BD Rhapsody™ ATAC-Seq Assays

Product overview and performance data

BD

Chapter I:
Value proposition

End-to-end single-cell multiomic solution

- BD Rhapsody™ ATAC-Seq Assays seamlessly plug into the established BD workflow for single-cell sorting, preserving and multiomic analysis.

BD Rhapsody™ ATAC-Seq Assays applications

- Run our assay to:

Single Cell ATAC-seq

1. *Gain insight into genome-wide native chromatin landscape at the single-cell level*
2. *Identify regulatory regions driving cell-type specific gene expression*
3. *Explore epigenetic heterogeneity within cell populations*

Single Cell Multiomic ATAC-seq + RNA-seq

1. *Uncover direct links between regulatory regions and transcriptional outputs at the single-cell level*
2. *Reveal multilayered regulatory dynamics underlying cellular identities and functions*
3. *Gain a multiomic view of heterogeneity within complex populations*

Applications in immunology

- Single-cell ATAC-seq maps epigenomic regulation and chromatin accessibility in immune cells, enabling high-resolution dissection of heterogeneity and dysregulated gene programs driving immunological disease states.

Year	Title	Methods summary	Outcome
2023	Multimodal single-cell datasets characterize antigen-specific CD8+ T cells across SARS-CoV-2 vaccination and infection	Uses single-cell ATAC-seq and RNA-seq analyses to study immune cells from COVID-19 vaccinated and naturally infected individuals.	COVID-19 vaccination and natural infection elicit distinct antigen-specific $CD8^+$ T cell landscapes, highlighting potential targets for improved vaccine design.
2023	Integrated single-cell chromatin and transcriptomic analyses of human scalp identify gene-regulatory programs and critical cell types for hair and skin diseases	Uses single-cell ATAC-seq and RNA-seq analyses to analyze human scalp tissue from healthy controls and patients with alopecia areata.	Integrated single-cell data pinpoints key cell types and regulatory mechanisms underlying hair and skin diseases, offering potential therapeutic targets.
2023	Drivers of heterogeneity in synovial fibroblasts in rheumatoid arthritis	Uses single-cell ATAC-seq and RNA-seq, imaging and spatial transcriptomics to study drivers of cellular heterogeneity in rheumatoid arthritis.	Reveals distinct patterns in synovial fibroblast types in joints, driven by varying immune cell-derived cytokine exposure, offering insights into disease progression.
2022	Profound phenotypic and epigenetic heterogeneity of the HIV-1-infected $CD4^+$ T cell reservoir	Uses flow cytometry and single cell ATAC-seq to study the phenotypic and epigenetic diversity in HIV-infected cells, aiming at identifying eradication strategies.	Reveals the remarkable heterogeneity of HIV-infected cells of $CD4^+$ T cells, suggesting hurdles of eradication and paving the way for more targeted strategies.

Applications in cancer research

- Single-cell ATAC-seq reveals epigenomic dysregulation in tumor microenvironments and cancer cells, identifying regulatory mechanisms underlying heterogeneity, metastasis and drug resistances in cancer.

Year	Title	Methods summary	Outcome
2023	Epigenetic regulation during cancer transitions across 11 tumour types	Uses single-cell ATAC-seq to analyze epigenetic changes across 11 tumor types to reveal drivers specific to cancer initiation and metastasis.	Pinpoints distinct drivers for tumor initiation and progression across different cancer types, highlighting potential targets for improved targeted therapies.
2023	Epigenetic plasticity cooperates with cell-cell interactions to direct pancreatic tumorigenesis	Uses single-cell ATAC-seq, RNA-seq and spatial transcriptomics to study how cell-to-cell interactions can lead to pancreatic tumor development and its progression.	Identifies distinct cellular populations and interactions driving disease initiation as well as plasticity-associated gene programs, offering early intervention strategies.
2023	FOXP1 and KLF2 reciprocally regulate checkpoints of stem-like to effector transition in CAR T cells	Uses single-cell ATAC-seq and RNA-seq to investigate how FOXP1 and KLF2 transcription factors regulate the transition of CAR T cells to effector states.	Shows what distinct roles these two transcription factors play in regulating the transition of CAR T cells to an exhausted state, potentially impacting therapy efficacy.
2022	Single-cell multiomics analysis reveals regulatory programs in clear cell renal cell carcinoma	Uses single-cell ATAC-seq to identify key regulatory programs in clear cell renal cell carcinoma and potentially therapeutic targets by analyzing tumor.	Unveils four key transcription factors that play a key regulatory role in tumor cell behavior and offers potential therapeutic targets based on the factors identified.

Applications in developmental biology

- Single-cell ATAC-seq elucidates chromatin dynamics across cell lineages during development, linking regulatory elements to gene network that orchestrate cell fate decisions and tissue formation.

Year	Title	Methods summary	Outcome
2023	Organization of the human intestine at single-cell resolution	Uses single-cell ATAC-seq to study the cellular organization of the human intestine to map different cell types and their functions.	Paints a comprehensive picture of the human intestine at the cellular level, revealing diverse cell populations and their roles, crucial for understanding health and disease.
2023	A human fetal lung cell atlas uncovers proximal-distal gradients of differentiation and key regulators of epithelial fates	Uses single-cell ATAC-seq and RNA-seq to create a cell atlas of the developing human lung and study regulatory mechanisms guiding lung formation.	Provides a comprehensive map of developing human lung cell types and their regulators, offering insights into lung development and potential targets for treating lung diseases.
2023	Multimodal characterization of murine gastruloid development	Uses single-cell ATAC-seq, RNA-seq and spatial imaging to study murine gastruloid development and cell states and types associated with this embryonic stage.	Reveals diverse cell states and spatial dynamics during murine gastruloid development, offering insights into early embryonic pattern formation.
2022	Single-cell roadmap of human gonadal development	Uses flow cytometry, ATAC-seq, RNA-seq and spatial analyses to chart development of human gonads and the different cell types associated with that process.	Unveils diverse cell types and developmental trajectories in human testes and ovaries, providing insights into gonadogenesis and potential targets for reproductive disorders.

Applications in neuroscience

- Single-cell ATAC-seq maps chromatin landscapes across cell subtypes, offering an epigenomic basis for neuronal and glial diversity, connectivity, plasticity and dysfunction critical in understanding brain in health and neurological disease.

Year	Title	Methods summary	Outcome
2023	Massively parallel functional dissection of schizophrenia-associated noncoding genetic variants	Uses single-cell ATAC-seq and CRISPR interference screening to analyze how non-coding genetic variants in schizophrenia impact gene expression in neuronal cells.	Reveals how non-coding regions impact gene expression, suggesting potential targets beyond protein-coding regions commonly focused on in drug development.
2023	A cell-type-specific error-correction signal in the posterior parietal cortex	Uses single-cell ATAC-seq in combination with electrophysiology to analyze cell types and signals crucial for error-correction during navigation.	Offers a deeper understanding of how specific neuronal populations in the posterior parietal cortex contribute to navigational error correction.
2023	Epigenomic dissection of Alzheimer's disease pinpoints causal variants and reveals epigenome erosion	Uses single-cell ATAC-seq, RNA-seq and bulk analyses at a large-scale to delve into the epigenomic and transcriptional landscapes of Alzheimer's disease.	Reveals distinct cell-type specific epigenomic dysregulation in microglia, suggesting cellular targets for treatment and highlighting an epigenome erosion in late-stage brains.
2022	Human prefrontal cortex gene regulatory dynamics from gestation to adulthood at single-cell resolution	Uses single-cell ATAC-seq and RNA-seq across various developmental stages to map gene expression and regulatory dynamics in the human prefrontal cortex.	Sheds light on healthy brain development and implicate potential disruptions in distinct cell trajectories and regulatory networks in neurological disorders.

BD Rhapsody™ Single Cell ATAC-Seq Assays

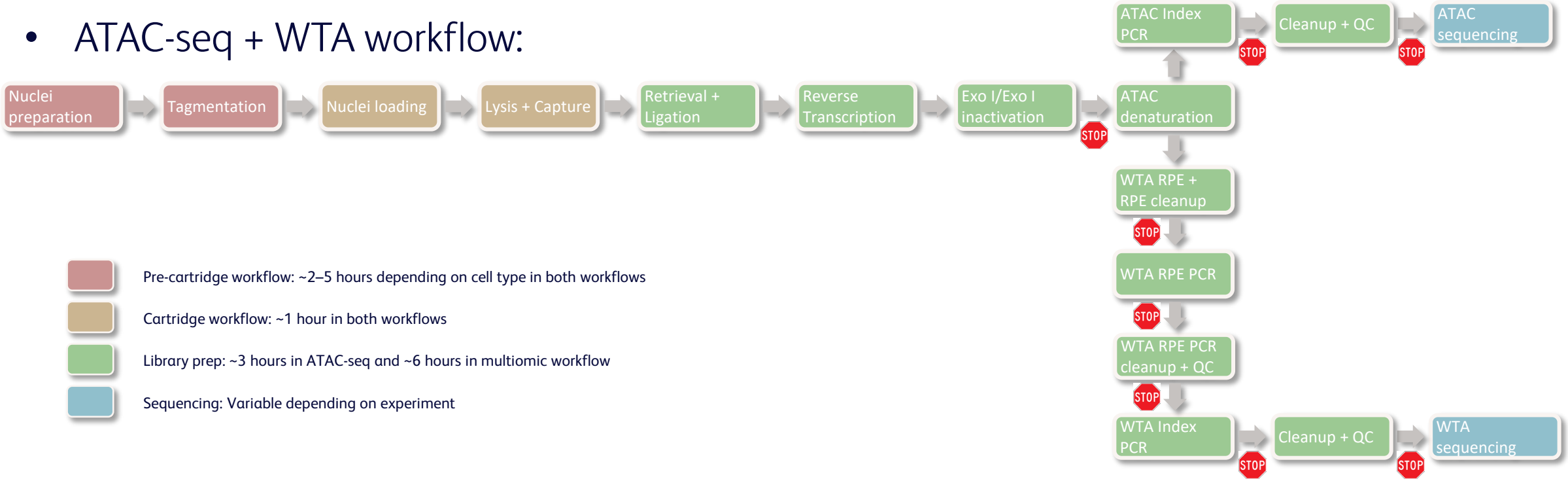
- BD Rhapsody™ ATAC-Seq Assays enable the generation of either standalone open chromatin profiling data from single nuclei or multiomic datasets of open chromatin accessibility and transcriptome of single nuclei in one experiment.
- Key product features include:

Reliable performance	High sensitivity and specificity across different experimental conditions.
Scalable input	Capable of accommodating a wide range of cell inputs.
Multiomic analysis	Integrated epigenomic and transcriptomic characterization on the same cells.
Sample tagging enabled	Compatibility with Custom BD® Nuclear Antibody-Oligonucleotide Conjugates.

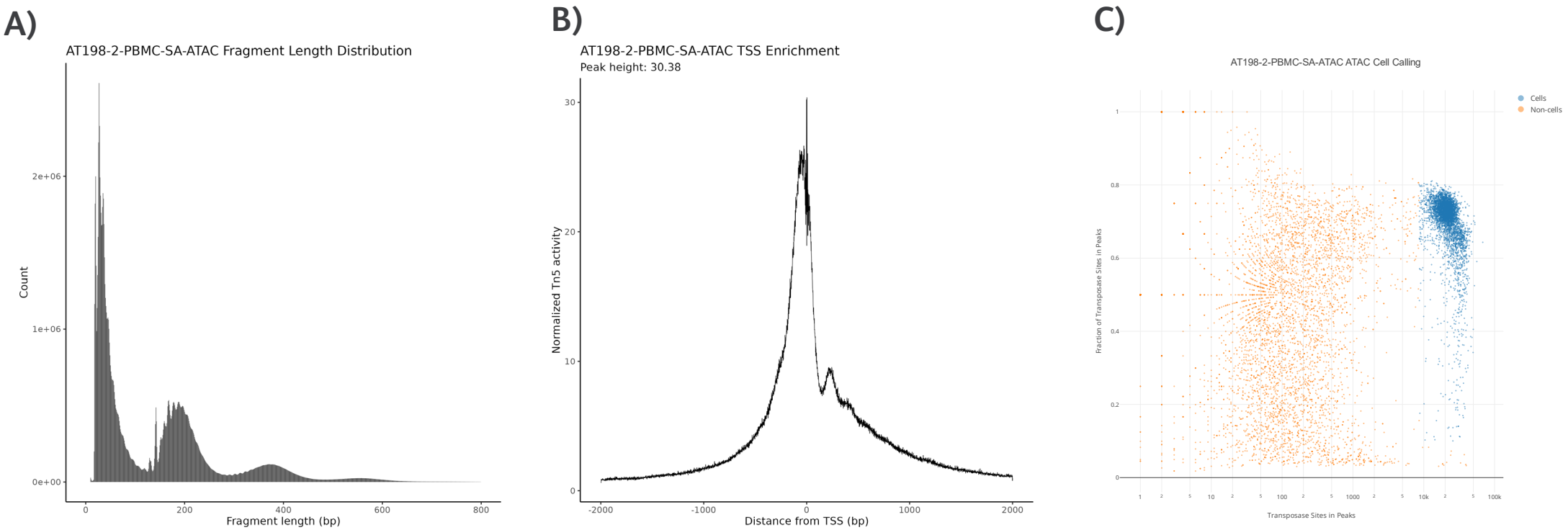
BD Rhapsody™ Single Cell ATAC-Seq Assays Workflow

- ATAC-seq workflow:

- ATAC-seq + WTA workflow:

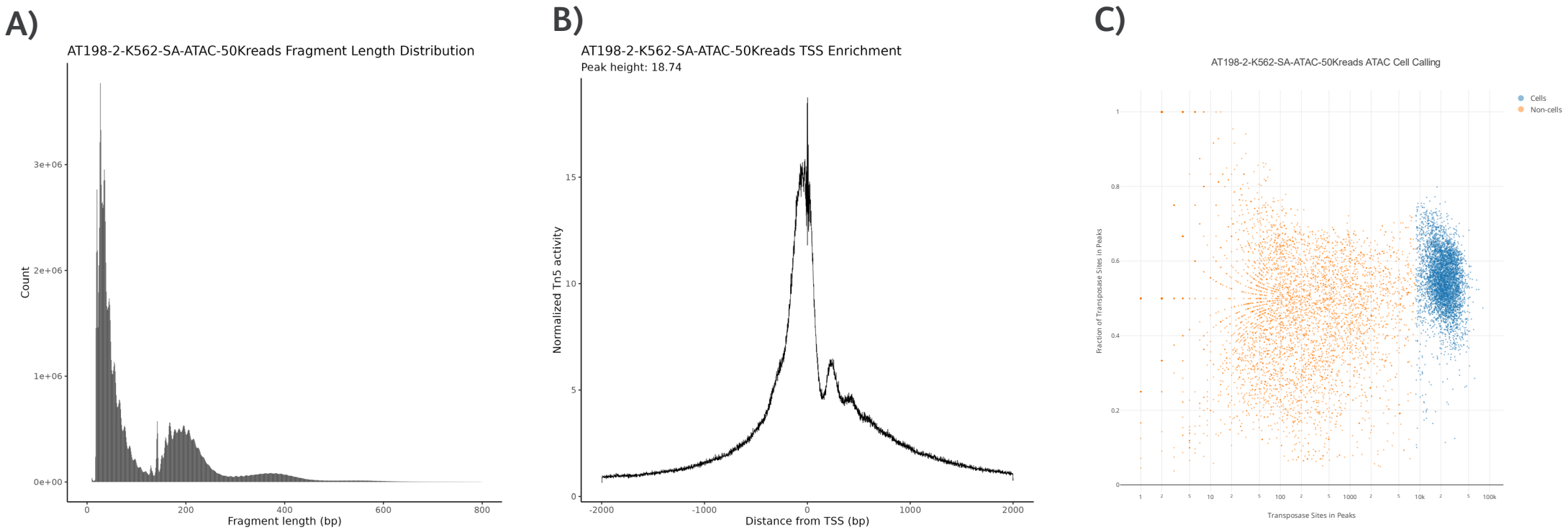


BD Rhapsody™ ATAC-Seq Assay performance—PBMCs



Sensitivity and specificity metrics for the BD Rhapsody™ Single-Cell ATAC-Seq Assay experiment with human PBMCs. A) Fragment size distribution plot demonstrating a preferential transposition of the nucleosome-free regions of open chromatin (large peak at <200 bp) and further peaks reflecting the ~200 bp repeating pattern of nucleosome positioning. **B)** Transcription start site (TSS) enrichment plot, an indicator of signal-to-noise ratio, from the same experiment, showing the aggregated read density around TSS over the genomic background that indicates the preferential accessibility of promoter regions. **C)** Scatter plot from the same experiment, showing the FriP (fraction of reads in promoters) score, a measure of specificity, against the number of transposase sites in peaks, a measure of sensitivity at roughly 50,000 mean raw read pairs per putative cell.

