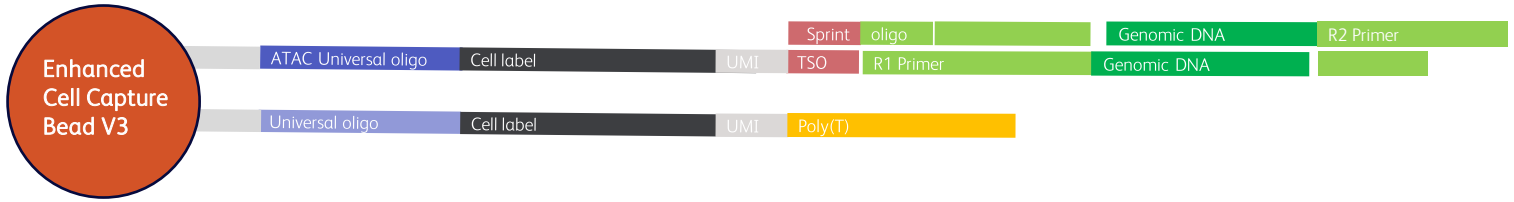
BD Rhapsody™ Enhanced Cell Capture Bead V3 layout

Splint Bead: Splint oligonucleotide is added to the beads to assist capturing of genomic DNA via capture oligo.

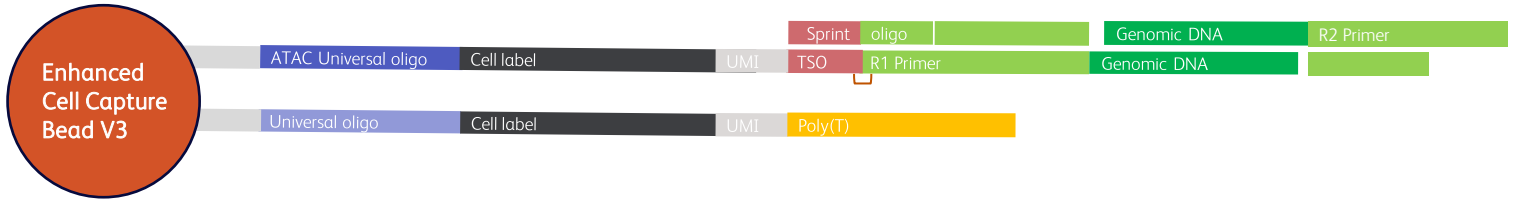
Tagmentation: During the bulk in situ tagmentation process, nuclei are exposed to a Tagmentation Mix, containing tagmentase. This enzyme targets accessible genomic regions (open chromatin areas), cutting the DNA and simultaneously attaching pre-loaded adapter sequences to the ends of each DNA fragment.

Library preparation steps (Single Cell ATAC-Seq)

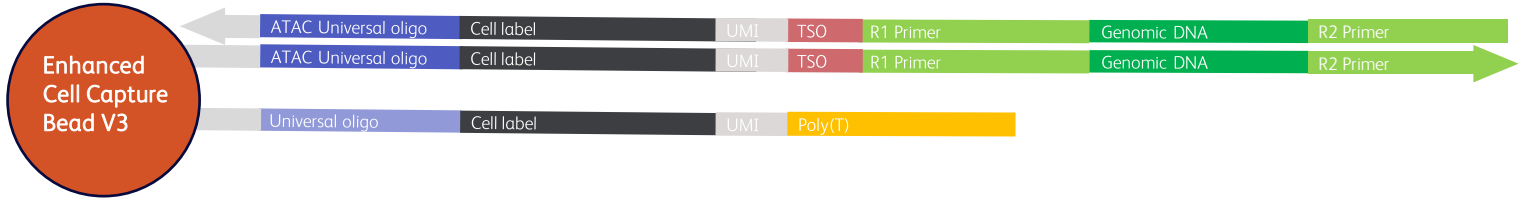
Single-cell capture:
Cell lysis in microwell. The genomic DNA is captured by the Splint-oligo bonded strands.



Ligation: The BD Rhapsody™ Bead oligo and tagmented genomic DNA are ligated by DNA ligase.

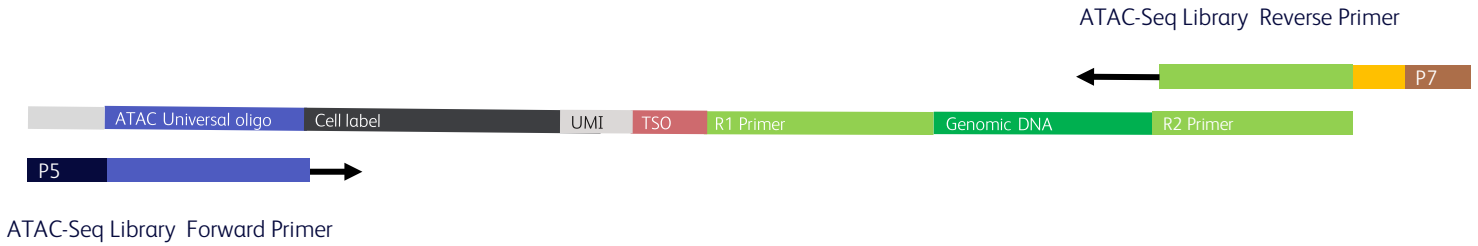


ATAC Fragment Gap Filling: ATAC fragment gap filling and extension to beads oligo



Library preparation steps (Single Cell ATAC-Seq)

ATAC fragment denaturation: Supernatant: The genomic DNA template is denatured off the bead. Illumina™ adapters and indices are added, and the ATAC product undergoes amplification.



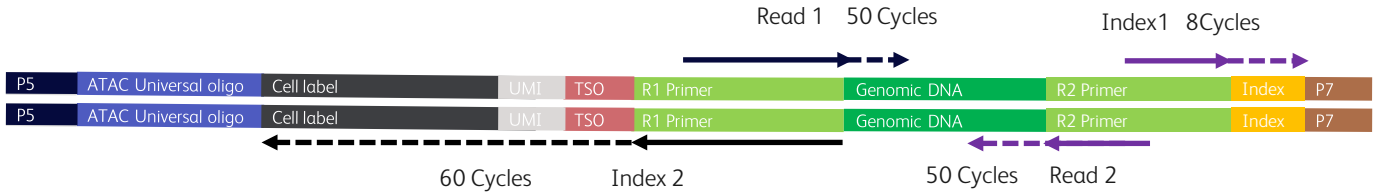
Sequencing:

Read 1: 50 cycles;

Read 2: 50 cycles

Index 1: 8 cycles;

Index 2: 60 cycles



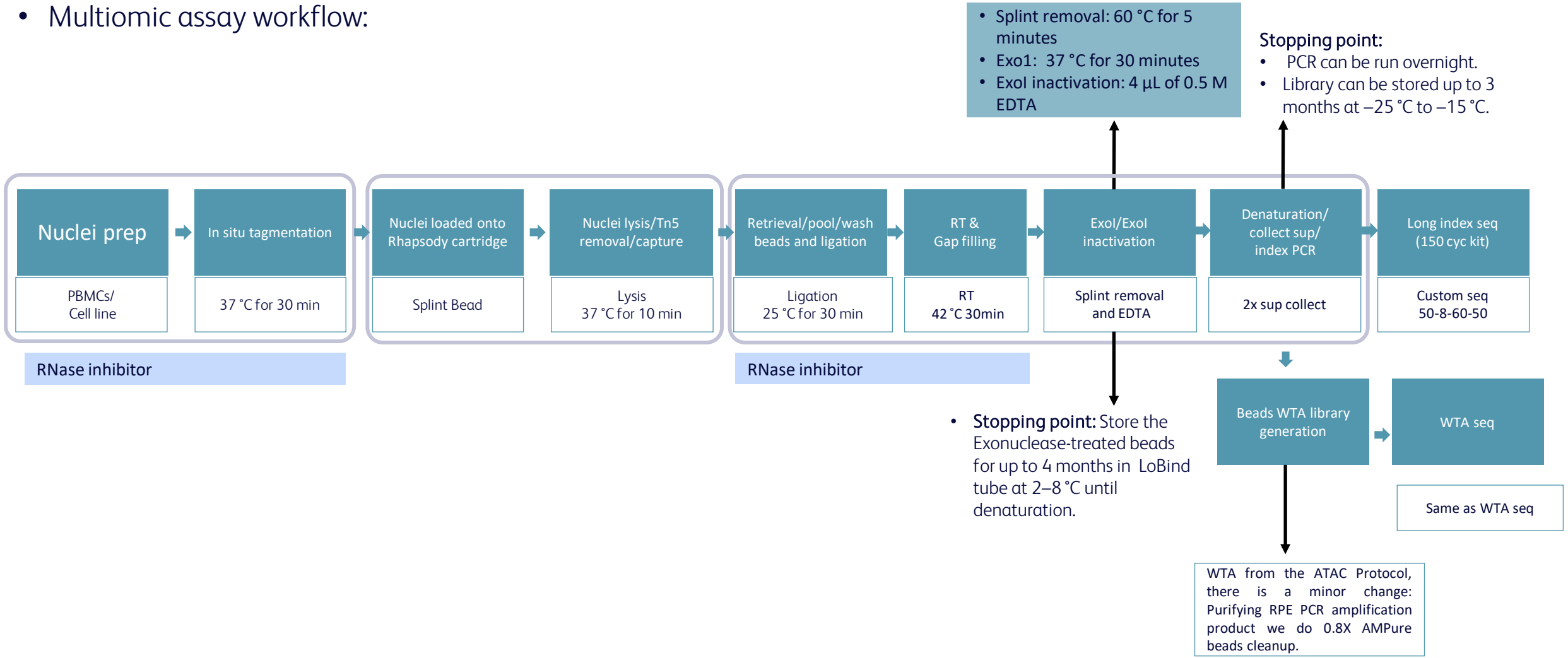
BD Rhapsody™ Multiomic ATAC-Seq Assay Workflow

BD Rhapsody™ ATAC-Seq vs BD Rhapsody™ Multiomic ATAC-Seq Assays

- Major differences between the two protocols:
    - Multiomics workflow uses reverse transcription (RT) for gap filling
    - RNase inhibitor needs to be added to buffers starting from nuclei prep
    - It is important to always keep the isolated nuclei on ice and use RNase Inhibitor to keep RNA intact.
    - We need modified cold Sample Buffer with RNase Inhibitor at concentration of 25 µL/1 mL
    - ExoI step after the Gap-filling
    - After the denaturation step supernatant goes to the ATAC library and beads are used for the WTA library generation.
    - All the other steps are same for WTA with a minor change on the Purifying RPE PCR amplification product step, we do 0.8X AMPure beads cleanup.

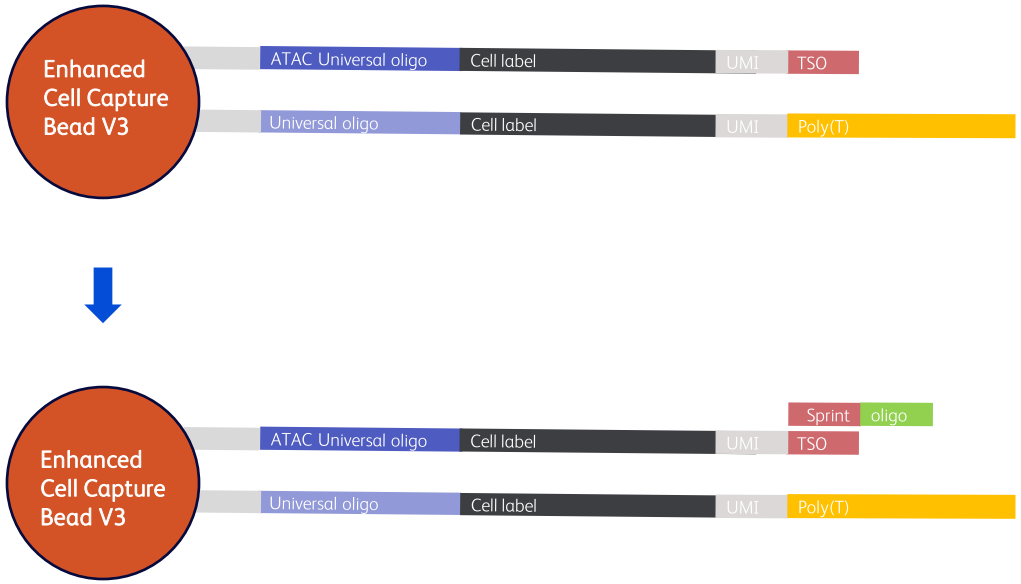
BD Rhapsody™ Multiomic ATAC-Seq Assay

- Multiomic assay workflow:



Library preparation steps (Single Cell Multiomic ATAC-Seq)

BD Rhapsody™ Enhanced Cell Capture Bead V3 layout



Splint Bead:

Splint oligonucleotide is added to the beads to assist capture of genomic DNA via the capture oligo.
Poly(dT) to capture mRNA at 3' end

Tagmentation: During the bulk in situ tagmentation process, nuclei are exposed to a Tagmentation Mix, containing Tagmentase. This enzyme targets accessible genomic regions (open chromatin areas), cutting the DNA and simultaneously attaching pre-loaded adapter sequences to the ends of each DNA fragment.

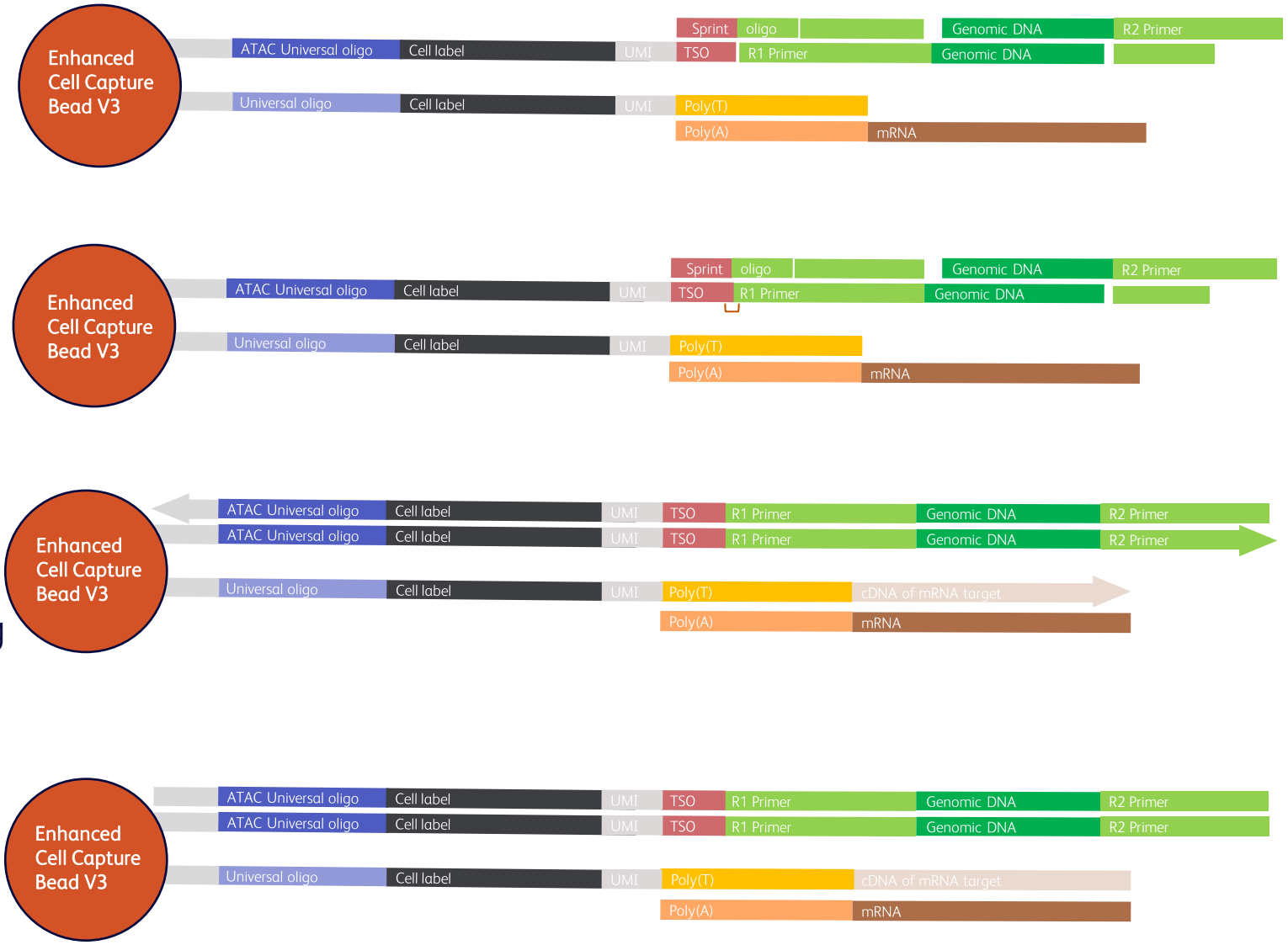
Library preparation steps (Single Cell Multiomic ATAC-Seq)

Single-cell capture: Cell lysis in microwell; the genomic DNA is captured by the Splint oligo bonded strand and mRNA is captured by poly(T).

Ligation: The BD Rhapsody™ Bead oligo and tagmented genomic DNA are ligated by DNA ligase.

