BD Rhapsody™ ATAC-Seq Assays

Data supporting sample multiplexing and compatibility with BD® OMICS-Guard Sample Preservation Buffer

BD

Chapter I

- Sample multiplexing with BD Rhapsody™ ATAC-Seq Assays

- Sample multiplexing with ATAC-Seq assays
- BD® Nuclear Sample Multiplexing Kit
- Ordering information
- Overview of performance data

Sample multiplexing with ATAC-Seq assays

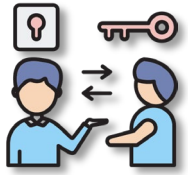
- Single-cell ATAC-seq is a rapid and scalable method to:
  - Gain insight into genome-wide native chromatin landscape
  - Uncover links between regulatory regions and transcriptional outputs

- Current methods, however, face several key limitations:

1. Experimental design flexibility and throughput limitations
2. Batch effects impacting data quality and comparative analyses
3. High per-sample cost when profiling samples individually

Sample multiplexing with ATAC-Seq assays

- To address these problems, we have validated a sample multiplexing workflow that:
  - Enables tagging and pooling of up to 6 samples per lane
  - Works with both standalone and multiomic assays



- This capability affords higher throughput at a lower per-sample cost:

1

Increased throughput and flexibility in experimental design

2

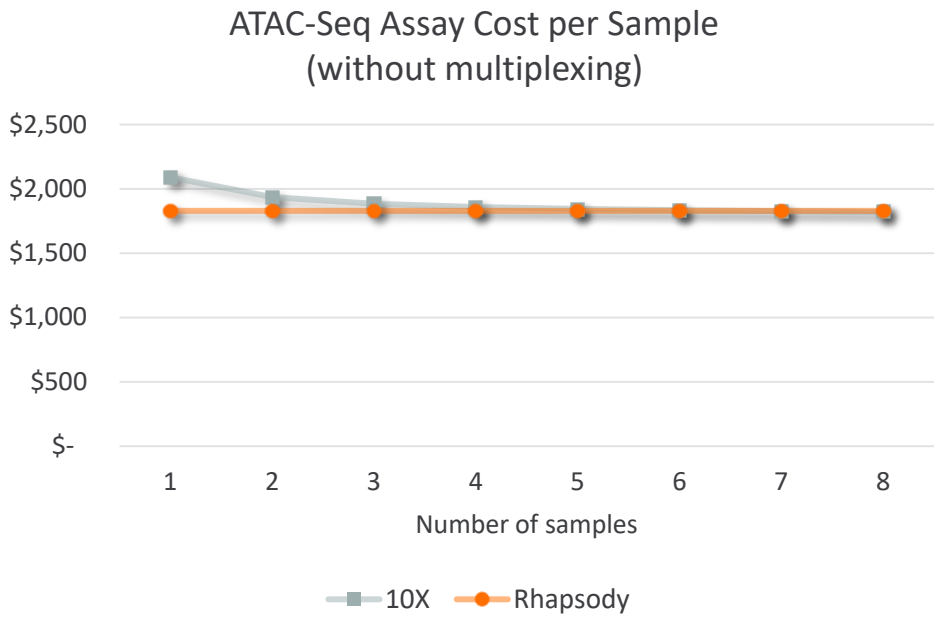
Minimized batch effects for robust comparative analyses

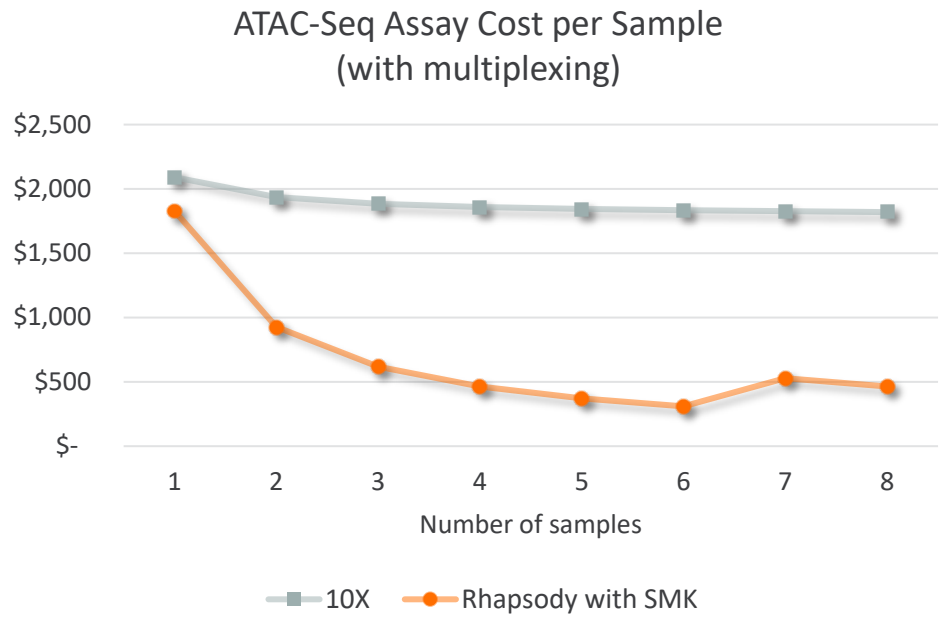
3

Lower per-sample cost without compromising assay performance

Sample multiplexing with ATAC-Seq assays

- Standalone workflow cost per sample for BD vs 10x Genomics assay:
  - Cost is based on list price (no discount) for all reagents needed to prepare a sequencing-ready library.
  - Since Chromium Next GEM Chip H is single use, cost for 10x varies according to # of samples.
  - Cost per sample is based on the cost for preparing 1 library for experiments with 1 up to 6 samples and preparing 2 libraries for experiment with 7 or 8 samples.
  - Average cost per sample for 10x ATAC is $1,887 (up to 8 samples) and $688 for the BD assay.

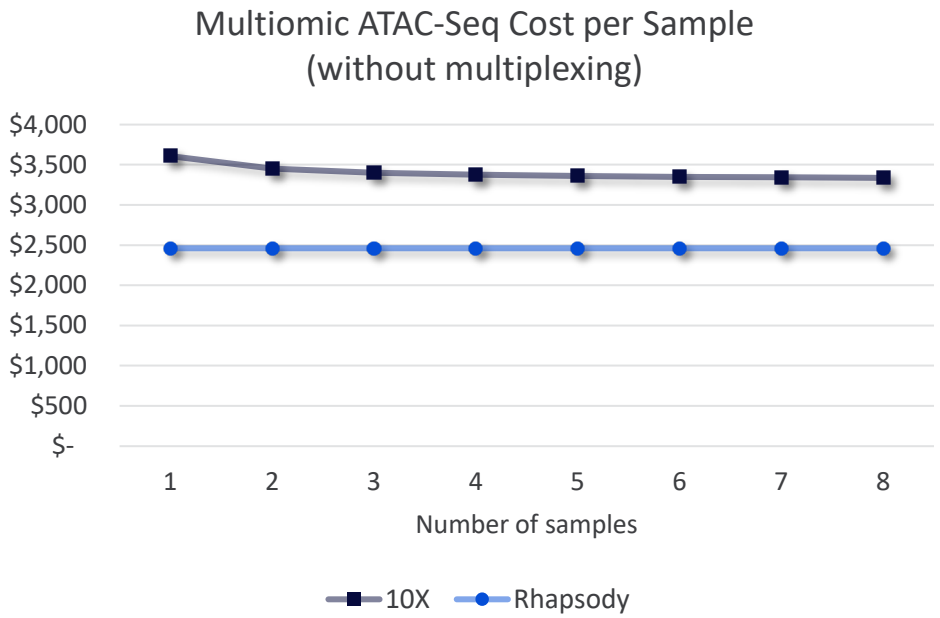


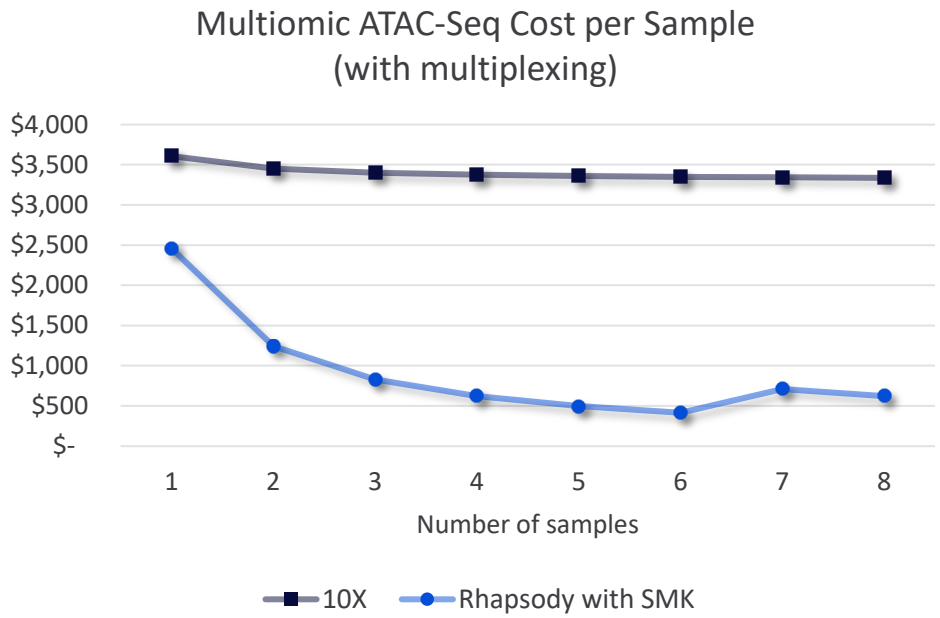


Rhapsody refers to the BD Rhapsody™ ATAC-Seq Assay.
Rhapsody with SMK refers to the BD Rhapsody™ ATAC-Seq Assay with sample multiplexing with the BD® Single-Cell Multiplexing Kit.

Sample multiplexing with ATAC-Seq assays

- Multiomic workflow cost per sample for BD multiomic assay vs 10x Genomics Multiome:
  - Cost is based on list price (no discount) for all reagents needed to prepare a sequencing-ready library.
  - Since Chromium Next GEM Chip J is single use, cost for 10x varies according to # of samples.
  - Cost per sample is based on the cost for preparing 1 library for experiments with 1 up to 6 samples and preparing 2 libraries for experiment with 7 or 8 samples.
  - Average cost per sample for 10x Multiome ATAC is $3,404 (up to 8 samples) and $925 for the BD assay.





Rhapsody refers to the BD Rhapsody™ ATAC-Seq Assay.
Rhapsody with SMK refers to the BD Rhapsody™ ATAC-Seq Assay with sample multiplexing with the BD® Single-Cell Multiplexing Kit.

BD® Nuclear Sample Multiplexing Kit

BD® Nuclear Sample Multiplexing Kit is a bundle of six anti-nucleoporin sample tags that are validated for use with the standalone and multiomic BD Rhapsody™ ATAC-Seq Assays.

Product configuration:

- Product: Anti-nucleoporin sample tags (antibody-oligonucleotide conjugates)
- Application: Single nuclei analysis on the BD Rhapsody™ Single-Cell Analysis System
- Clone: 53/Nucleoporin p62
- Clone reactivity: Human (QC testing), Mouse, Rat, Chicken (tested in development)
- Pipeline analysis: Multiplexing Settings → Sample Tags Version → Single-Cell Multiplex Kit – Mouse

Cat. No.	Material Description	U.S. List Price	Product Config.	Barcode
460291	MS NUCLEOPORIN P62 SAMPLE TAG 1 53 25Tst	$585/vial	25 tests/vial, 2 µL/test	AAGAGTCGACTGCCATGTCCCCTCCGCGGGTCCGTGCCCCCCAAG
460293	MS NUCLEOPORIN P62 SAMPLE TAG 5 53 25Tst	$585/vial	25 tests/vial, 2 µL/test	GGCAAGGTGTCACATTGGGCTACCGCGGGAGGTCGACCAGATCCT
460294	MS NUCLEOPORIN P62 SAMPLE TAG 7 53 25Tst	$585/vial	25 tests/vial, 2 µL/test	ACCGGAGGCGTGTGTACGTGCGTTTCGAATTCCTGTAAGCCCACC
460295	MS NUCLEOPORIN P62 SAMPLE TAG 8 53 25Tst	$585/vial	25 tests/vial, 2 µL/test	TCGCTGCCGTGCTTCATTGTCGCCGTTCTAACCTCCGATGTCTCG
460296	MS NUCLEOPORIN P62 SAMPLE TAG 9 53 25Tst	$585/vial	25 tests/vial, 2 µL/test	GCCTACCCGCTATGCTCGTCGGCTGGTTAGAGTTTACTGCACGCC
460297	MS NUCLEOPORIN P62 SAMPLE TAG 10 53 25Tst	$585/vial	25 tests/vial, 2 µL/test	TCCCATTCGAATCACGAGGCCGGGTGCGTTCTCCTATGCAATCCC

List of six anti-nucleoporin sample tags that are validated for use with the standalone and multiomic BD Rhapsody™ ATAC-Seq Assays.