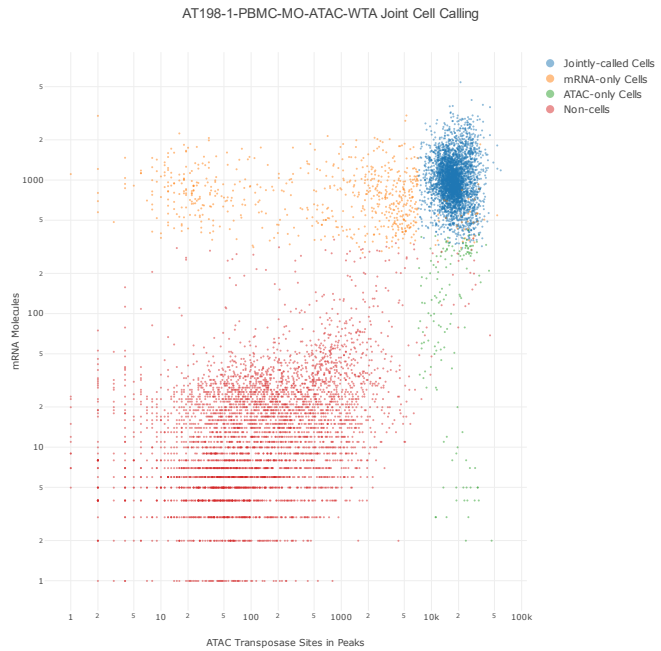
BD Rhapsody™ ATAC-Seq Assay performance—K562



Sensitivity and specificity metrics for the BD Rhapsody™ Single-Cell ATAC-Seq Assay experiment with K562 cell line. A) Fragment size distribution plot demonstrating a preferential transposition of the nucleosome-free regions of open chromatin (large peak at <200 bp) and further peaks reflecting the ~200 bp repeating pattern of nucleosome positioning. **B)** Transcription start site (TSS) enrichment plot, an indicator of signal-to-noise ratio, from the same experiment, showing the aggregated read density around TSS over the genomic background that indicates the preferential accessibility of promoter regions. **C)** Scatter plot from the same experiment, showing the FriP (fraction of reads in promoters) score, a measure of specificity, against the number of transposase sites in peaks, a measure of sensitivity at roughly 50,000 mean raw read pairs per putative cell.

BD Rhapsody™ Single Cell Multiomic ATAC-Seq Assay performance—PBMCs and K562

A)



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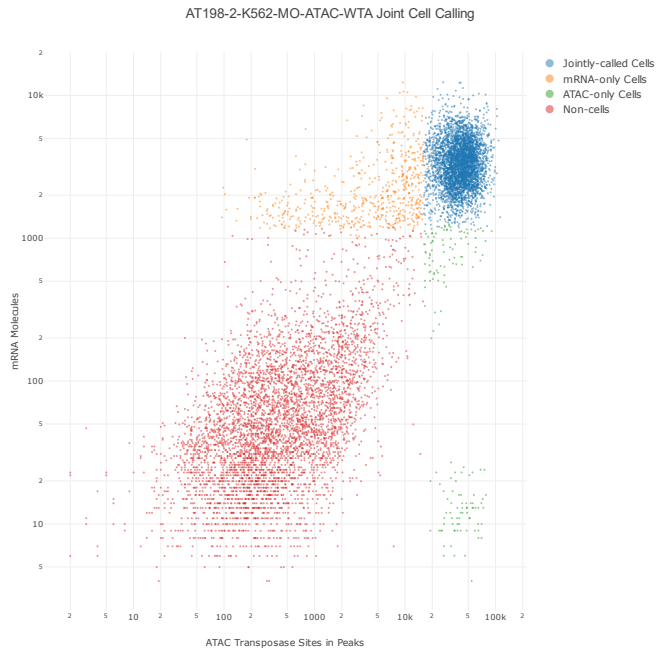
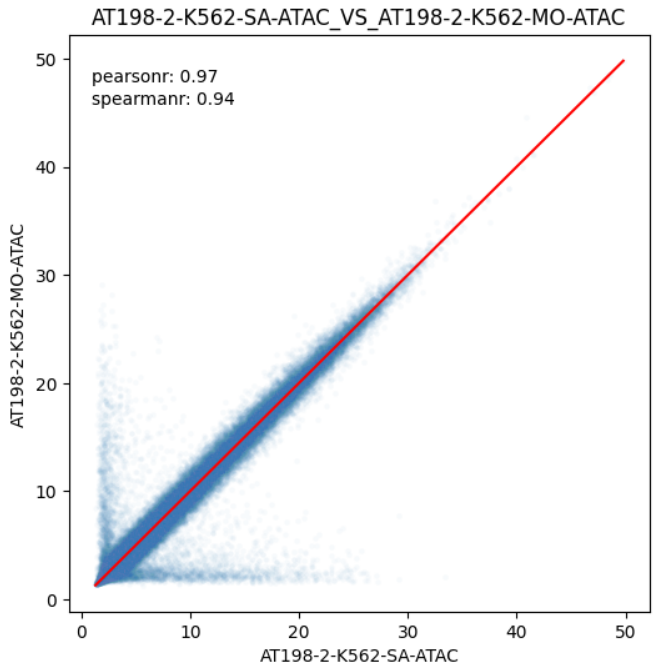


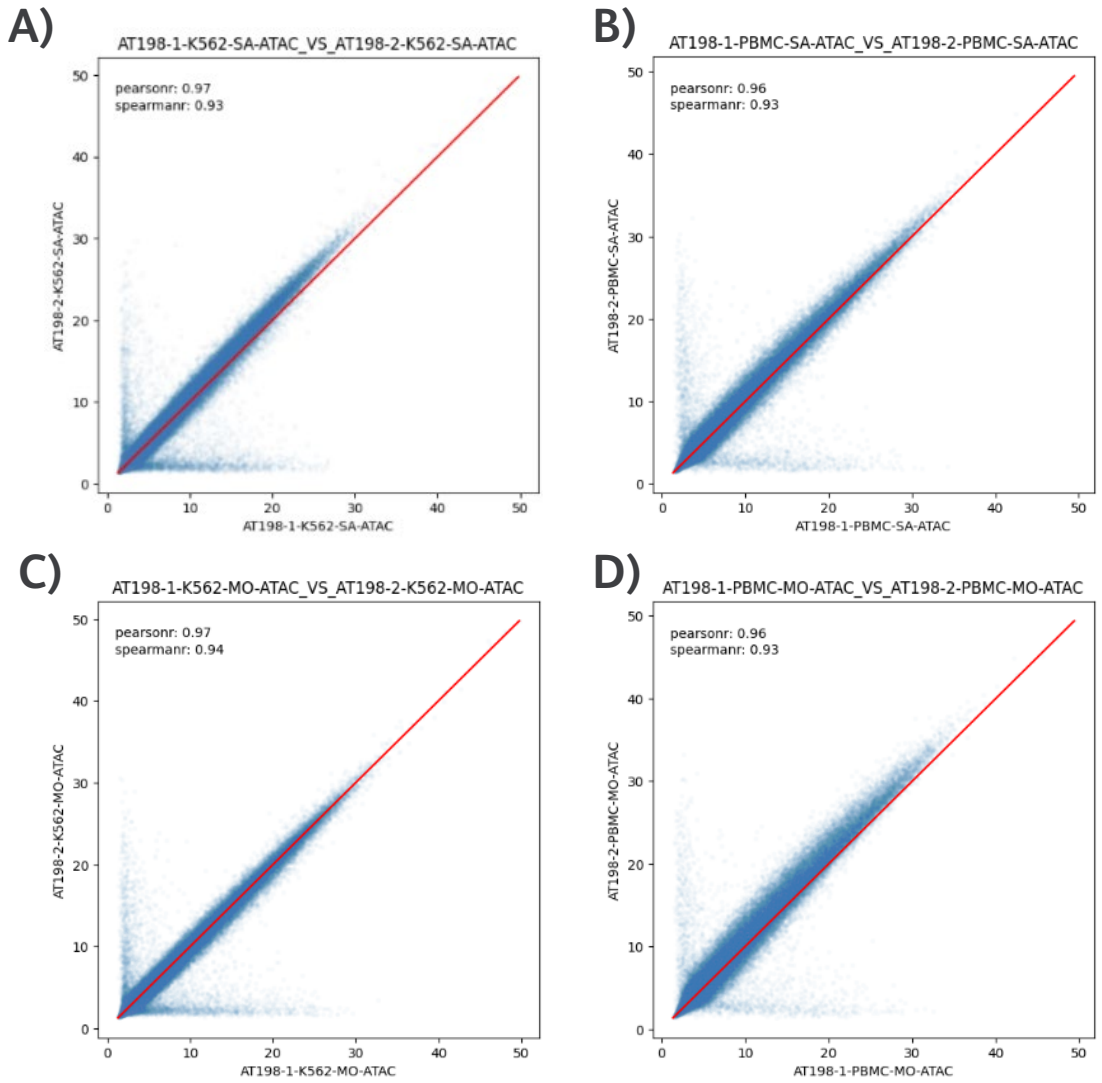
C)



Sensitivity and specificity metrics for the BD Rhapsody™ Single-Cell Multiomic ATAC-Seq Assay. A) Joint calling scatter plot from a multiomic experiment with PBMCs where each cell is represented as a dot and RNA molecules are plotted against the ATAC-seq transposition events. **B)** The same plot with the K562 cell line. The clear separation between cell and non-cell events indicates the ability to effectively distinguish real cells from non-cell barcodes as well as a successful integration and co-analysis of the chromatin accessibility and transcriptomic data at the single-cell level. Both samples were sequenced up to ~50,000 mean raw read pairs per putative cell. **C)** High correlation between the ATAC-seq data from a standalone BD Rhapsody™ ATAC-Seq experiment vs a multiomic BD Rhapsody™ ATAC-Seq experiment with K562 cells as shown by the high peak signal correlation plot.

BD Rhapsody™ ATAC-Seq Assay reproducibility—PBMCs and K562

High reproducibility with BD Rhapsody™ ATAC-Seq and Multiomic ATAC-Seq Assay. The peak signal correlation across two different standalone BD Rhapsody™ ATAC-Seq Assay experiments with the K562 cell line (**A**) and two different standalone BD Rhapsody™ ATAC-Seq Assay experiments with human PBMCs (**B**). The peak signal correlation across two different multiomic BD Rhapsody™ ATAC-Seq and WTA Assay experiments with the K562 cell line (C) and human PBMCs (D).

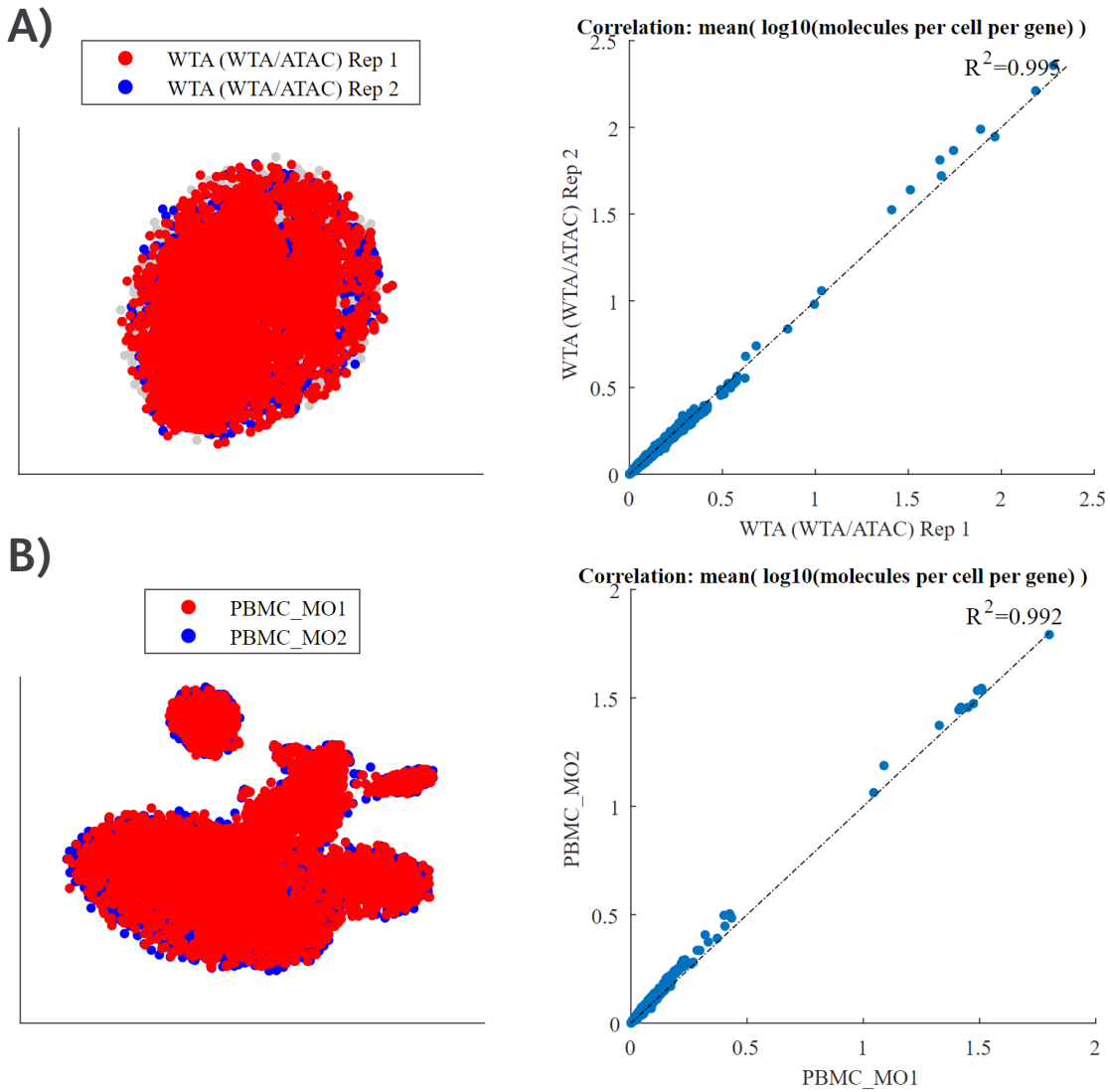


ATAC-seq	Number of Peaks	Number of Common	%Common
K562 Exp 1	260,373	227,714	87%
K562 Exp 2	252,839	229,662	91%
PBMC Exp 1	128,983	117,956	91%
PBMC Exp 2	129,136	118,017	91%

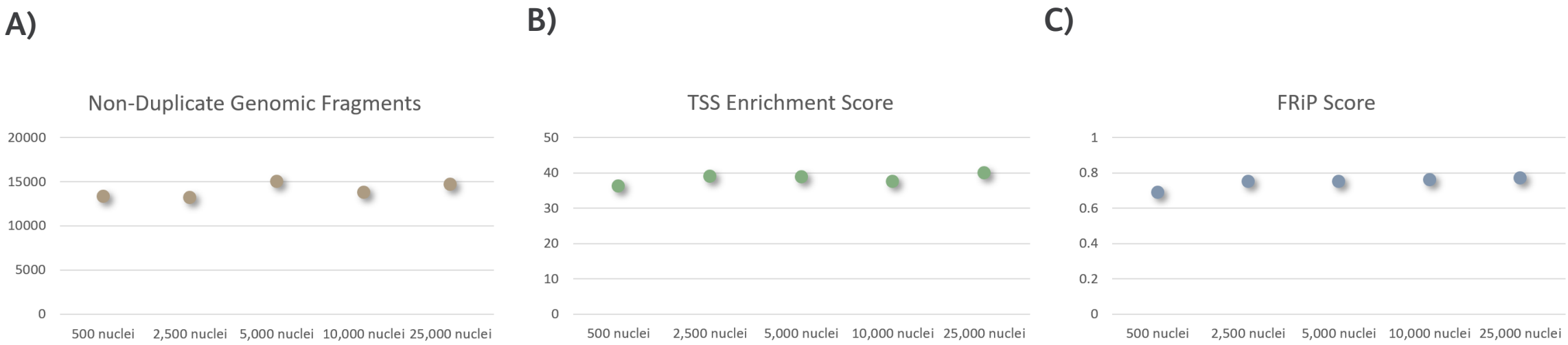
Multiomic ATAC-seq	Number of Peaks	Number of Common	%Common
K562 Exp 1	249,702	221,817	89%
K562 Exp 2	246,111	222,865	91%
PBMC Exp 1	122,707	109,687	89%
PBMC Exp 2	120,204	109,924	91%

WTA reproducibility in multiomic workflow—PBMCs and K562

High reproducibility in whole transcriptome analysis with BD Rhapsody™ Single-Cell Multiomic ATAC-Seq Assay. Clustering and gene expression correlation analysis for the WTA samples in the multiomic BD Rhapsody™ ATAC-Seq Assay, indicating no batch effect in WTA libraries made across two different experiments with the K562 cell line (A) and human PBMCs (B).



