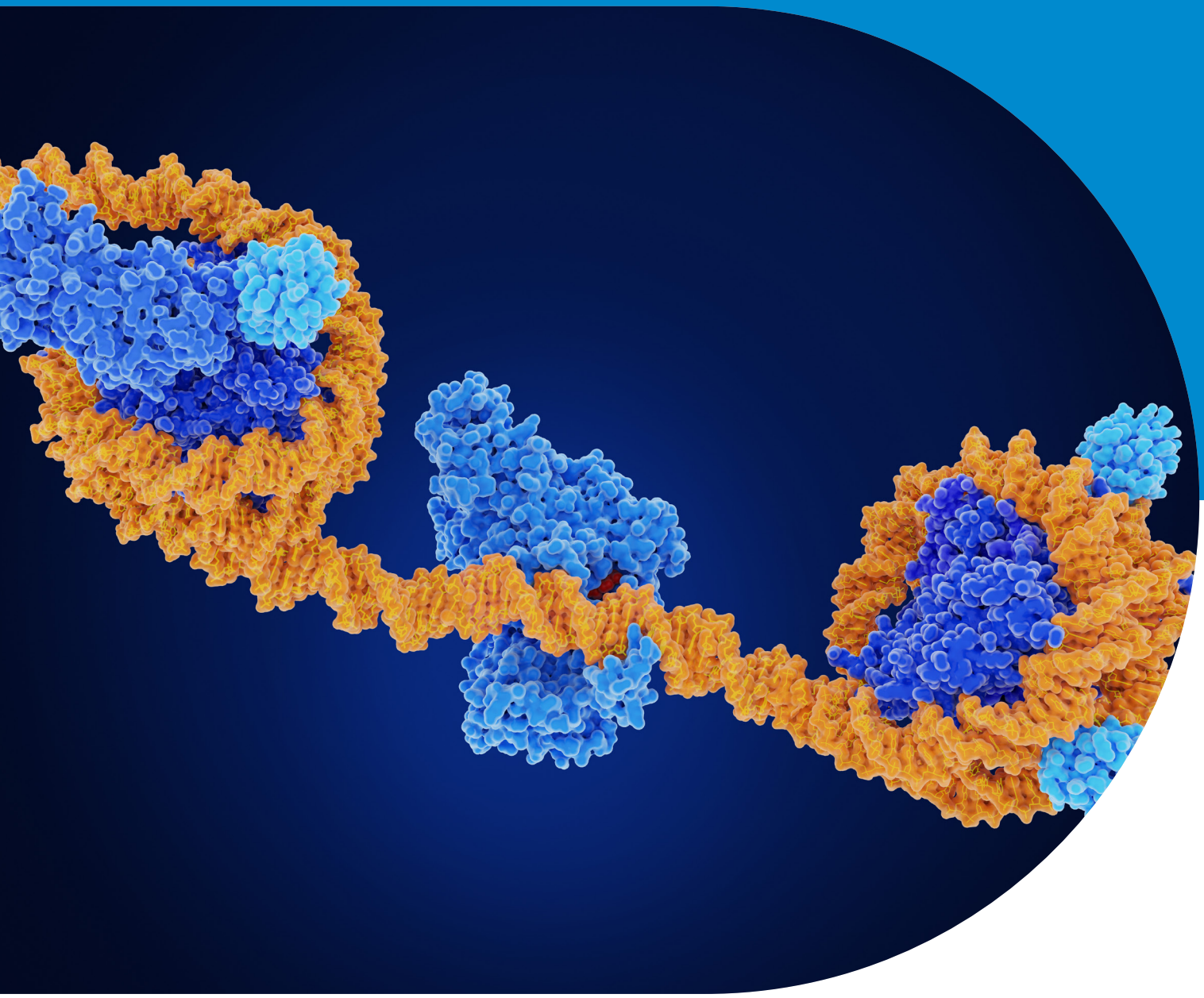


BD Rhapsody™ ATAC-Seq Assay

Genome-wide epigenomic and transcriptomic analysis on the same cell

BD

Uncover new regulatory connections shaping cellular identity and function

Mapping open chromatin regions at the single-cell level elucidates cell-to-cell variability in regulatory landscapes that drives cell fate decisions and responses to stimuli. Assay for transposase-accessible chromatin using sequencing (ATAC-seq) excels at this by providing an unbiased, genome-wide readout of chromatin accessibility with higher sensitivity than its predecessors.

ATAC-seq utilizes a hyperactive Tn5 transposase to fragment accessible chromatin and insert adapters at the cleaved sites. The adapters permit amplification and sequencing of the captured fragments, the data from which are used to create genome-wide maps of chromatin accessibility, delineating the location of active regulatory elements like promoters, enhancers and transcription factor binding sites.

When combined with single-cell transcriptomic data from the same cells, the resulting multiomic view enables direct associations of the regulatory chromatin state with gene expression output in each cell, affording even deeper mechanistic understanding of cell state in different conditions.

BD Rhapsody™ ATAC-Seq Assays enable you to either reproducibly generate standalone open chromatin profiling data or perform a multiomic analysis of open chromatin accessibility and transcriptome of single cells in one experiment on the BD Rhapsody™ Single-Cell Analysis System.

The BD Rhapsody™ ATAC-Seq Assay workflow: Single nuclei are isolated and tagmented in bulk with Tn5 transposase loaded with sequencing adapters, simultaneously fragmenting the DNA and inserting adapters into regions of open chromatin. The tagmented nuclei are then loaded onto the BD Rhapsody™ Cartridge to capture the tagged DNA fragments on BD Rhapsody™ Beads. Next, beads are retrieved and their captured fragments are PCR-amplified to generate a sequencing library. The library is sequenced, with the resulting reads mapped to the genome to identify accessible chromatin regions across individual nuclei.

Key features

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.

Compatible with preservation
Suitable for use with preserved cells and BD® OMICS-Guard Sample Preservation Buffer

Key applications

- Immunology research: Uncover heterogeneity and dysregulated gene programs driving immunological disease states.
- Cancer research: Reveal regulatory elements driving intra-tumor heterogeneity and therapeutic resistance.
- Developmental biology: Chart chromatin dynamics across cell lineages to pinpoint regulatory elements orchestrating cell fate decisions.
- Neuroscience: Illuminate regulatory landscapes underlying neuronal identities, plasticity and disease states.