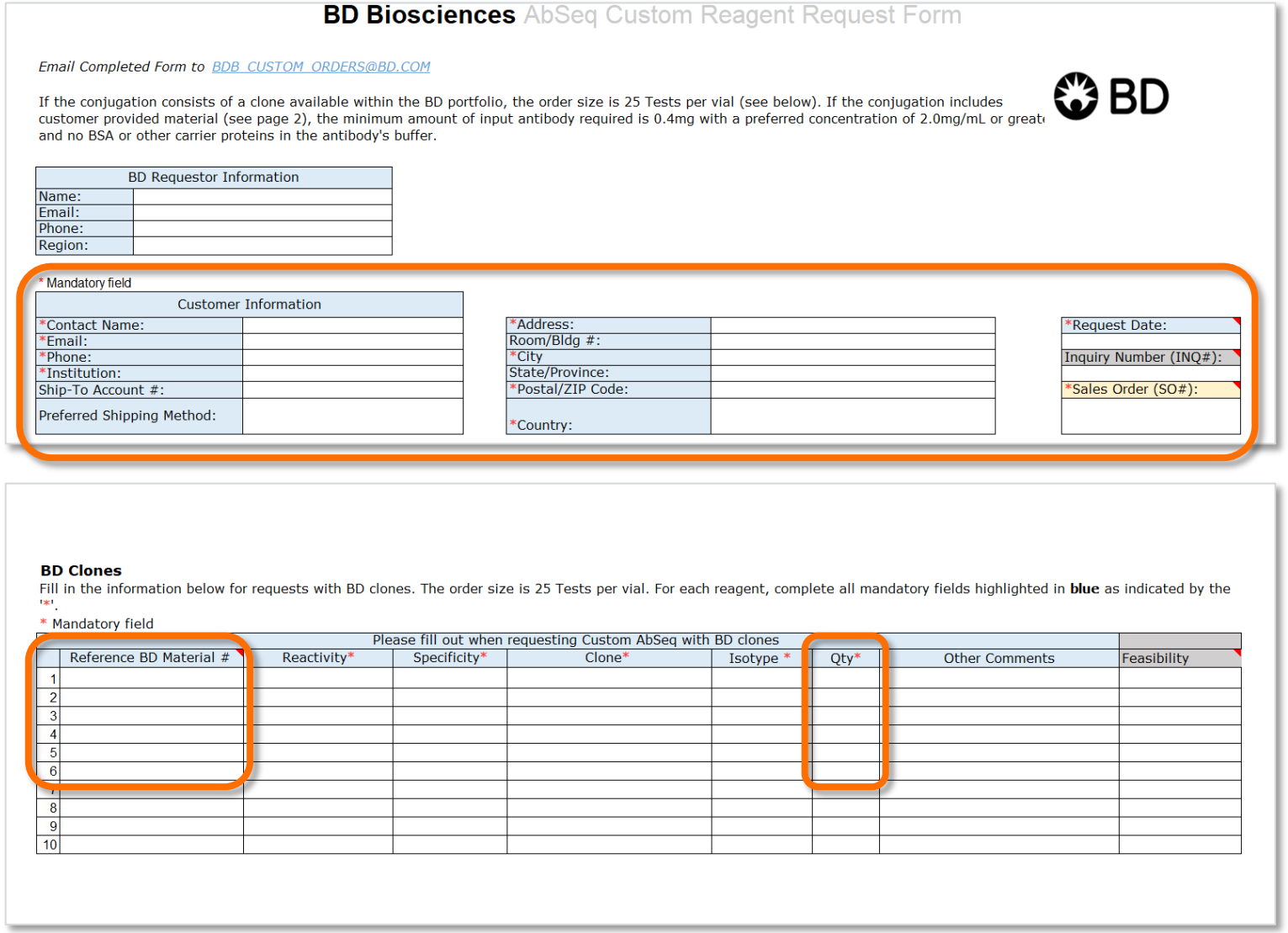
Ordering information

To order the BD® Nuclear Sample Multiplexing Kit or any number of the tags listed in the previous slide, please fill out the form below and send it to BDB custom orders@bd.com:

AbSeq Custom Reagent Request Form v4.xlsx

- On Page 1, fill out the Customer Information section.
- On the same page, add the requested tags information to the **BD Clones** table:
  - Add the Cat. No. of the tags that you would like to purchase in the **Reference BD Material #** column.
  - Add the quantity of interest for each tag in the **Qty** column. Please note that quantity of 1 for a given tag is sufficient for 25 tests.
  - Leave other columns blank.

For more information, feel free to contact us at SCOMIX@bdscomix.bd.com.



BD Biosciences AbSeq Custom Reagent Request Form

Email Completed Form to BDB_CUSTOM_ORDERS@BD.COM

If the conjugation consists of a clone available within the BD portfolio, the order size is 25 Tests per vial (see below). If the conjugation includes customer provided material (see page 2), the minimum amount of input antibody required is 0.4mg with a preferred concentration of 2.0mg/mL or greate and no BSA or other carrier proteins in the antibody's buffer.

BD Requestor Information	
Name:	
Email:	
Phone:	
Region:	

* Mandatory field

Customer Information	
*Contact Name:	
*Email:	
*Phone:	
*Institution:	
Ship-To Account #:	
Preferred Shipping Method:	

*Address:	
Room/Bldg #:	
*City	
State/Province:	
*Postal/ZIP Code:	
*Country:	

*Request Date:	
Inquiry Number (INQ#):	
*Sales Order (SO#):	

BD Clones

Fill in the information below for requests with BD clones. The order size is 25 Tests per vial. For each reagent, complete all mandatory fields highlighted in **blue** as indicated by the '*'.

* Mandatory field

	Please fill out when requesting Custom AbSeq with BD clones							
	Reference BD Material #	Reactivity*	Specificity*	Clone*	Isotype *	Qty*	Other Comments	Feasibility
1								
2								
3								
4								
5								
6								
8								
9								
10								

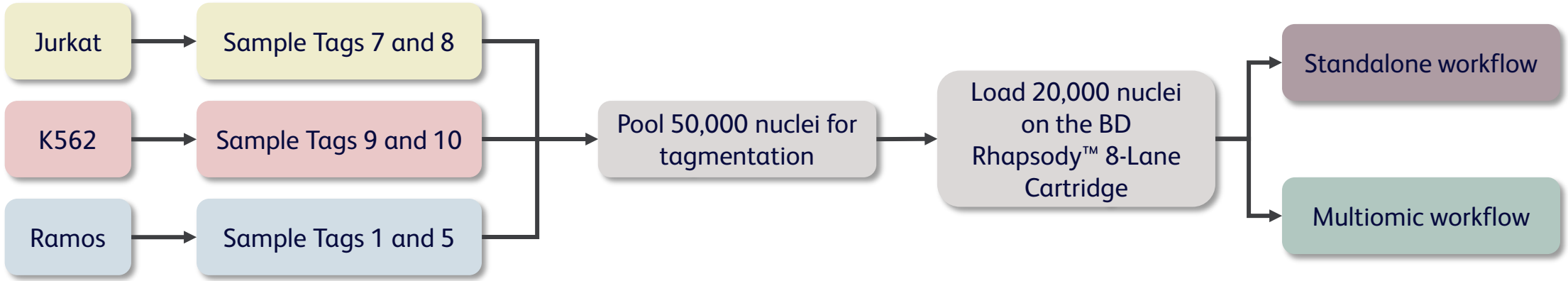
Overview of performance data

In a representative experiment, three different cell lines were stained using six BD® Nuclear Sample Multiplexing Ab-Oligos, pooled at different ratios, run using standalone and multiomic ATAC-Seq assays (using a BD Rhapsody™ 8-Lane Cartridge) and analyzed against unstained control samples.

For both stained and unstained samples, 50,000 nuclei were pooled for the tagmentation reaction, 20,000 of which were loaded onto the 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.

BD Rhapsody™ ATAC-Seq Assay configurations:

1. ATAC (Control)
2. ATAC + SMK (Sample Tag Stained)
3. ATAC + WTA (Control)
4. ATAC + WTA + SMK (Sample Tag Stained)



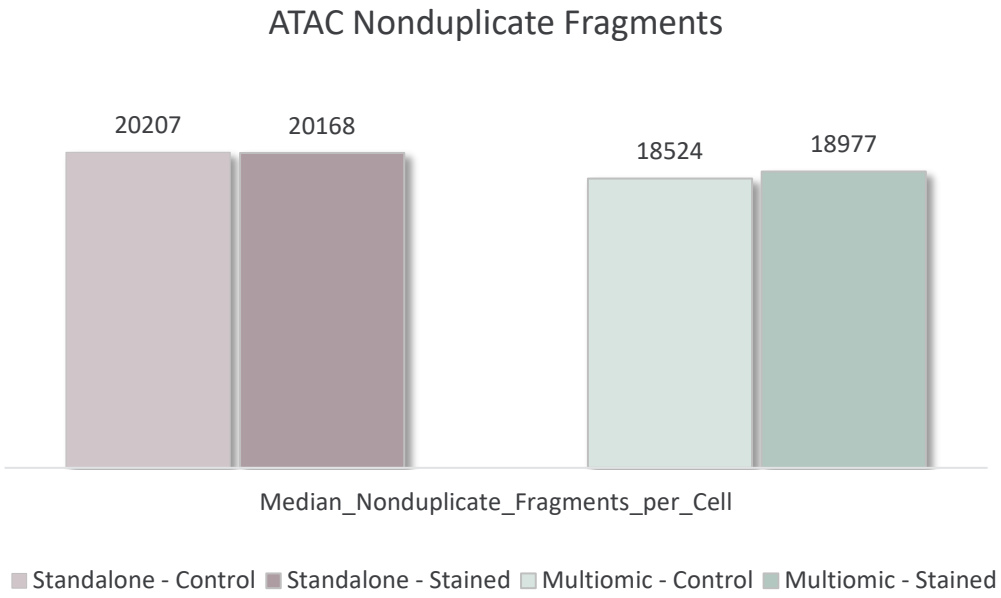
Schematic of the six Sample Tag experiment with 20,000 nuclei of mixed sample types loaded on the BD Rhapsody™ 8-Lane Cartridge.

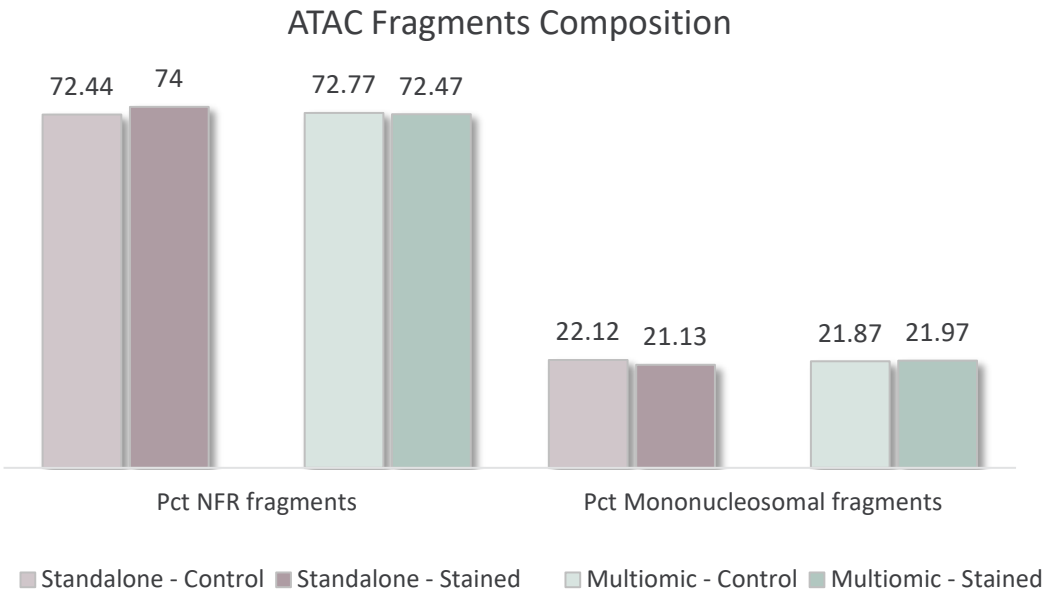
Overview of performance data

Sensitivity metrics from the same experiments showing similarly high values for *Median Nonduplicate Fragments per Cell* at ~50,000 mean read pairs per cell and high cumulative fraction of *nucleosome-free* and *mono-nucleosomal fragments* across different assay combinations.

BD Rhapsody™ ATAC-Seq Assay configurations:

1. ATAC (Control)
2. ATAC + SMK (Sample Tag Stained)
3. ATAC + WTA (Control)
4. ATAC + WTA + SMK (Sample Tag Stained)





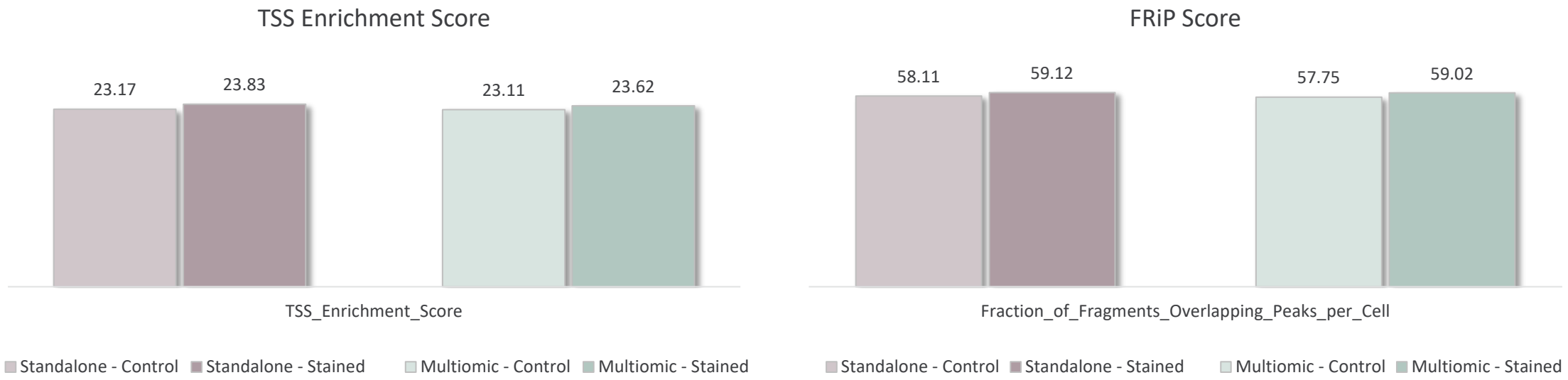
Median nonduplicate fragments at ~50,000 mean read pairs per cell and fragment composition for stained vs control samples in standalone and multiomic ATAC-Seq experiments.

Overview of performance data

Specificity metrics from the same experiments showing similarly high values for *TSS Enrichment Score* and *Fraction of Fragments Overlapping Peaks per Cell* (*FRiP score*) across different assay combinations.