BD Rhapsody™ Single Cell Multiomic ATAC-Seq Assay reproducibility—PBMCs and K562



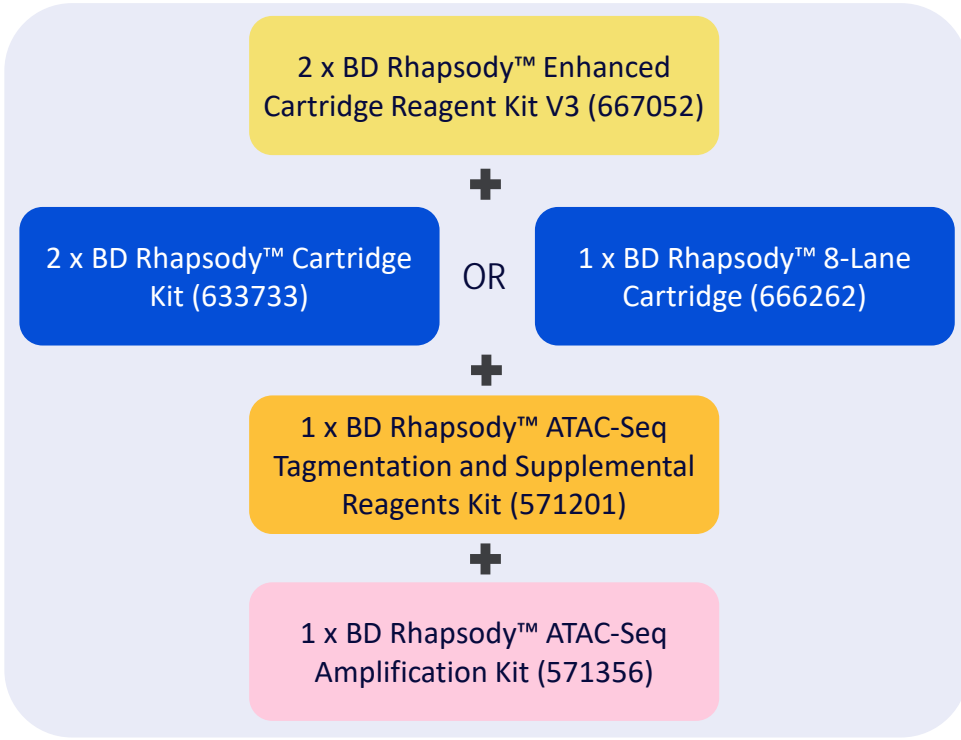
Robust performance across different cell input amounts. BD Rhapsody™ ATAC-Seq Assays enable scalable profiling of open chromatin and gene expression analysis from limited cell inputs to thousands of single cells in one experiment, as demonstrated by the data from a series of BD Rhapsody™ ATAC-Seq Assay experiments with 500, 2,500, 5,000, 10,000, and 25,000 nuclei from human PBMCs captured in a single lane on the BD Rhapsody™ 8-Lane Cartridge. A. Median nonduplicate fragments per putative cell at roughly 50,000 mean raw read pairs per putative cell. B. TSS enrichment plots from the same experiments. C. FriP scores from the same experiments, all pointing to a robust and reproducible performance irrespective of the nuclei number.

Chapter II:
Product overview

BD Rhapsody™ ATAC-Seq Assays reagents

- Reagent bundles needed to enable ATAC-Seq and Multiomic ATAC-Seq for 8 reactions.

Single-Cell ATAC-Seq Assay



Existing kit

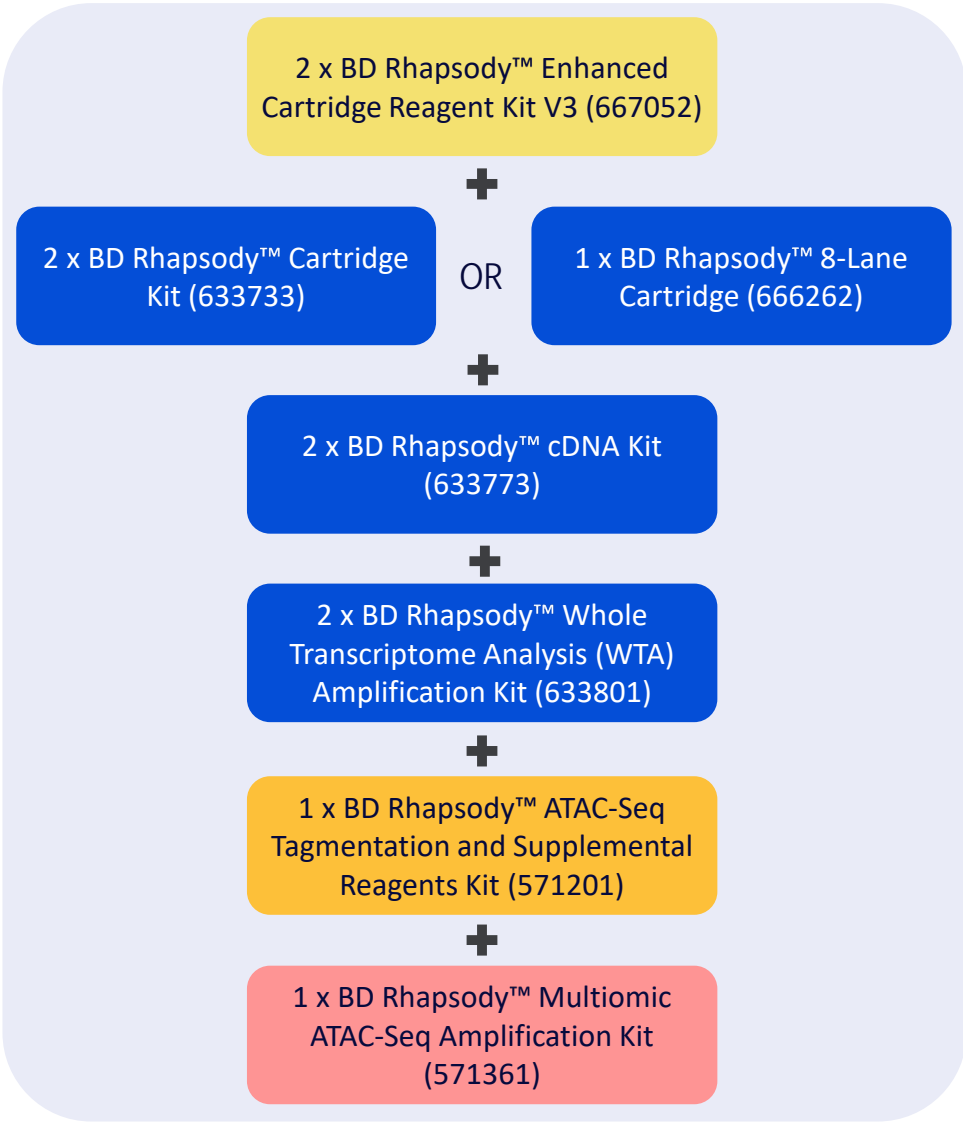
New kit, TCR/BCR Next and ATAC-Seq assays

New kit, Standalone and Multiomic assays

New kit, Standalone assay

New kit, Multiomic assay

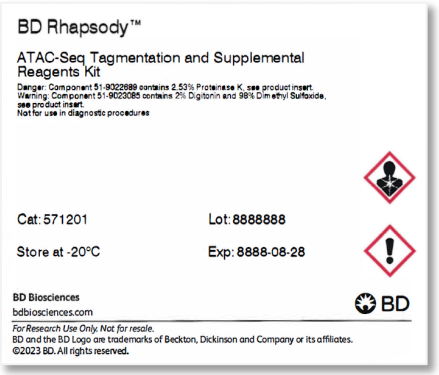
Single-Cell Multiomic ATAC-Seq Assay



BD Rhapsody™ ATAC-Seq Tagmentation and Supplemental Reagents Kit

- Tagmentation and Supplemental Reagents Kit (used in both workflows)

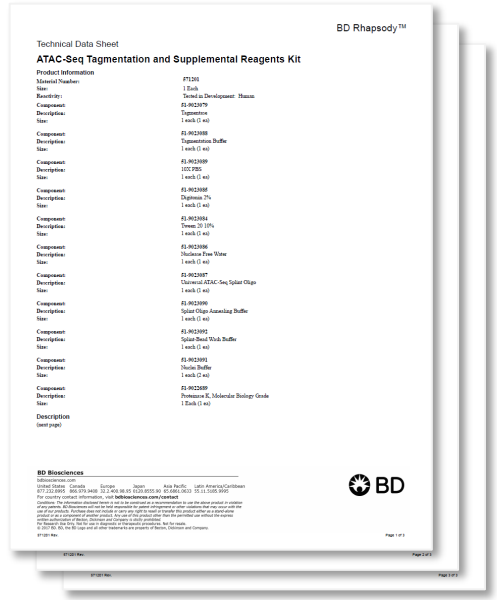
Description	PN	Storage temperature	Shelf life
BD Rhapsody™ ATAC-Seq Tagmentation and Supplemental Reagents Kit	571201	–20°C	1.5 years



ATAC-Seq Tagmentation and Supplemental Reagents Kit

Cap Color	Name	Quantity
●	Tagmentase	1
●	Tagmentation Buffer	1
●	10X PBS	1
●	Digitonin 2%	1
●	Tween 20 10%	1
○	Nuclease Free Water	1

Cap Color	Name	Quantity
●	Universal ATAC-Seq Splint Oligo	1
●	Splint Oligo Annealing Buffer	1
●	Splint-Bead Wash Buffer	1
●	Nuclei Buffer	2
○	Proteinase K, Molecular Biology Grade	1



- Kit contains printed Technical Data Sheet (TDS) (right).
- Safety Data Sheet (SDS) and TDS is available on the website.

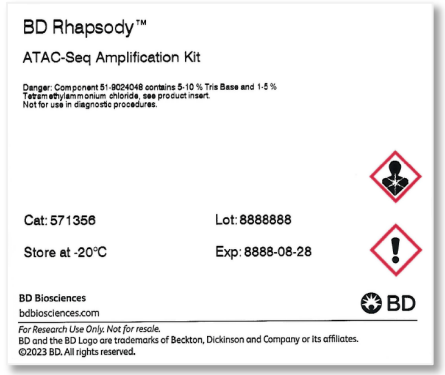
BD Rhapsody™ ATAC-Seq Amplification Kit

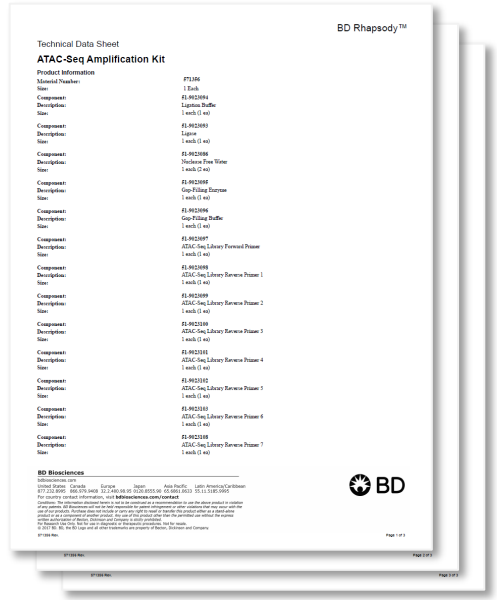
- ATAC-Seq Amplification Kit (used only in standalone workflow)

Description	PN	Storage temperature	Shelf life
BD Rhapsody™ ATAC-Seq Amplification Kit	571356	–20°C	1 year

ATAC-Seq Amplification Kit

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





- Kit contains printed Technical Data Sheet (TDS) (right).
- Safety Data Sheet (SDS) and TDS is available on the website.