Reverse transcription and ATAC fragments Gap Filling:
Complementary DNA (cDNA) is synthesized from captured mRNA. ATAC fragment gap filling and extension to beads oligo

Splint oligo removal and Exonuclease I treatment: To remove unused oligos from the beads



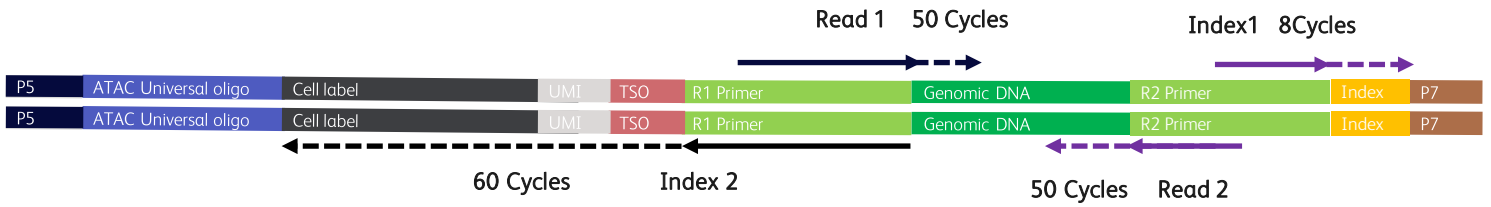
Library preparation steps (Single Cell Multiomic ATAC-Seq)

ATAC fragment denaturation:
Supernatant: The genomic DNA template is denatured off the bead. Illumina™ adapters and indices are added, and the ATAC product undergoes amplification.

Bead:
Beads will proceed through the WTA workflow.

Sequencing:

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

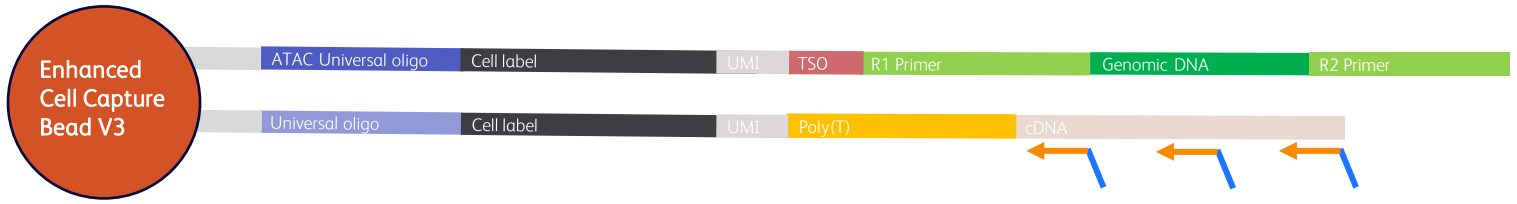


Library preparation steps (Single Cell Multiomic ATAC-Seq)

WTA library amplification workflow

WTA RPE: Random priming on the bead

Random Priming and Extension

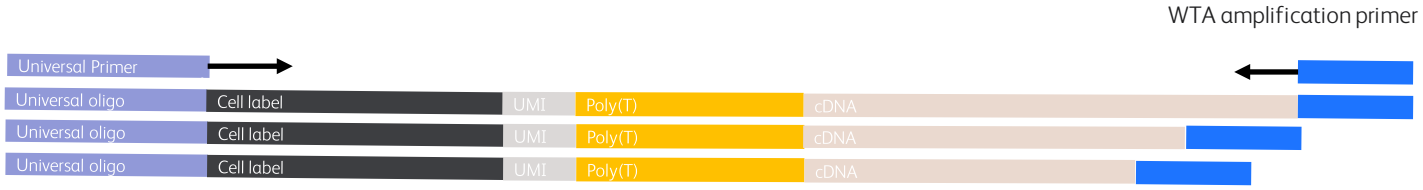


Denature off the RPE product

RPE product (ssDNA)

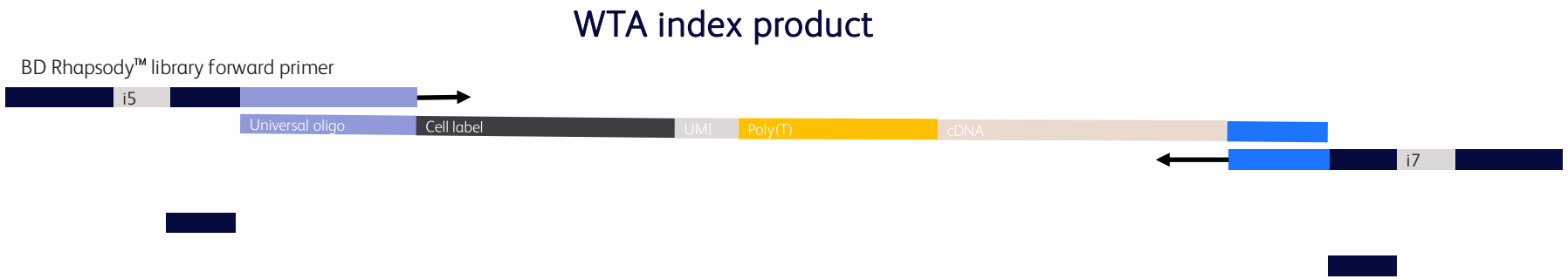
WTA RPE PCR: Amplify the RPE product

RPE PCR products (WTA)



Library preparation steps (Single Cell Multiomic ATAC-Seq)

WTA Index PCR: Add Illumina™ adapters and indices.



Final WTA Product

Sequencing recommendations (Single Cell Multiomic ATAC-Seq)

Examples of pooling:

Sample name (Enter)	Rhapsody Index Primer (Enter)	Qubit concentration (from instrument) (ng/uL) (Enter)	Converstion factor (µL/L)	dsDNA (bp mol/g)	Avg Size (from Bioanalyzer) (bp) (Enter)	Calculated Qubit concentration (nM)
ATAC-Seq Library						
AT198_1_PBMC_SA_ATAC	N703	6.86	1000000	660	486	21.4
AT198_2_PBMC_SA_ATAC	N707	4.65	1000000	660	480	14.7

Sample name (Enter)	Calculated Qubit (nM)	EBT vol (µL)	Sample vol (µL)	Final desired concentration (nM)
ATAC-Seq Library				
AT198_1_PBMC_SA_ATAC	21.4	17.4	4	4
AT198_2_PBMC_SA_ATAC	14.7	10.7	4	4

Sample name (Enter)	Cell number (Enter)	Number of reads/cell desired (Enter)	Total reads	Pooling ratio
Libraries				
AT198_1_PBMC_SA_ATAC	4000	50000	200000000	50.0%
AT198_2_PBMC_SA_ATAC	4000	50000	200000000	50.0%

Recommended sequencing depth: 50,000 read pairs per cells.

Loading concentration: Load the flow cell at a concentration between 1.5–1.8pM with 1% PhiX spike-in.

Parameter	Requirement
Platform	Illumina™
Paired-end reads	Recommend: 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)

Key considerations

Sample preparation

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

We have internally used the isolation protocols offered in the Omni-ATAC paper (DOI: 10.1038/nmeth.4396):

For suspending cells: Supplementary Protocol 1 – Omni-ATAC – An improved and broadly applicable ATAC-seq protocol

For tissue cells: Supplementary Protocol 2 – Isolation of nuclei from frozen tissues for ATAC-seq

Cell QC:

- For cell line: We would recommend using them after at least 3 passage post-thawing so that they are fully recovered from the freeze/thawing stress
- For PBMCs: Thawing step is as critical as nuclei isolation.
- For both cell types: Make sure that you have a good cell viability (>90%) to proceed to the next step.
- It is recommended to use a swinging-bucket centrifuge for pelleting cells.
- Use wide bore tips when handling nuclei.
- Measurements of <5% live input cells indicate proper nuclei isolation.

Before you begin

- Review the time consideration chart and plan accordingly.
- Prepare the Splint oligo loaded bead (This can be done prior to the experiment and store at 2 to 8 °C, up to a week). Never vortex the beads. Pipet-mix only.
- Prepare the buffer mixes on the same day of performing the nuclei isolation.
- Thaw reagents (not enzymes) at room temperature (15 °C to 25 °C), and then place on ice. Keep enzymes at –25 °C to –15 °C.
- NOTE: Only thaw the reagents needed for the day.
- Prepare Tagmentation Buffer with DMF: aliquot 200 µL of Tagmentation Buffer into a new 1.5-mL LoBind tube, then add 50 µL of 100% DMF into the tube and mix. Tagmentation Buffer with DMF can be stored at – 20°C for multiple usage.
- Dilute 2% digitonin to 1% digitonin with nuclease-free water.

Tagmentation reaction

- The number of nuclei per reaction will depend on the ATAC-seq experimental design. Successful tagmentation has been performed with 50,000 nuclei in a 50 µL reaction. When using less than 50,000 nuclei, proportionally scale down the reaction.
- Add the components in the order listed on the table.
- Add 50,000 nuclei and gently pipet-mix 10 times with a wide bore tip.

*If the nuclei concentration is less than 10,000 nuclei/µL, adjust the volume of nuclei and nuclease-free water.

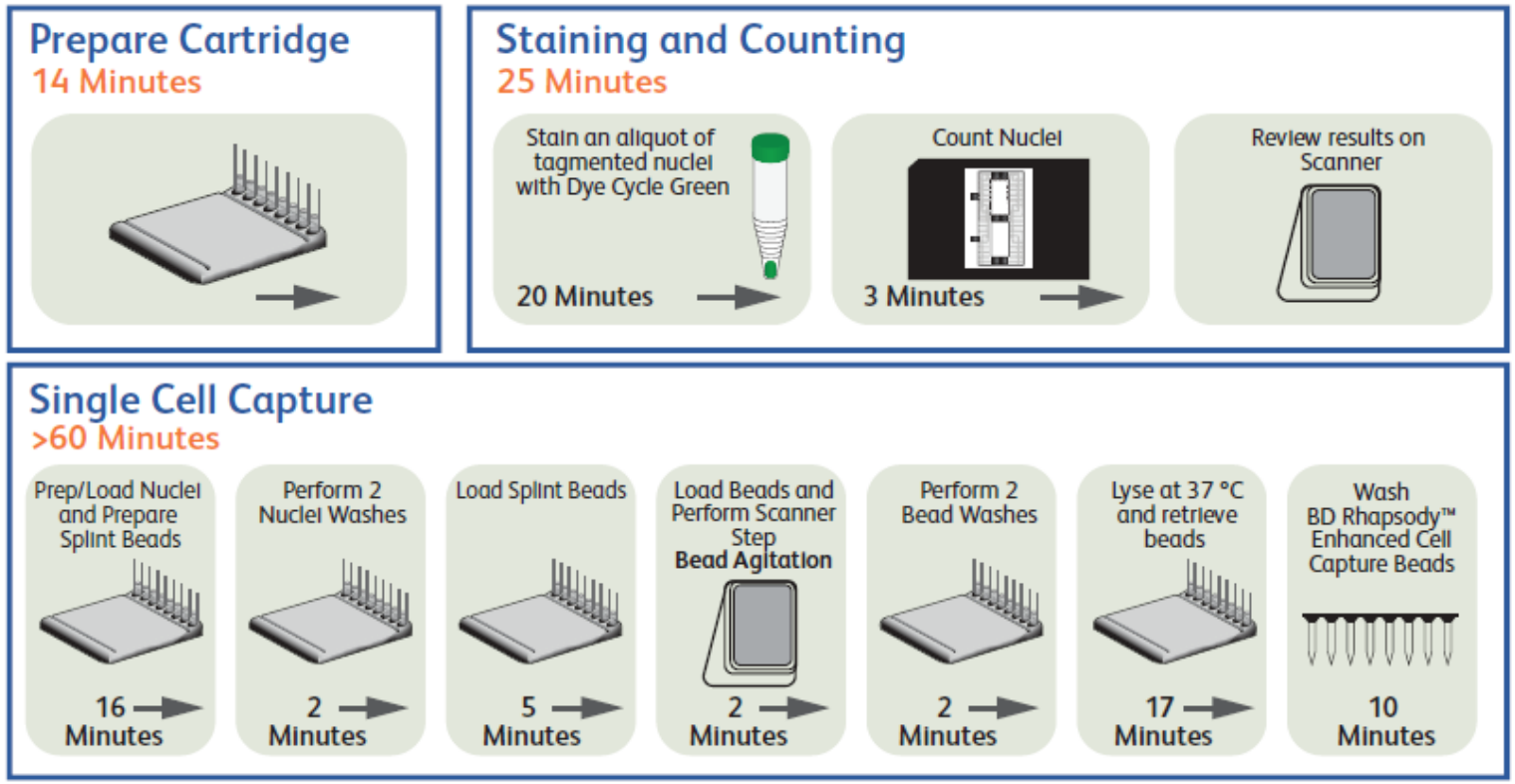
**1% Digitonin is diluted from 2% stock with nuclease-free water.

Kit component	Volume (µL)
Tagmentation Buffer with DMF†	25
Nuclease-free water	13*
10X PBS	2
Digitonin 1%**	0.5
Tween20, 10%	0.5
Tagmentase	4
Nuclei	5*

†100% Dimethylformamide (DMF) is not provided in the BD Rhapsody™ ATAC-Seq Tagmentation and Supplemental Reagents Kit (PN 571201)

BD Rhapsody™ Cartridge Workflow for ATAC-Seq

- The cartridge workflow for BD Rhapsody™ ATAC-Seq Assays do not have any scans other than what is indicated below.

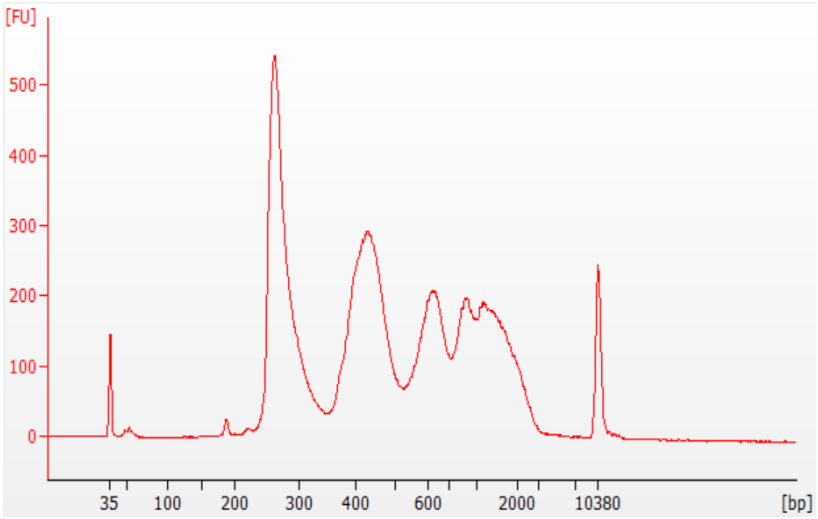


Pre-sequencing quality control

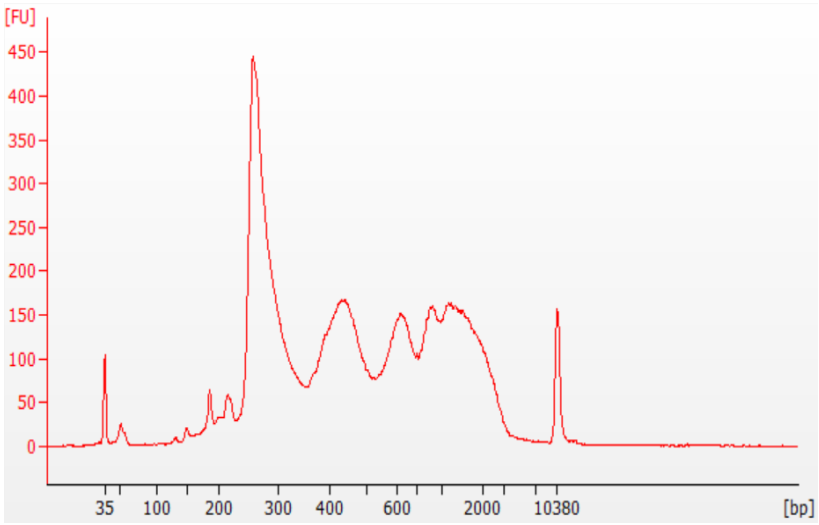
Measure the concentration of each ATAC-seq library by Qubit (2 µL of the final sequencing library)
Run Agilent 2100 Bioanalyzer:

- If the concentration is >5 ng/µL, dilute the library to ≤5 ng/µL with Elution Buffer.
- Measure the average fragment size of the ATAC-seq libraries within the size range of 200–1,000 bp

Representative scATAC-seq library trace is shown here with majority of the fragments distributed between ~200–2,000 bp.