BD Rhapsody™ ATAC-Seq Assay configurations:

1. ATAC (Control)
2. ATAC + SMK (Sample Tag Stained)
3. ATAC + WTA (Control)
4. ATAC + WTA + SMK (Sample Tag Stained)

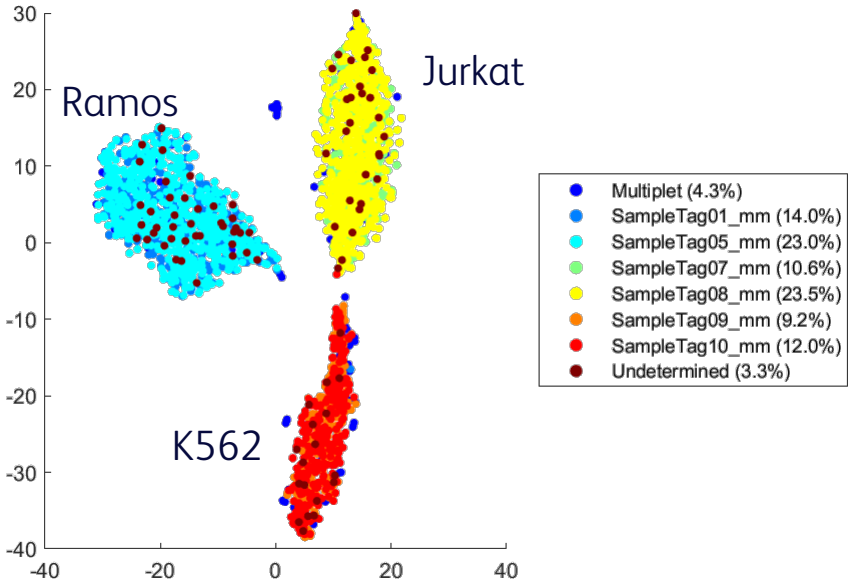


TSS Enrichment Score and Fraction of Fragments Overlapping Peaks per Cell (FRiP score) for stained vs control samples in standalone and multiomic ATAC-Seq experiments.

Overview of performance data

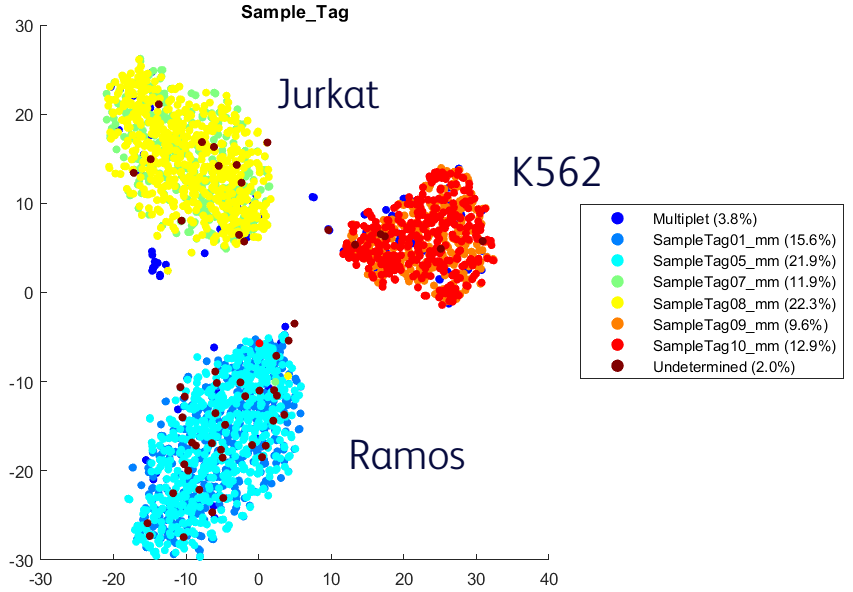
Sensitivity (% of undetermined tags) and specificity (total % of cells with expected tag) from the same experiments showing high sensitivity (*less than 5% undetermined cells*) and specificity (*>95% cells detected with expected tags*) after removing multiplets and undetermined cells.

ATAC + SMK



	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%

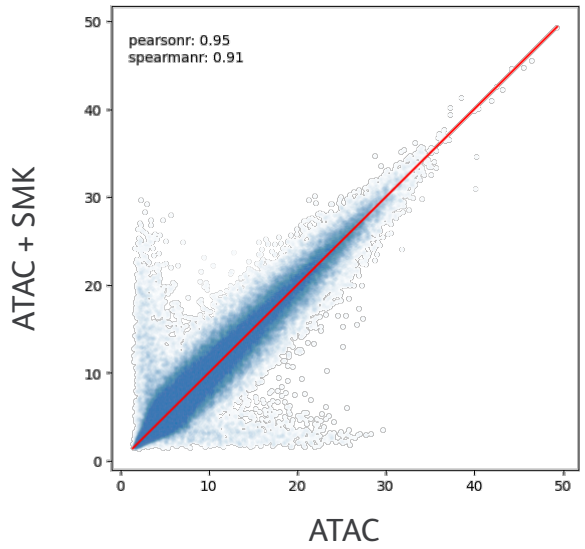
ATAC + WTA + SMK

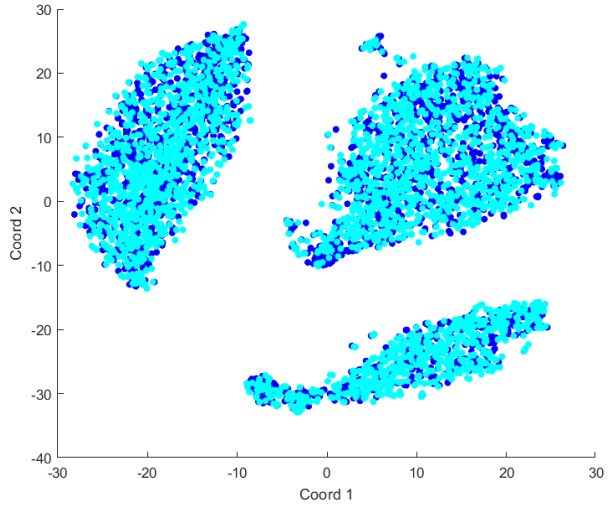


	Jurkat	K562	Ramos
SampleTag01 mm	0%	0%	41.42%
SampleTag05 mm	0%	0%	58.27%
SampleTag07 mm	34.81%	0%	0.1%
SampleTag08 mm	65.19%	0%	0.1%
SampleTag09 mm	0%	42.88%	0%
SampleTag10 mm	0%	57.12%	0.1%
Specificity	100%	100%	99.69%

Overview of performance data

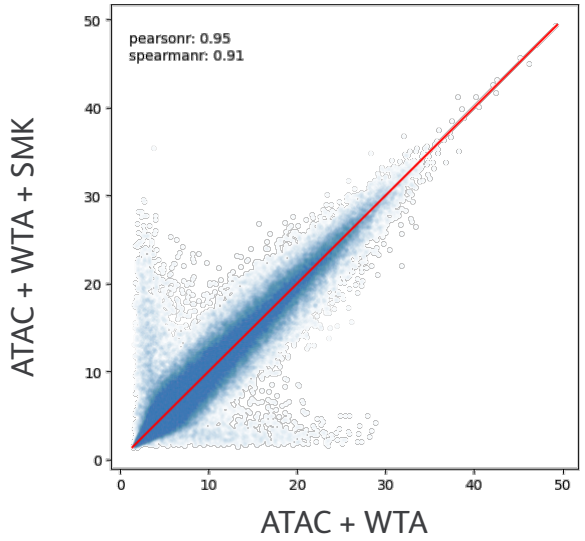
ATAC peak correlation plot and expression analysis plotted by *gene-by-cell* files for standalone ATAC-Seq experiments with stained sample vs control, showing no difference between the ATAC + SMK and ATAC libraries.

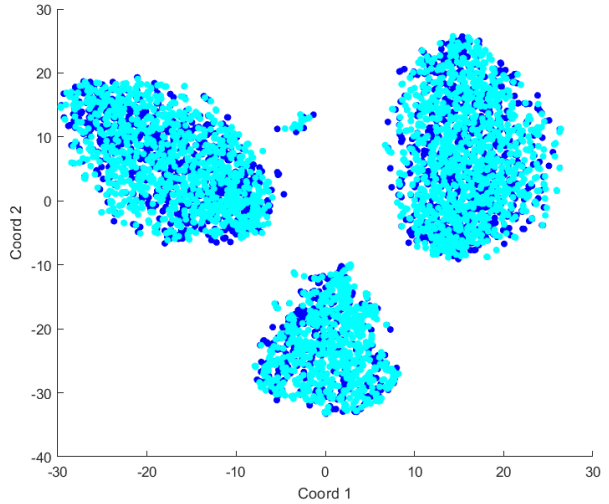




Standalone - Control
Standalone - Stained

ATAC peak correlation plot and expression analysis plotted by *RSEC-mols-per-cell* files for multiomic ATAC-Seq experiments with stained sample vs control, showing no difference between the ATAC + WTA + SMK and ATAC + WTA libraries.



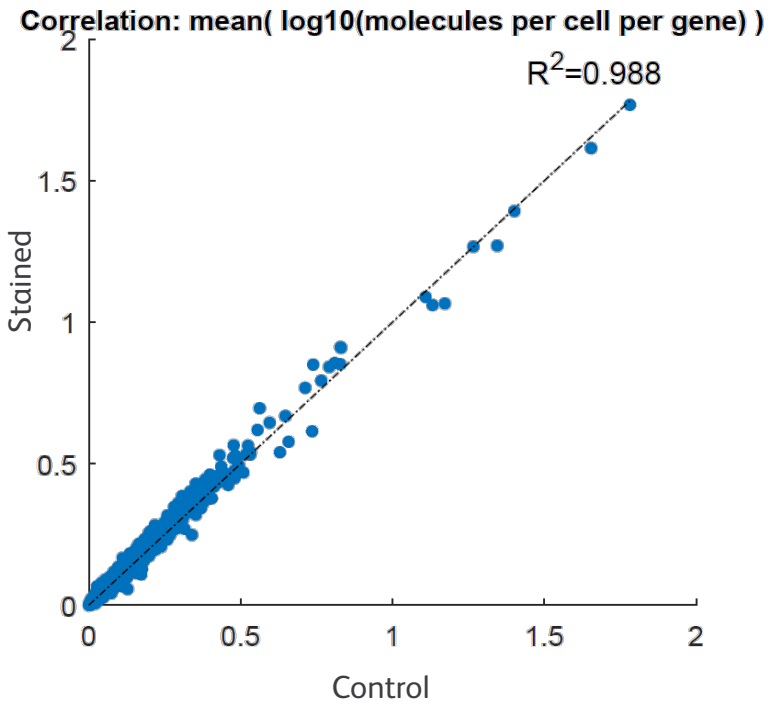


Multiomic - Control
Multiomic - Stained

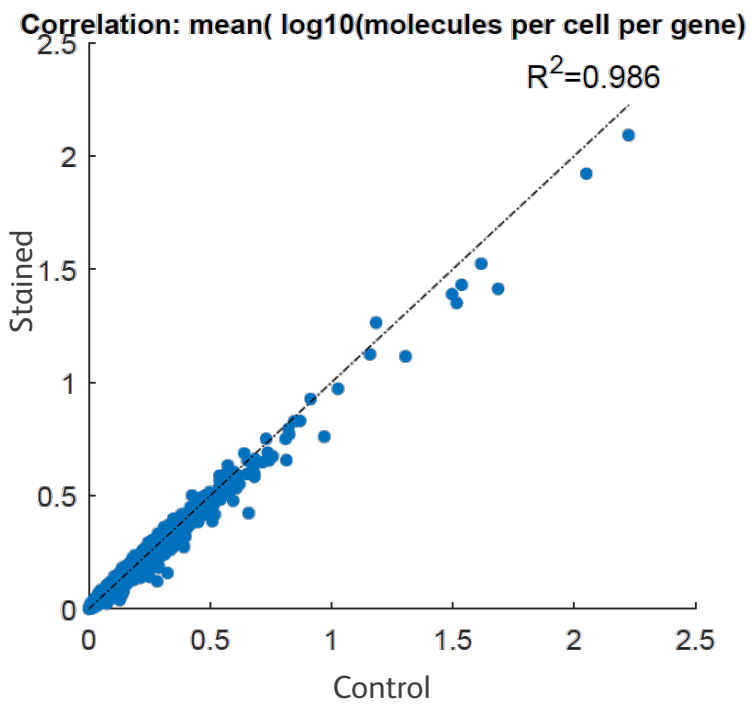
Overview of performance data

Gene expression correlation plots from multiomic ATAC-Seq experiments with stained sample vs control, showing high correlation for differential gene expression between stained samples vs control.

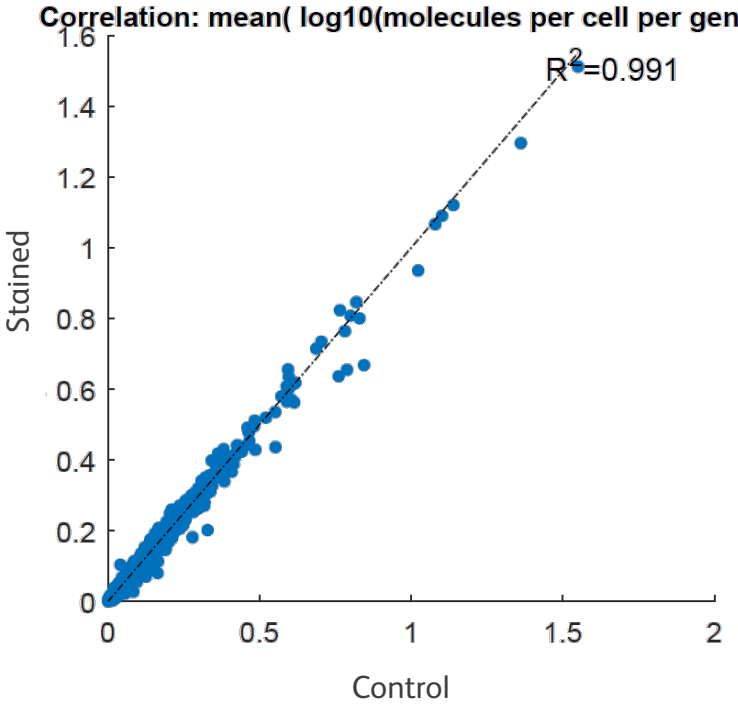
Jurkat



K562



Ramos



Chapter II

- Compatibility with BD® OMICS-Guard Sample Preservation Buffer

- BD® OMICS-Guard Sample Preservation Buffer
- Overview of performance data

BD® OMICS-Guard Sample Preservation Buffer

- Studies involving multi-site and/or timepoint sample collection currently struggle with:

1. Maintaining sample viability across sites and timepoints
2. Preserving nuclei's integrity for ATAC-Seq analysis

- To address these challenges, ATAC-Seq assays are validated for use with cells preserved in BD® OMICS-Guard Sample Preservation Buffer.