Unveil epigenomic heterogeneity with precision

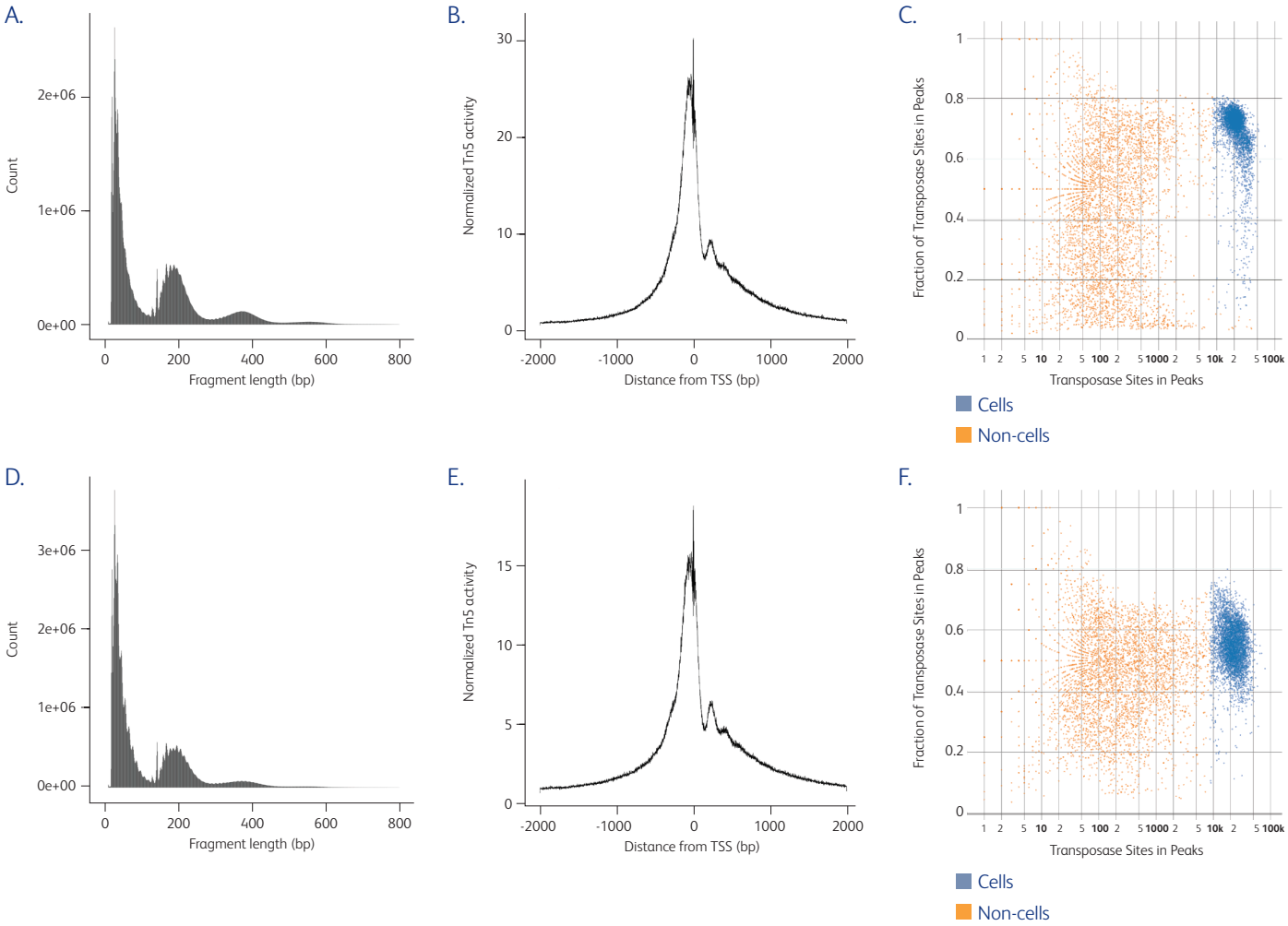


Figure 1. Sensitivity and specificity metrics for the BD Rhapsody™ Single-Cell ATAC-Seq Assay. A) Fragment size distribution from a BD Rhapsody™ ATAC-Seq Assay experiment with human peripheral blood mononuclear cells (PBMCs), demonstrating a preferential transposition of the nucleosome-free regions of 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 peaks) 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. **D–F)** The same plots from a BD Rhapsody™ ATAC-Seq Assay experiment with the K562 cell line sequenced to the same depth.