BD Rhapsody™ ATAC-Seq Assay Library - Bioanalyzer High Sensitivity DNA trace

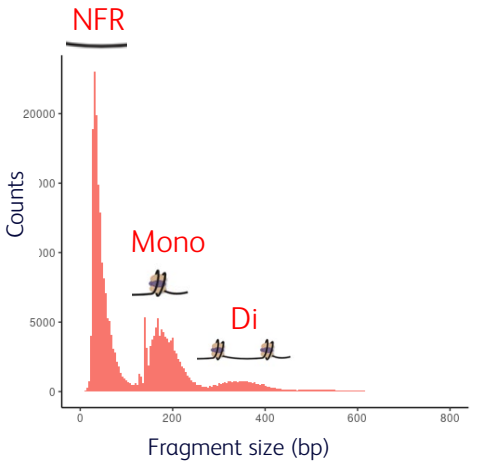


BD Rhapsody™ Multiomic ATAC-Seq Assay Library - Bioanalyzer High Sensitivity DNA trace

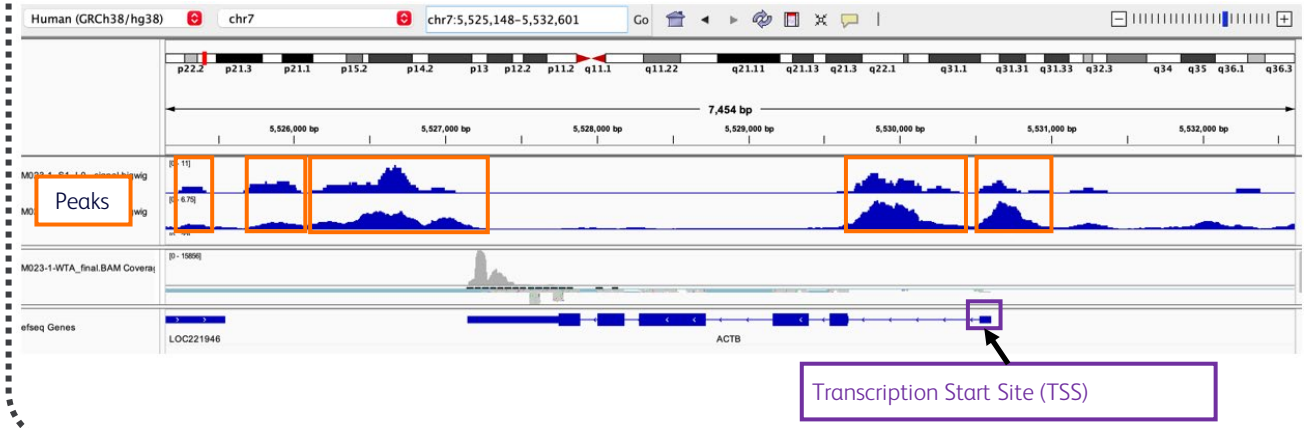
How to interpret ATAC-seq data

What do we expect to get from ATAC-seq?

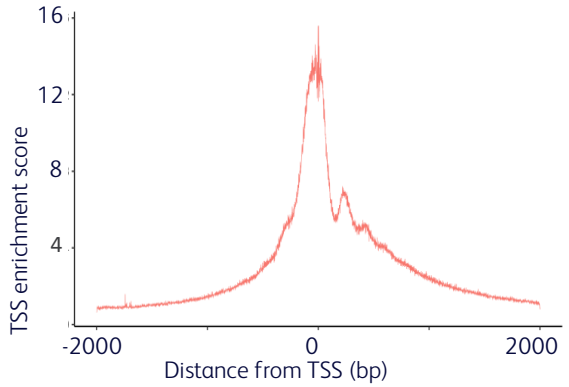
Fragment length distribution



Fraction of Reads in Peaks (FRiP)



TSS enrichment



Sensitivity

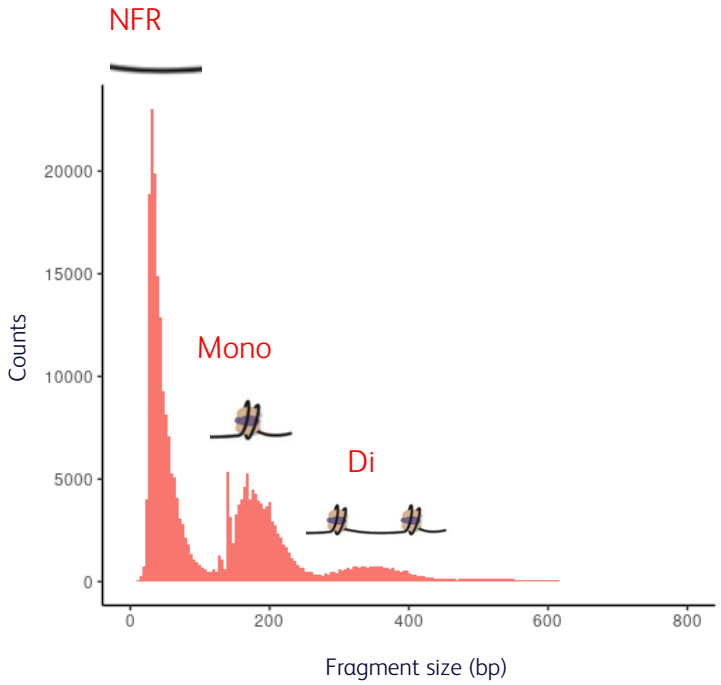
- Obtain as many unique fragments as possible
- Majority (>65%) of fragments should be from nucleosome-free regions (NFR) and mononucleosomal regions (Mono)

Specificity

- Fragments should compile in certain regions of open chromatins → peaks in ATAC
- Fraction of Reads in Peaks (FRiP) should be higher than 20%
- Fragments should be enriched around Transcription Start Site (TSS), TSS enrichment score >15

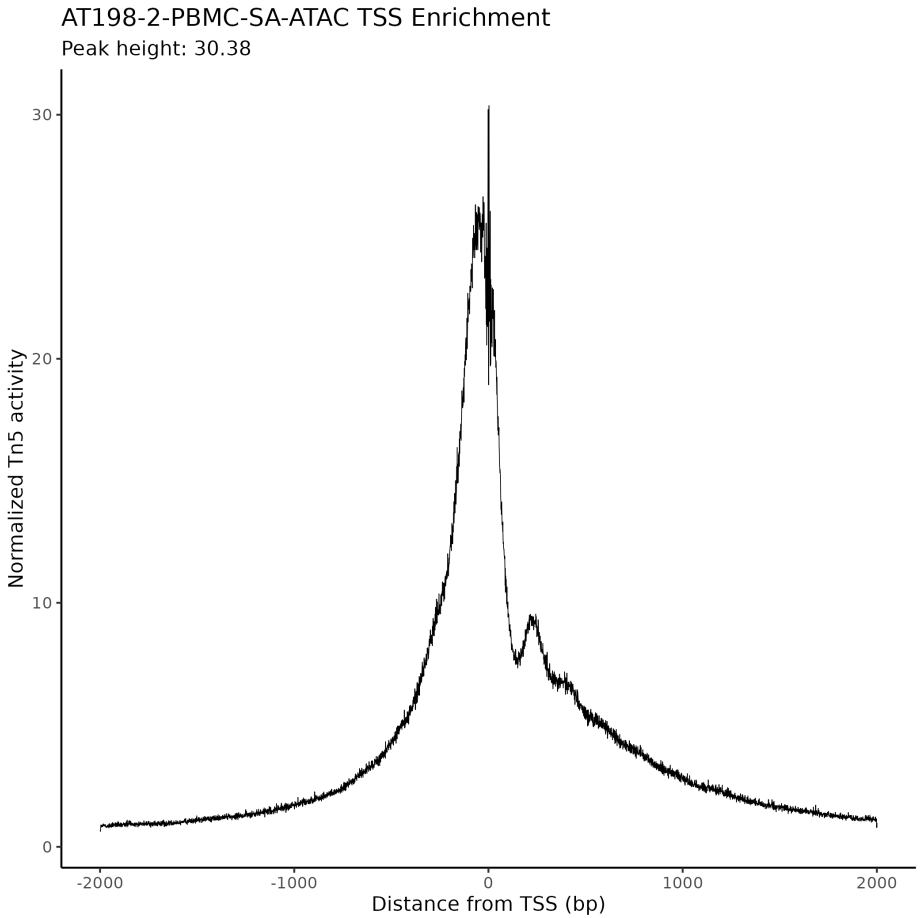
Fragment size distribution

- ATAC-seq paired-end reads provides detailed information about nucleosome positioning, with the insert size distribution showing a periodicity equal to the helical pitch of DNA.
- Different functional classes of chromatin typically shows distinct enrichment pattern in the size distribution, suggesting characteristic accessibility for each class.
- For example, transcription factors are enriched for NFR fragments, while transcription start sites (TSSs) are depleted for mono-, di- and tri-nucleosome-associated fragments.
- Transcribed regions are enriched for longer multi-nucleosomal fragments, indicating a less accessible structure compared to TSS.
- Repressed regions are depleted for short fragments and enriched for large multi-nucleosomal inserts consistent with their inaccessible state.
- Having most fragments from NFR and mono-nucleosomal regions indicates high sensitivity in detecting accessible chromatin, which is crucial for identifying regulatory elements.



Transcription Start Site (TSS) enrichment

- TSS enrichment score provides an indication of how well ATAC-seq signals are enriched around the transcription start size of genes.
- A high TSS enrichment score suggest that the ATAC-seq data effectively captures open chromatin regions around gene promoters and transcription start sites, which are typically associated with regulatory elements like promoters and enhancers.
- A low TSS enrichment score may indicate that the ATAC-seq experiment did not efficiently capture open chromatin regions near transcription start sites, pointing to low data quality.
- A high TSS score is crucial for downstream analyses like identifying cell type-specific accessible regions or interfering gene regulatory networks.



Sensitivity and specificity

- Sensitivity in ATAC-seq refers to the ability to comprehensively detect accessible chromatin regions across the genome. High sensitivity means capturing even small or subtle regions of open chromatin.
- Specificity in the context of ATAC-seq refers to the ability to accurately identify regions of open chromatin while minimizing signal from closed chromatin regions.
- Both sensitivity and specificity are important metrics for ATAC-seq. However, whether sensitivity or specificity is more critical depends on the specific goals of the user:

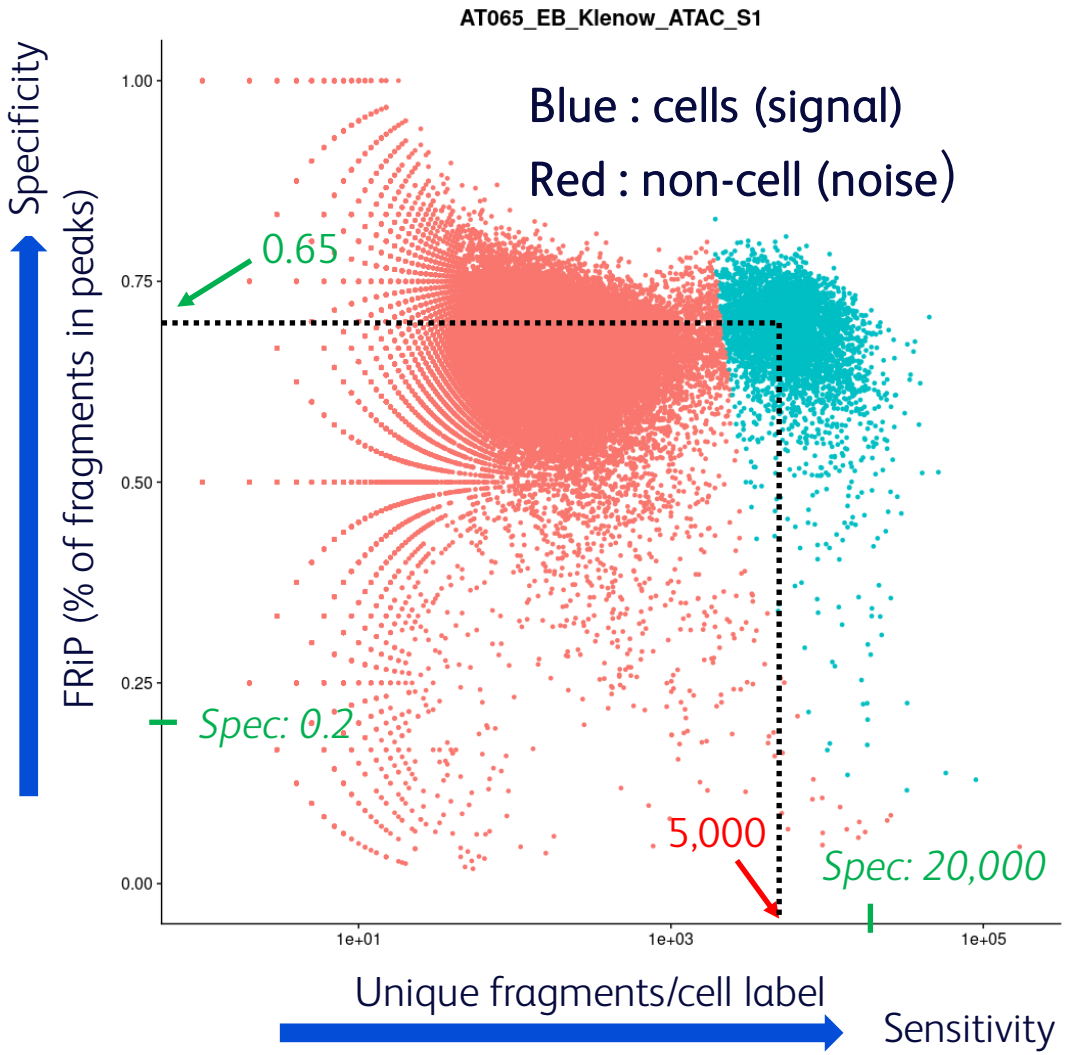
- Sensitive assay is particularly useful when:

  - The goal is to comprehensively map all open chromatin regions in the genome. Capturing even small or weak sites requires high sensitivity.
  - Profiling subtle changes in chromatin accessibility across conditions. High sensitivity ensures differential sites are not missed.
  - Analyzing complex tissues or heterogeneous cell populations. Detecting sites in minor subpopulations requires high sensitivity.
  - Identifying distal regulatory elements like enhancers. These tend to have lower accessibility signals requiring high sensitivity.

- Specific assay is particularly useful when:

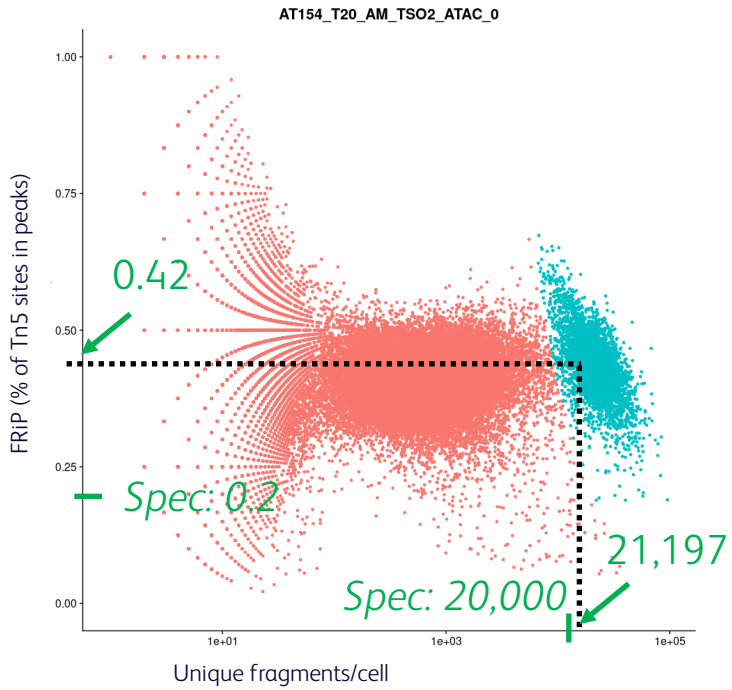
  - Accurately calling peaks and assigning them to genes/features is critical. High specificity reduces false positives.
  - Comparing chromatin accessibility across defined cell types. Stringent specificity allows meaningful comparisons.
  - Validating known open chromatin sites like promoters. Specificity ensures assay reliability.
  - Studying samples with low cellular input or poor quality. Reducing background signal is important for accuracy.

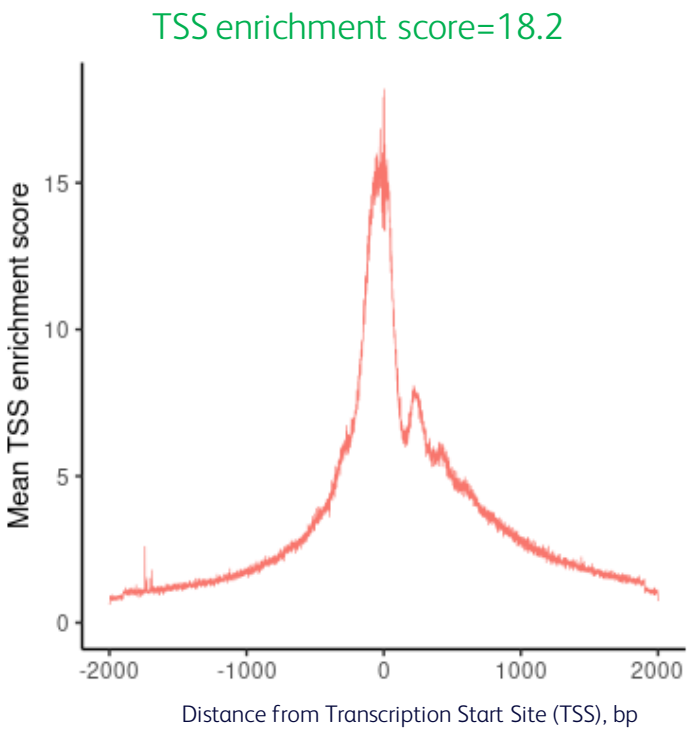
Assay performance with the K562 cell line



- High specificity ✓
- But low sensitivity

Improved assay performance with the K562 cell line





- High sensitivity: >20,000 unique fragments per cell at 50,000 reads/cell
- Good specificity: most fragments are from peak regions and near transcription start sites