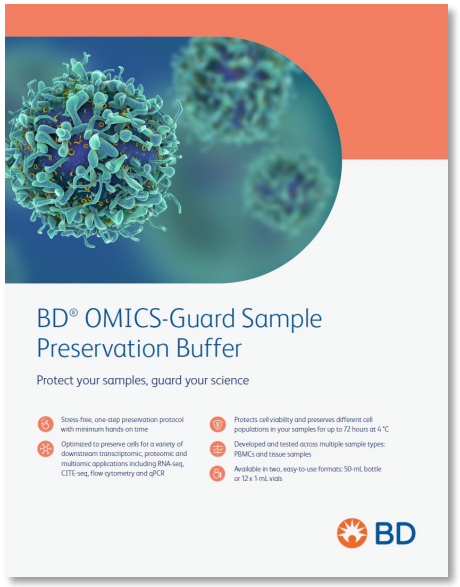
BD® OMICS-Guard Sample Preservation Buffer

- Experiments (standalone and multiomic) using nuclei from cells preserved up to 72 hours in BD® OMICS-Guard Sample Preservation Buffer showed:
  - No or minimal impact on ATAC sensitivity and specificity
  - No or minimal impact on WTA sensitivity and specificity

- For more information, refer to:

  BD® OMICS-Guard Sample Preservation Buffer

- To order BD® OMICS-Guard Sample Preservation Buffer and Kit, refer to: Order Now



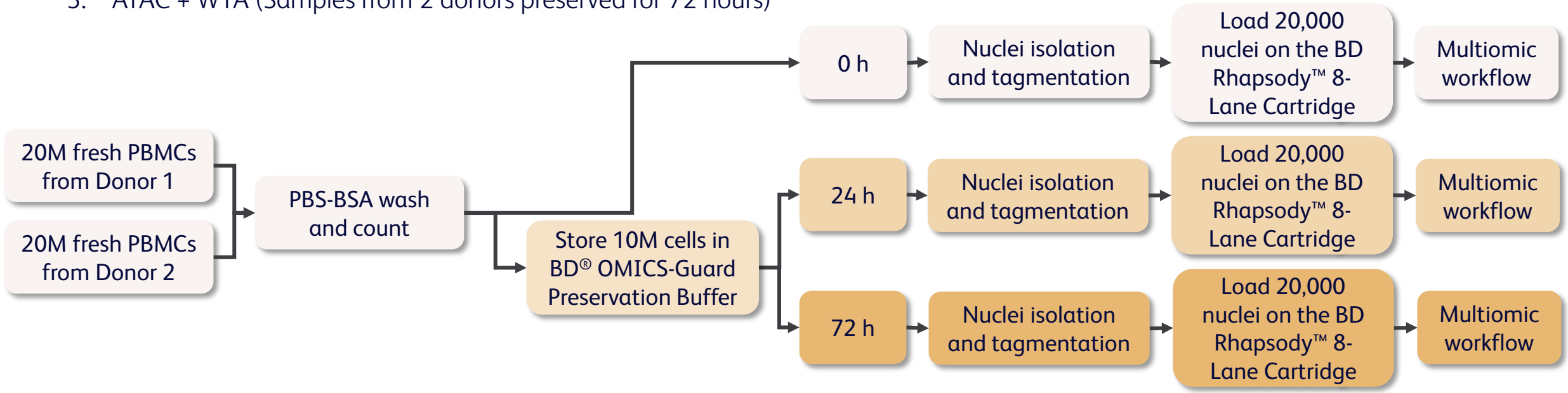
Overview of performance data

In a representative experiment, fresh PBMC samples 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.

Cells from both donors, in complete media, went through a PBS-BSA wash and then were counted using Calcein AM and DRAQ7™ dyes before being divided for fresh experiments and storage in BD® OMICS-Guard Sample Preservation Buffer for experiments carried out at different time points.

BD Rhapsody™ ATAC-Seq Assay configurations:

1. ATAC + WTA (Fresh control samples from 2 donors)
2. ATAC + WTA (Samples from 2 donors preserved for 24 hours)
3. ATAC + WTA (Samples from 2 donors preserved for 72 hours)

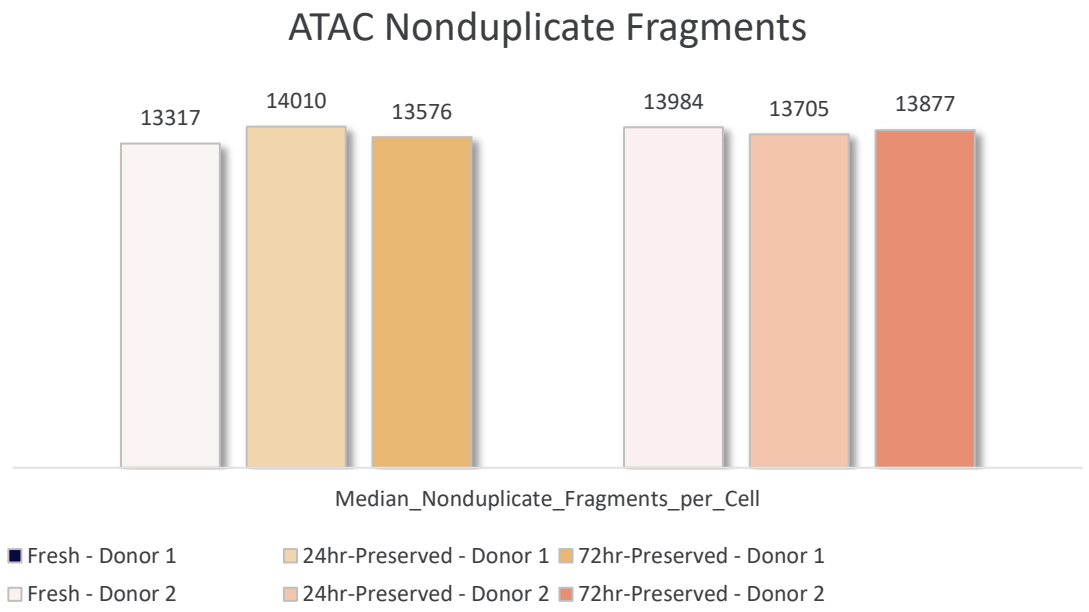


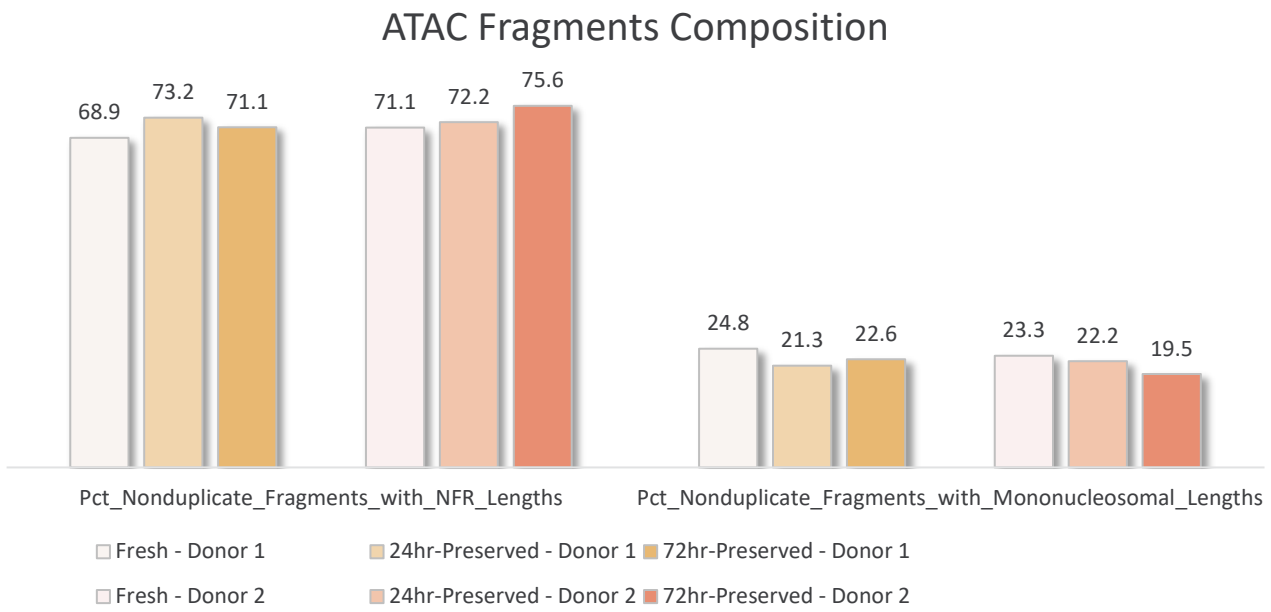
Overview of performance data

Sensitivity metrics from the same experiments showing similarly high values for *Median Nonduplicate Fragments per Cell* at ~52,000 mean read pairs per cell and high cumulative fraction of *nucleosome-free* and *mono-nucleosomal fragments* across different assay combinations.

BD Rhapsody™ ATAC-Seq Assay configurations:

1. ATAC + WTA (Fresh control samples from 2 donors)
2. ATAC + WTA (Samples from 2 donors preserved for 24 hours)
3. ATAC + WTA (Samples from 2 donors preserved for 72 hours)





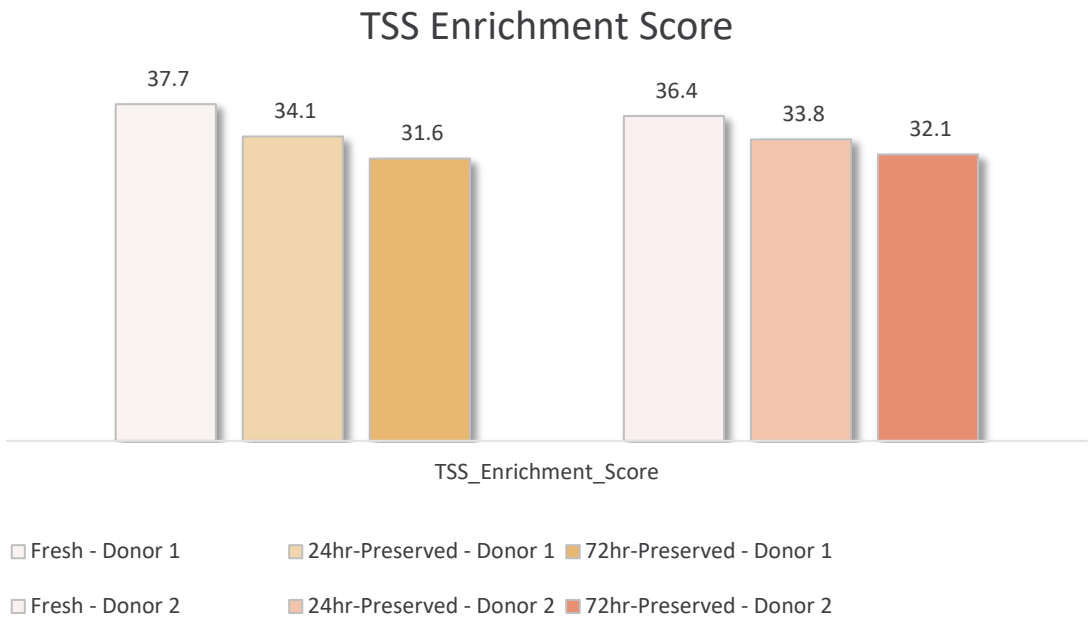
Median nonduplicate fragments at ~52,000 mean read pairs per cell and fragment composition for fresh vs preserved samples in multiomic ATAC-Seq experiments.

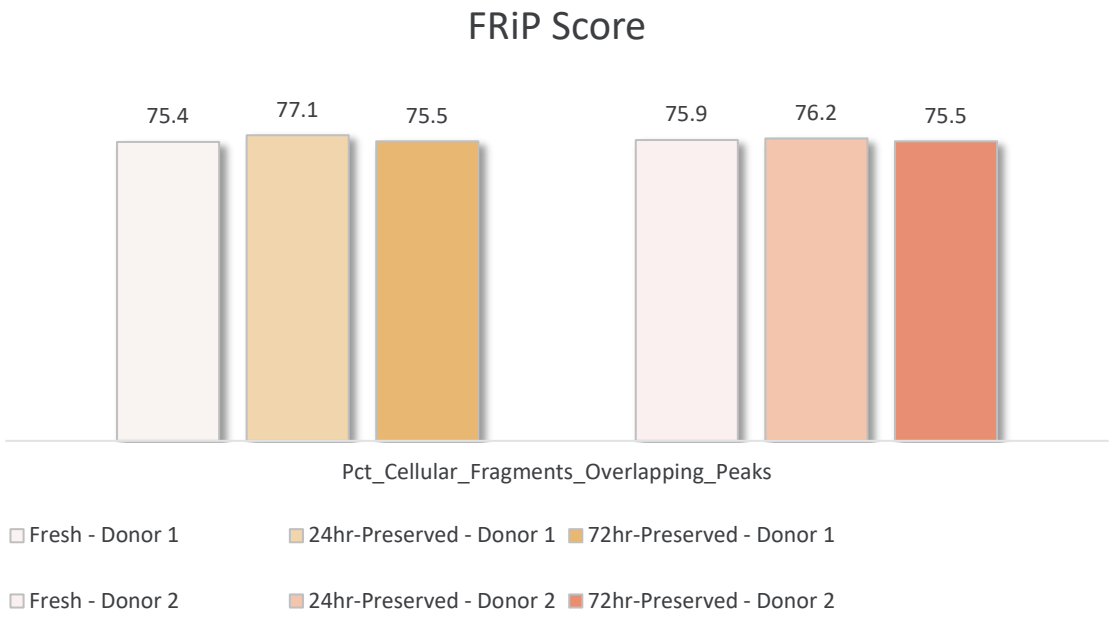
Overview of performance data

Specificity metrics from the same experiments showing similarly high values for *TSS Enrichment Score* and *Fraction of Fragments Overlapping Peaks per Cell* (*FRiP score*) across different assay combinations.