Uncover ties between gene regulation and expression at the single-cell level

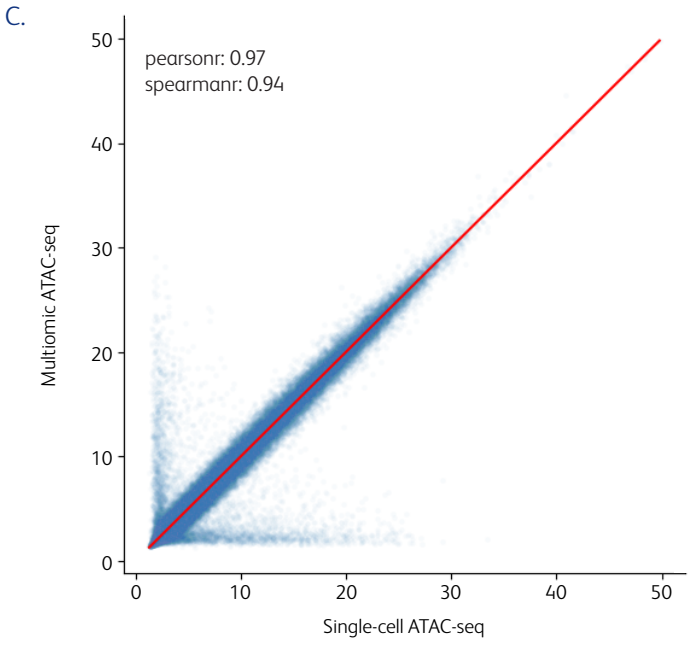


Figure 2. Sensitivity and specificity metrics for the BD Rhapsody™ Single-Cell Multiomic ATAC-Seq Assay. A) Joint calling scatter plot from a multiomic BD Rhapsody™ ATAC-Seq Assay experiment with human PBMCs where each cell is represented as a dot and RNA molecules from each cell are plotted against the ATAC-seq transposition events. **B)** The same plot from a multiomic BD Rhapsody™ ATAC-Seq Assay experiment with the K562 cell line. The clear separation between cell and non-cell events indicates the ability to effectively distinguish real cells from ambient RNA on non-cell barcodes as well as successful co-analysis of the chromatin accessibility and transcriptomic data at the single-cell level. Both samples were sequenced up to roughly 50,000 mean raw read pairs per putative cell. **C)** High correlation between the ATAC-seq data from a standalone BD Rhapsody™ ATAC-Seq Assay experiment vs a multiomic BD Rhapsody™ ATAC-Seq Assay experiment with the K562 cell line as shown by the high peak signal correlation plot.

Generate reproducible results across different samples and studies

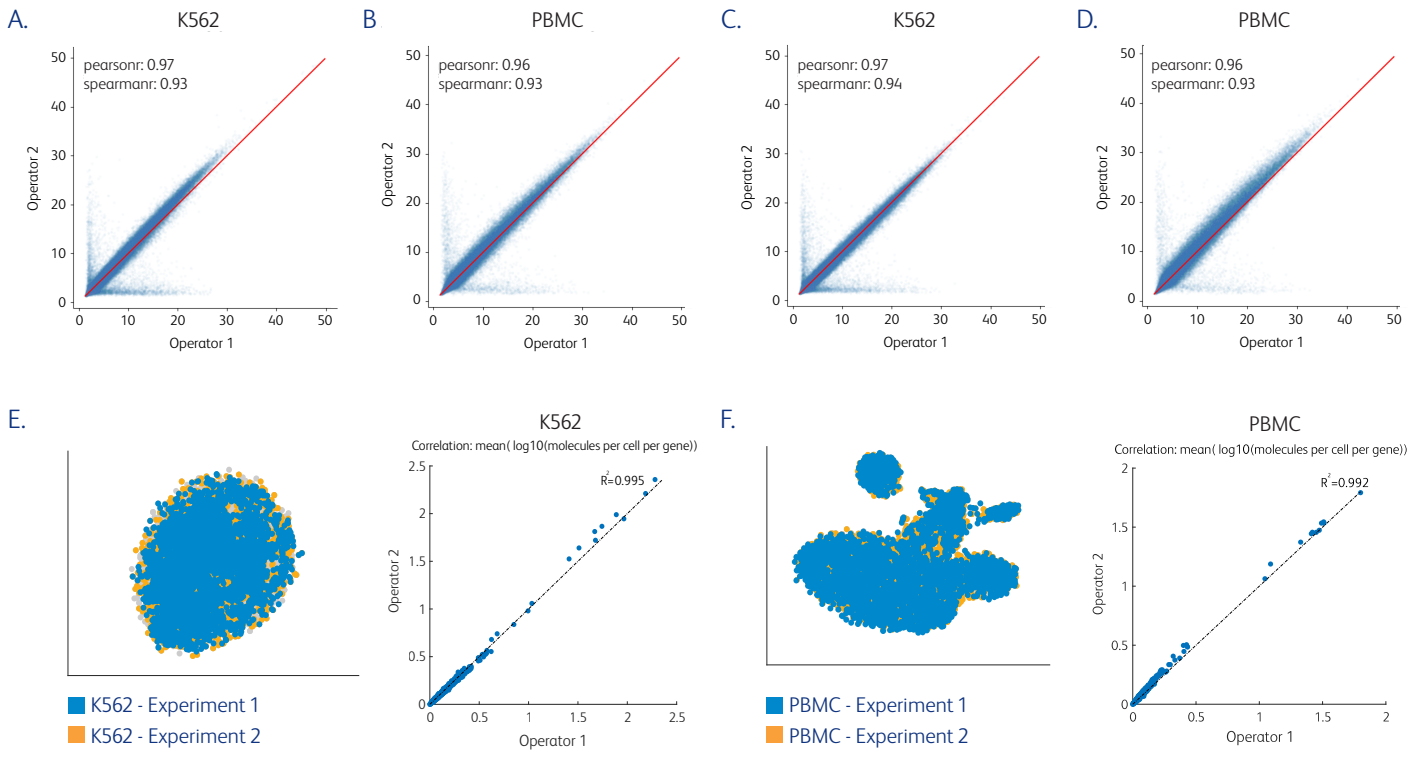


Figure 3. High reproducibility with BD Rhapsody™ ATAC-Seq Assays. The peak signal correlation across two different standalone BD Rhapsody™ ATAC-Seq Assay experiments with the K562 cell line **(A)** and two different standalone BD Rhapsody™ ATAC-Seq Assay experiments with human PBMCs **(B)**. The peak signal correlation across two different multiomic BD Rhapsody™ ATAC-Seq and WTA Assay experiments with the K562 cell line **(C)** and human PBMCs **(D)**. 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 **(E)** and human PBMCs **(F)**.