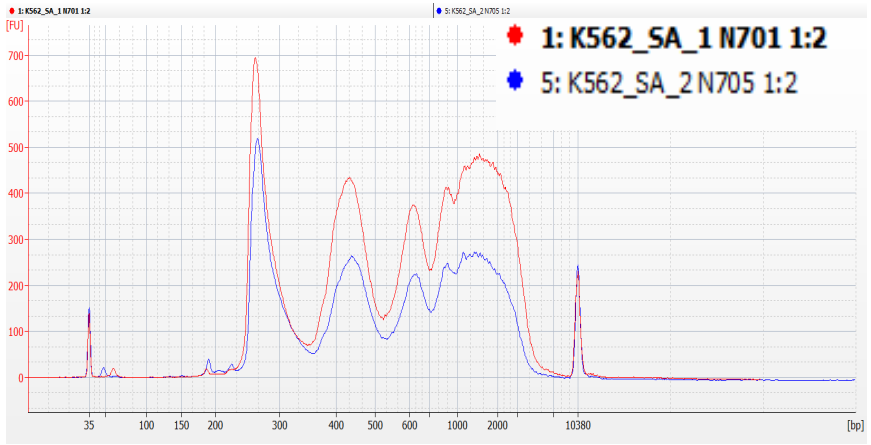
Performance overview

BD Rhapsody™ ATAC-Seq Assay metrics summary

Metric	PBMCs1K cell	PBMCs 5K cell	PBMCs 10K cell	PBMCs 20K cell
Percent input reads assigned cell label	96.1	95.1	95.4	95.6
Percent clean reads assigned cell label	97.22	97.6	97.7	97.8
Percent cell-labeled reads aligned confidently	88.59	87.9	88.9	88.1
Percent mitochondrial reads	0.84	2.1	1.1	2.4
Fraction of nonduplicate fragments with NFR lengths	71.67	0.75	0.72	0.70
Fraction of nonduplicate fragments with mononucleosomal lengths	20.06	0.19	0.20	0.22
%NFR + monosome	91.73	0.94	0.92	0.92
Total number of peaks called	83682	111254	121429	129514
Total number of putative cells	452	2366	4083	7591
Fraction of nonduplicate fragments associated with putative cells	72.16	0.70	0.72	0.66
Fraction of Tn5 transposase sites in Peak regions from putative cells	68.64	0.75	0.75	0.76
TSS enrichment (peak value)	36.18	39.0	38.8	37.5
Total raw read pairs	21967843	96416927	191844156	344116869
Average raw read pairs per putative cell	48601	40751	46986	45332
Median proper read pairs per putative cell	27693	22724	27892	23566
Median nonduplicate fragments per putative cell	13326	13172	15001	13772
Median ratio of fragments to proper read pairs per putative cell	48.42	0.57	0.54	0.58

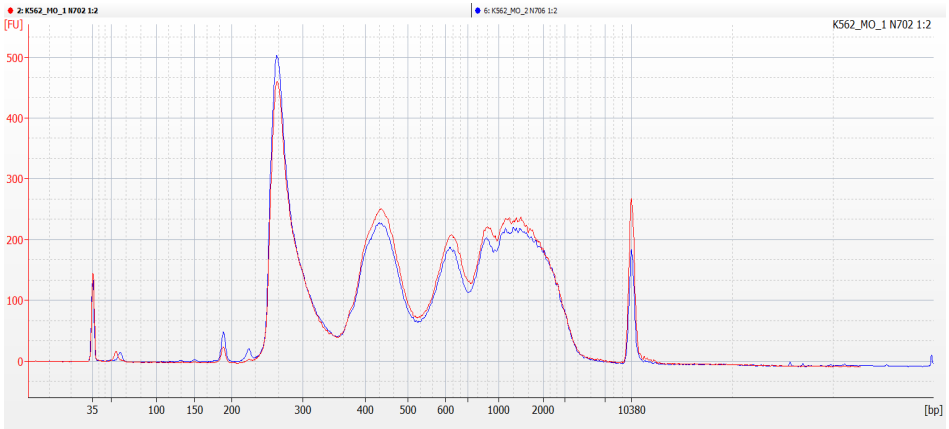
Performance metrics—BD Rhapsody™ ATAC-Seq Assay library quality

Standalone assay



Sample Name	Qubit (ng/µL)	Average Size (bp) 150 – 1,000 bp
K562_SA_1 N701	23.2	482
K562_SA_2 N705	17.3	463
PBMC_SA_1 N703	6.86	486
PBMC_SA_2 N707	4.65	480

Multiomic assay



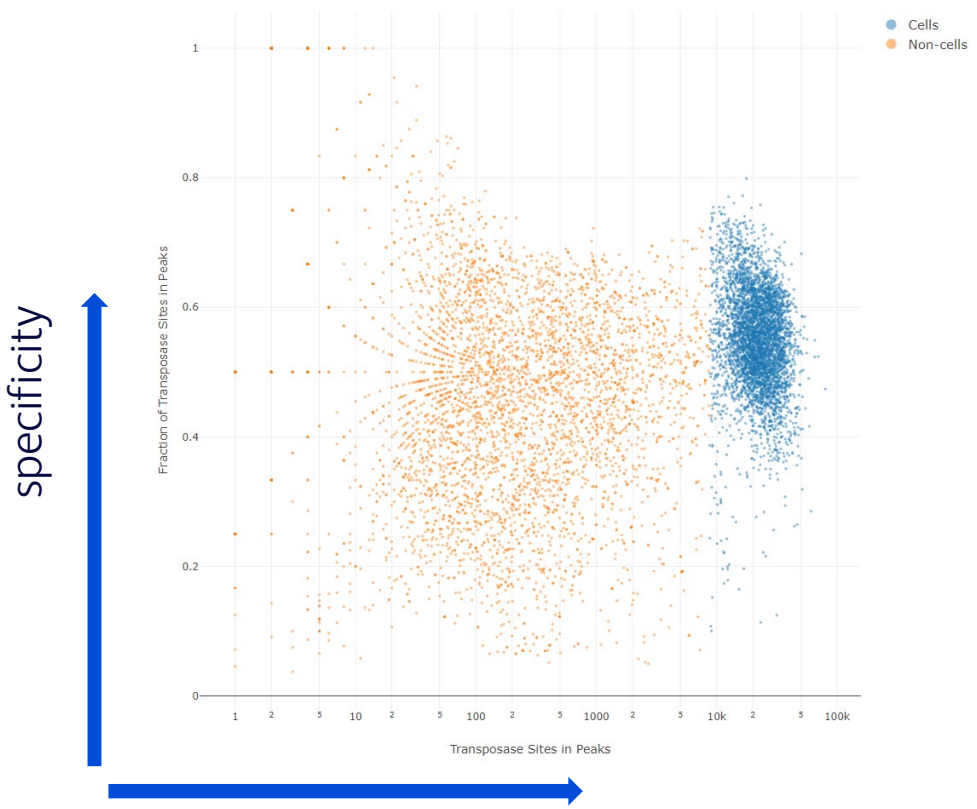
Sample Name	Qubit (ng/µL)	Average Size (bp) 150 – 1,000 bp
K562_MO_1 N702	13.1	480
K562_MO_2 N706	14.9	472
PBMC_MO_1 N704	3.85	488
PBMC_MO_2 N708	3.53	474

Bioanalyzer traces:

- Look for the distinct fragment size peaks from NFR, mono, di, tri nucleosome size fragments.
- ~2 fold higher peak from ~260 bp (NFR) size fragment compared to 420 bp (monosome) fragment is expected and correlated with high specificity (FRiP) of data.

BD Rhapsody™ ATAC-Seq Assay visualization

K562 cell line



Cell scatter plot to show the sensitivity and specificity

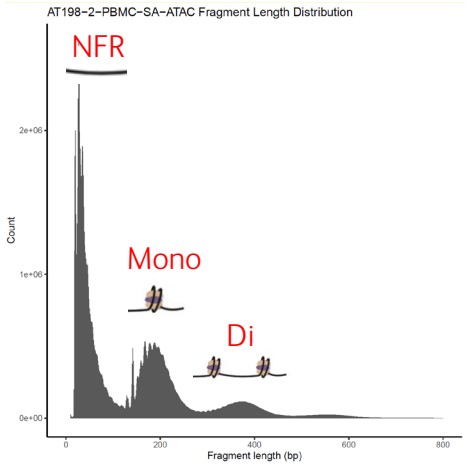
PBMCs

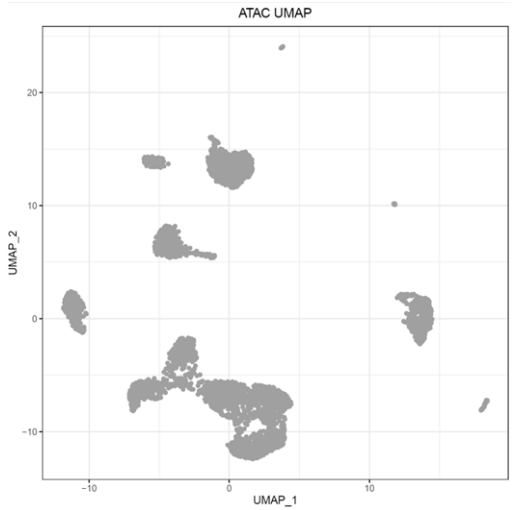
~50,000 mean raw read pairs per putative cell for both samples

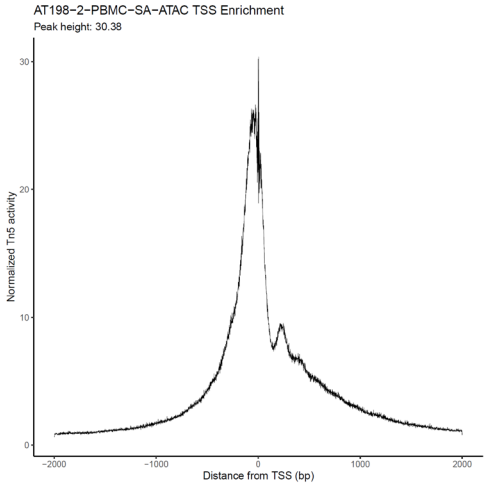
BD Rhapsody™ ATAC-Seq Assay visualization

Fragment length distribution

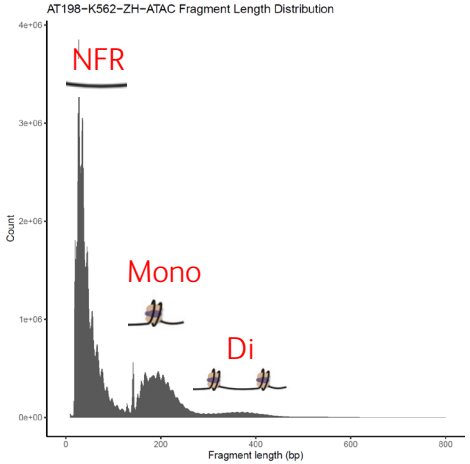
PBMCs

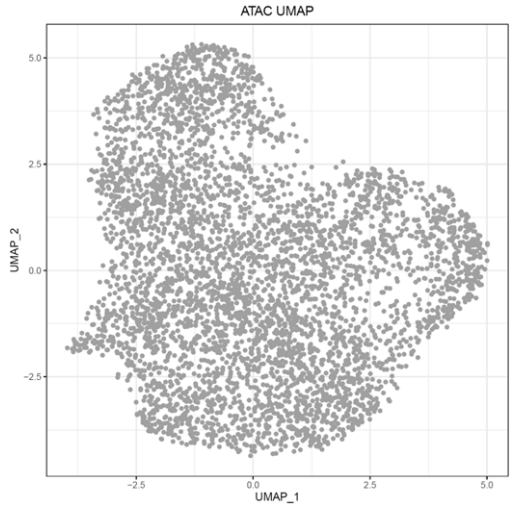


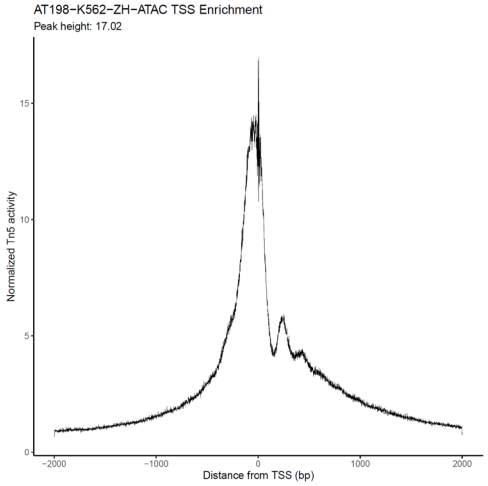




K562







BD Rhapsody™ Multiomic ATAC-Seq Assay visualization (joint cell calling)

K562 cell line

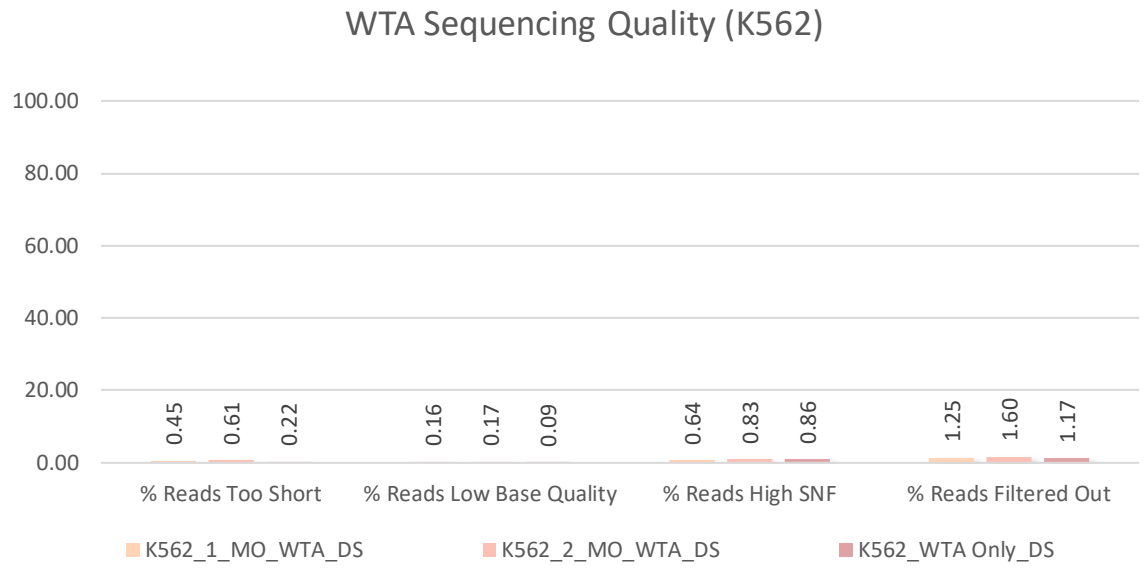
PBMCs

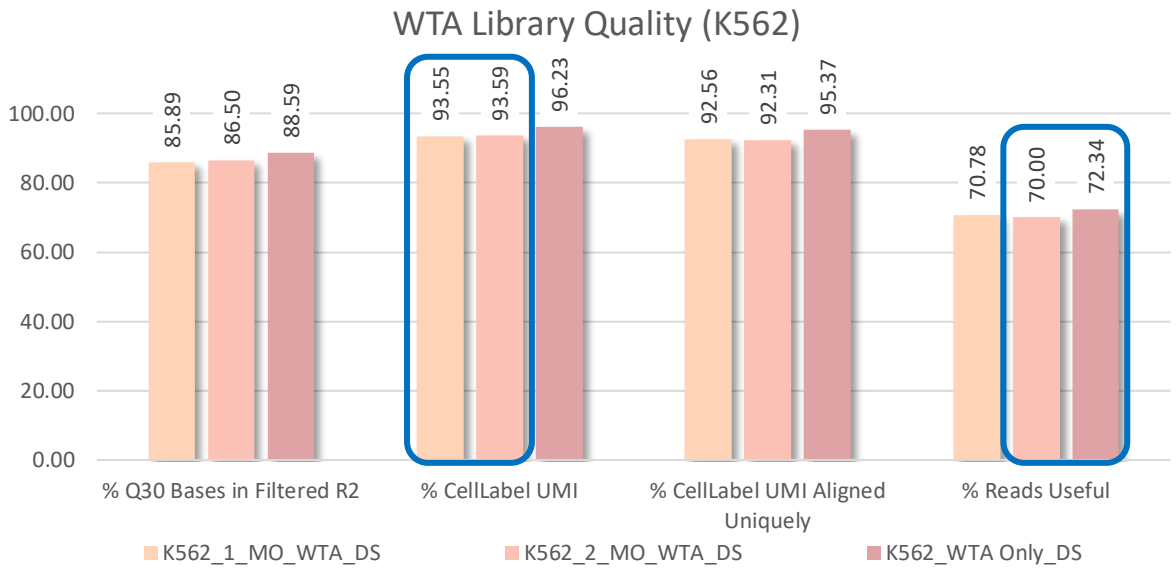
- Jointly called cells
- WTA-only cells
- ATAC-only cells
- Non cells

~50,000 mean raw read pairs per putative cell for both samples

Performance metrics—WTA sequencing and library quality

Multiomic experiment with K562 cell line:



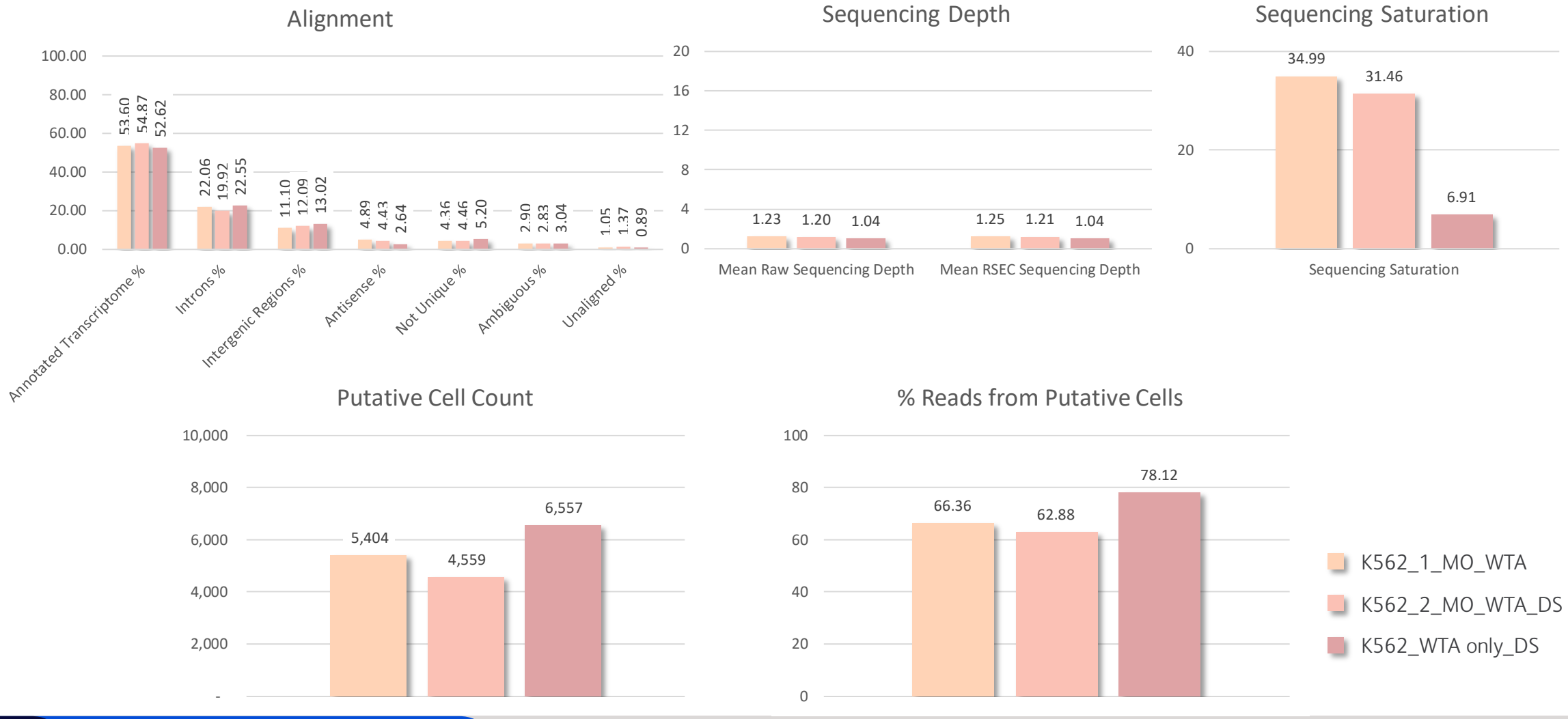


Product requirement:

- The assay shall generate cell line libraries with the total % of mapped reads (read 1) corresponding to WTA library in the multiomic ATAC-Seq to be in the range of 85–95%.
- The assay shall generate cell line libraries with the total % of mapped reads (read 2) corresponding to WTA library in the multiomic ATAC-Seq to be in the range of 50–60%.