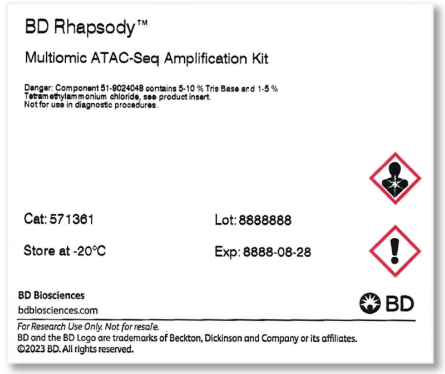
BD Rhapsody™ Multiomic ATAC-Seq Amplification Kit

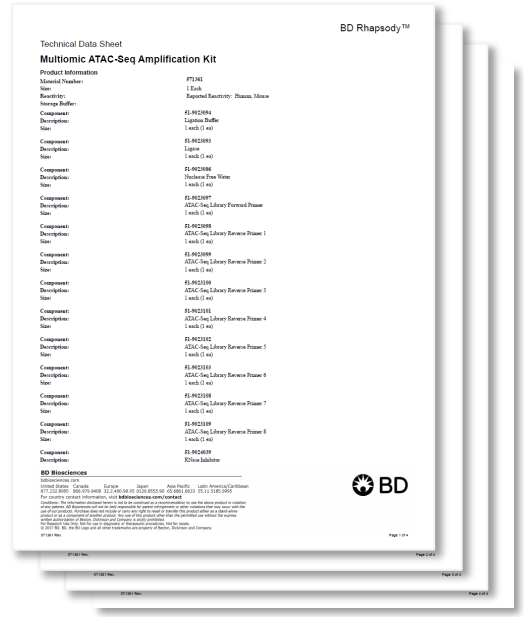
- Multiomic ATAC-Seq Amplification Kit (used only in multiomic workflow)

Description	PN	Storage temperature	Shelf life
BD Rhapsody™ Multiomic ATAC-Seq Amplification Kit	571361	–20°C	1 year

Multiomic ATAC-Seq Amplification Kit

Cap Color	Name	Quantity	Cap Color	Name	Quantity
Green	Ligation Buffer	1	Red	ATAC-Seq Library Reverse Primer 6	1
Green	Ligase	1	Red	ATAC-Seq Library Reverse Primer 7	1
White	Nuclease Free Water	1	Red	ATAC-Seq Library Reverse Primer 8	1
White	ATAC-Seq Library Forward Primer	1	Green	RNase Inhibitor	1
Red	ATAC-Seq Library Reverse Primer 1	1	White	0.1M DTT, Molecular Biology Grade	1
Red	ATAC-Seq Library Reverse Primer 2	1	White	Bead Resuspension Buffer	1
Red	ATAC-Seq Library Reverse Primer 3	1	Purple	Elution Buffer	1
Red	ATAC-Seq Library Reverse Primer 4	1	Blue	Splint oligo Removal Buffer	2
Red	ATAC-Seq Library Reverse Primer 5	1	White	PCR MasterMix	1





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Workflow protocols

- Label-approved library preparation protocols and bioinformatics user guide:

Doc ID	Doc Title	Workflow
23-24473	BD Rhapsody™ System Single-Cell ATAC-Seq Library Preparation Protocol	ATAC-seq
23-24474	BD Rhapsody™ System Single-Cell ATAC-Seq and mRNA Whole Transcriptome Analysis Library Preparation Protocol	ATAC-seq + WTA
23-24580	BD Rhapsody™ Sequence Analysis Pipeline User Guide	All Rhapsody assays

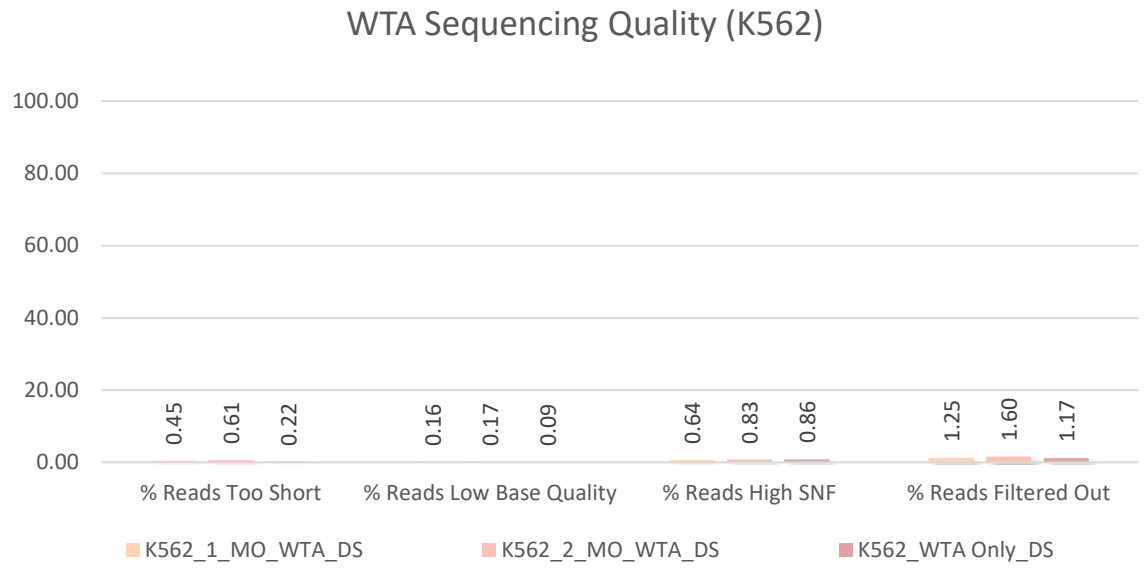
- SMK protocols will be released after launch:

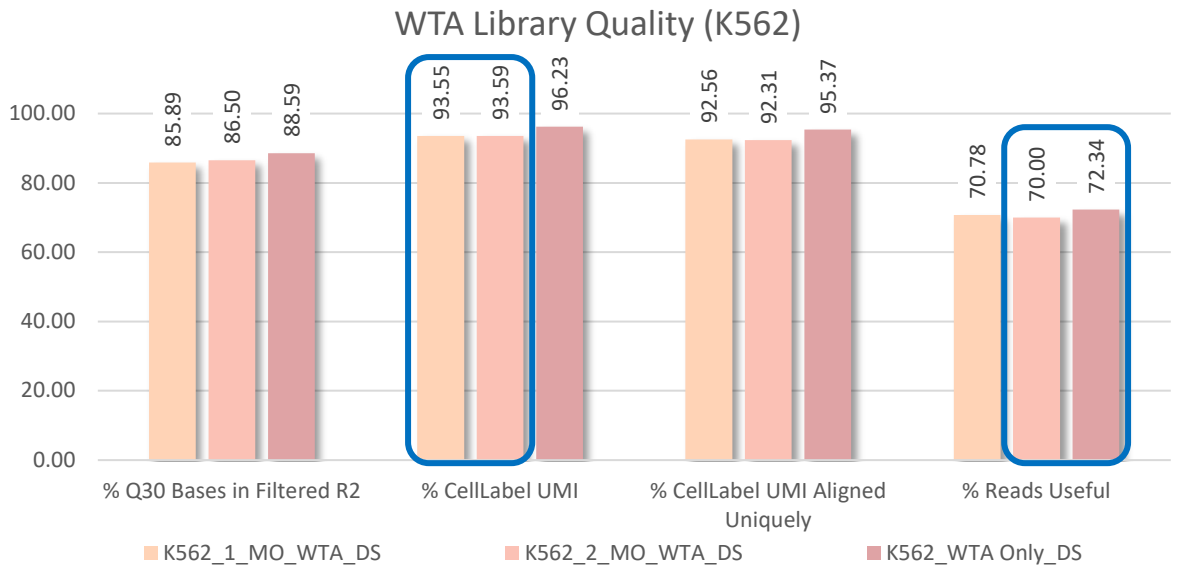
Doc ID	Doc Title	Workflow
23-24798	BD Rhapsody™ System Single-Cell ATAC-Seq and Sample Tag Library Preparation Protocol	ATAC-seq + SMK
23-24799	BD Rhapsody™ System Single-Cell ATAC-Seq, mRNA Whole Transcriptome Analysis, and Sample Tag Library Preparation Protocol	ATAC-seq + WTA + SMK

Chapter III:
Supplementary data

Performance metrics—whole transcriptome analysis (WTA) sequencing and library quality

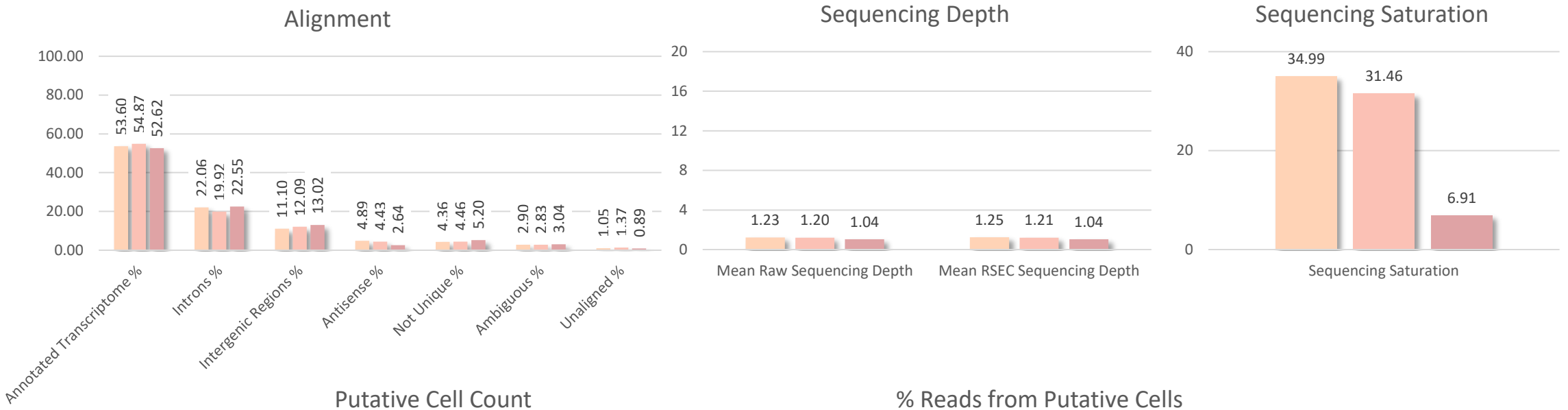
Multiomic (MO) ATAC-Seq experiment with K562 cell line:

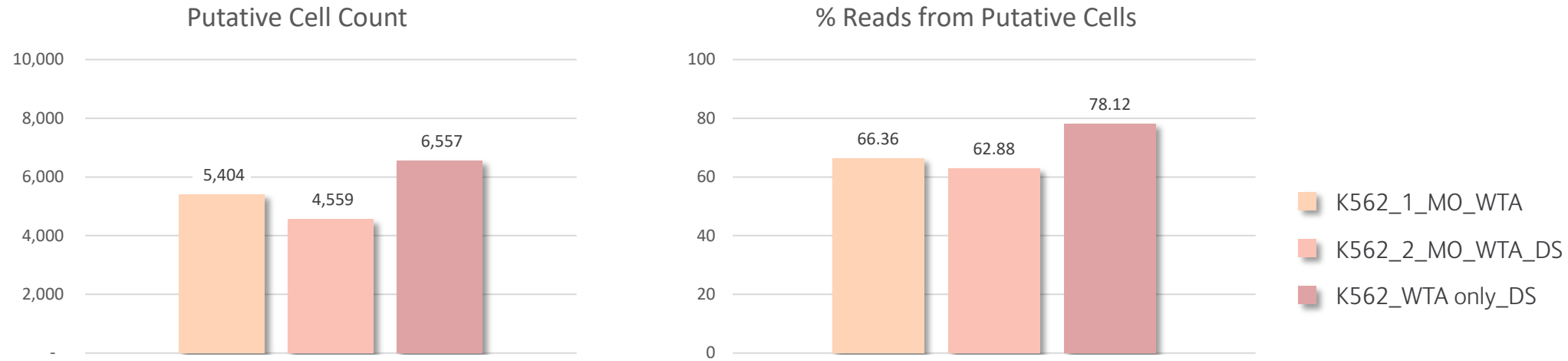




Performance metrics—WTA metrics

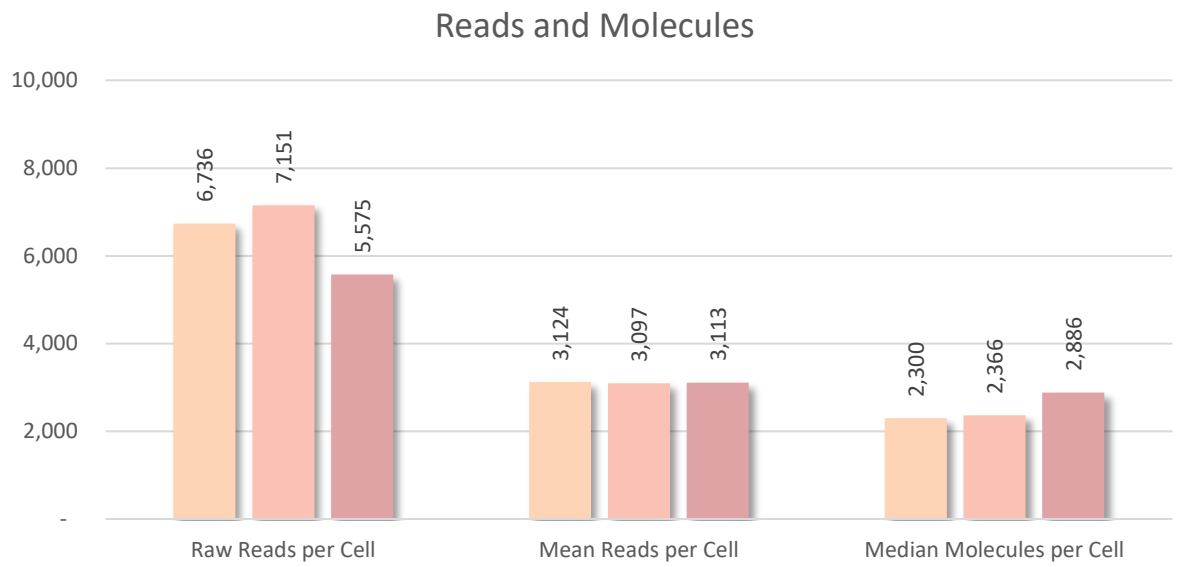
Multiomic ATAC-Seq experiment with K562 cell line:

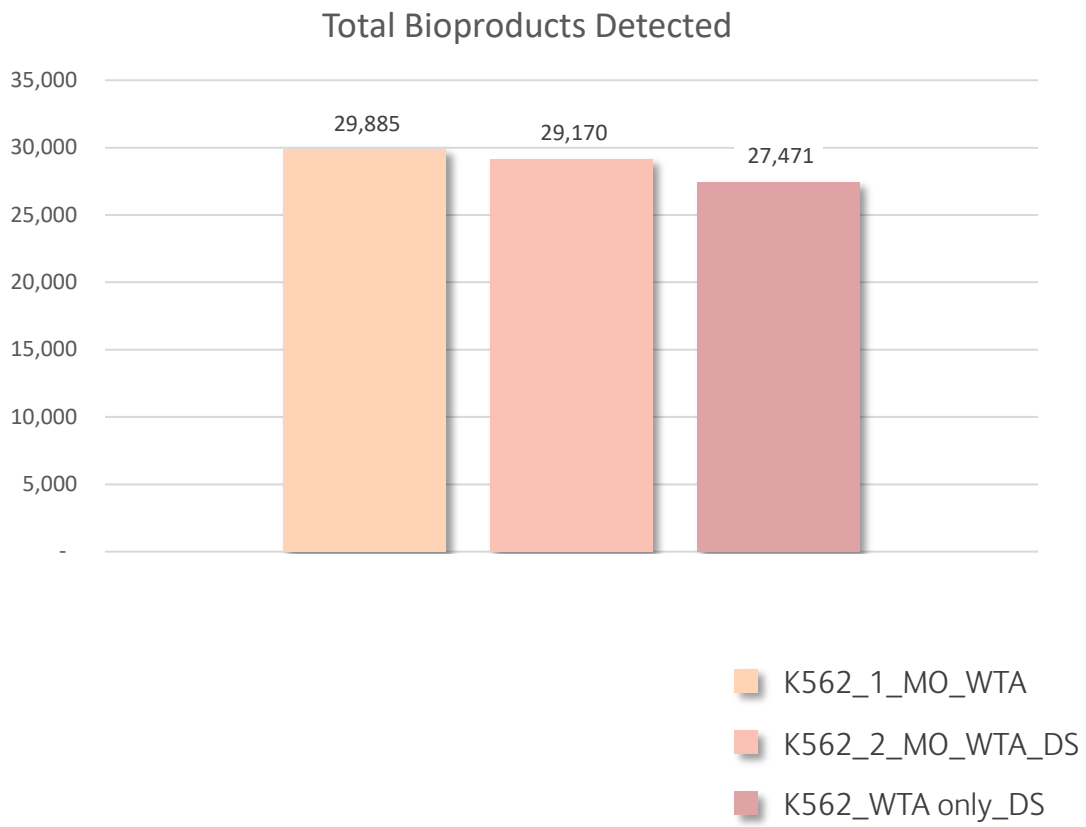




Performance metrics—WTA sensitivity

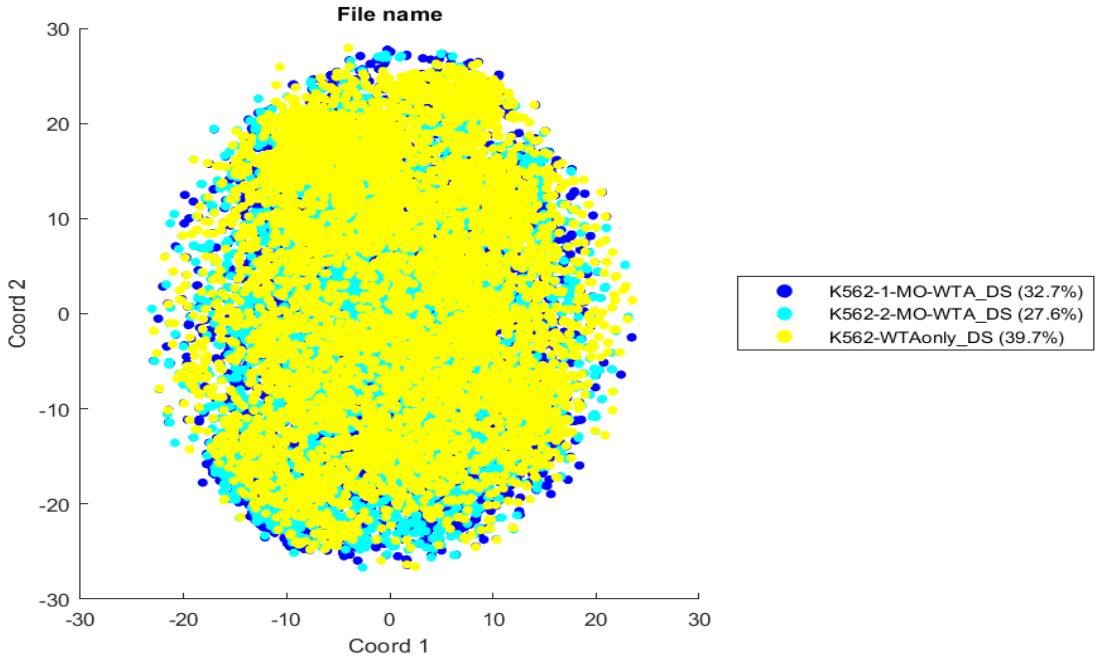
Multiomic ATAC-Seq experiment with K562 cell line:
Post down-sampling to similar mean reads/cell