BD Rhapsody™ ATAC-Seq Assay configurations:

1. ATAC + WTA (Fresh control samples from 2 donors)
2. ATAC + WTA (Samples from 2 donors preserved for 24 hours)
3. ATAC + WTA (Samples from 2 donors preserved for 72 hours)





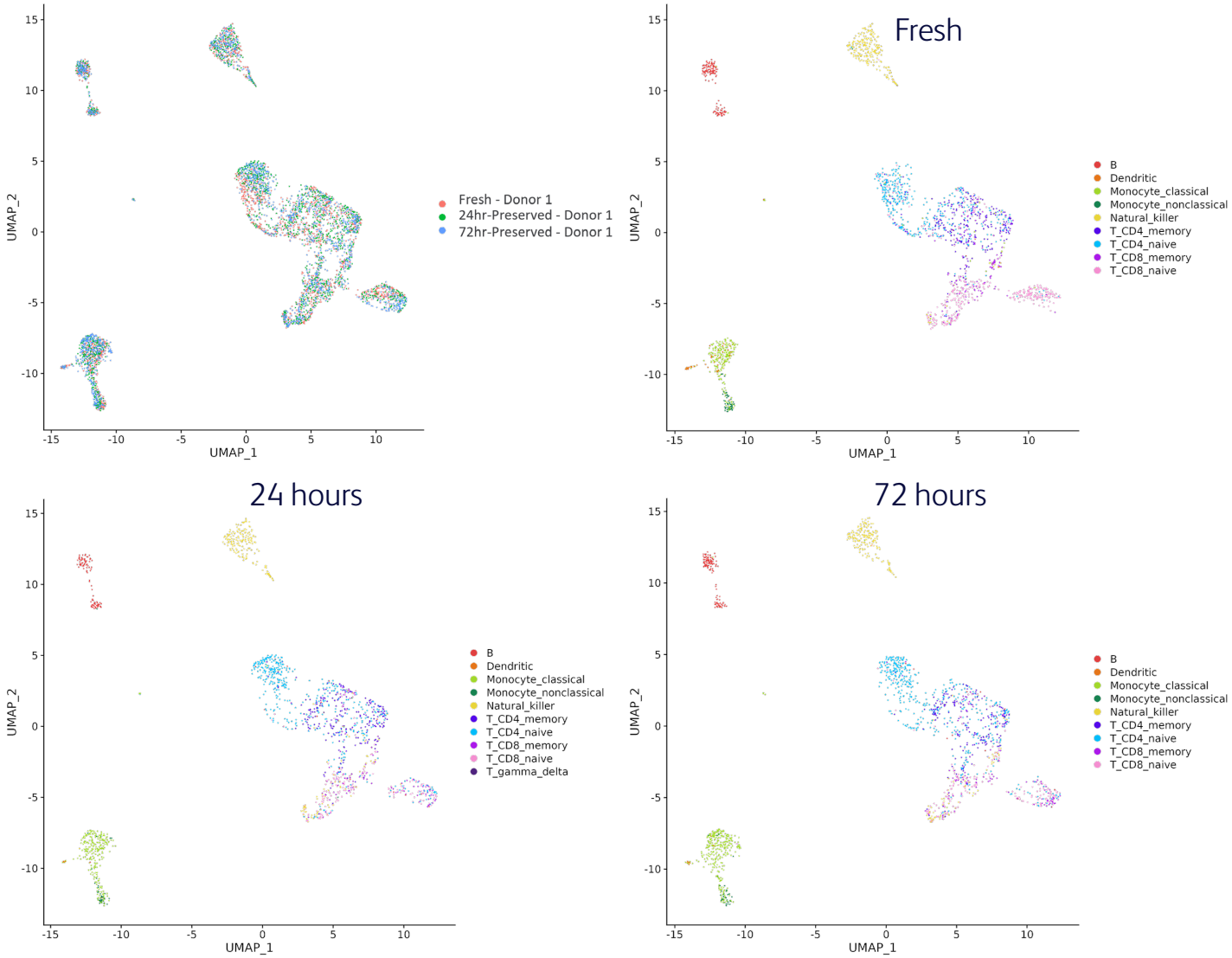
TSS Enrichment Score and Fraction of Fragments Overlapping Peaks per Cell (FRiP score) for fresh vs preserved samples in multiomic ATAC-Seq experiments.

Overview of performance data

Uniform Manifold Approximation and Projection (UMAP) analysis using both the ATAC and WTA data from the multiomic experiments with the sample from Donor 1 showing that the original sample biology (genes and chromatin accessibility) is retained after preservation in BD® OMICS-Guard Sample Preservation Buffer even after 72 hours.

BD Rhapsody™ ATAC-Seq Assay configurations:

1. ATAC + WTA (Fresh control sample from Donor 1)
2. ATAC + WTA (Sample from Donor 1 preserved for 24 hours)
3. ATAC + WTA (Sample from Donor 1 preserved for 72 hours)

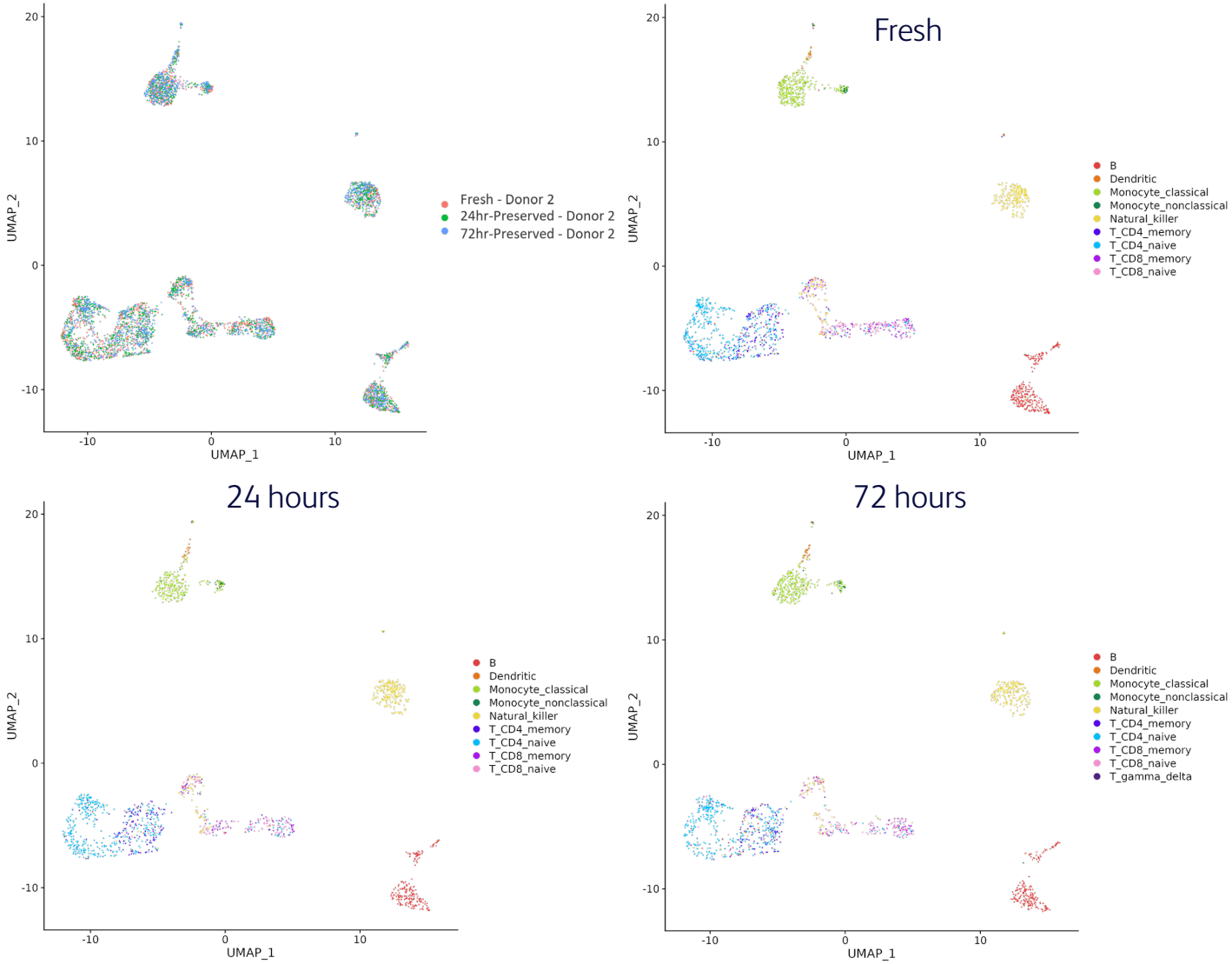


Overview of performance data

UMAP analysis using both the ATAC and WTA data from the multiomic experiments with the sample from Donor 2 showing that the original sample biology (genes and chromatin accessibility) is retained after preservation in BD® OMICS-Guard Sample Preservation Buffer even after 72 hours.

BD Rhapsody™ ATAC-Seq Assay configurations:

1. ATAC + WTA (Fresh control sample from Donor 2)
2. ATAC + WTA (Sample from Donor 2 preserved for 24 hours)
3. ATAC + WTA (Sample from Donor 2 preserved for 72 hours)



Appendix (Part I)

- Sample multiplexing experiments with BD Rhapsody™ ATAC-Seq Assays
- Compatibility with BD® OMICS-Guard Sample Preservation Buffer
- Sample multiplexing with BD® OMICS-Guard Buffer-preserved samples

Sample multiplexing with BD Rhapsody™ ATAC-Seq Assays

ATAC-Seq pipeline metrics summary for the experiments with and without Sample Tag staining (using a BD Rhapsody™ 8-Lane Cartridge) showing similarly high sensitivity (*Median Nonduplicate Fragments per Cell*) and specificity (*Fraction of Fragments Overlapping Peaks per Cell* and *TSS Enrichment Score*) across different assay combinations.

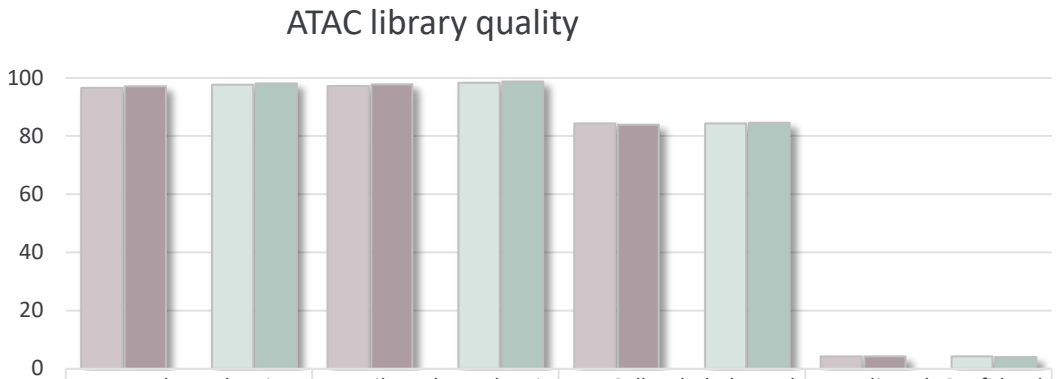
BD Rhapsody™ ATAC-Seq Assay configurations:

1. ATAC (Control)
2. ATAC + SMK (Sample Tag Stained)
3. ATAC + WTA (Control)
4. ATAC + WTA + SMK (Sample Tag Stained)

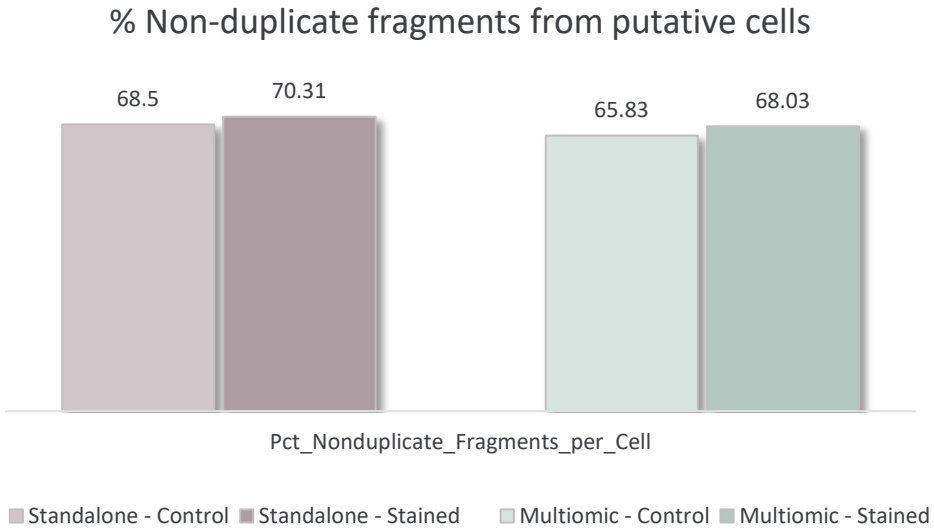
	Standalone ATAC-Seq		Multiomic ATAC-Seq	
Pipeline Metric	**1. Control**	**2. Stained**	**3. Control**	**4. Stained**
Pct Total Read Pairs with Cell Label	96.52	97.10	97.66	98.04
Pct Filtered Read Pairs with Cell Label	97.22	97.73	98.33	98.75
Pct Cell Labeled Reads Aligned Confidently	84.28	83.93	84.28	84.50
Pct Aligned Confidently Mitochondrial	4.25	4.25	4.27	3.98
Pct Nonduplicate Fragments with NFR Lengths	72.44	74.00	72.77	72.47
Pct Nonduplicate Fragments with Mononucleosomal Lengths	22.12	21.13	21.87	21.97
Fraction of Fragments Overlapping Peaks per Cell	**58.11**	**59.12**	**57.75**	**59.02**
TSS Enrichment Score	**23.17**	**23.83**	**23.11**	**23.62**
Total Peaks	166,270	179,854	161,326	178,956
Total Putative Cells	2,135	2,600	1,860	2,653
Percent nonduplicate fragments associated with putative cells	68.50	70.31	65.83	68.03
Mean Read Pairs per Cell	50,003	49,833	50,020	49,860
Median Nonduplicate Fragments per Cell	**20,207**	**20,168**	**18,524**	**18,977**
Pct Duplicate Fragments	17.31	16.06	18.95	19.15

