BD Rhapsody™ TCR/BCR Next Multiomic Assays

Overview of performance data

BD

Chapter I

- **BD Rhapsody™ TCR/BCR Next Multiomic Assays performance:**
- Reproducibility and repeatability data
- Compatibility with BD® OMICS-Guard Sample Preservation Buffer

Performance across different assay configurations:

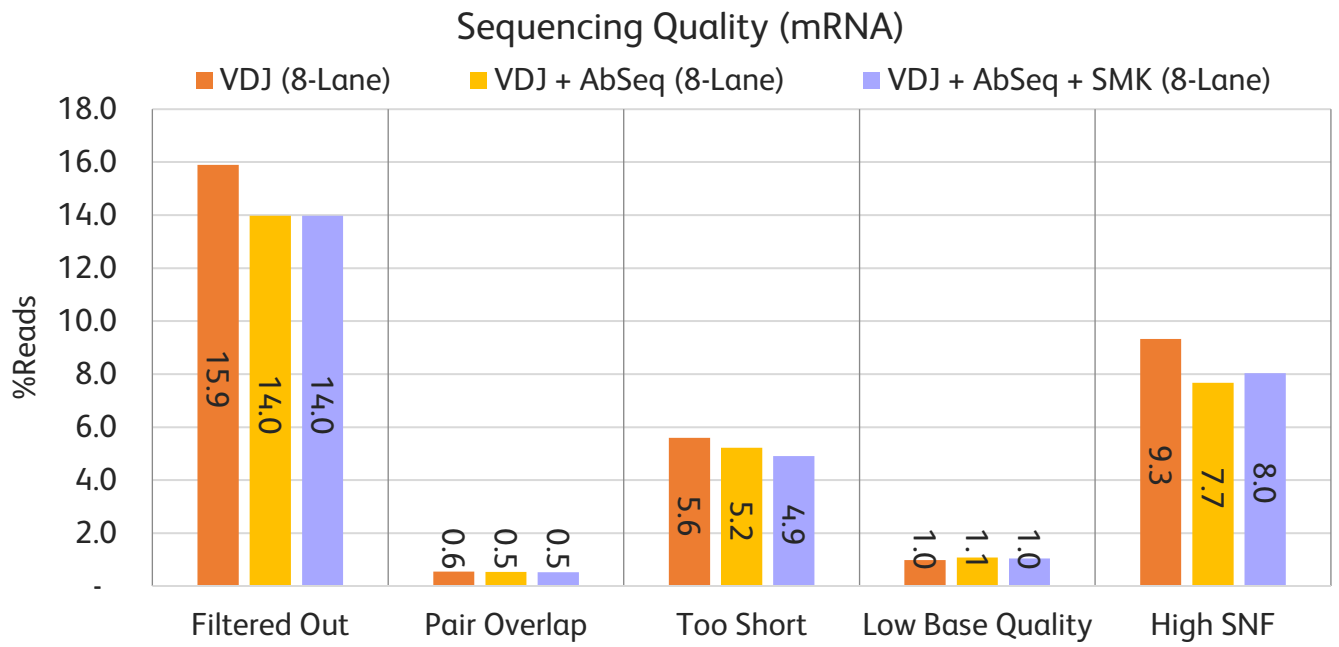
1. WTA + VDJ workflow
2. WTA + VDJ + AbSeq workflow
3. WTA + VDJ + AbSeq + SMK workflow

Performance metrics—sequencing quality

BD Rhapsody™ Whole Transcriptome Analysis (WTA) Assay mRNA sequencing quality in three different assays (using a BD Rhapsody™ 8-Lane Cartridge) showing consistent performance across different assay combinations.