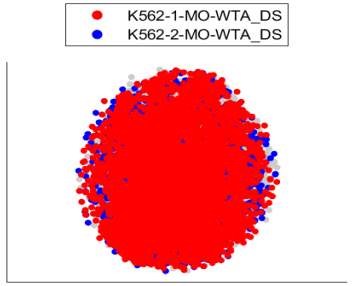


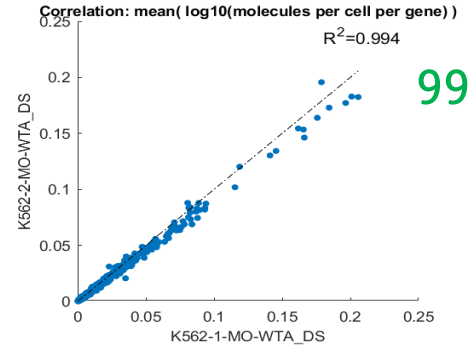


WTA data visualization

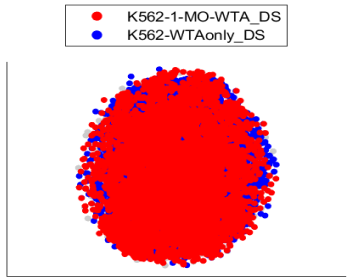
Multiomic experiment with K562 cell line:
Analysis based on highly variable genes

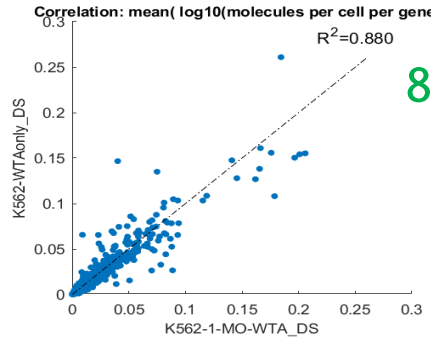




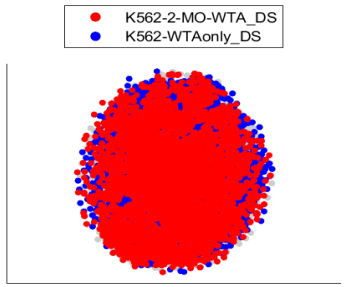


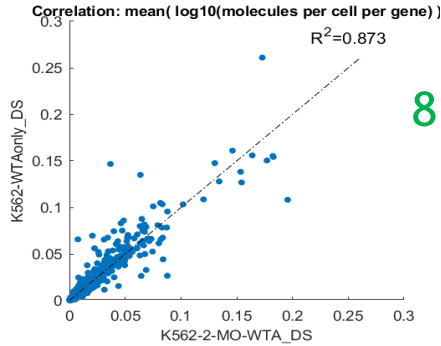
99.4%





88.0%



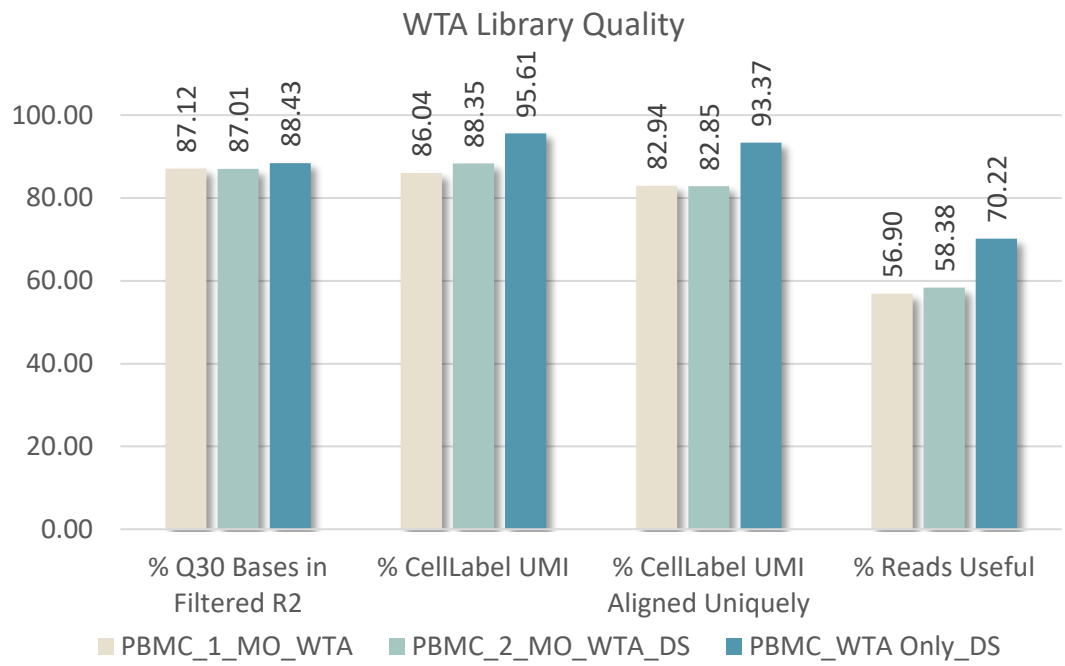


87.3%

WTA gene expression correlation R^2> 80%.

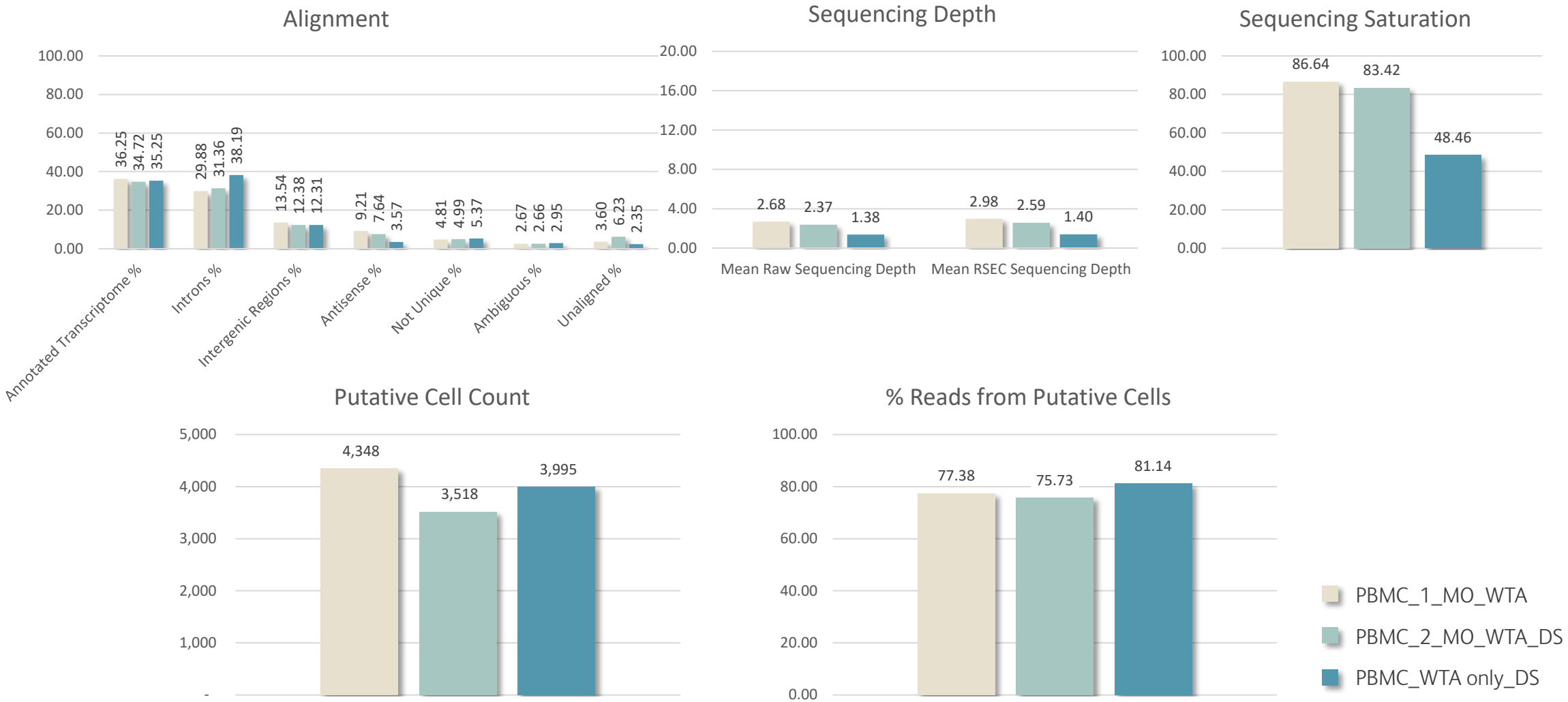
Performance metrics—whole transcriptome analysis (WTA) sequencing and library quality

Multiomic (MO) ATAC-Seq experiment with PBMCs:



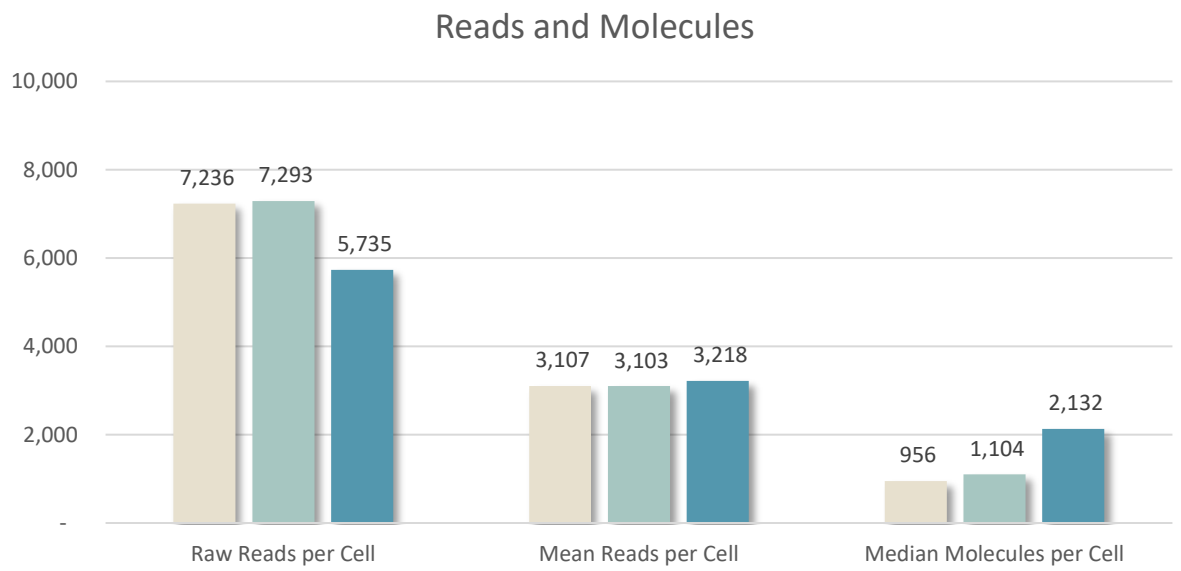
Performance metrics—WTA metrics

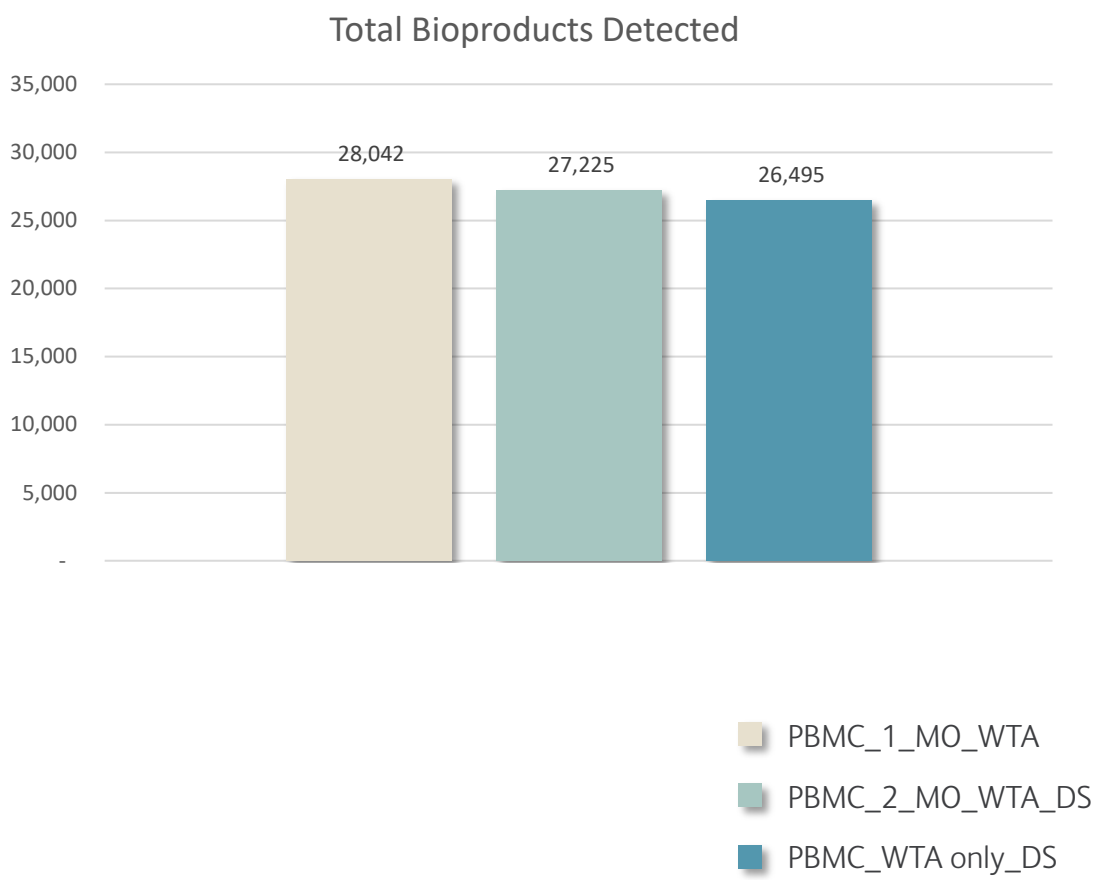
Multiomic ATAC-Seq experiment with PBMCs:



Performance metrics—WTA metrics

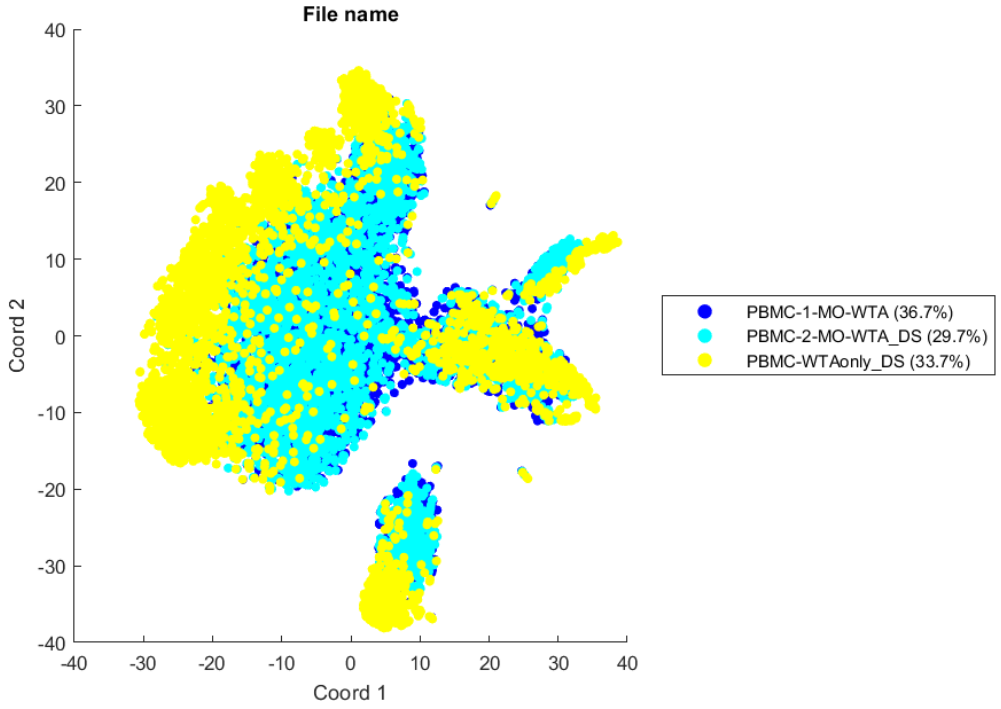
Multiomic ATAC-Seq experiment with PBMCs:
Post down-sampling to similar mean reads/cell



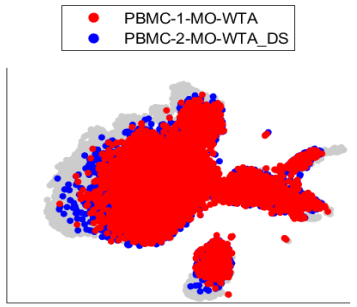


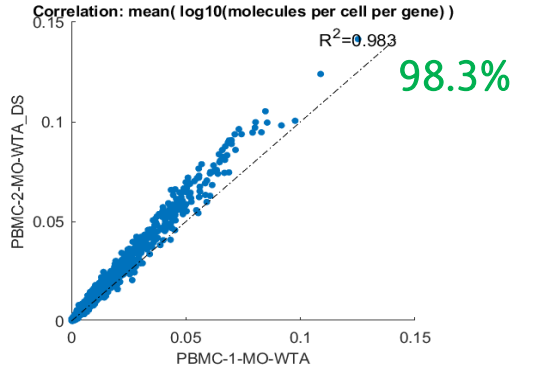
WTA data visualization

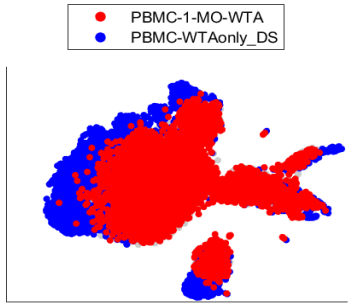
Multiomic experiment with PBMCs:
Analysis based on highly variable genes

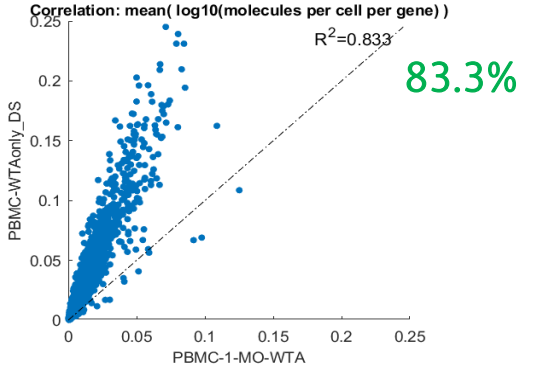


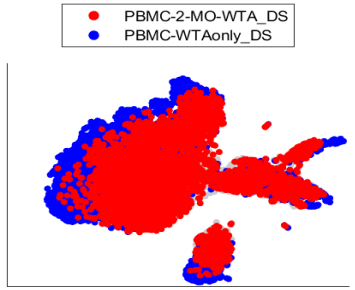
WTA gene expression correlation R^2> 80%.

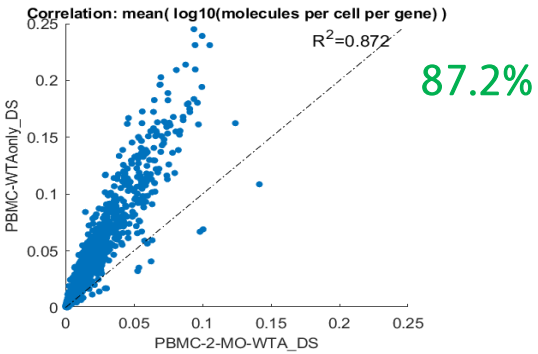












Performance metrics—repeatability

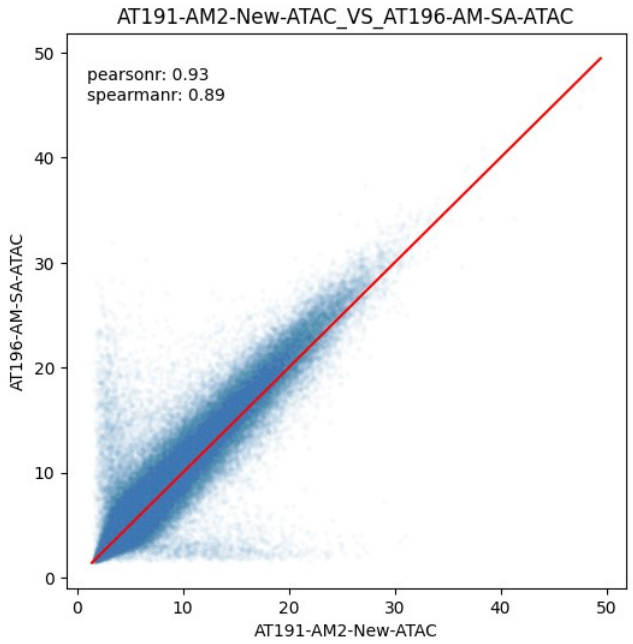
Standalone (SA) and multiomic (MO) experiments with K562 cell line:

Peak Overlap by Genomic Location

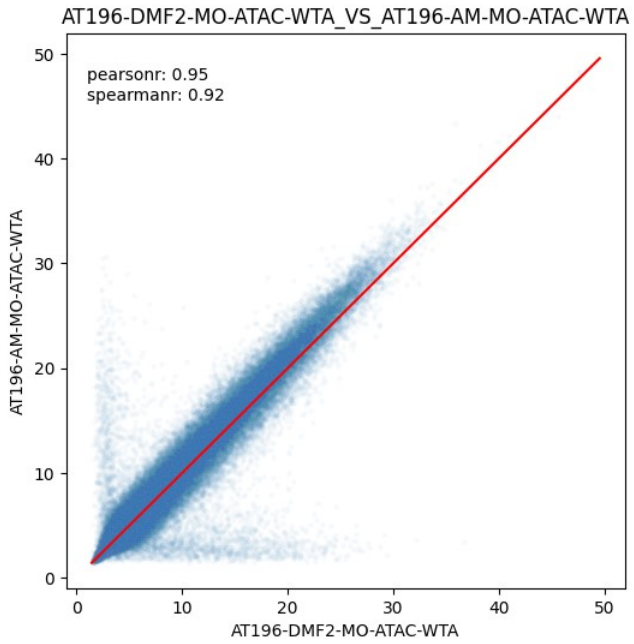
Experiment	# Peaks	# Comm	%Common
K562-SA_PN_AT191	183,268	158,007	**86%**
K562-SA_PN_AT196	190,801	157,272	**82%**
K562-MO_PN_AT196_1	189,017	166,226	**88%**
K562-MO_PN_AT196_2	189,941	167,181	**88%**

Peak Signal Correlation

K562 (Standalone)

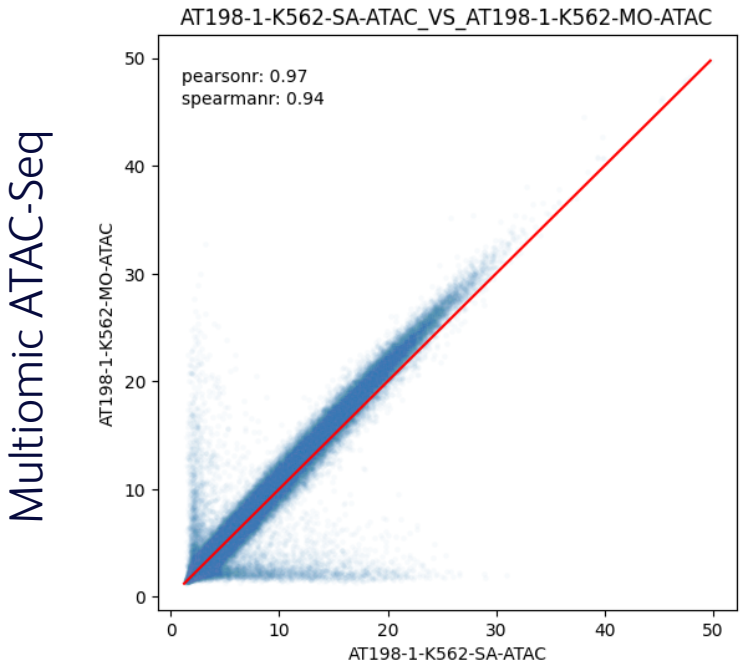


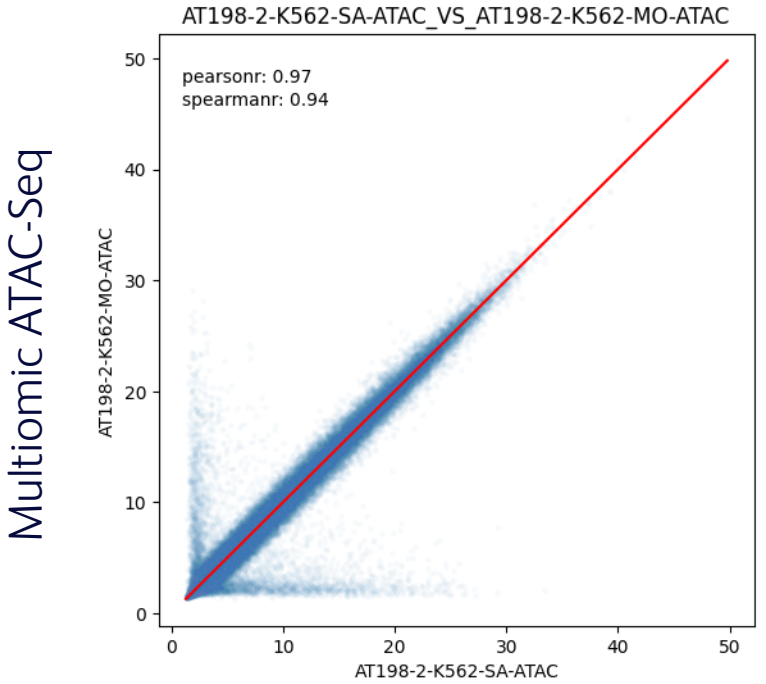
K562 (Multiomic)



Performance metrics—repeatability

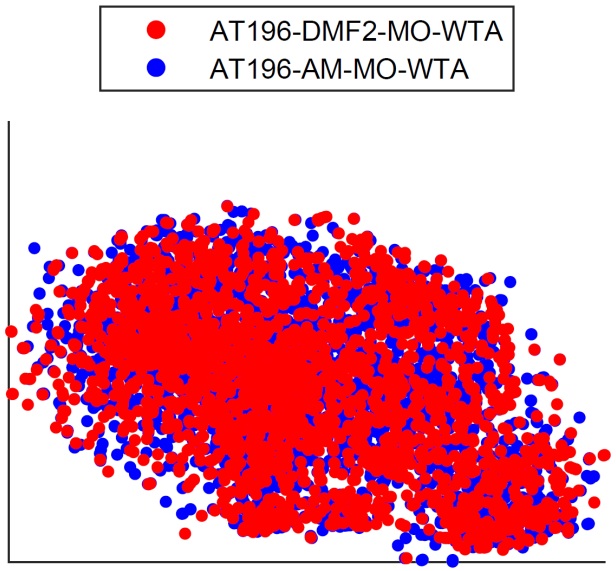
Standalone and multiomic experiments with K562 cell line:

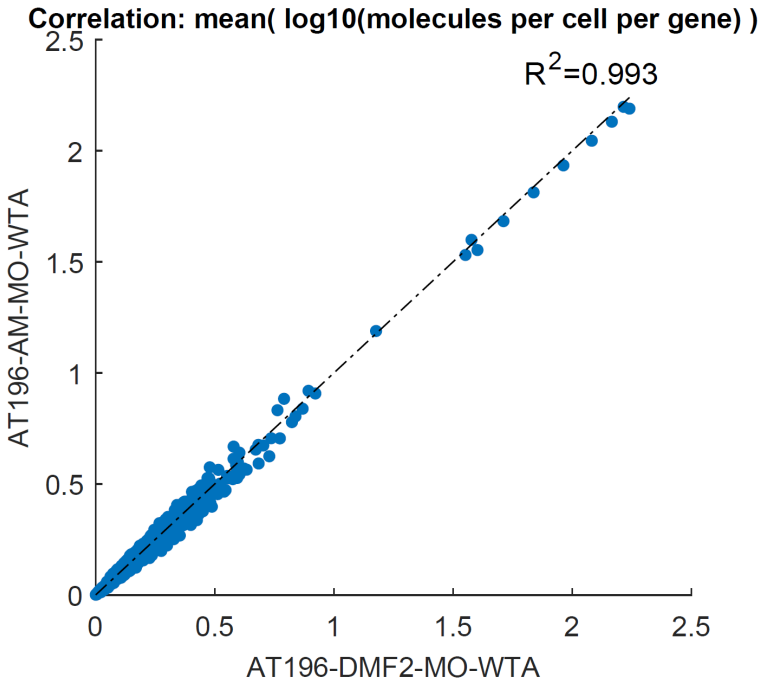




Performance metrics—repeatability

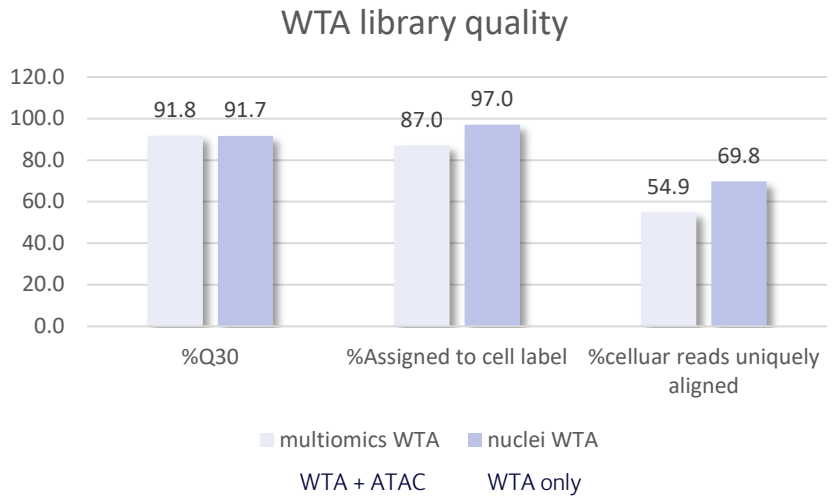
WTA repeatability in multiomic assay:

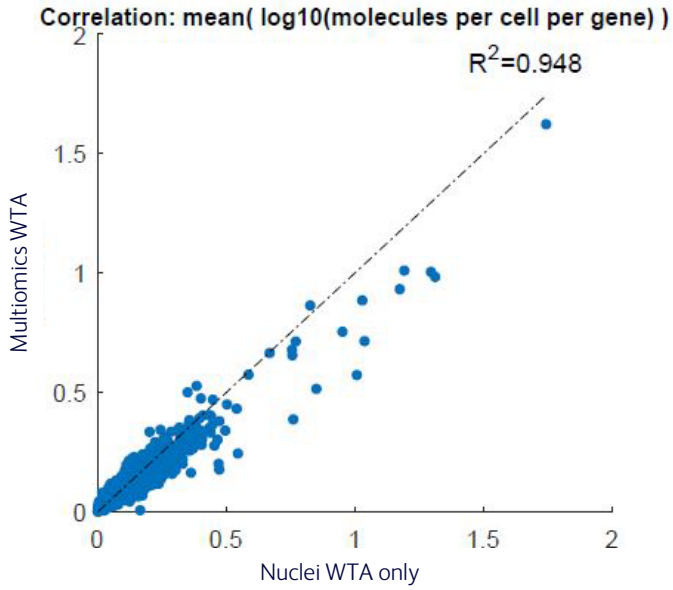




Performance metrics—WTA performance

Multiomic experiment with K562 cell line:



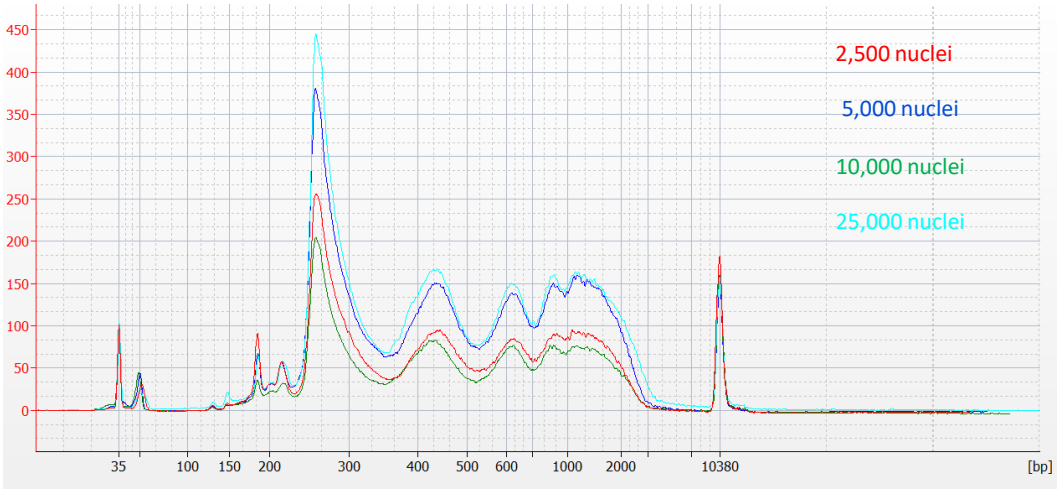


- WTA (Whole Transcriptome Analysis) quality in multiomics ATAC+WTA assay is comparable to nuclei WTA standalone assay
- RNA-seq quality is not significantly impacted by ATAC-seq

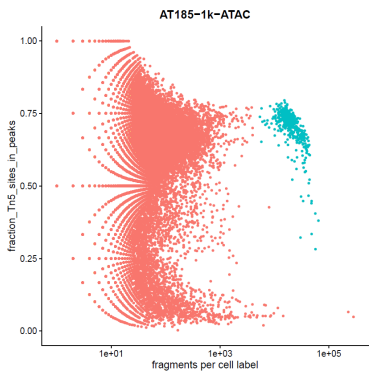
Performance metrics—cell input experiments

Standalone experiments with PBMCs:

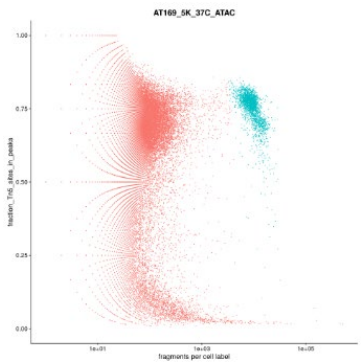
Target nuclei	PCR cycles	Qubit (ng/uL)
500 nuclei	16	4.62
2,500 nuclei	13	3.59
5,000 nuclei	13	5.55
10,000 nuclei	11	3.23
25,000 nuclei	11	6.68



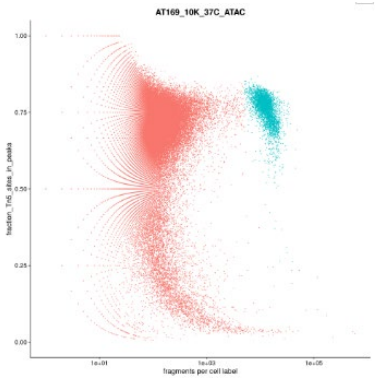
500 nuclei



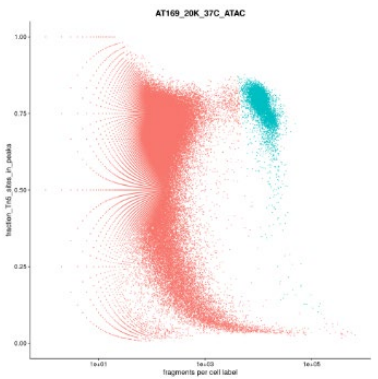
2,500 nuclei



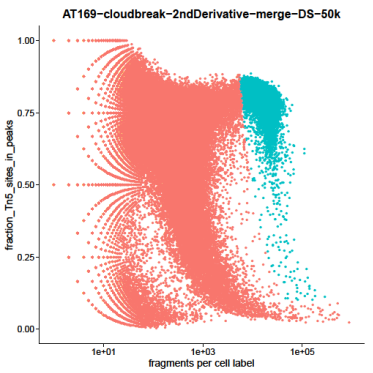
5,000 nuclei



10,000 nuclei

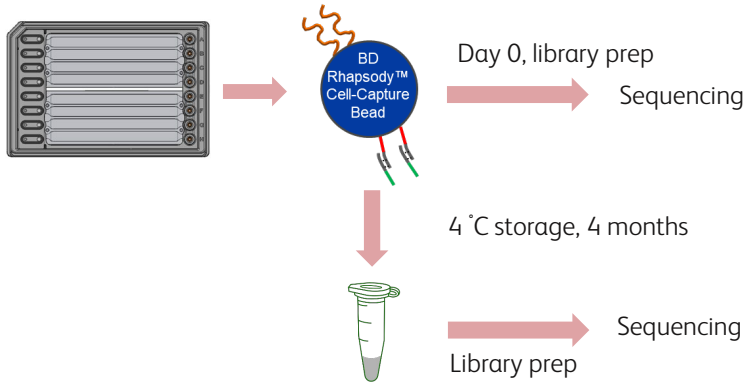


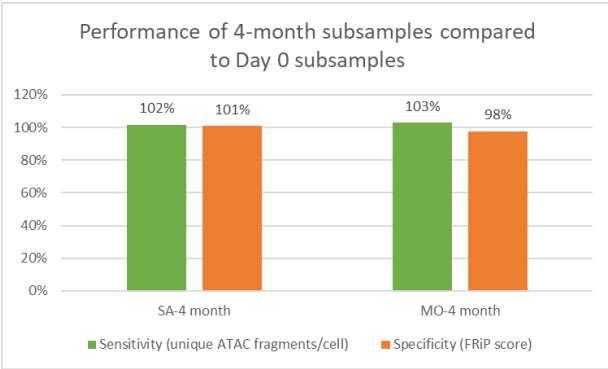
25,000 nuclei



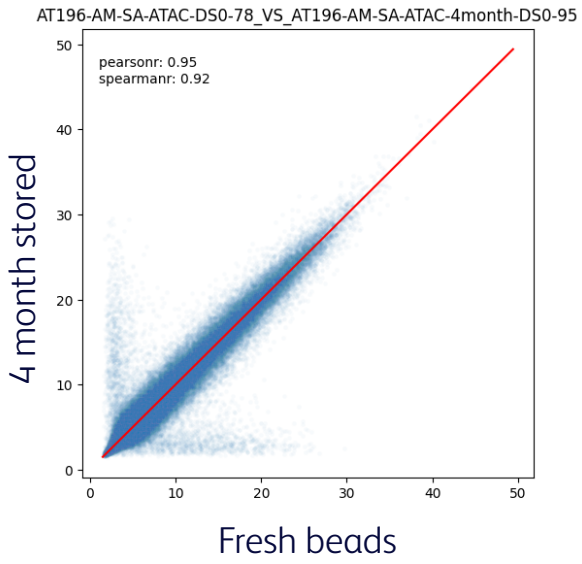
Performance metrics—bead storage

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

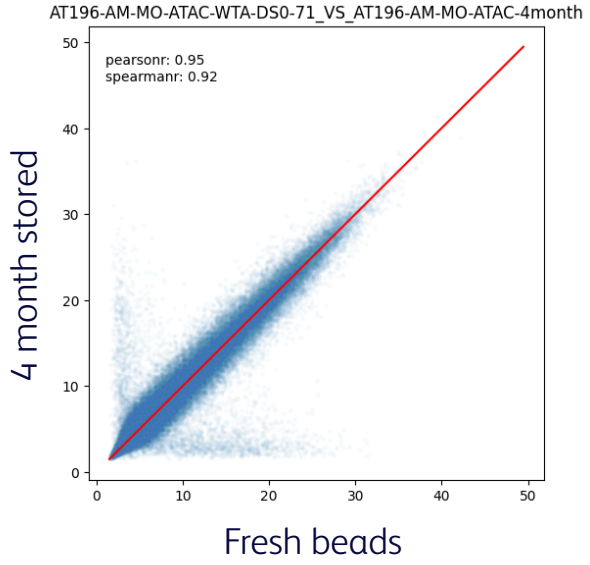




Standalone ATAC-Seq



Multiomic ATAC-Seq

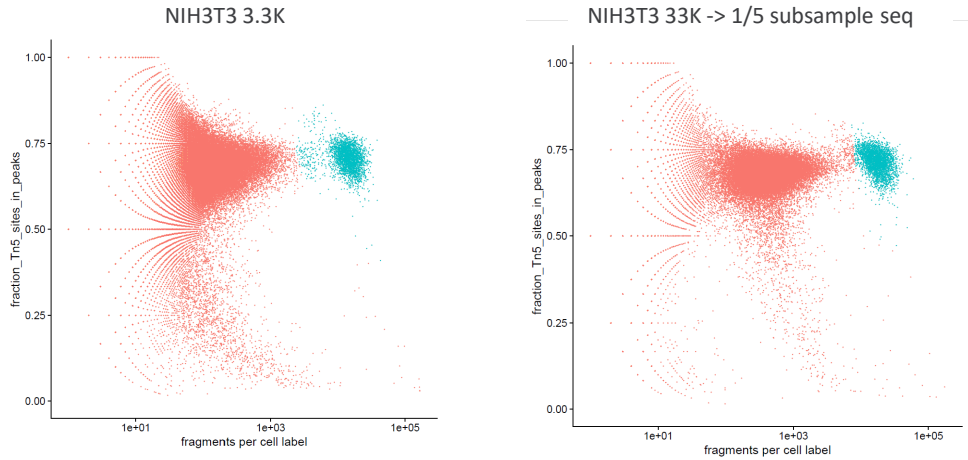


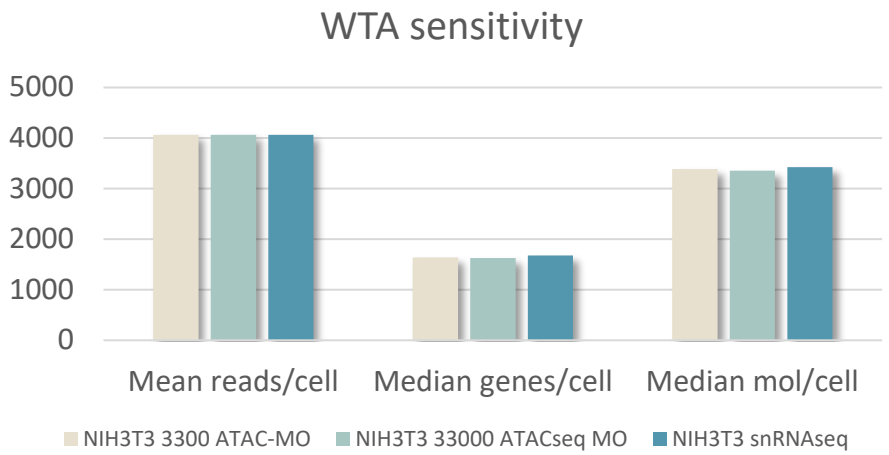
	Standalone Day 0	Standalone 4-month	Multiomic Day 0	Multiomic 4-month
Median proper read pairs per putative cells	20,871	21,390	20,057	21,232
Median nonduplicate fragments per putative cells	18,275	18,573	16,979	17,492

Performance metrics—mouse NIH3T3 cell line

Multiomic ATAC-Seq experiments with mouse NIH3T3 cell line:

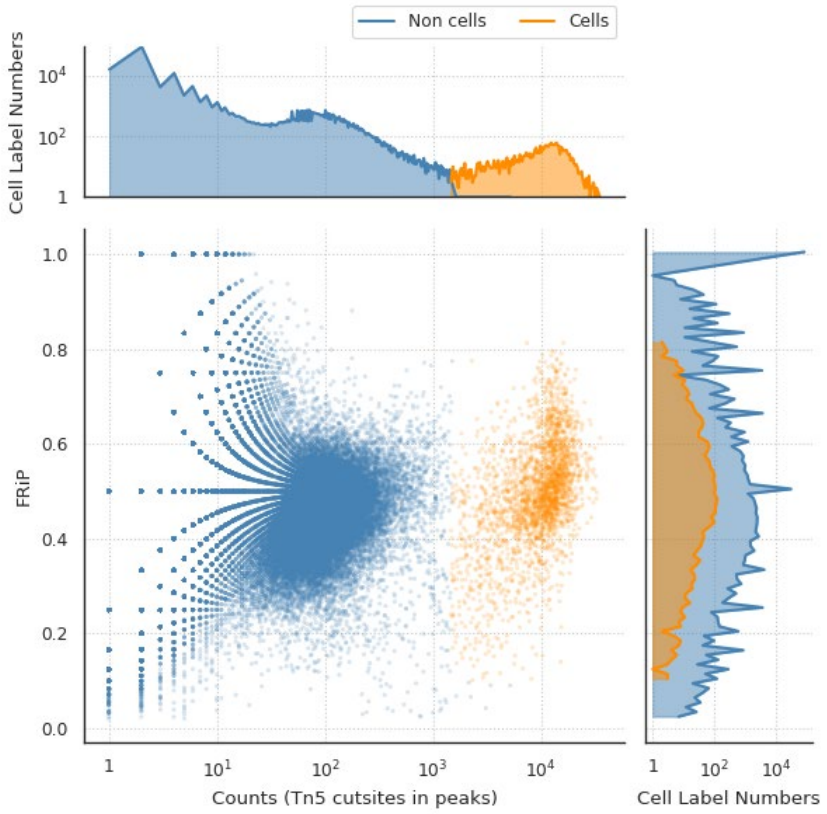
Metric	NIH3T3 3300	NIH3T3 33000 -> 1/5 sequenced
Percent input reads assigned cell label	96.97	96.97
Percent clean reads assigned cell label	98.52	98.44
Percent cell-labeled reads aligned confidently	91.2	90.87
Percent mitochondrial reads	4.15	4.85
Fraction of nonduplicate fragments with NFR lengths	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