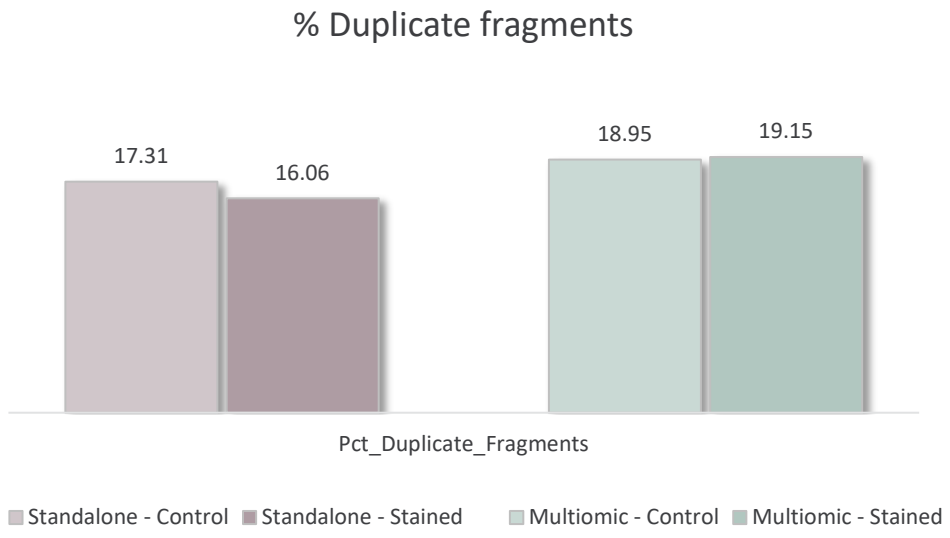
Sample multiplexing with BD Rhapsody™ ATAC-Seq Assays

Library quality metrics along with *% Non-duplicate fragments from putative cells* and *% Duplicate fragments* from ATAC-Seq libraries in stained and control samples.



	Pct_Total_Read_Pairs_with_Cell_Label	Pct_Filtered_Read_Pairs_with_Cell_Label	Pct_Cell_Labeled_Reads_Aligned_Confidently	Pct_Aligned_Confidently_Mitochondrial
Standalone - Control	96.52	97.22	84.28	4.25
Standalone - Stained	97.1	97.73	83.93	4.25
Multiomic - Control	97.66	98.33	84.28	4.27
Multiomic - Stained	98.04	98.75	84.5	3.98



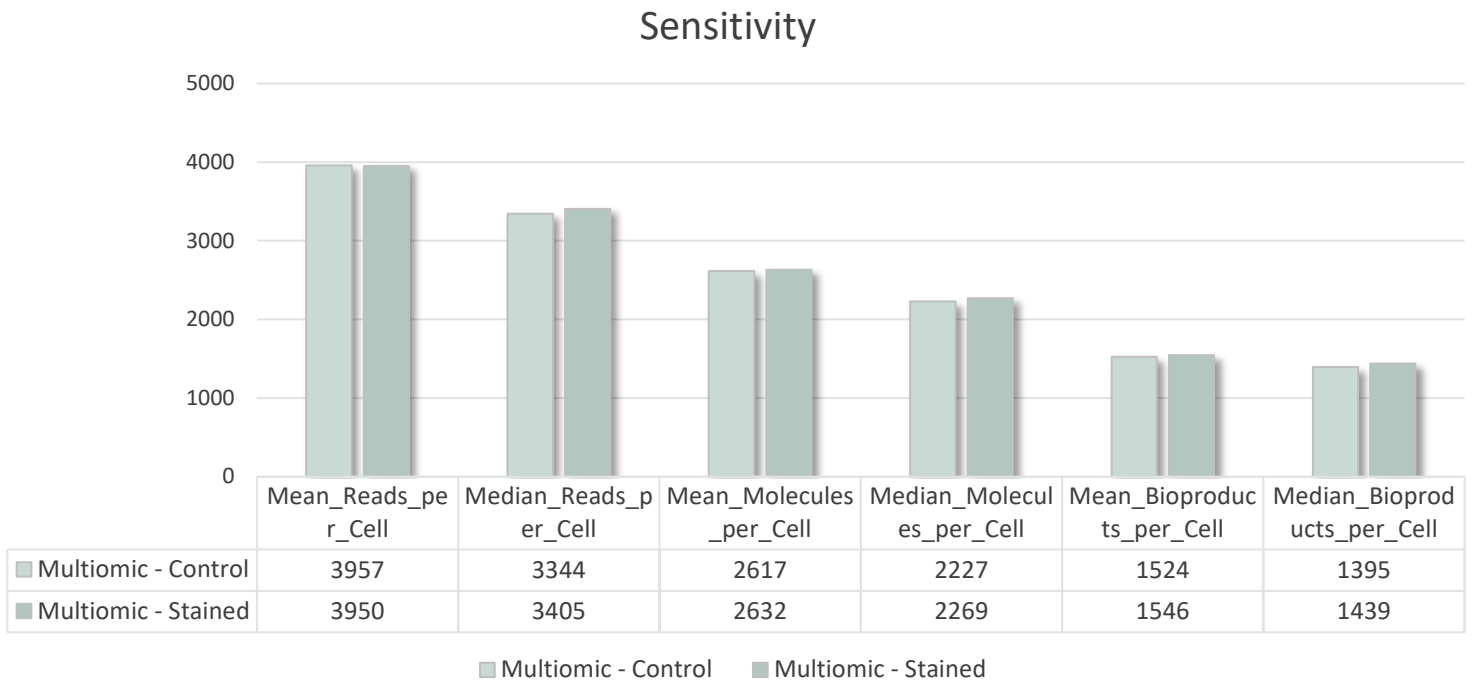


Sample multiplexing with BD Rhapsody™ ATAC-Seq Assays

Sensitivity metrics for the WTA libraries sequenced at a shallow depth of ~4,000 read pairs per cell in the multiomic ATAC-Seq experiments (using a BD Rhapsody™ 8-Lane Cartridge) showing similar per cell values for stained samples vs control.

BD Rhapsody™ ATAC-Seq Assay configurations:

1. ATAC + WTA (Control)
2. ATAC + WTA + SMK (Sample Tag Stained)



	Mean_Reads_per_Cell	Median_Reads_per_Cell	Mean_Molecules_per_Cell	Median_Molecules_per_Cell	Mean_Bioproducts_per_Cell	Median_Bioproducts_per_Cell
Multiomic - Control	3957	3344	2617	2227	1524	1395
Multiomic - Stained	3950	3405	2632	2269	1546	1439

Appendix (Part II)

- Sample multiplexing experiments with BD Rhapsody™ ATAC-Seq Assays
- Compatibility with BD® OMICS-Guard Sample Preservation Buffer
- Sample multiplexing with BD® OMICS-Guard Buffer-preserved samples

Compatibility with BD® OMICS-Guard Sample Preservation Buffer

ATAC-Seq pipeline metrics summary for the experiments with and without preservation in BD® OMICS-Guard Sample Preservation Buffer showing similarly high sensitivity (*Median Nonduplicate Fragments per Cell*) and specificity (*Fraction of Fragments Overlapping Peaks per Cell* and *TSS Enrichment Score*) across different assay combinations.

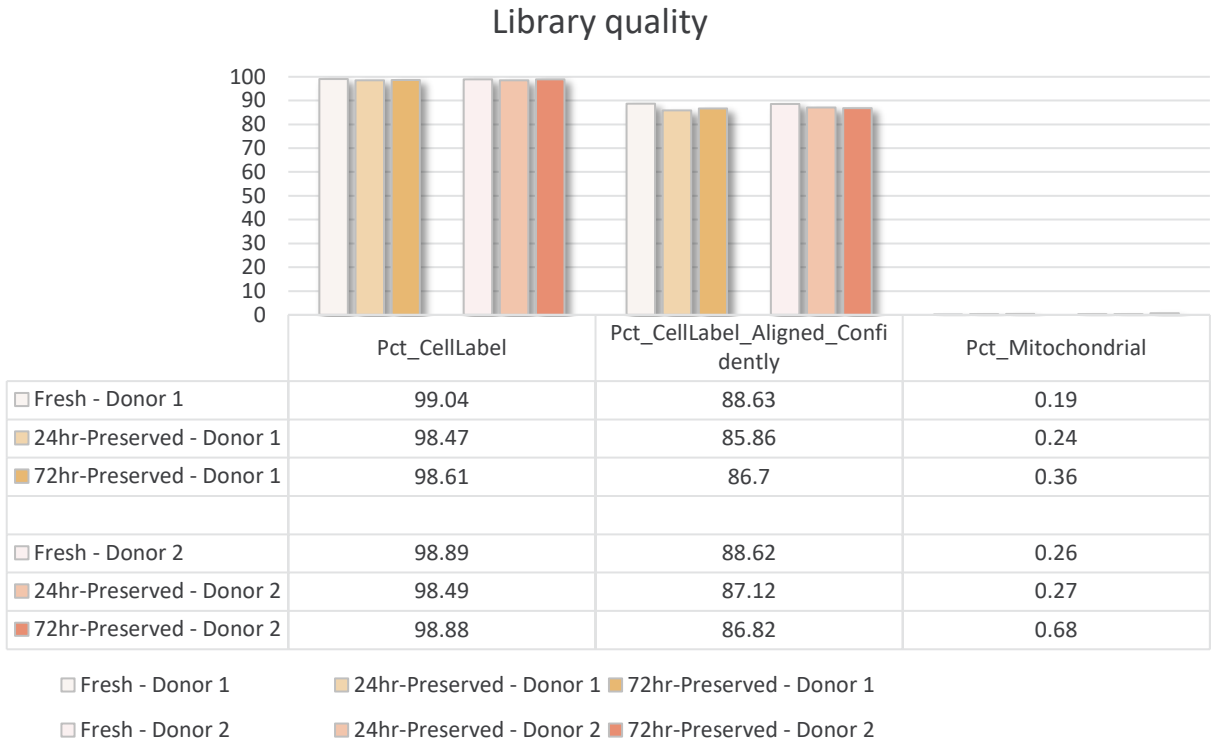
BD Rhapsody™ ATAC-Seq Assay configurations:

1. ATAC + WTA (Fresh control samples from 2 donors)
2. ATAC + WTA (Samples from 2 donors preserved for 24 hours)
3. ATAC + WTA (Samples from 2 donors preserved for 72 hours)

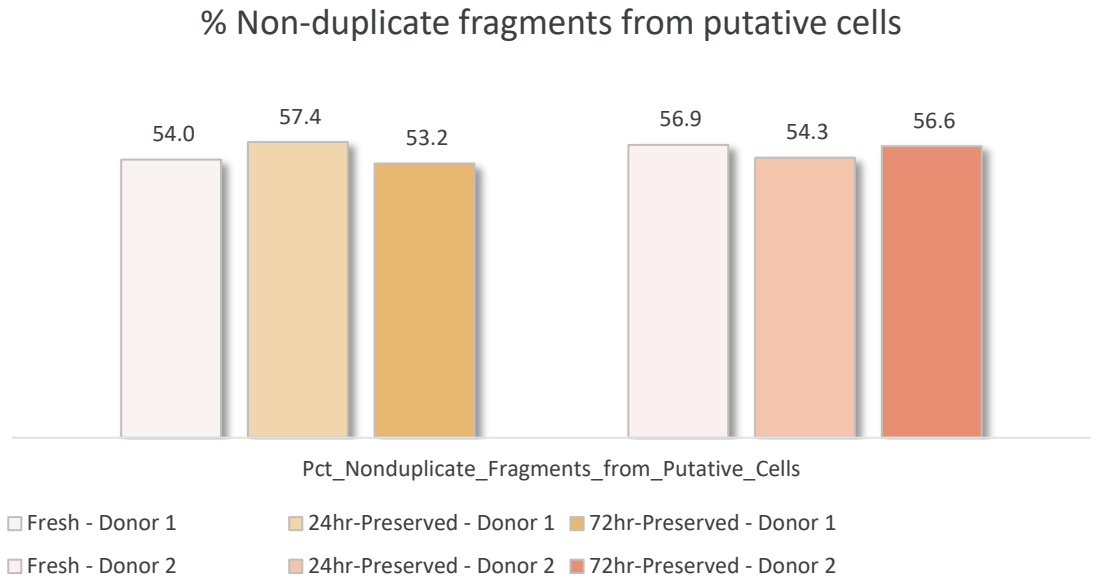
	Fresh sample		24-h preserved sample		72-h preserved sample	
Metrics	**Donor 1**	**Donor 2**	**Donor 1**	**Donor 2**	**Donor 1**	**Donor 2**
Pct Cell Label	99.04	98.89	98.47	98.49	98.61	98.88
Pct Cell Label Aligned Confidently	88.63	88.62	85.86	87.12	86.7	86.82
Pct Mitochondrial	0.19	0.26	0.24	0.27	0.36	0.68
Pct Nonduplicate Fragments with NFR Lengths	68.94	71.06	73.16	72.21	71.1	75.61
Pct Nonduplicate Fragments with Mononucleosomal Lengths	24.8	23.33	21.26	22.2	22.62	19.49
Pct Cellular Fragments Overlapping Peaks	**75.37**	**75.85**	**77.05**	**76.22**	**75.5**	**75.5**
TSS Enrichment Score	**37.73**	**36.38**	**34.09**	**33.79**	**31.62**	**32.09**
Total Peaks	110,924	119,130	110,206	111,954	111,283	116,880
Total Putative Cells	1944	2084	1672	1611	1749	1626
Pct Nonduplicate Fragments from Putative Cells	**54.00**	**56.85**	**57.37**	**54.34**	**53.21**	**56.58**
Mean Reads per Cell	**50,968**	**52,142**	**52,254**	**51,335**	**52,575**	**52,415**
Median Nonduplicate Fragments per Cell	**13,317**	**13,984**	**14,010**	**13,705**	**13,576**	**13,877**
Pct Duplicate Fragments	38.73	39.38	39.27	36.34	37.4	37.24