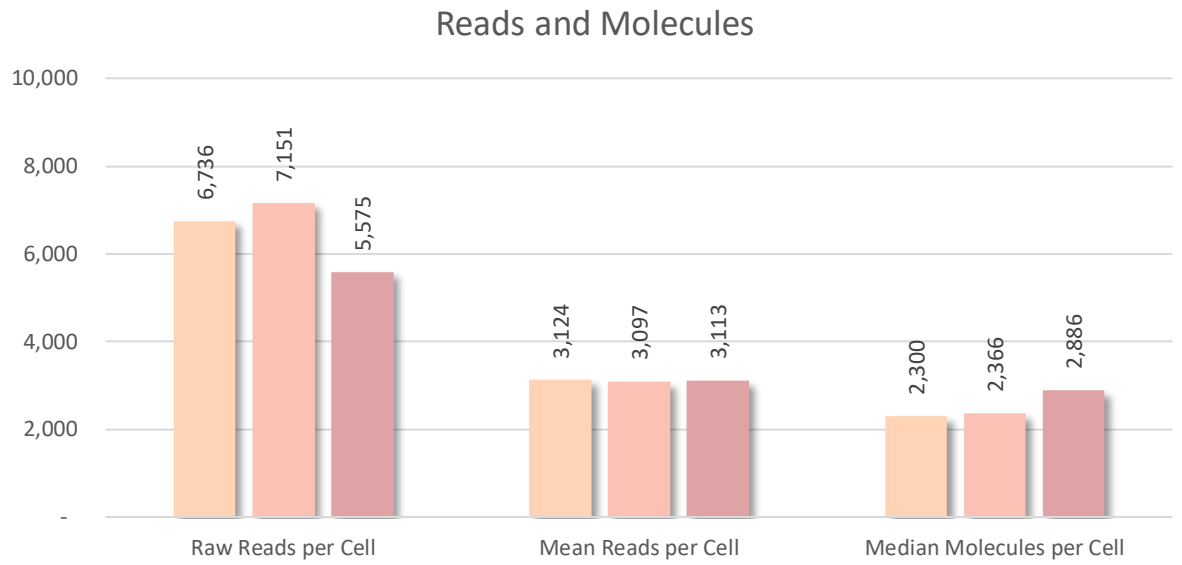
Performance metrics—WTA metrics

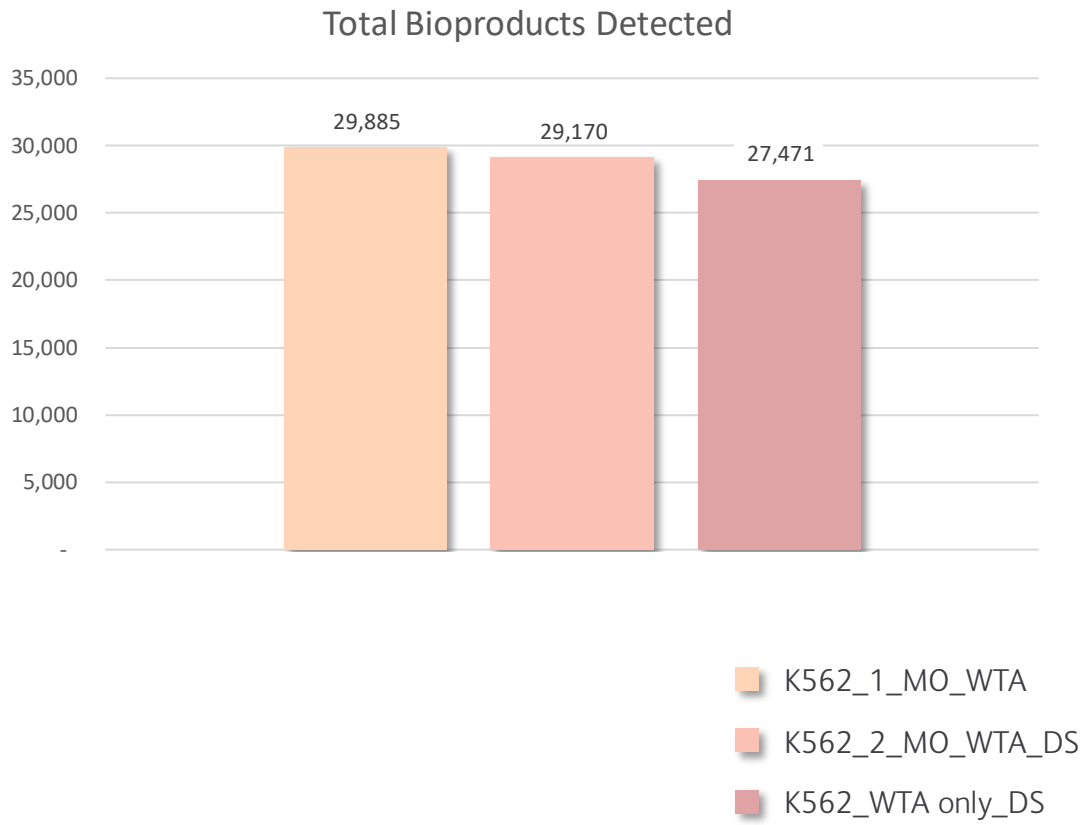
Multiomic experiment with K562 cell line:



Performance metrics—WTA sensitivity

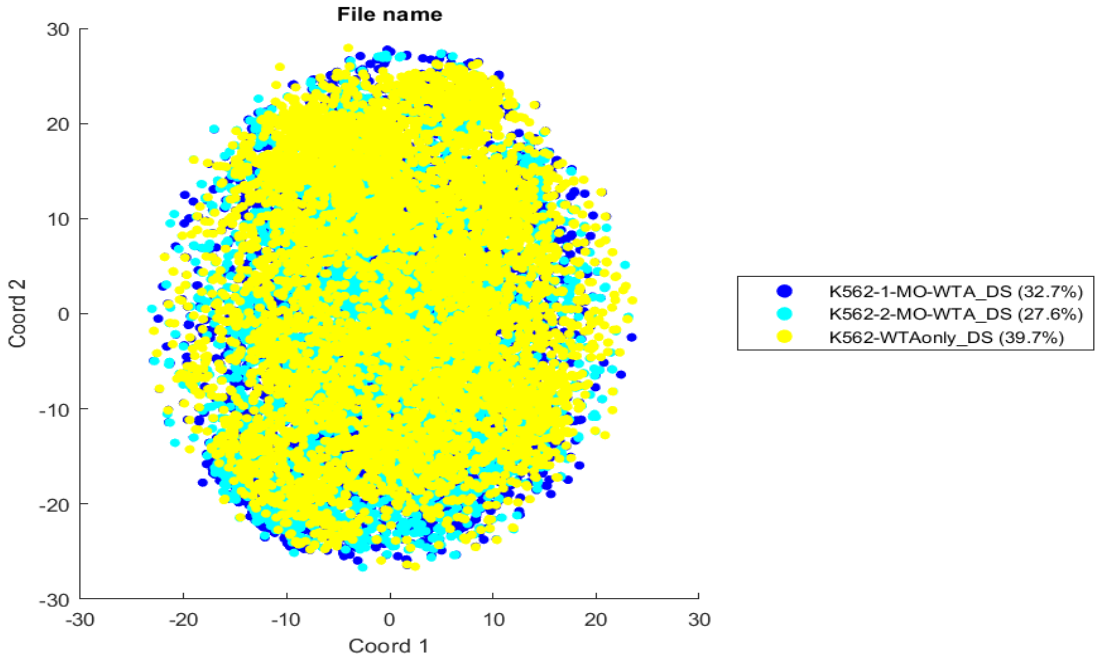
Multiomic experiment with K562 cell line:
Post down-sampling to similar mean reads/cell

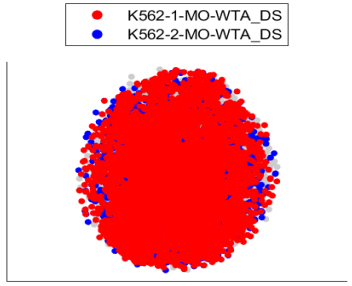


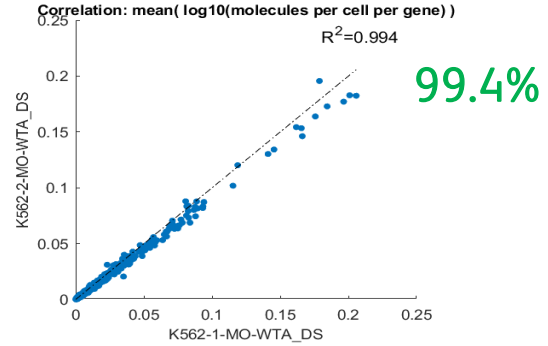


WTA data visualization

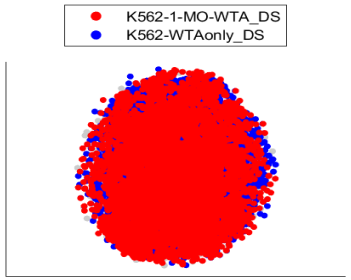
Multiomic experiment with K562 cell line:
Analysis based on highly variable genes

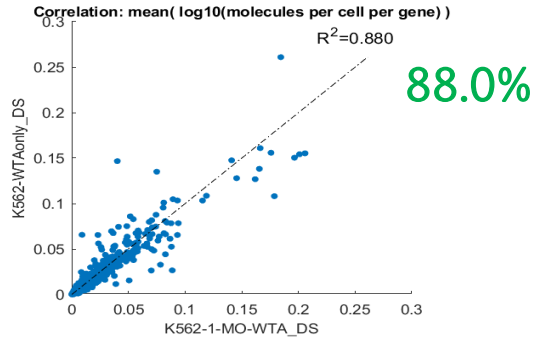




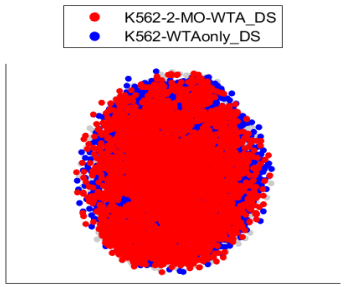


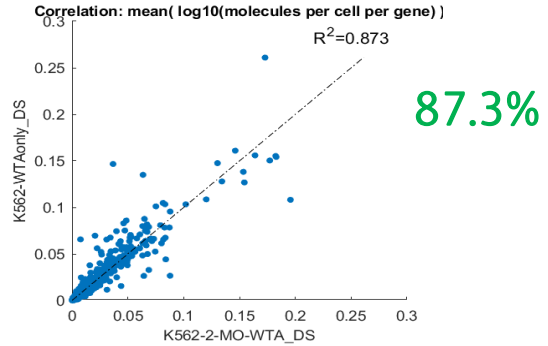
99.4%





88.0%



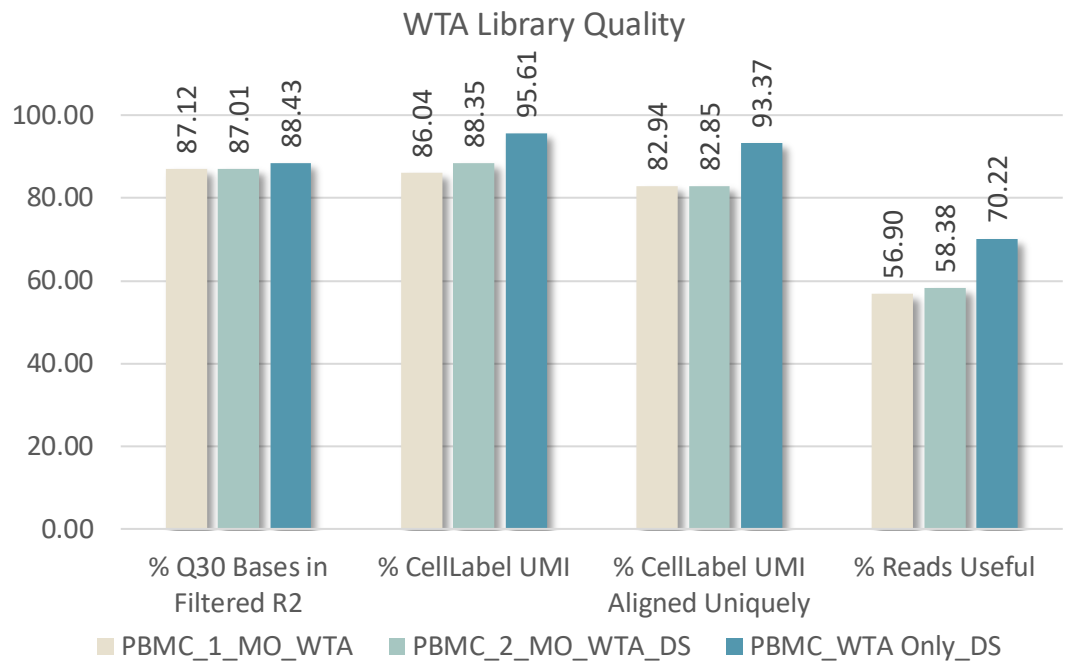


87.3%

WTA gene expression correlation R^2> 80%.