BD Rhapsody™ TCR/BCR Next Assay configurations:

1. WTA + VDJ (PBMCs)
2. WTA + VDJ + AbSeq (PBMCs)
3. WTA + VDJ + AbSeq + SMK (Enriched T + B cells)

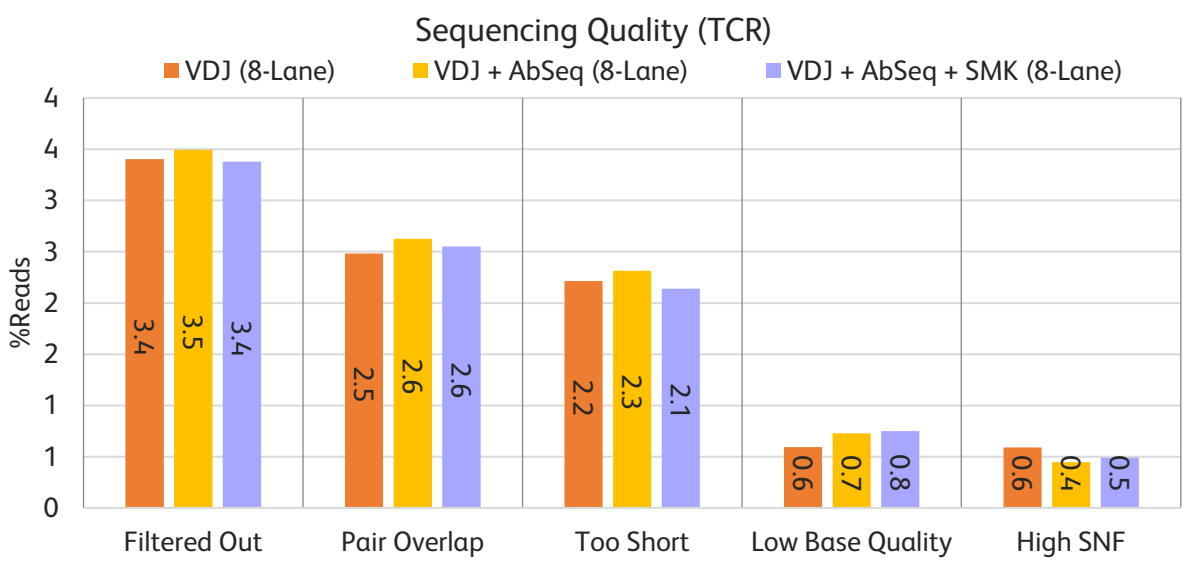


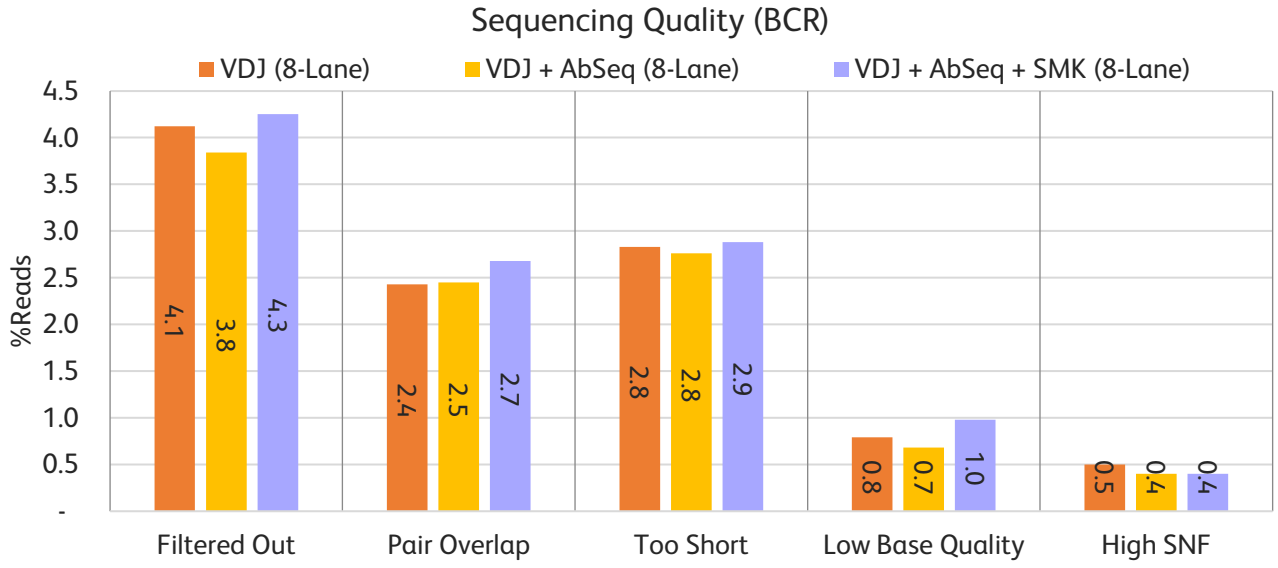
Performance metrics—sequencing quality

TCR and BCR sequencing quality in three different assays (using a BD Rhapsody™ 8-Lane Cartridge) showing consistent performance across different assay combinations.