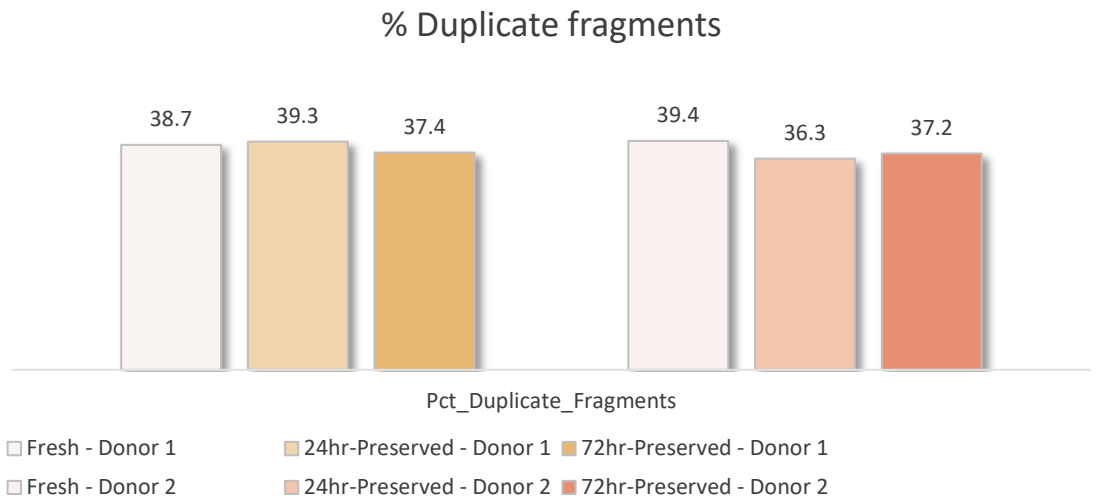
Compatibility with BD® OMICS-Guard Sample Preservation Buffer

Library quality metrics along with *% Non-duplicate fragments from putative cells* and *% Duplicate fragments* from ATAC-Seq libraries in fresh and preserved samples.



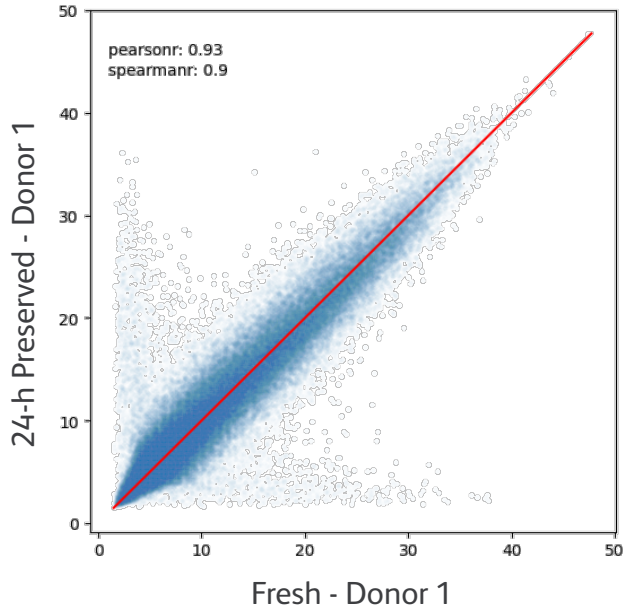
	Pct_CellLabel	Pct_CellLabel_Aligned_Confidently	Pct_Mitochondrial
Fresh - Donor 1	99.04	88.63	0.19
24hr-Preserved - Donor 1	98.47	85.86	0.24
72hr-Preserved - Donor 1	98.61	86.7	0.36
Fresh - Donor 2	98.89	88.62	0.26
24hr-Preserved - Donor 2	98.49	87.12	0.27
72hr-Preserved - Donor 2	98.88	86.82	0.68

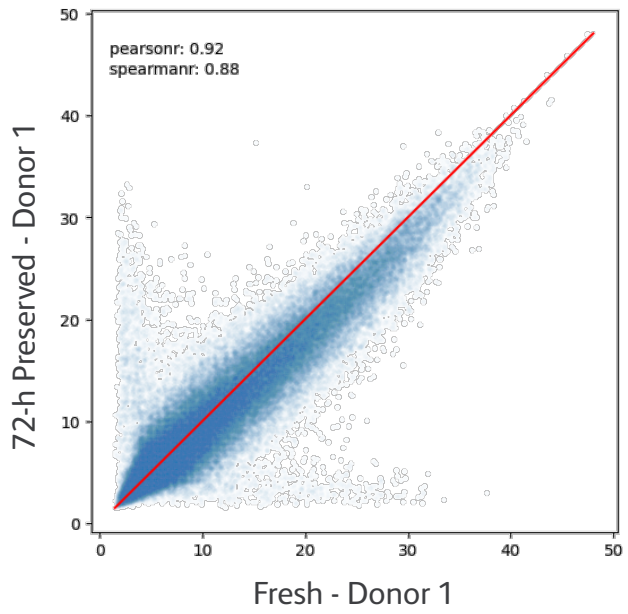


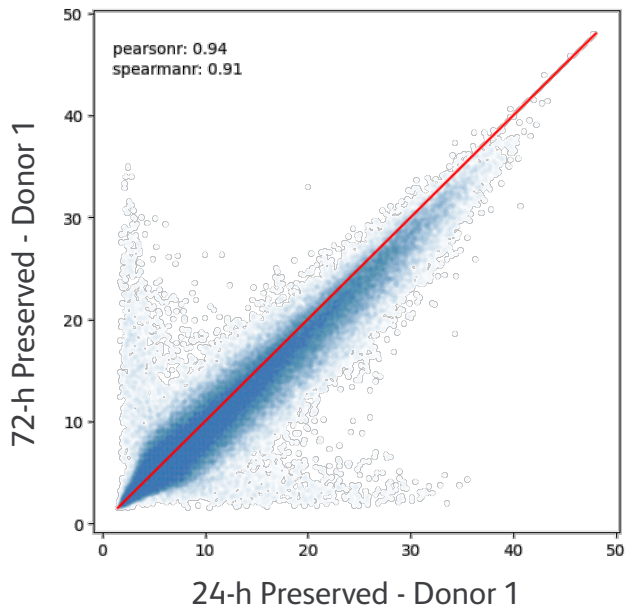


Compatibility with BD® OMICS-Guard Sample Preservation Buffer

ATAC peak correlation plots multiomic ATAC-Seq experiments with preserved samples vs fresh control, showing no difference between the libraries made at different timepoints for Donor 1.







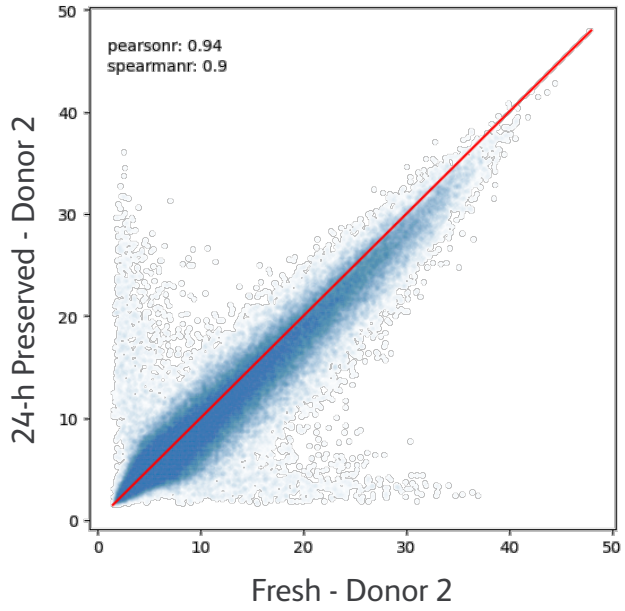
Donor 1	Fresh	%	24 h	%
# Peaks	110,749		110,060	
# Common	98,769	89.2	98,204	89.2
# Unique	11,980	10.8	11,856	10.8

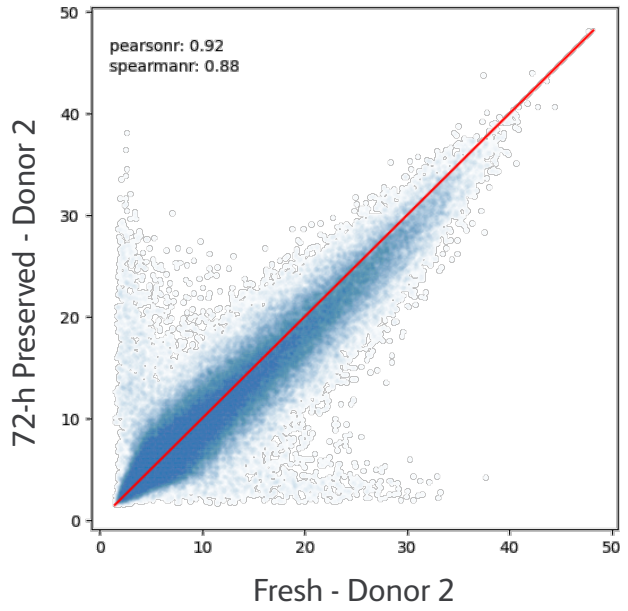
Donor 1	Fresh	%	72 h	%
# Peaks	110,749		111,102	
# Common	98,769	88.3	97,047	87.3
# Unique	11,980	11.6	14,055	12.9

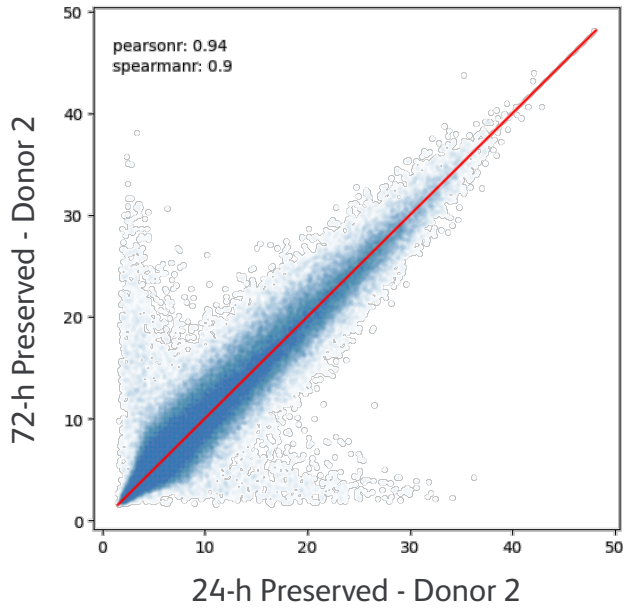
Donor 1	24 h	%	72 h	%
# Peaks	110,060		111,102	
# Common	99,804	90.7	99,446	89.5
# Unique	10,256	9.3	11,656	10.5

Compatibility with BD® OMICS-Guard Sample Preservation Buffer

ATAC peak correlation plots multiomic ATAC-Seq experiments with preserved samples vs fresh control, showing no difference between the libraries made at different timepoints for Donor 2.