Performance metrics—WTA sequencing and library quality

Multiomic experiment with PBMCs:

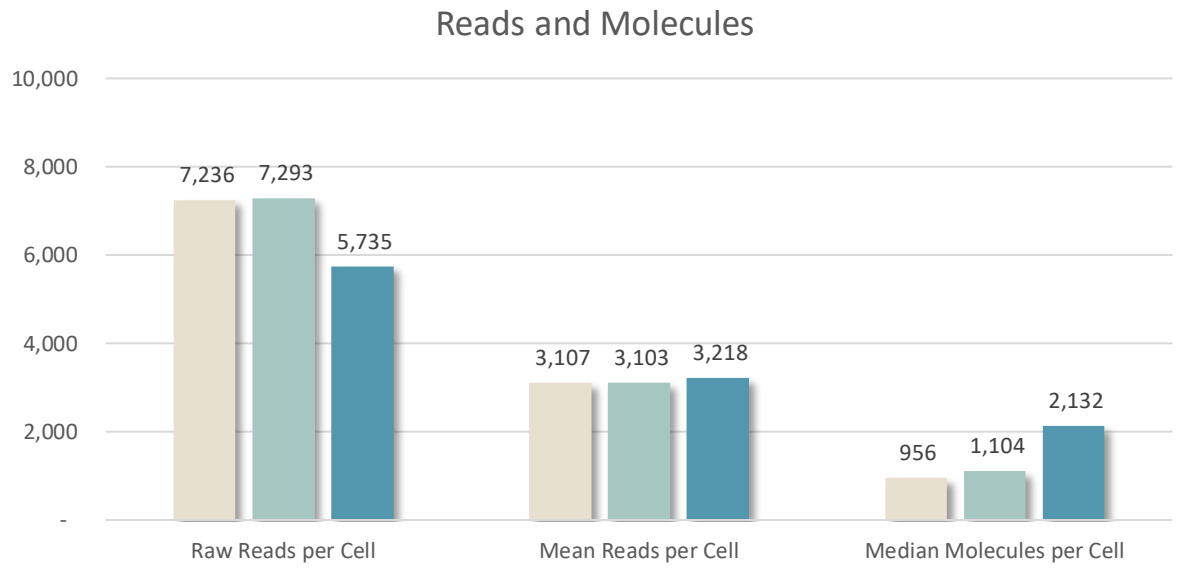


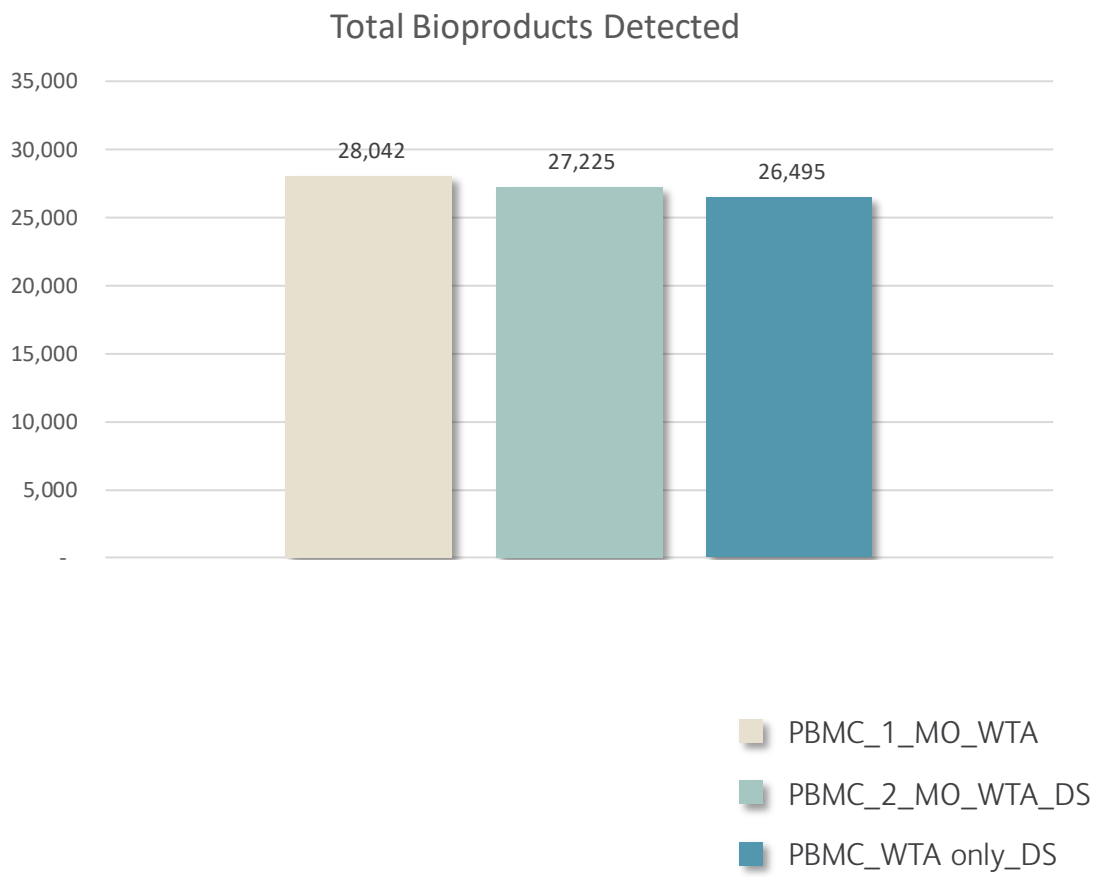
Performance metrics—WTA metrics

Multiomic experiment with PBMCs:

Performance metrics—WTA metrics

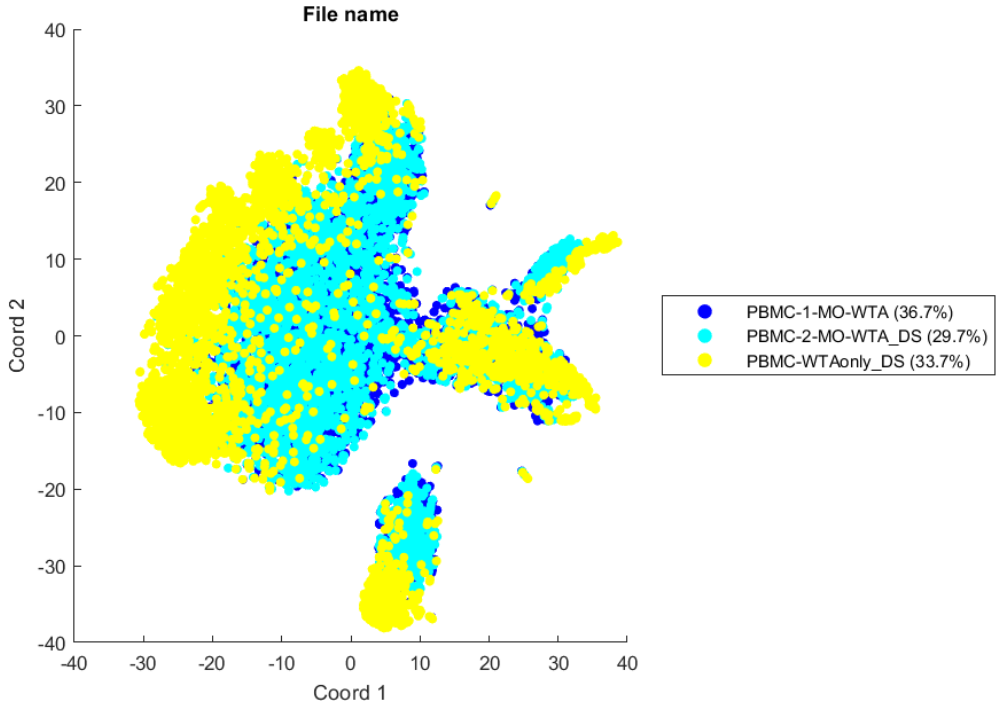
Multiomic experiment with PBMCs:
Post down-sampling to similar mean reads/cell



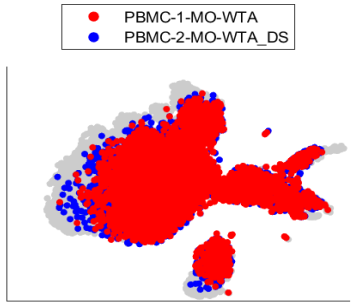


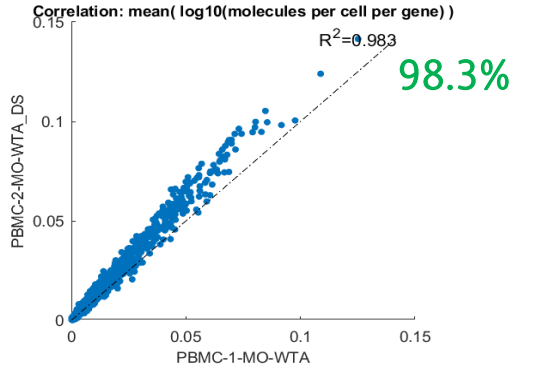
WTA data visualization

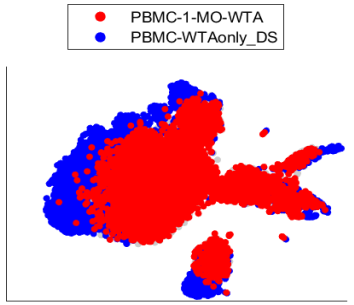
Multiomic experiment with PBMCs:
Analysis based on highly variable genes

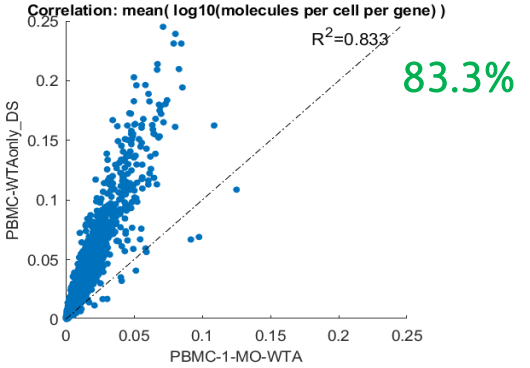


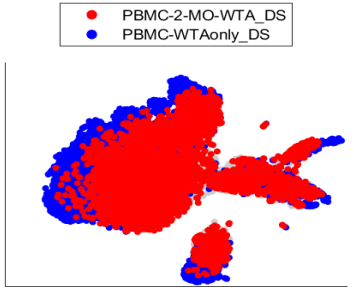
WTA gene expression correlation R^2> 80%.

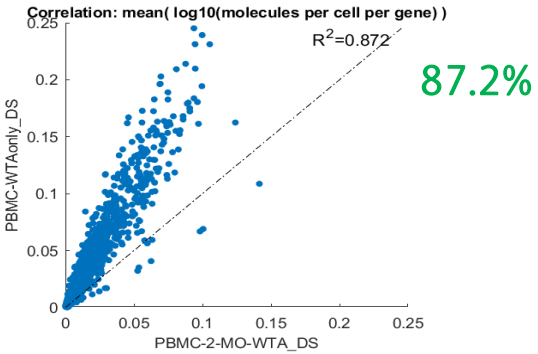










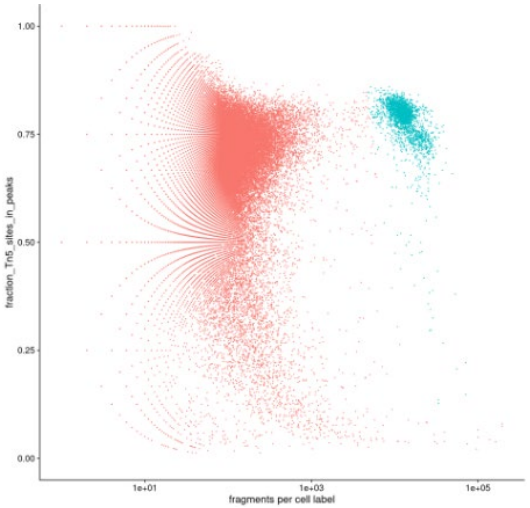


Performance metrics: 8-lane vs single-lane cartridge

Multiomic experiment with PBMCs:

Metric	8-Lane Cart	Single-Lane Cart
Percent input reads assigned cell label	96.6	93.5
Percent clean reads assigned cell label	98.1	98.0
Percent cell-labeled reads aligned confidently	87.5	88.2
Percent mitochondrial reads	3.6	5.1
Fraction of nonduplicate fragments with NFR lengths	0.74	0.78
Fraction of nonduplicate fragments with mononucleosomal lengths	0.18	0.19
%NFR+monosome	0.92	0.97
Total number of peaks called	109554	99788
Total number of putative cells	2588	1444
Fraction of nonduplicate fragments associated with putative cells	0.76	0.62
Fraction of Tn5 transposase sites in Peak regions from putative cells	0.77	0.73
TSS enrichment (peak value)	41	37
Average raw read pairs per putative cell	50009	35380
Median proper read pairs per putative cell	29192	15679
Median nonduplicate fragments per putative cell	12760	7439.5
Median ratio of fragments to proper read pairs per putative cell	0.44	0.48

8-lane cartridge



Single-lane cartridge

Data are sequenced in different depth so that we cannot compare the sensitivity side by side but the overall metrics are very compatible. One thing we constantly noticed is that the “Fraction of nonduplicate fragments associated with putative cells” is better in the HT cartridge than single lane; probably one of the metrics we improved on in the HT cartridge.

Performance metrics—reproducibility

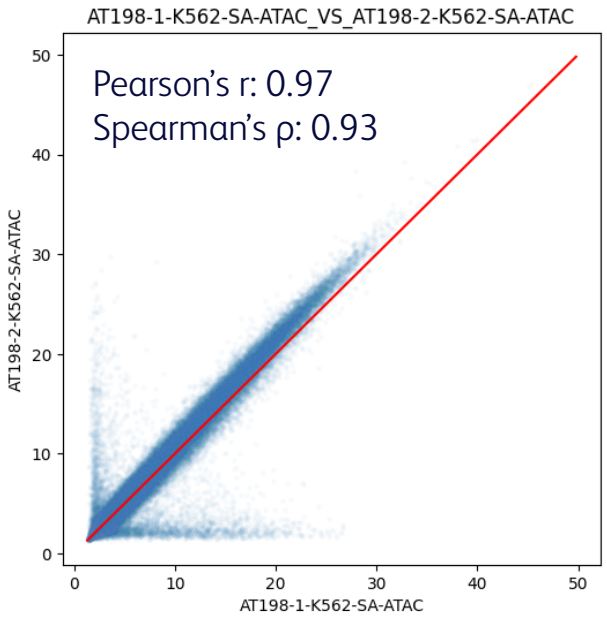
Standalone experiment with K562 and PBMCs:

Peak Overlap by Genomic Location

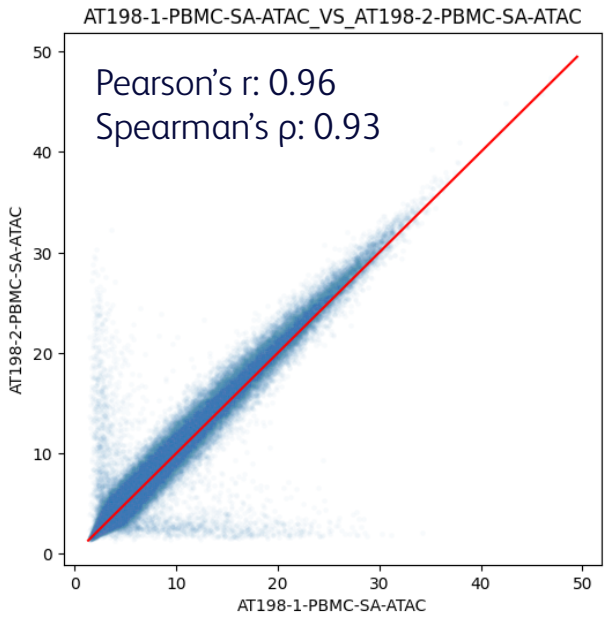
Experiment	# Peaks	# Comm	%Common
K562-SA_EXP_1	260,373	227,714	**87%**
K562-SA_EXP_2	252,839	229,662	**91%**
PBMC-SA_EXP_1	128,983	117,956	**91%**
PBMC-SA_EXP_2	129,136	118,017	**91%**

Peak Signal Correlation

K562



PBMC



Performance metrics—reproducibility

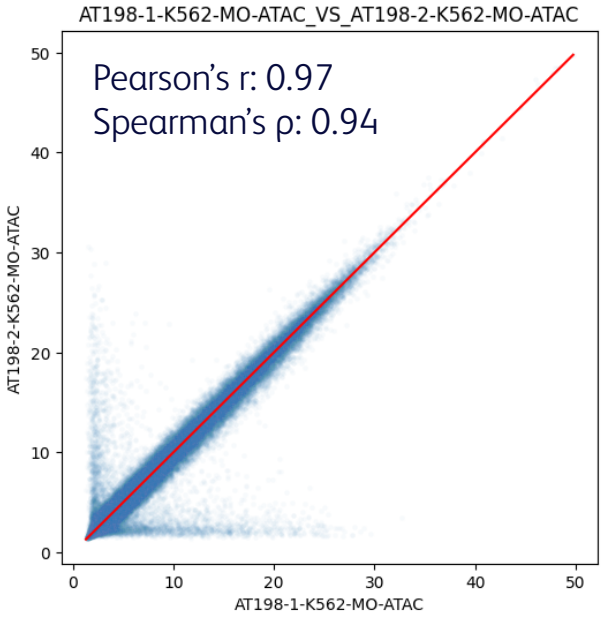
Multiomic experiment with K562 and PBMCs:

Peak Overlap by Genomic Location

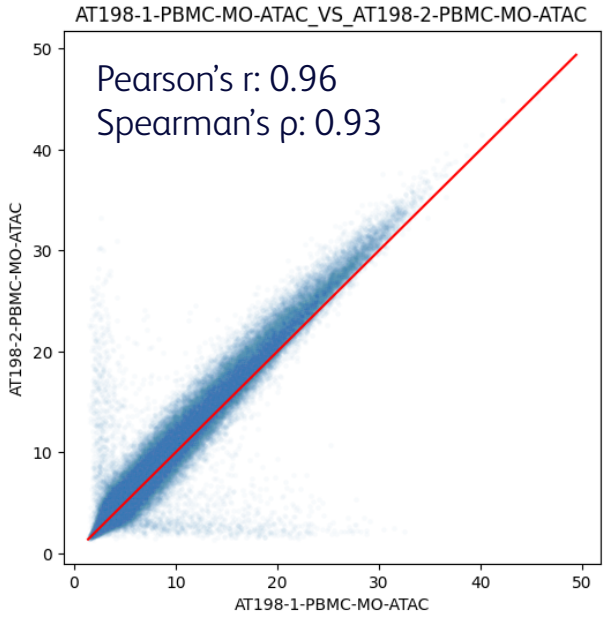
Experiment	# Peaks	# Comm	%Common
K562-MO_EXP_1	249,702	221,817	**89%**
K562-MO_EXP_2	246,111	222,865	**91%**
PBMC-MO_EXP_1	122,707	109,687	**89%**
PBMC-MO_EXP_2	120,204	109,924	**91%**