Generate consistent, high-quality data across a wide range of cell inputs

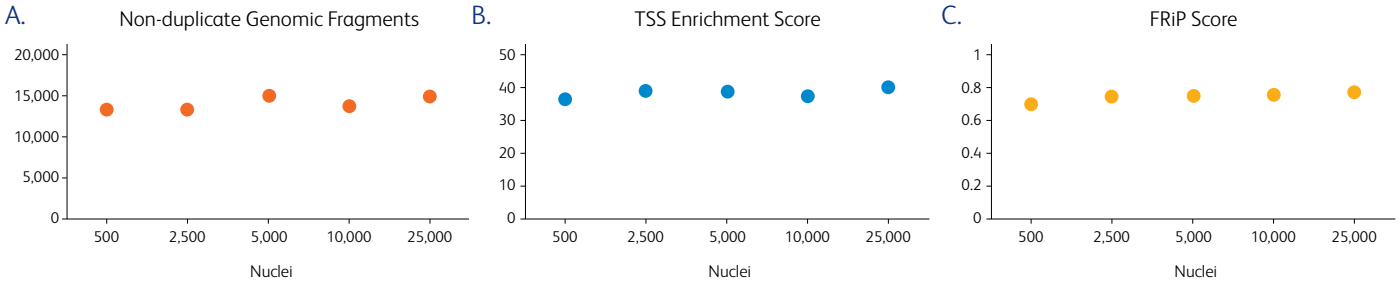
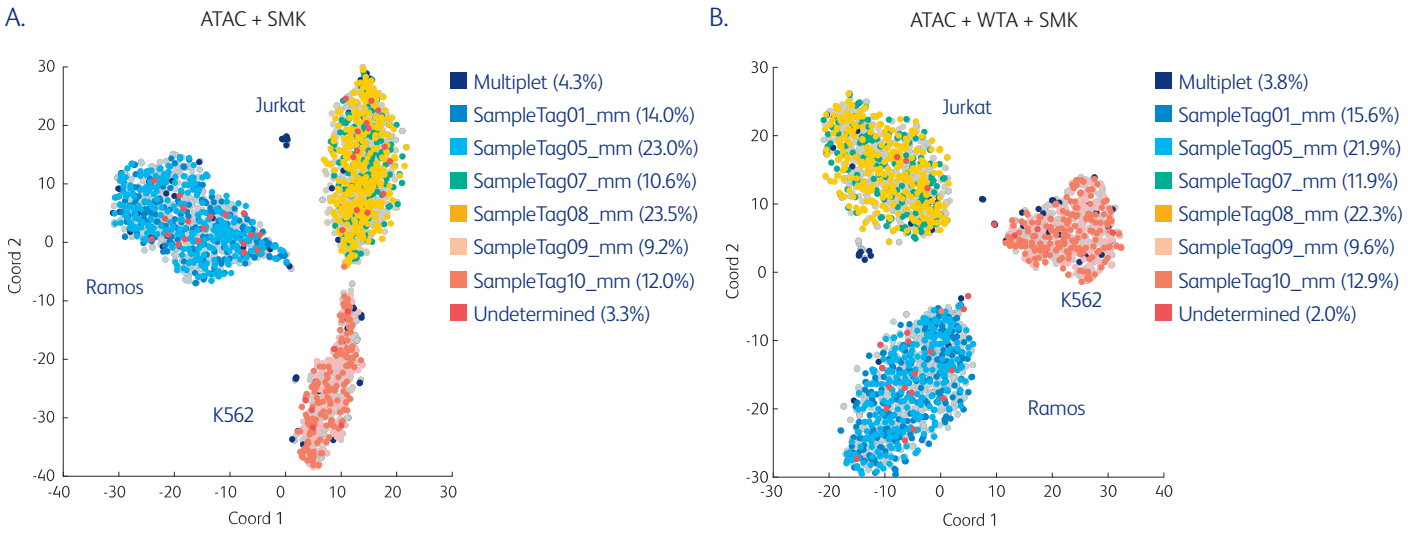


Figure 4. Robust performance across different cell input amounts. BD Rhapsody™ ATAC-Seq Assays enable scalable profiling of open chromatin and gene expression analysis from limited cell inputs to thousands of single cells in one experiment, as demonstrated by the data from a series of BD Rhapsody™ ATAC-Seq 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 number of 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.

Multiplex your ATAC-Seq experiments to increase data throughput and efficiency



	Jurkat	K562	Ramos
SampleTag01_mm	0%	0.18%	37.71%
SampleTag05_mm	0%	0%	62.29%
SampleTag07_mm	31.04%	0%	0%
SampleTag08_mm	68.85%	0%	0%
SampleTag09_mm	0%	43.48%	0%
SampleTag10_mm	0.11%	56.34%	0%
Specificity	**99.89%**	**99.82%**	**100%**

	Jurkat	K562	Ramos
SampleTag01_mm	0%	0%	41.42%
SampleTag05_mm	0%	0%	58.27%
SampleTag07_mm	34.81%	0%	0.1%
SampleTag08_mm	65.19%	0%	0.1%
SampleTag09_mm	0%	42.88%	0%
SampleTag10_mm	0%	57.12%	0.1%
Specificity	**100%**	**100%**	**99.69%**

Figure 5. Sample multiplexing with BD Rhapsody™ ATAC-Seq Assays. tSNE visualization of cell clusters from two representative multiplexed ATAC-Seq experiments with three different cell lines (Jurkat, K562, and Ramos) stained using six BD® Nuclear Sample Multiplexing Ab-Oligos, pooled at different ratios, and run against unstained control samples using standalone and multiomic ATAC-Seq assays. For both stained and unstained samples, 50,000 nuclei were pooled for the tagmentation reaction, 20,000 of which were loaded onto a BD Rhapsody™ 8-Lane Cartridge, followed by lysis at 37 °C for 10 min. After ligation, the samples were halved for running the standalone and multiomic experiments. The sensitivity (% of undetermined tags) and specificity (total % of cells with expected tag) analysis from these experiments show high sensitivity (less than 5% undetermined cells) and specificity (>95% cells detected with expected tags) after removing multiplets in both assay configurations. **A)** St andalone BD Rhapsody™ ATAC-Seq Assay experiment. **B)** Multiomic BD Rhapsody™ ATAC-Seq Assay experiment.