Donor 2	Fresh	%	24 h	%
# Peaks	118,952		111,788	
# Common	102,733	86.4	102,795	92.0
# Unique	16,219	13.6	8,993	8.0

Donor 2	Fresh	%	72 h	%
# Peaks	118,952		116,742	
# Common	102,733	87.9	103,936	89.0
# Unique	16,219	12.1	12,806	11.0

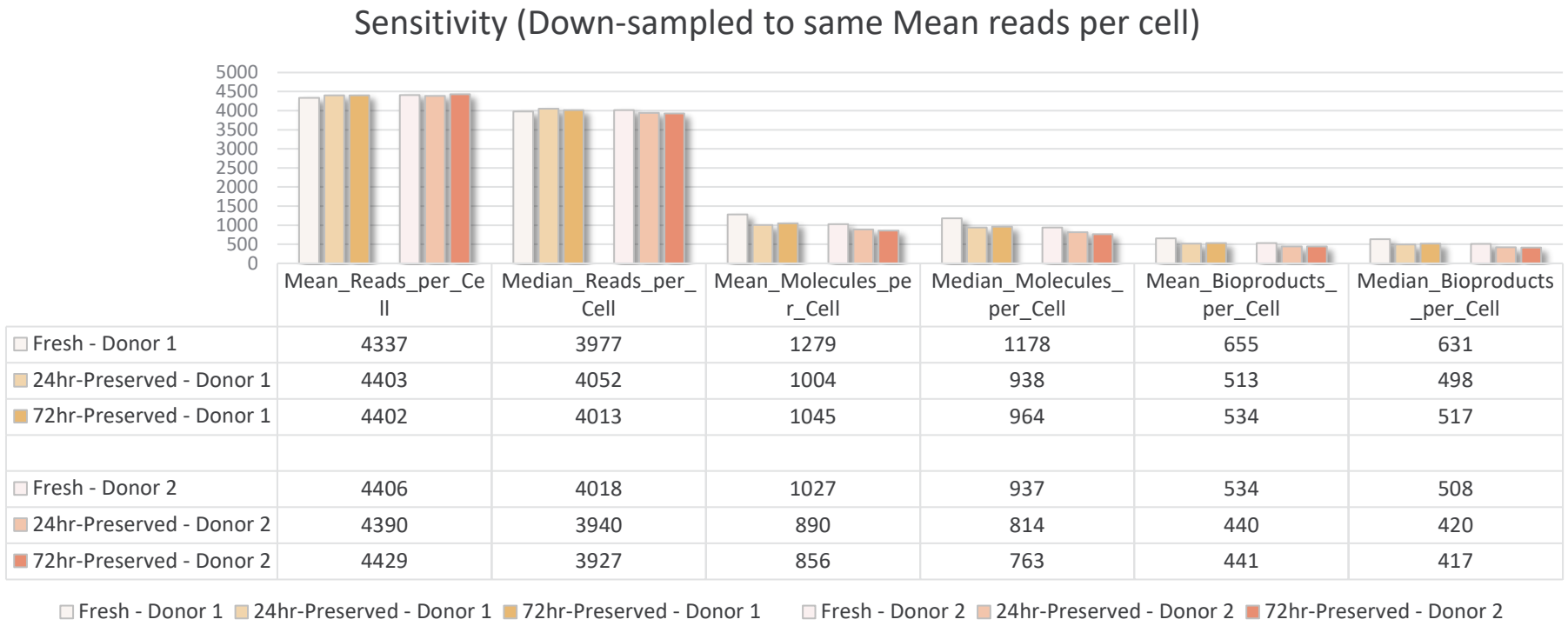
Donor 2	24 h	%	72 h	%
# Peaks	111,788		116,742	
# Common	102,992	92.1	102,219	87.6
# Unique	8,796	7.9	14,523	12.4

Compatibility with BD® OMICS-Guard Sample Preservation Buffer

Sensitivity metrics for the WTA libraries from the same experiments (using a BD Rhapsody™ 8-Lane Cartridge) showing minimal impact on the per cell values for stained samples vs control.

BD Rhapsody™ ATAC-Seq Assay configurations:

1. ATAC + WTA (Fresh control samples from 2 donors)
2. ATAC + WTA (Samples from 2 donors preserved for 24 hours)
3. ATAC + WTA (Samples from 2 donors preserved for 72 hours)



	Mean_Reads_per_Cell	Median_Reads_per_Cell	Mean_Molecules_per_Cell	Median_Molecules_per_Cell	Mean_Bioproducts_per_Cell	Median_Bioproducts_per_Cell
Fresh - Donor 1	4337	3977	1279	1178	655	631
24hr-Preserved - Donor 1	4403	4052	1004	938	513	498
72hr-Preserved - Donor 1	4402	4013	1045	964	534	517
Fresh - Donor 2	4406	4018	1027	937	534	508
24hr-Preserved - Donor 2	4390	3940	890	814	440	420
72hr-Preserved - Donor 2	4429	3927	856	763	441	417

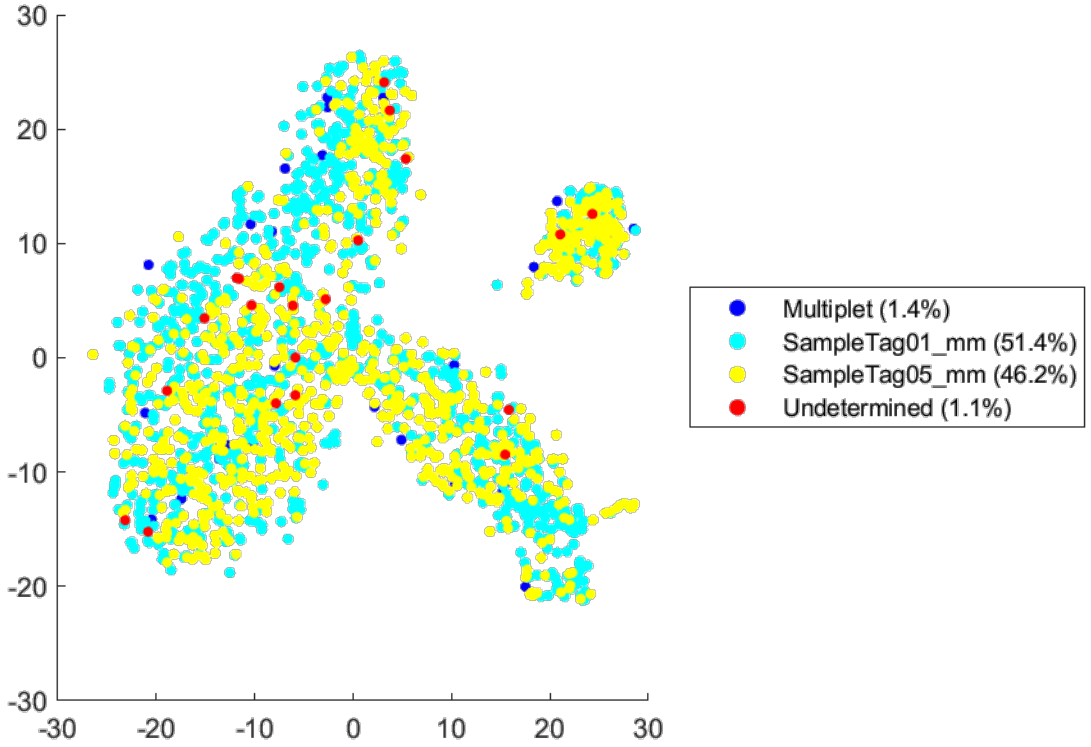
Appendix (Part III)

- Sample multiplexing experiments with BD Rhapsody™ ATAC-Seq Assays
- Compatibility with BD® OMICS-Guard Sample Preservation Buffer
- Sample multiplexing with BD® OMICS-Guard Buffer-preserved samples

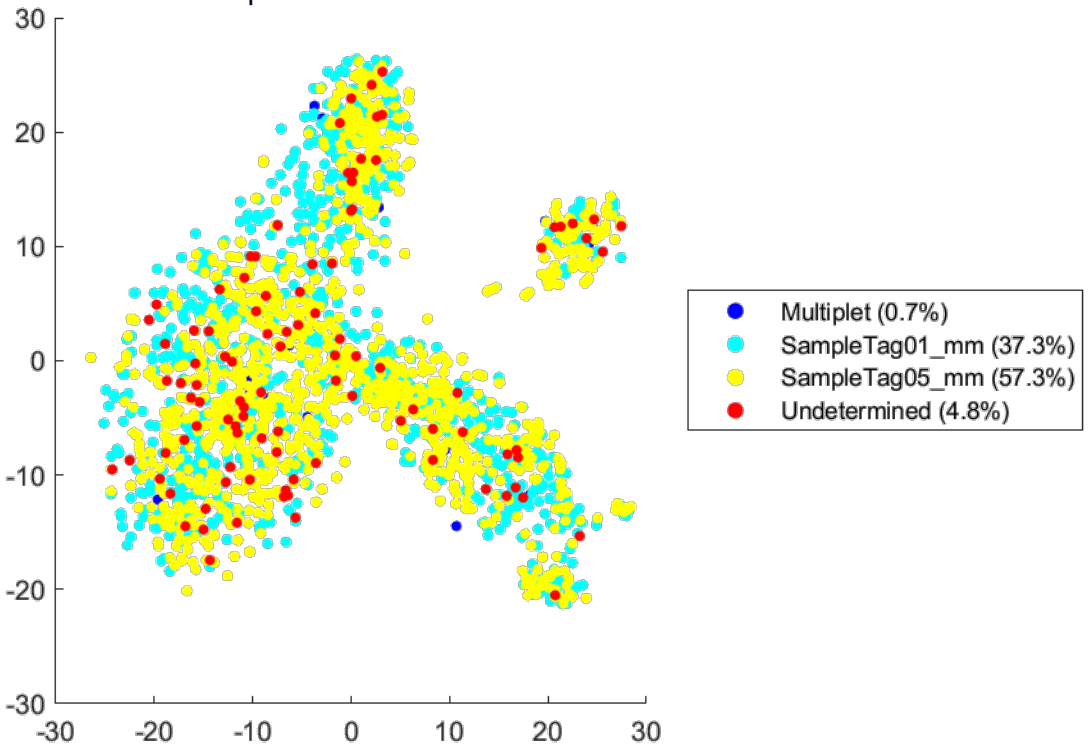
Sample multiplexing with BD® OMICS-Guard Buffer-preserved samples

High sensitivity (% of undetermined tags) for SMK libraries in multiomic ATAC-Seq assays performed using fresh PBMCs and PMBCs that were preserved for 24 h in BD® OMICS-Guard Sample Preservation Buffer.

ATAC + WTA + SMK
Fresh PMBCs



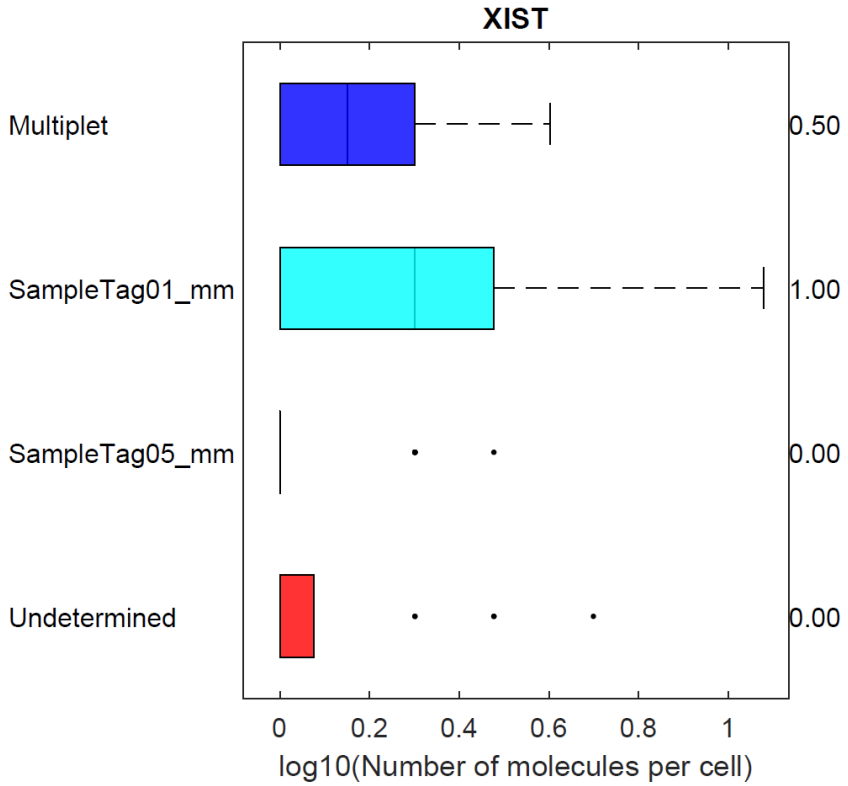
ATAC + WTA + SMK
PMBCs preserved for 24 h in BD® OMICS-Guard Buffer



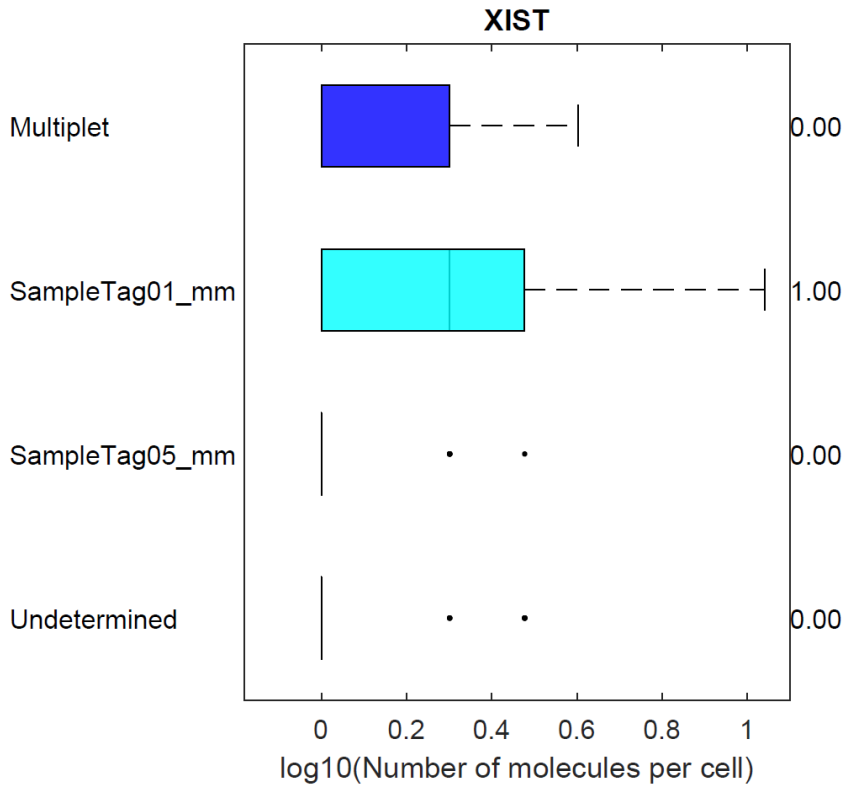
Sample multiplexing with BD® OMICS-Guard Buffer-preserved samples

Box plots showing gender-specific detection of XIST gene only in the sample collected from Donor 1 (female) and stained using Sample Tag 1, indicating an analysis free of any cross contamination using ATAC-Seq assay and BD® Nuclear Sample Multiplexing Ab-Oligos Conjugates even after preservation for 24 hours in BD® OMICS-Guard Sample Preservation Buffer. Donor 2 (male) sample was stained using Sample Tag 5.

ATAC + WTA + SMK
Fresh PMBCs



ATAC + WTA + SMK
PMBCs preserved for 24 h in BD® OMICS-Guard Buffer



Thank you