Peak Signal Correlation

K562



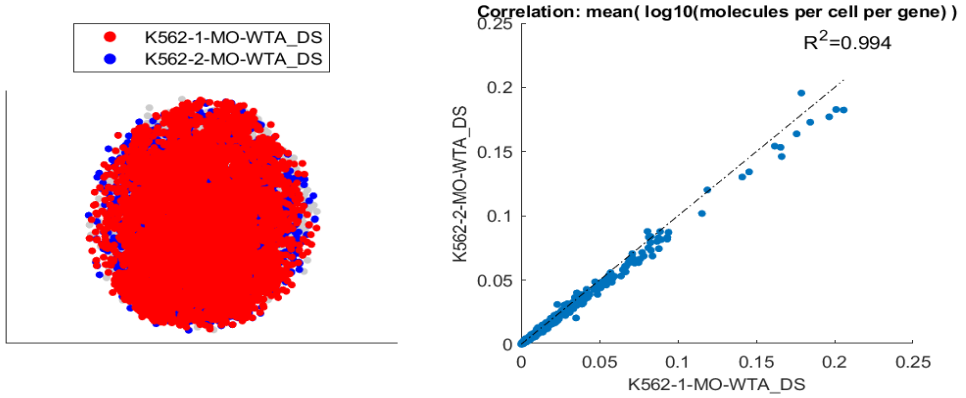
PBMC



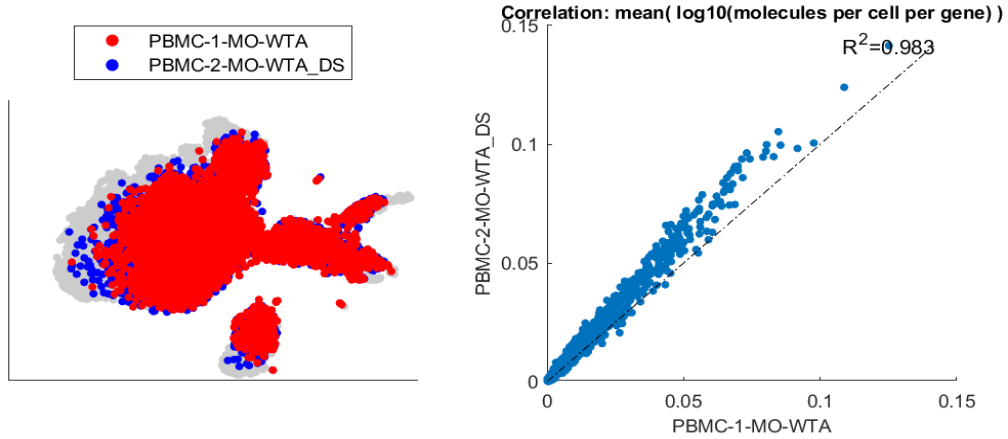
Performance metrics—reproducibility

Reproducibility of WTA in multiomic assay:

K562 WTA (EXP1 vs EXP2)



PBMC WTA (EXP1 vs EXP2)



Performance metrics—repeatability

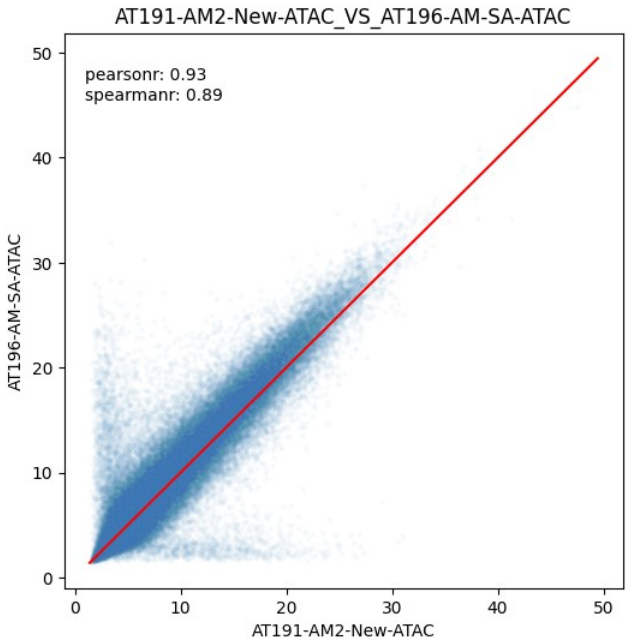
Standalone and multiomic experiments with the K562 cell line:

Peak Overlap by Genomic Location

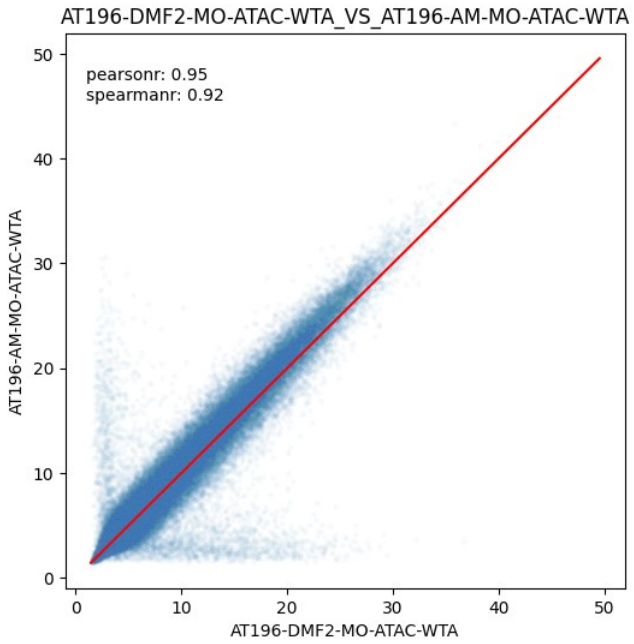
Experiment	# Peaks	# Comm	%Common
K562-SA_PN_AT191	183,268	158,007	**86%**
K562-SA_PN_AT196	190,801	157,272	**82%**
K562-MO_PN_AT196_1	189,017	166,226	**88%**
K562-MO_PN_AT196_2	189,941	167,181	**88%**

Peak Signal Correlation

K562 (Standalone)

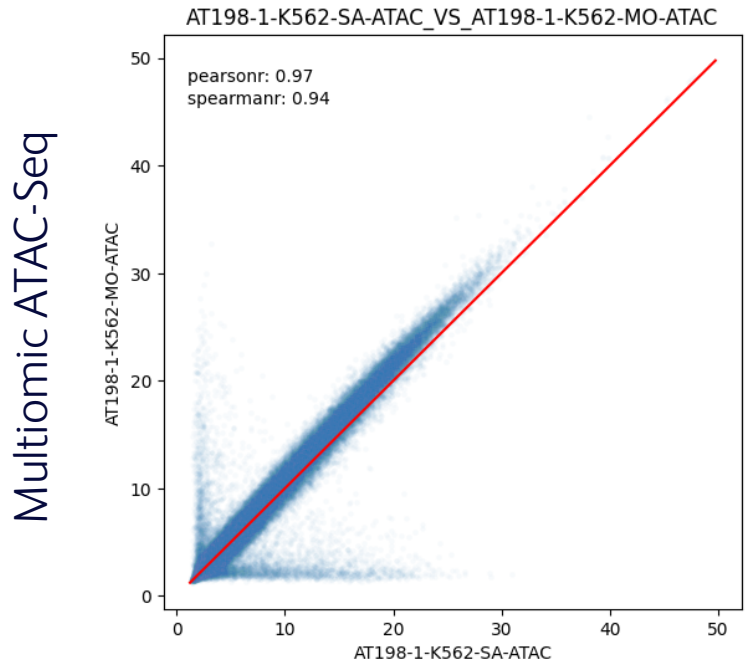


K562 (Multiomic)



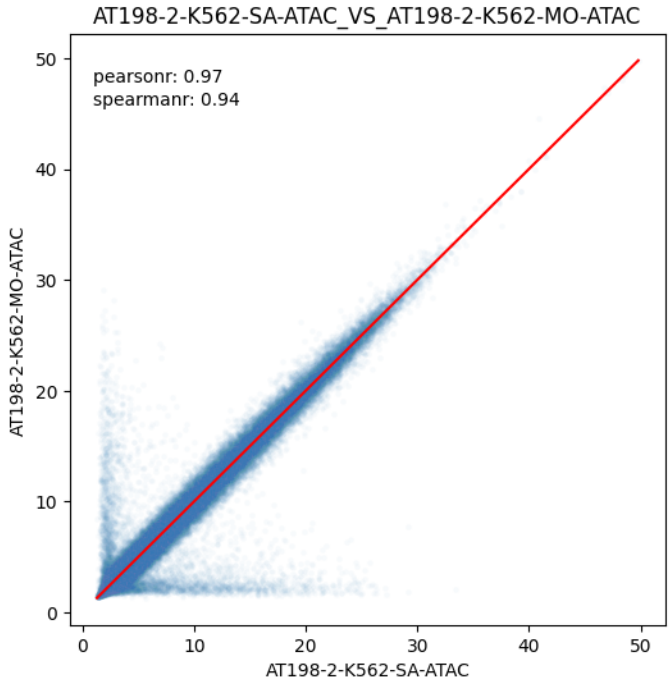
Performance metrics—repeatability

Standalone and multiomic experiments with the K562 cell line:



Multiomic ATAC-Seq

Standalone ATAC-Seq

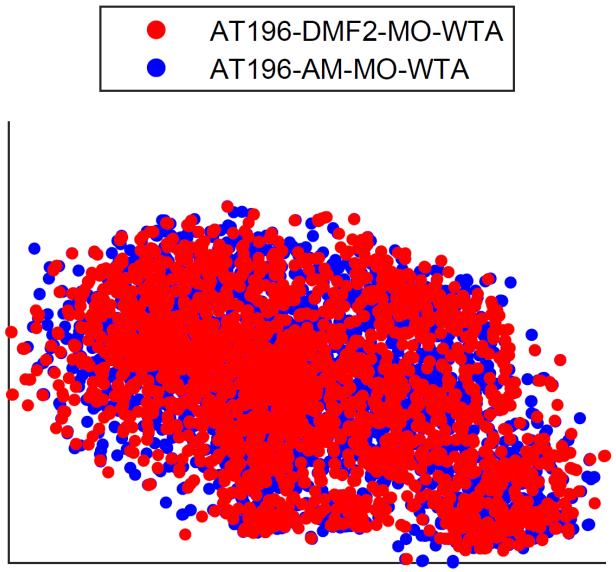


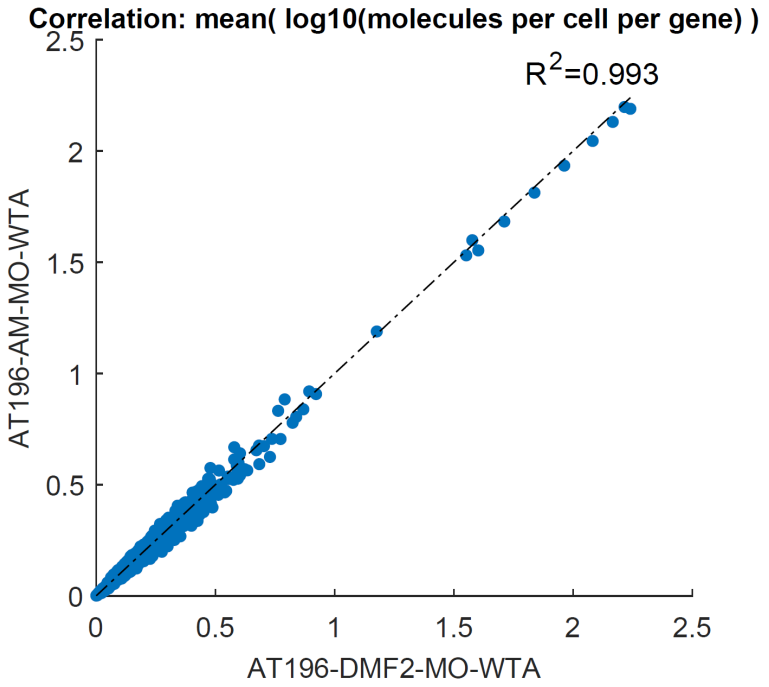
Multiomic ATAC-Seq

Standalone ATAC-Seq

Performance metrics—repeatability

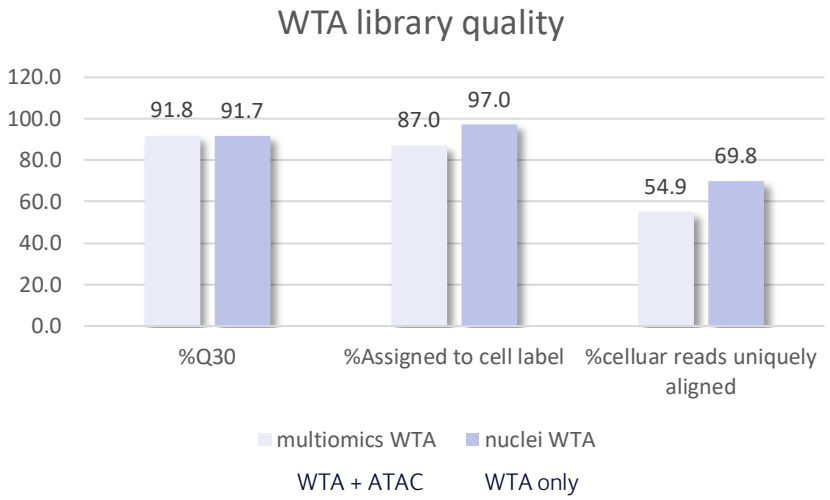
WTA repeatability in the multiomic assay:

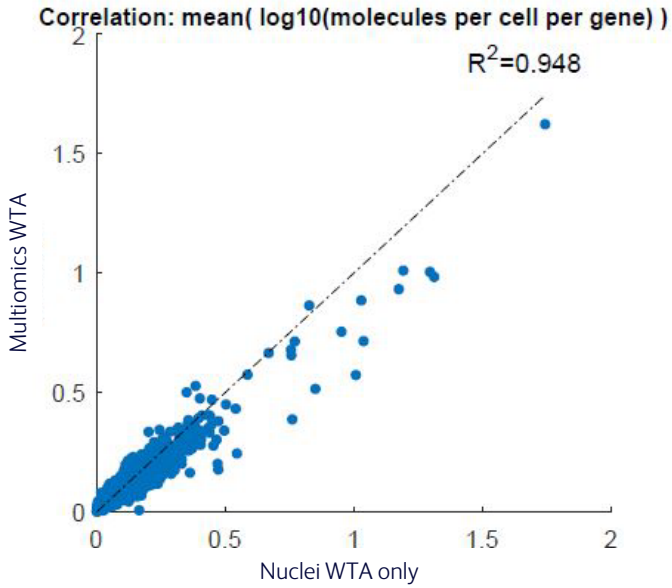




Performance metrics—WTA performance

Multiomic experiment with the K562 cell line:



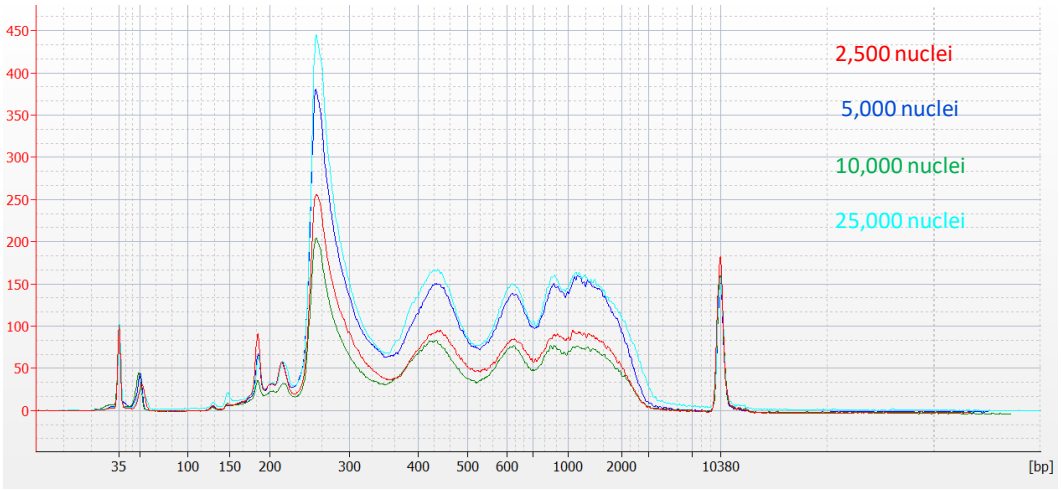


- WTA (Whole Transcriptome Analysis) quality in multiomics ATAC+WTA assay is comparable to nuclei WTA standalone assay
- RNA-seq quality is not significantly impacted by ATAC-seq

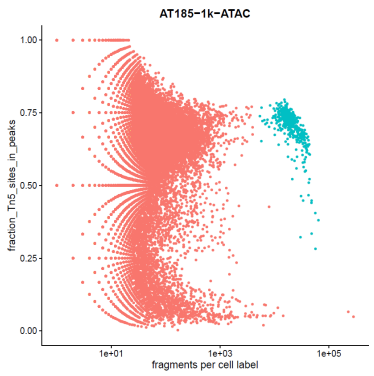
Performance metrics—cell input experiments

Standalone experiments with PBMCs:

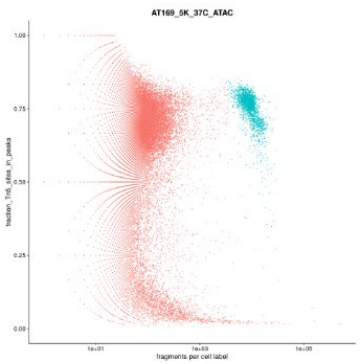
Target nuclei	PCR cycles	Qubit (ng/uL)
500 nuclei	16	4.62
2,500 nuclei	13	3.59
5,000 nuclei	13	5.55
10,000 nuclei	11	3.23
25,000 nuclei	11	6.68