Minimize sample loss by preserving your samples upstream of the ATAC-Seq workflow

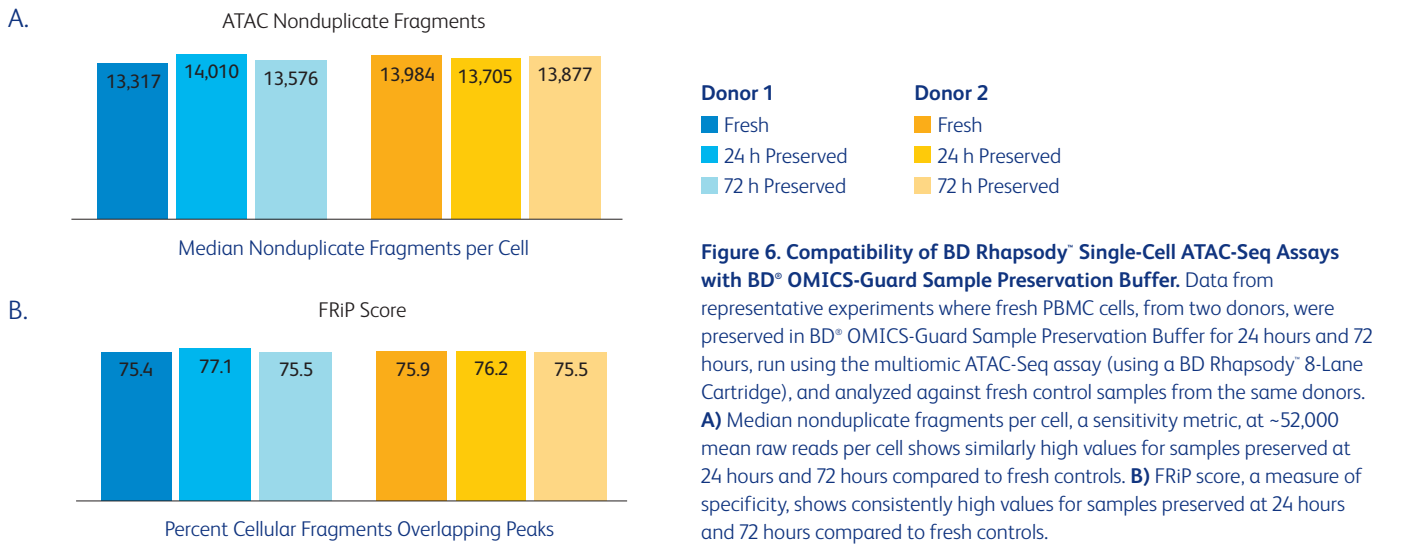
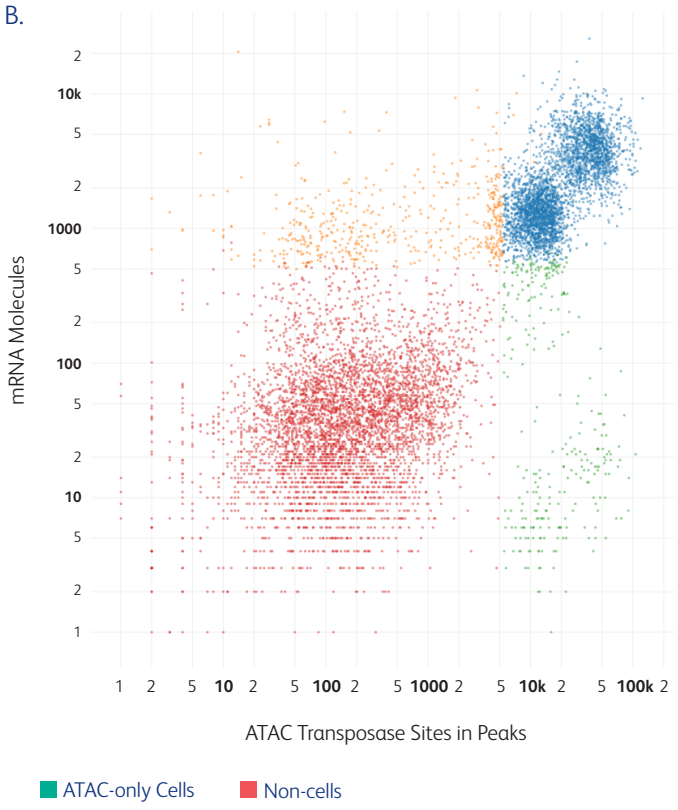


Figure 6. Compatibility of BD Rhapsody™ Single-Cell ATAC-Seq Assays with BD® OMICS-Guard Sample Preservation Buffer. Data from representative experiments where fresh PBMC cells, from two donors, were preserved in BD® OMICS-Guard Sample Preservation Buffer for 24 hours and 72 hours, run using the multiomic ATAC-Seq assay (using a BD Rhapsody™ 8-Lane Cartridge), and analyzed against fresh control samples from the same donors. **A)** Median nonduplicate fragments per cell, a sensitivity metric, at ~52,000 mean raw reads per cell shows similarly high values for samples preserved at 24 hours and 72 hours compared to fresh controls. **B)** FRiP score, a measure of specificity, shows consistently high values for samples preserved at 24 hours and 72 hours compared to fresh controls.

Obtain high-quality ATAC-Seq datasets from tissue samples

A.

B.



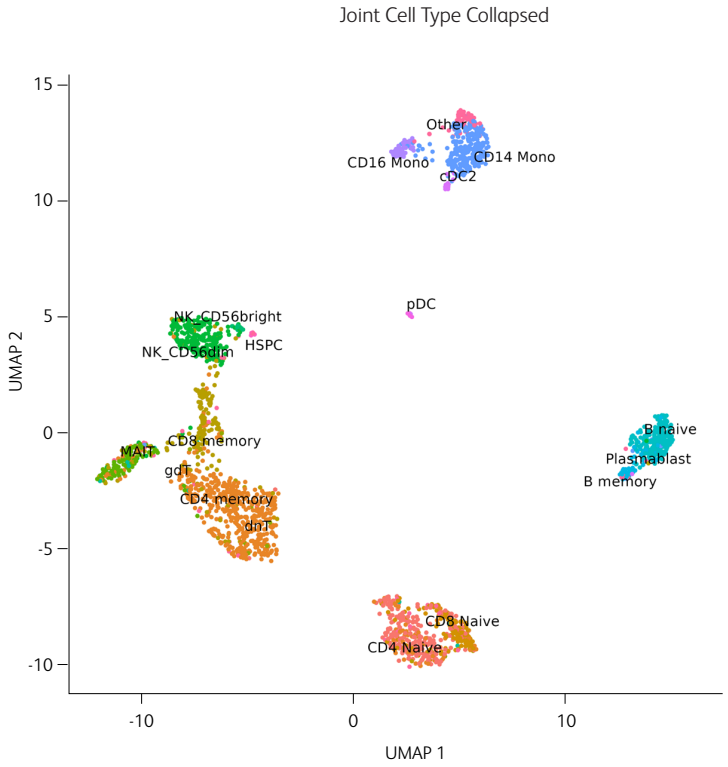
Jointly-called Cells mRNA-only Cells ATAC-only Cells Non-cells

C.