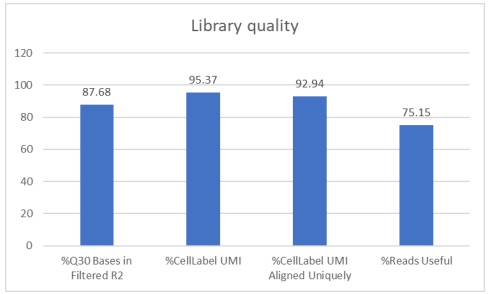


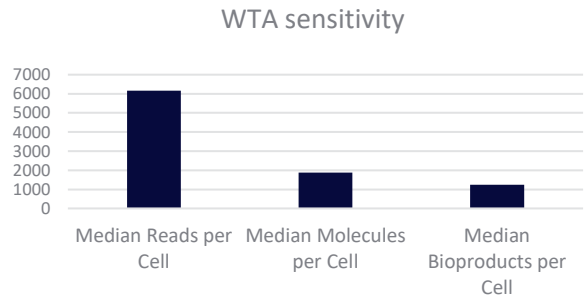
Performance metrics—mouse frozen kidney cells

Multiomic ATAC-Seq experiments with mouse frozen kidney cells:



WTA Sequencing quality and library quality





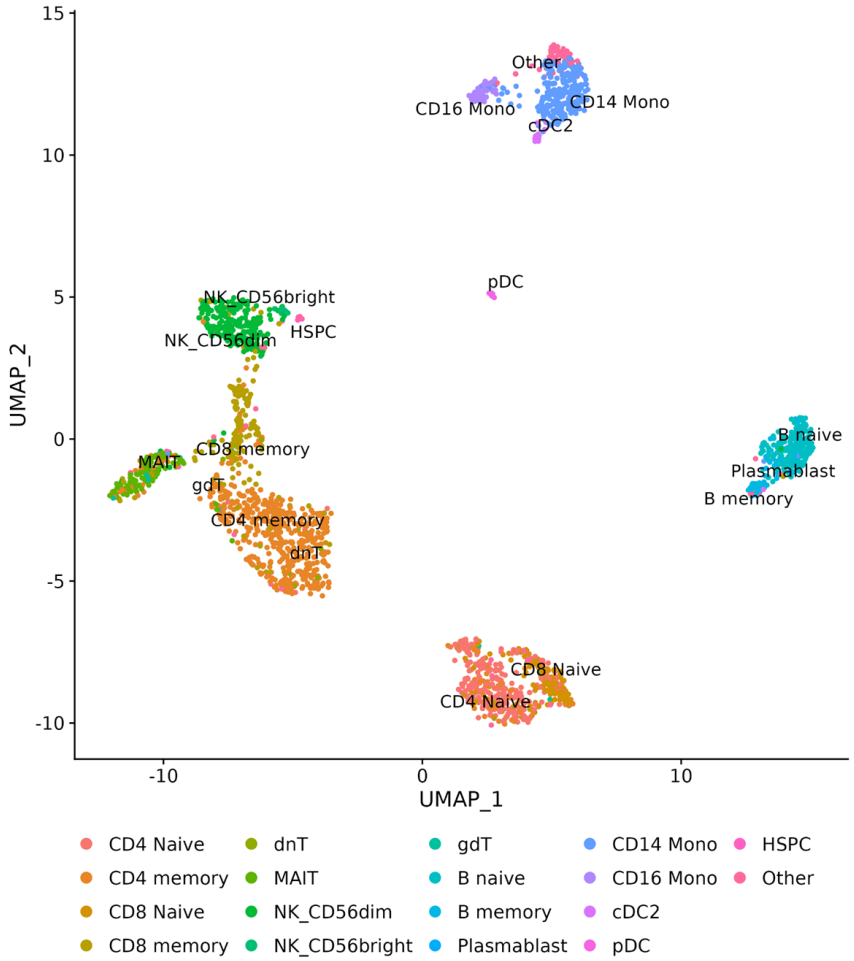
	Value
Median proper read pairs per putative cells	51,018
Median nonduplicate fragments per putative cells	10,830

Chapter IV:
Downstream analysis

Downstream analysis—sample dataset

- Comprehensive picture of cellular identities with the BD Rhapsody™ Single-Cell Multiomic ATAC-Seq Assay:

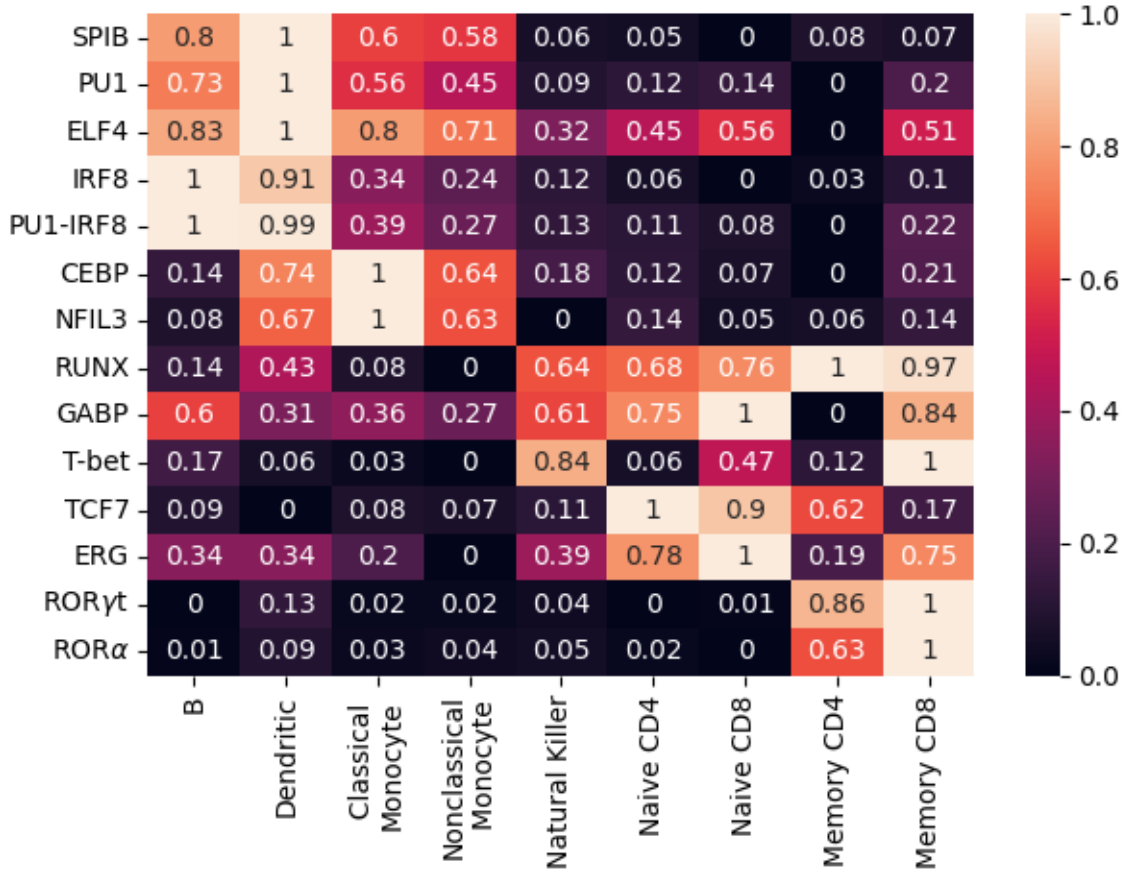
Cell-type annotation using the data from a BD Rhapsody™ Single-Cell Multiomic ATAC-Seq Assay. Representative data from BD Rhapsody™ Single-Cell Multiomic ATAC-Seq and WTA Assays, where 2,190 nuclei from human PBMCs were analyzed, followed by a joint WTA and ATAC-seq dimensionality reduction performed using Uniform Manifold Approximation and Projection (UMAP) and cell type annotation using the WTA data from a PBMC reference atlas.



Downstream analysis—sample dataset

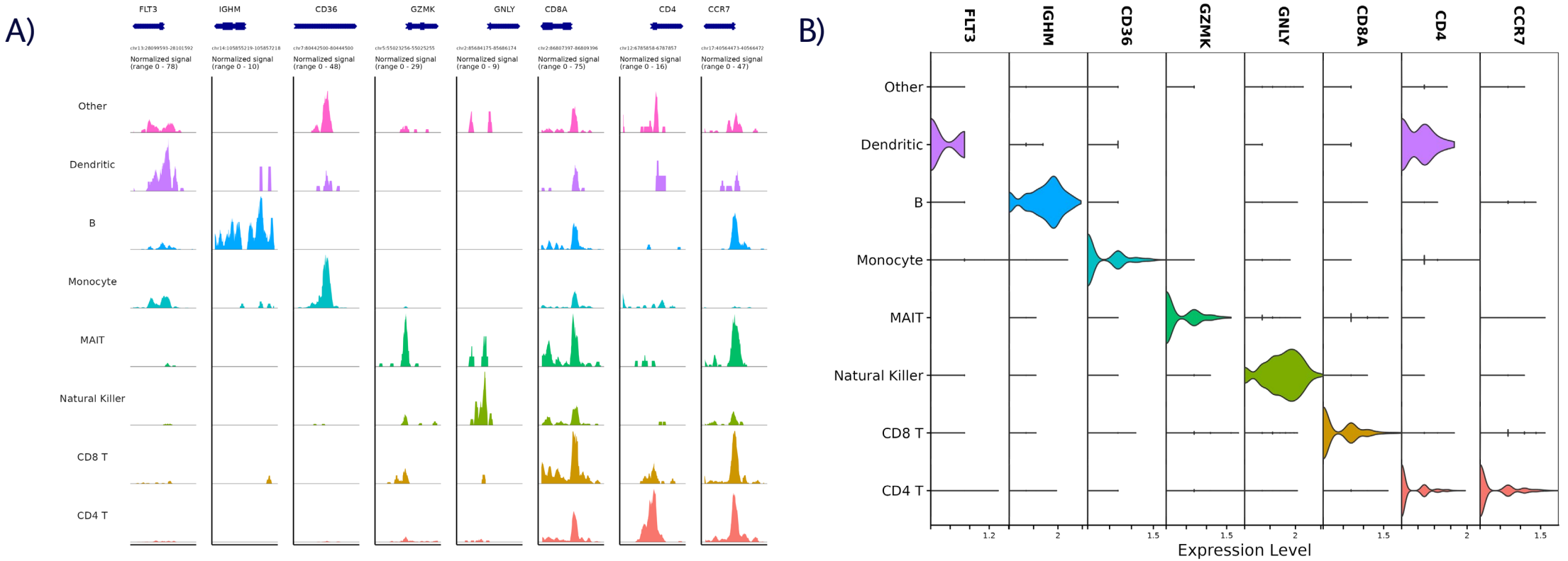
- Enrichment of cell type-specific transcription factor motifs in PBMCs:

Cell types and their specific motifs revealed using the BD Rhapsody™ Single-Cell Multiomic ATAC-Seq Assay. A heat map showing normalized enrichment scores of cell type–specific transcription factor motifs in PBMCs. Motif scores were calculated using a binomial distribution, determining the relative enrichment of each motif in differentially accessible regions of a given cell type compared to GC-matched background regions. The scores were then normalized across cell types per motif on a 0–1 scale, where 0 indicates least enrichment and 1 indicates highest enrichment of each motif.



Downstream analysis—sample dataset

- Multiomic epigenomic and transcriptomic data illuminate how epigenetic regulatory landscapes drive the transcriptional programs specifying distinct cell types within PBMCs:

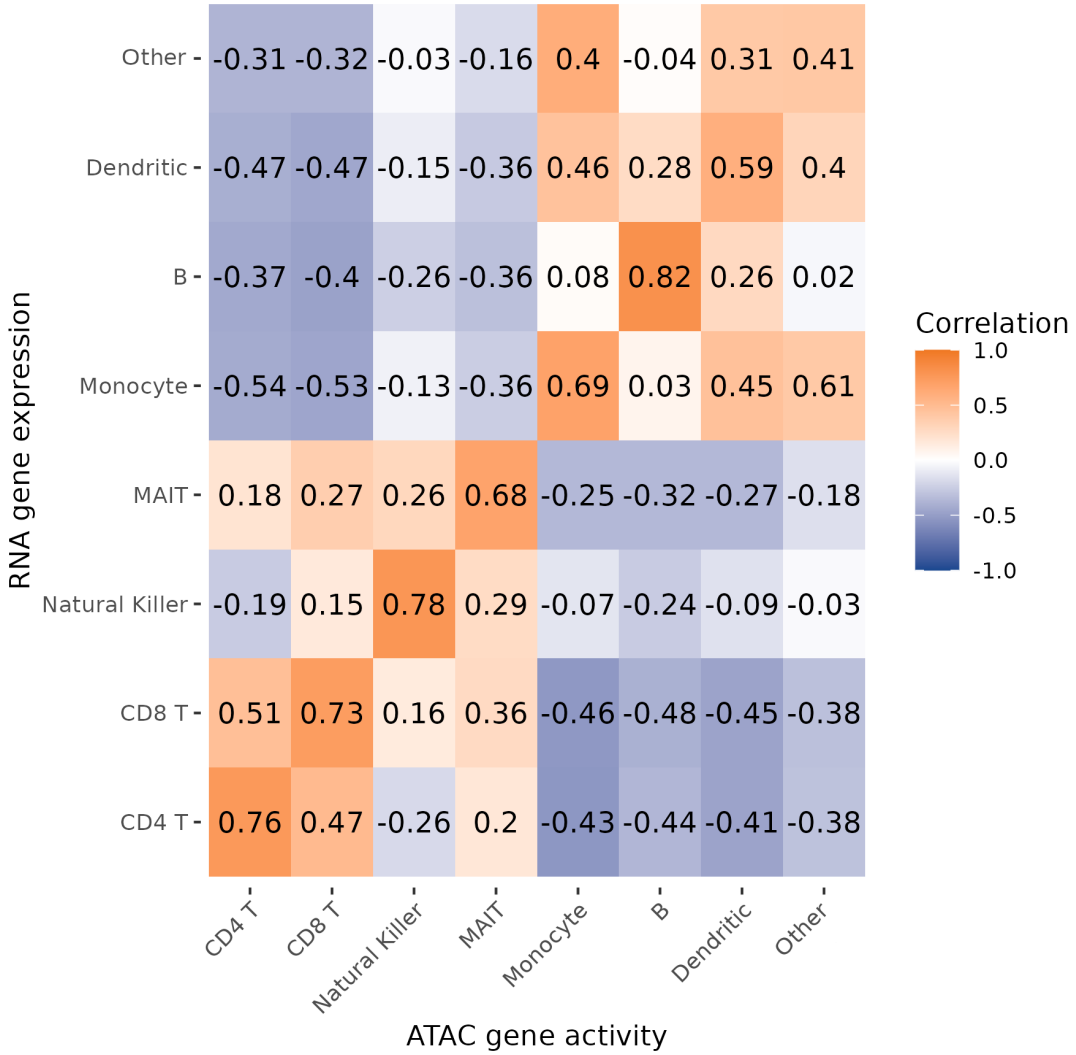


Comparison between single-cell ATAC-seq and WTA data with PBMCs. A) Read density across each ATAC-seq cluster at the transcription start sites of cell type marker genes. **B)** Violin plots showing cell type-specific gene expression in WTA data.

Downstream analysis—sample dataset

- Cell type-specific correlation of ATAC-seq gene activity and gene expression values:

Concordance between gene expression and inferred gene activity score. A heatmap showing Pearson's correlation coefficients between ATAC-seq gene activity scores and gene expression values in PBMCs, with each row representing a cell type in WTA data and each column a cell type in ATAC-seq data.



Thank you