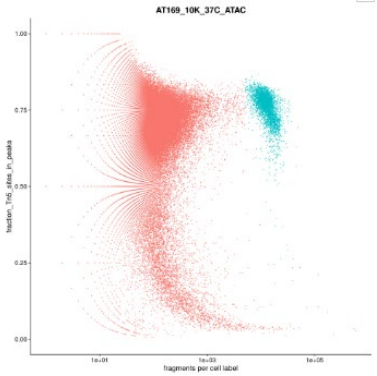
500 nuclei



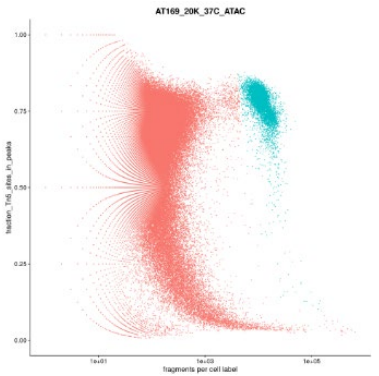
2,500 nuclei



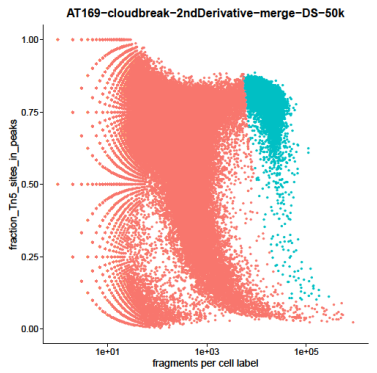
5,000 nuclei



10,000 nuclei

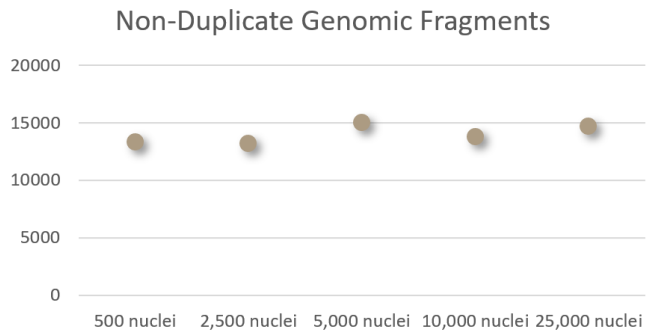


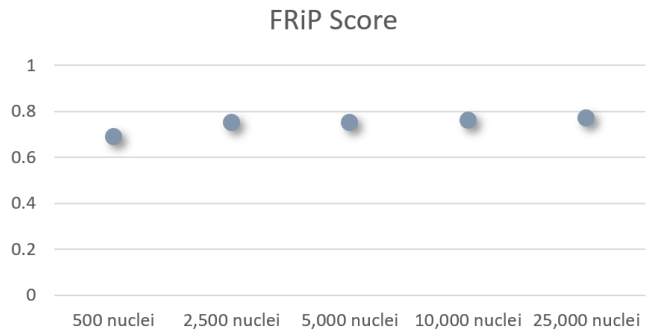
25,000 nuclei

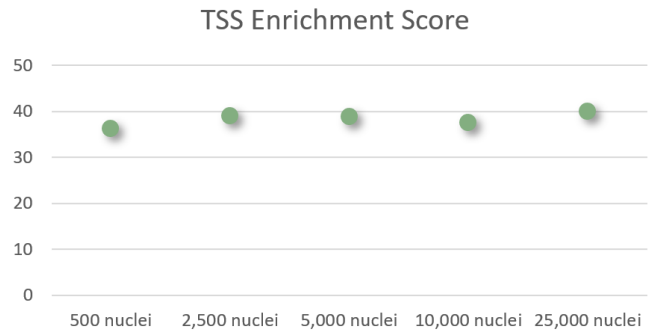


Performance metrics—cell input experiments

Standalone experiments with PBMCs:





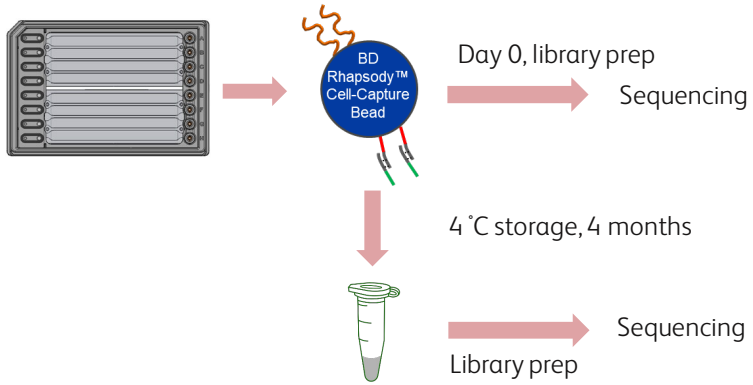


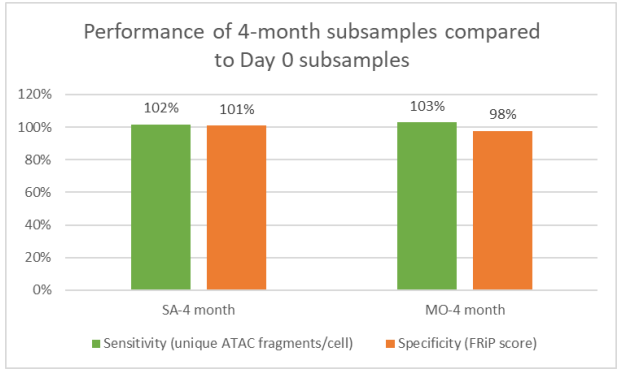
Median nonduplicate fragments per putative cell at roughly 50,000 mean raw read pairs per putative cell.

Robust and reproducible performance irrespective of the nuclei number.

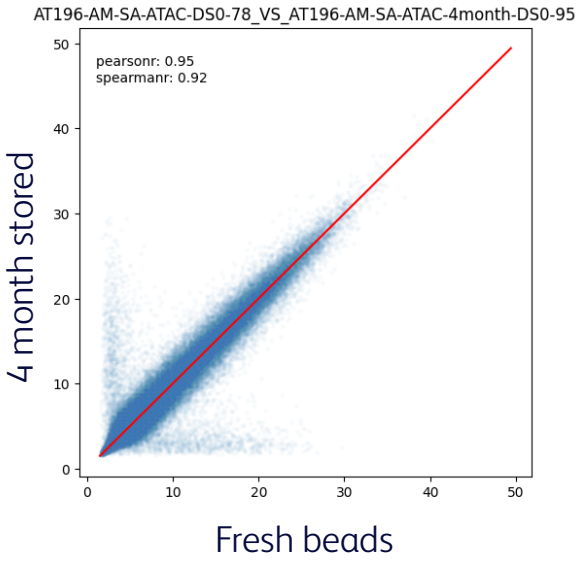
Performance metrics—bead claims

Standalone experiments with PBMCs: Uncompromised assay performance using subsampled beads after being stored at 4 °C for 4 months.

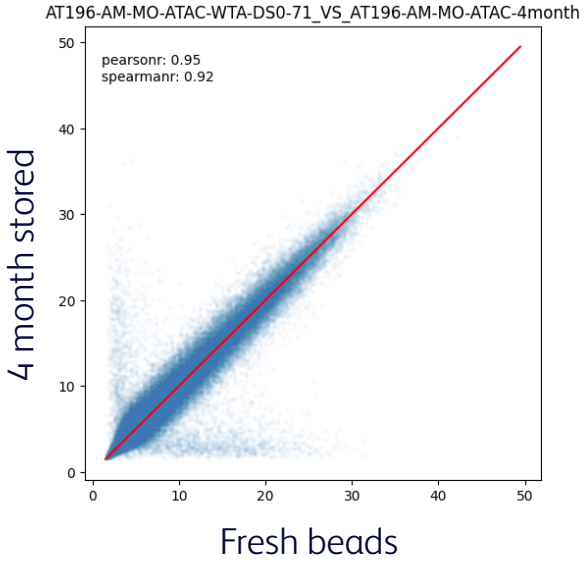




Standalone ATAC-Seq



Multiomic ATAC-Seq

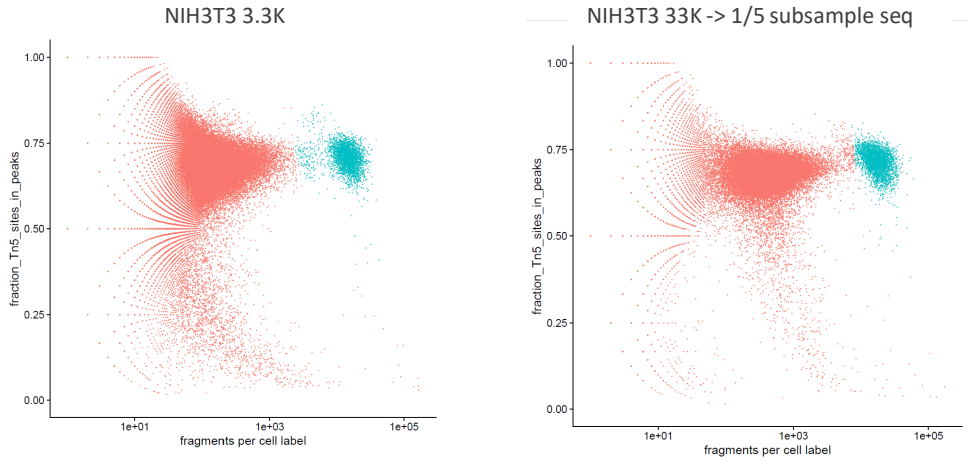


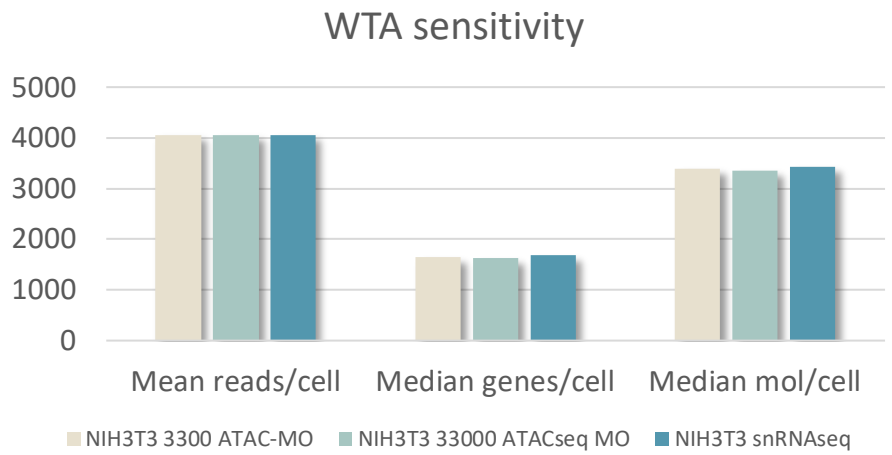
	Standalone Day 0	Standalone 4 month	Multiomic Day 0	Multiomic 4 month
Median proper read pairs per putative cells	20,871	21,390	20,057	21,232
Median nonduplicate fragments per putative cells	18,275	18,573	16,979	17,492

Performance metrics—mouse NIH3T3 cell line

Multiomic ATAC-Seq experiments with mouse NIH3T3 cell line:

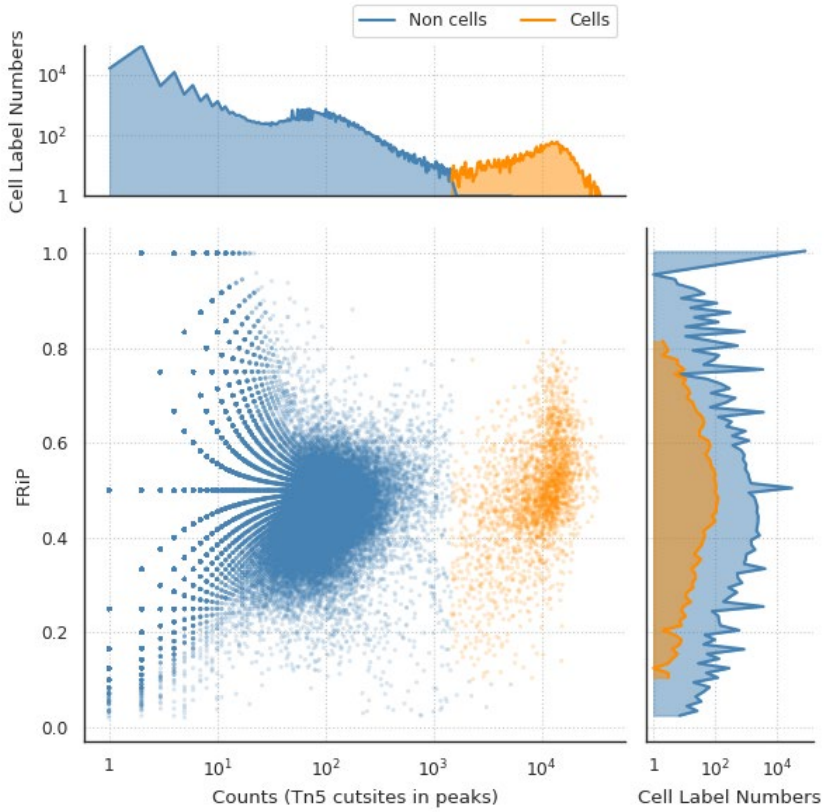
Metric	NIH3T3 3300	NIH3T3 33000 -> 1/5 sequenced
Percent input reads assigned cell label	96.97	96.97
Percent clean reads assigned cell label	98.52	98.44
Percent cell-labeled reads aligned confidently	91.2	90.87
Percent mitochondrial reads	4.15	4.85
Fraction of nonduplicate fragments with NFR lengths	76.28	76.16
Fraction of nonduplicate fragments with mono-nucleosomal lengths	16.98	16.99
%NFR + monosome	93.26	93.15
Total number of putative cells	2873	3115
Fraction of nonduplicate fragments associated with putative cells	72.8	73.3
Fraction of Tn5 transposase sites in Peak regions from putative cells	70.35	71.04
TSS enrichment (peak value)	23.57	25.24
Total raw read pairs	83672108	112606404
Average raw read pairs per putative cell	29123.6	36149.73
Median proper read pairs per putative cell	17638	21082
Median nonduplicate fragments per putative cell	14205	17445
Percent duplicate fragments	19.23	17.22



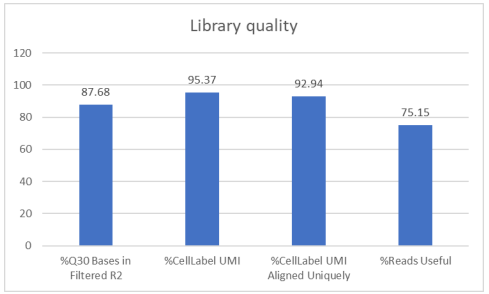


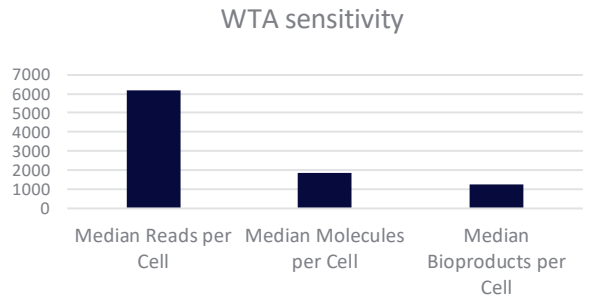
Performance metrics—mouse frozen kidney cells

Multiomic ATAC-Seq experiments with mouse frozen kidney cells:



WTA Sequencing quality and library quality





	Value
Median proper read pairs per putative cells	51,018
Median nonduplicate fragments per putative cells	10,830

Thank you

Glossary of terms

ATAC-seq: Assay for transposase accessible chromatin using sequencing is a method that uses an engineered enzyme known as transposase to integrate sequencing adapters into open regions of chromatin, allowing genome-wide mapping of chromatin.
Chromatin: Complex of DNA and proteins that constitutes the natural state of genetic material within the nucleus of eukaryotic cells.
FRiP score: Fraction of reads in promoter score is a quality metric that quantifies the percentage of ATAC-seq reads falling within known promoter regions, indicating the enrichment of regulatory signal over background noise. Used as a measure of assay specificity.
Histone: Group of proteins that provide structural support for DNA by acting as spools around which DNA wraps.
Non-duplicate fragments: Median number of genomic fragments that were associated with putative cells and found to be non-duplicate in ATAC-Seq data at a given sequencing depth. Uses as a measure of assay sensitivity.
Nucleosome: Basic unit of DNA packing, consisting of a segment of DNA wrapped around histone protein cores.
Promoter: Region of DNA that initiates transcription of a particular gene by providing a binding site for RNA polymerase and transcription factors.
Regulatory DNA: Non-coding genomic sequences that control the transcription of nearby genes by providing binding sites for transcription factors and other proteins.
Subnucleosomal fragments: Non-duplicate genomic fragments with nucleosome-free-region (NFR) lengths < 147 bp in ATAC-seq data. Used as a measure of assay specificity.
Transcription: Process by which the information encoded in a gene's DNA sequence is copied into a complementary RNA molecule.
Transcription factor: Protein that binds to specific DNA sequences and help regulate the transcription of genetic information from DNA to mRNA.
Transcription start site (TSS): Nucleotide position on a DNA sequence where transcription of a gene into mRNA begins.
Transposase: Enzyme that can cut and paste segments of DNA from one location to another within a genome. In ATAC-Seq, an engineered transposase fragments regions of open chromatin and integrates sequencing adapters into cleaved sites, allowing those sites to be identified through sequencing.
Transposase sites in peaks: Count of individual transposase integration events that fall within regions of open chromatin regions identified as peaks in ATAC-seq data. Used as a measure of assay sensitivity.
TSS enrichment score: Score that measures the enrichment or depletion of chromatin accessibility signals around transcription start sites, indicating potential regulatory activity near gene promoters. Used as a measure of assay specificity.