ATAC-Seq metric	Mouse brain	Mouse liver
Raw read pairs per cell	51,853	50,004
Median non-duplicate fragments per cell	25,099	21,558
TSS Enrichment Score	15.3	16.4
FRiP Score	40.9	57.2
WTA metric	**Mouse brain**	**Mouse liver**
Raw read pairs per cell	21,191	16,007
Median molecules per cell	4,658	1,728
Median bioproducts per cell	2,384	1,087

Figure 7. BD Rhapsody™ Single-Cell ATAC-Seq Assay performance with tissue samples. A) Scatter plot from a multiomic BD Rhapsody™ ATAC-Seq experiment with nuclei isolated from frozen mouse brain tissues isolated using the S2 Genomics Singulator™ Platform and prepared following the BD Rhapsody™ Nuclei Isolation Protocol, shows the FRiP 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. **B)** The same plots from a BD Rhapsody™ ATAC-Seq Assay experiment with nuclei isolated from frozen mouse liver tissues isolated and prepared following the BD Rhapsody™ Nuclei Isolation Protocol and sequenced to the same depth. The divide in the jointly called cell populations (in blue) represent two different hepatocytes and endothelial cells from the liver tissue, that exhibit different levels of gene expression and open chromatin accessibility. **C)** ATAC-Seq and WTA metrics from the same experiments.

Gain a comprehensive picture of cellular identities



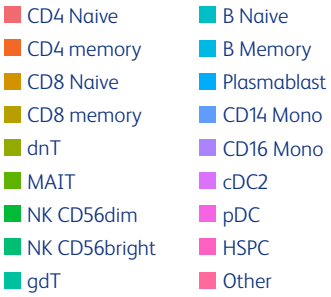


Figure 8. Cell-type annotation using the data from a BD Rhapsody™ Single-Cell Multiomic ATAC-Seq Assay. Representative data from BD Rhapsody™ Single-Cell Multiomic ATAC-Seq and WTA Assays, where 2,190 nuclei from human PBMCs were analyzed, followed by a joint WTA and ATAC-seq dimensionality reduction performed using Uniform Manifold Approximation and Projection (UMAP) and cell type annotation using the WTA data from a PBMC reference atlas.

Enrich cell type-specific transcription factor motifs

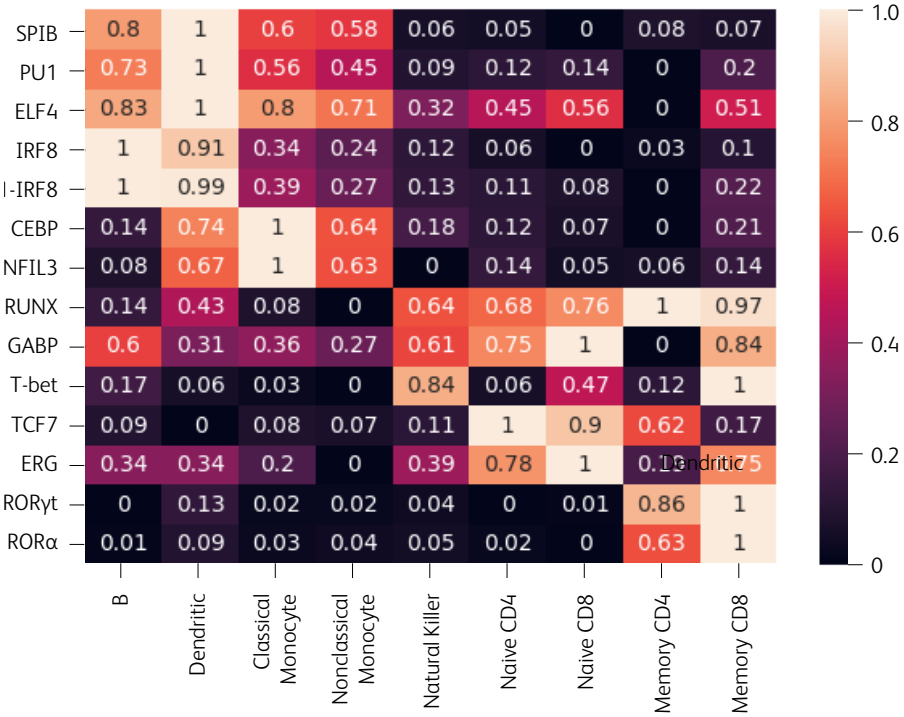


Figure 9. Cell types and their specific motifs revealed using the BD Rhapsody™ Single-Cell Multiomic ATAC-Seq Assay. A heat map showing normalized enrichment scores of cell type–specific transcription factor motifs in PBMCs. Motif scores were calculated using a binomial distribution, determining the 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.

Use multiomic epigenomic and transcriptomic data to illuminate epigenetic regulatory landscapes

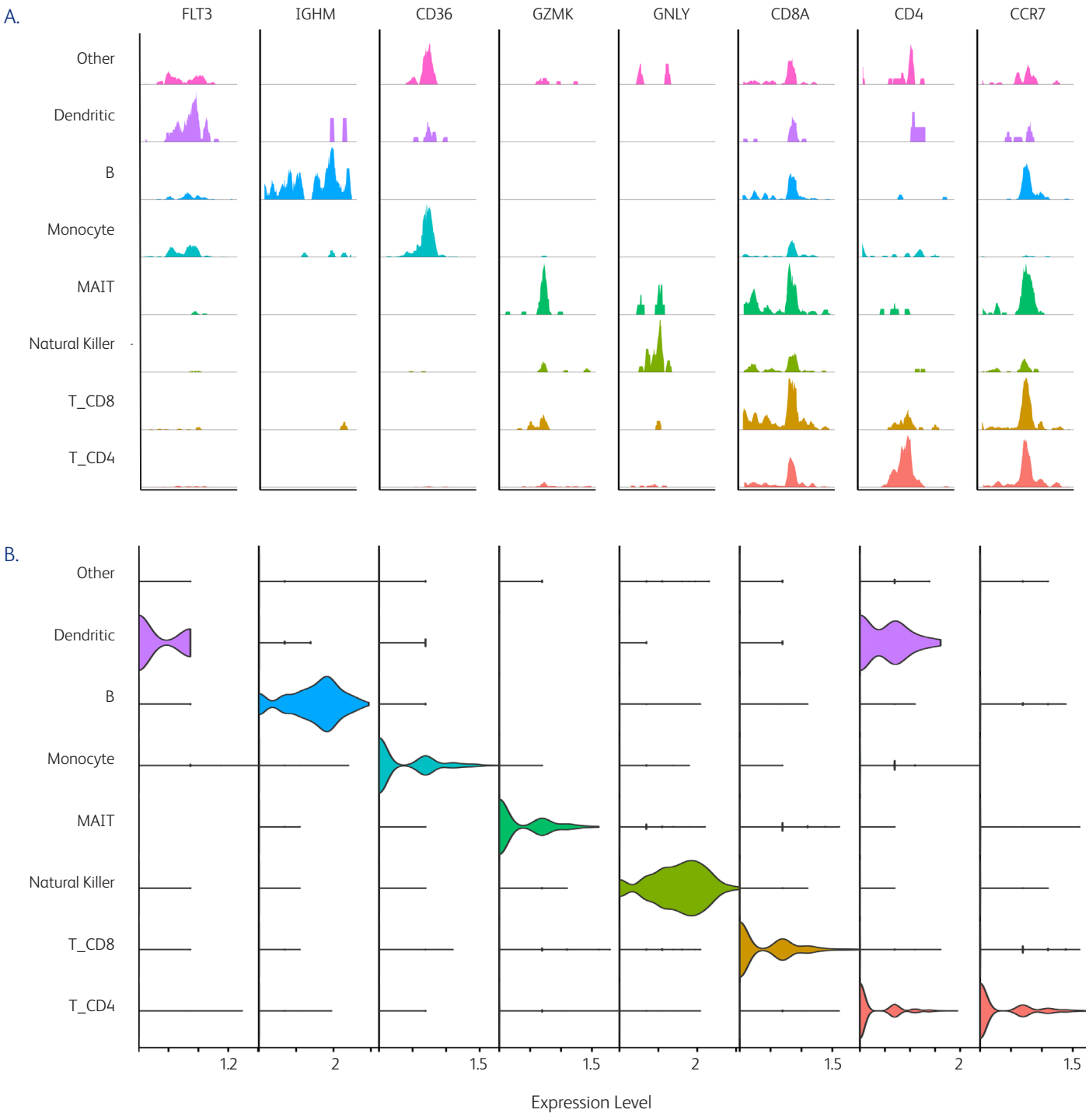


Figure 10. Comparison between single-cell ATAC-seq and WTA data with PBMCs. A) Read density across each ATAC-seq cluster at the transcription start sites of cell type marker genes. **B)** Violin plots showing cell type-specific gene expression in WTA data.

Obtain cell type-specific correlation of ATAC-seq gene activity and gene expression values

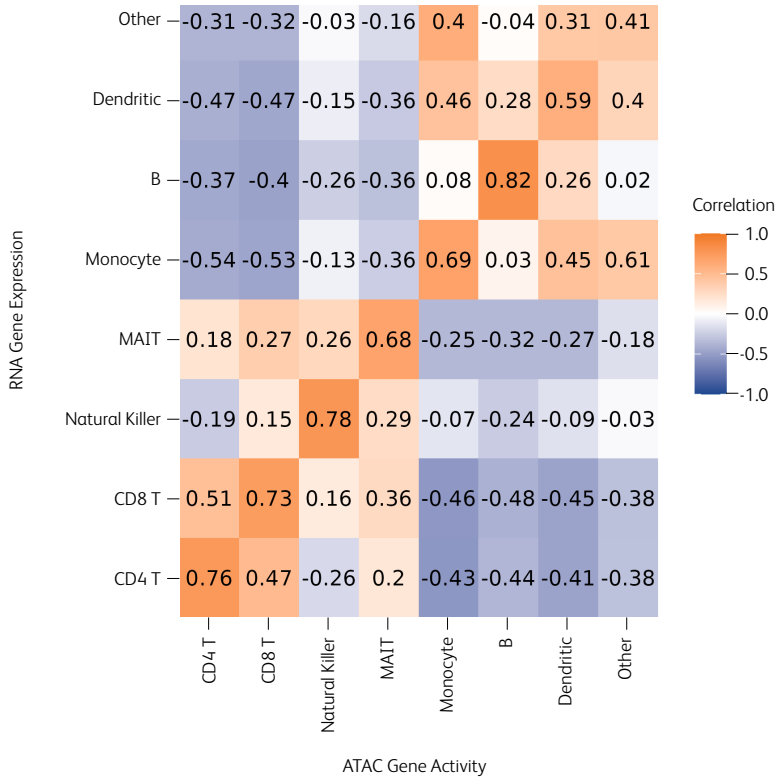
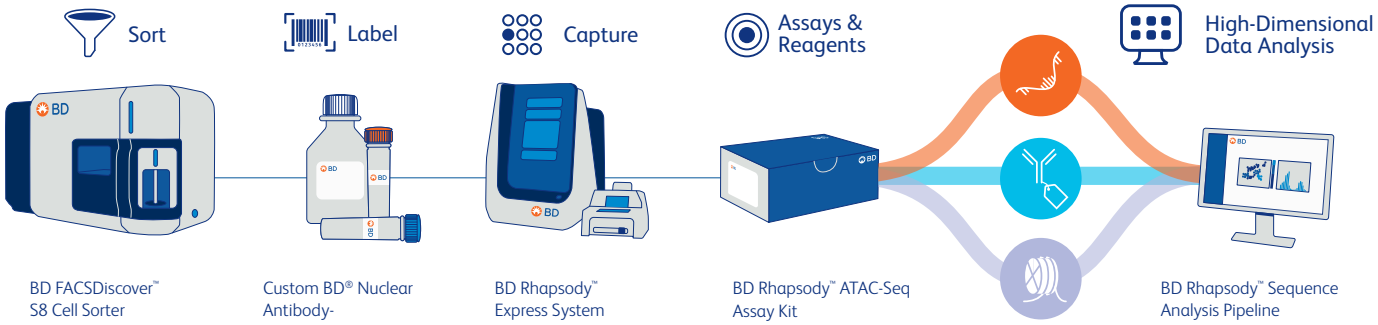


Figure 11. Concordance between gene expression and inferred gene activity score. A heatmap showing Pearson's correlation coefficients between ATAC-seq gene activity scores and gene expression values in PBMCs, with each row representing a cell type in WTA data and each column a cell type in ATAC-seq data.

Benefit from a complete single-cell multiomics workflow

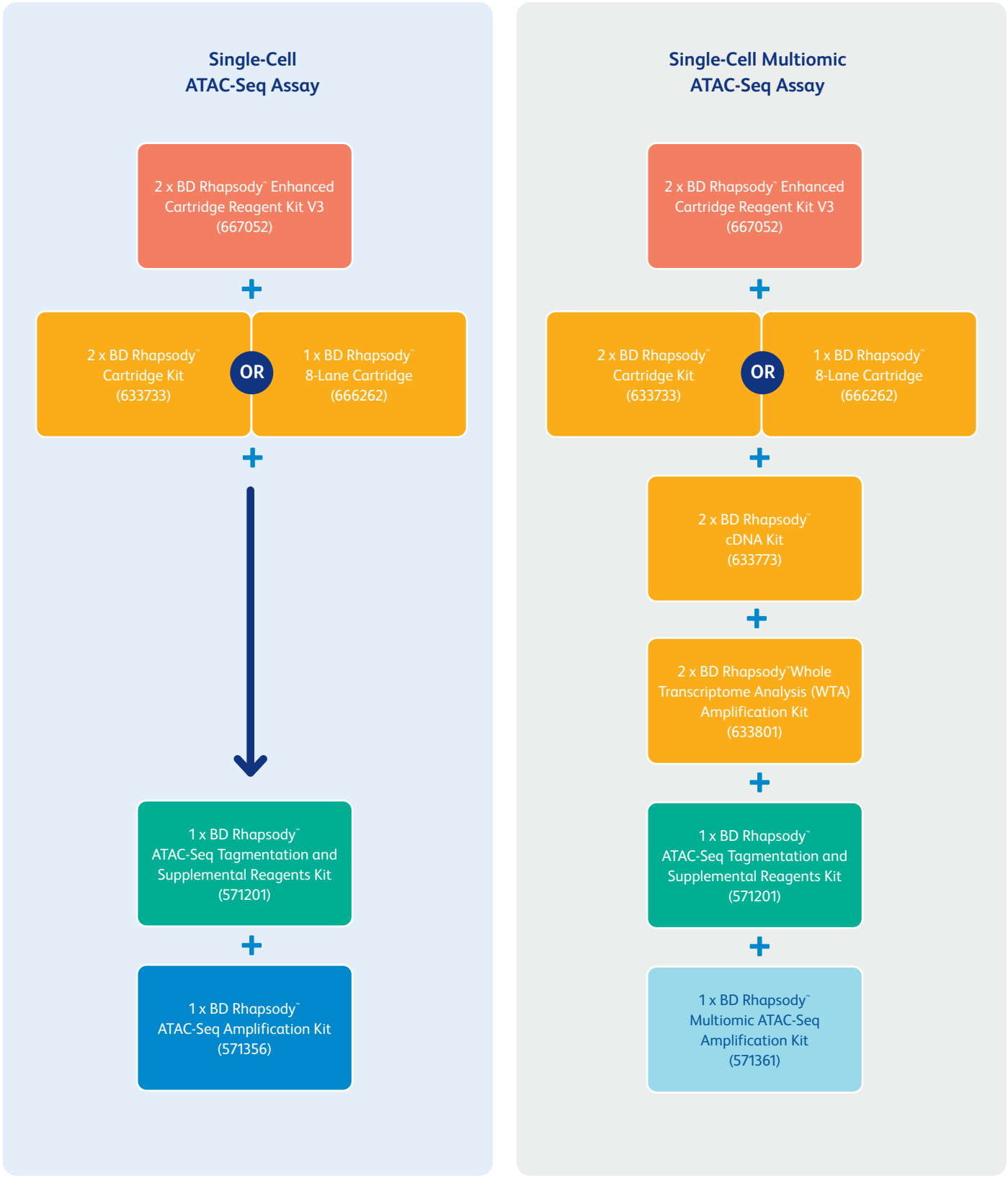


Utilize our expertise and insights for your single-cell experiments.

Reach out to your local BD sales representative or contact our help desk at **scomix@bdscomix.bd.com** to learn more about using BD Rhapsody™ ATAC-Seq Assays.

Product purchase guide for BD Rhapsody™ ATAC-Seq Assays

Reagent kits bundle for the BD Rhapsody™ Single-Cell ATAC-Seq Assay and BD Rhapsody™ Single-Cell Multiomic ATAC-Seq Assay. The number in each box represents the number of kits required to enable eight reactions in total with each workflow.



Ordering information

Individual kits	
Description	Cat. No.
BD Rhapsody™ ATAC-Seq Tagmentation and Supplemental Reagents Kit	571201
BD Rhapsody™ ATAC-Seq Amplification Kit	571356
BD Rhapsody™ Multiomic ATAC-Seq Amplification Kit	571361
Suggested companion instruments	
Description	Cat. No.
BD Rhapsody™ Single-Cell Analysis System	633701
BD Rhapsody™ Express Single-Cell Analysis System Package	633707
BD Rhapsody™ HT Xpress Package	666625
Suggested companion products	
Description	Cat. No.
BD Rhapsody™ Cartridge Kit	633733
BD Rhapsody™ 8 Lane Cartridge Kit	666262
BD Rhapsody™ Enhanced Cartridge Reagent Kit V3	667052
BD Rhapsody™ cDNA Kit	633773
BD Rhapsody™ Whole Transcriptome Analysis (WTA) Amplification Kit	633801
BD® OMICS-Guard Sample Preservation Buffer	570911
Nuclear Single-Cell Multiplexing Kit	Contact for more info

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