BD Rhapsody™ TCR/BCR Next Assay configurations:

1. WTA + VDJ (PBMCs)
2. WTA + VDJ + AbSeq (PBMCs)
3. WTA + VDJ + AbSeq + SMK (Enriched T + B cells)



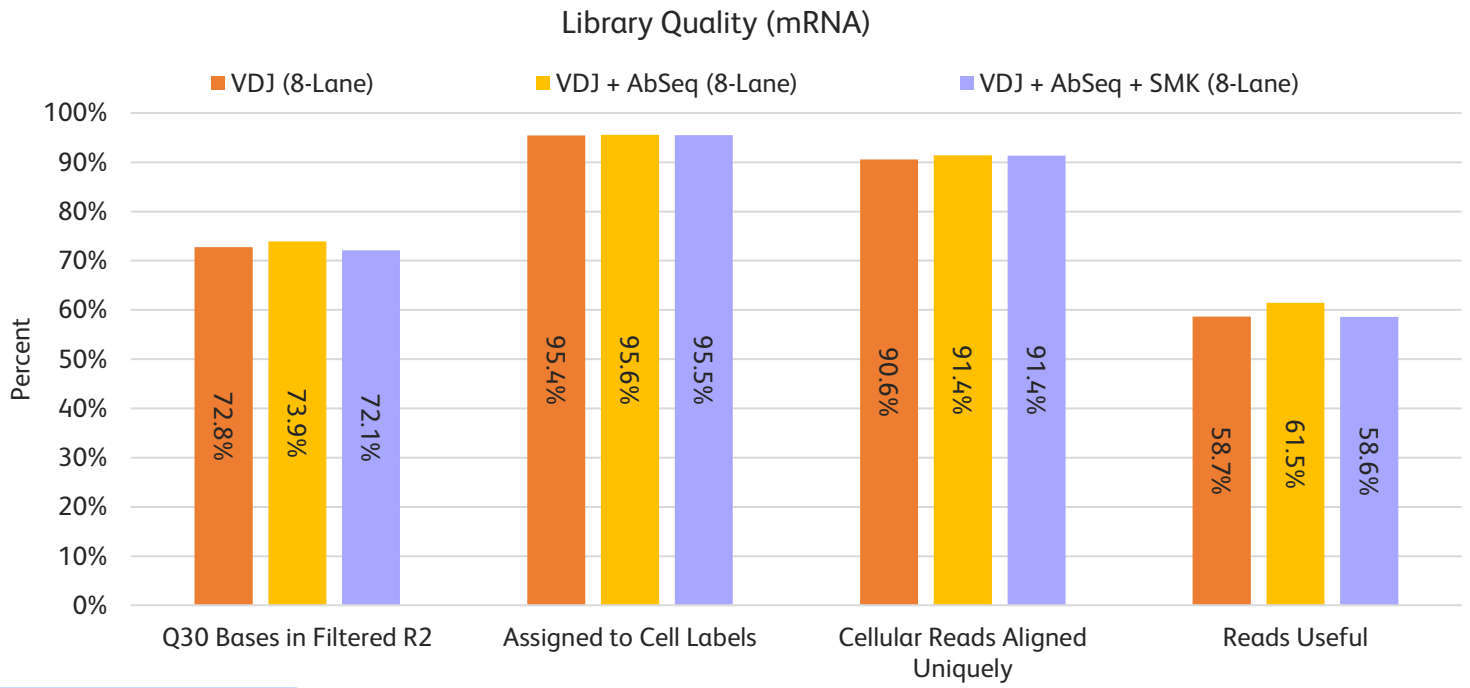


Performance metrics—library mapping

WTA mRNA library quality in three different assays (using a BD Rhapsody™ 8-Lane Cartridge) showing similar mapping across different assay combinations.

BD Rhapsody™ TCR/BCR Next Assay configurations:

1. WTA + VDJ (PBMCs)
2. WTA + VDJ + AbSeq (PBMCs)
3. WTA + VDJ + AbSeq + SMK (Enriched T + B cells)

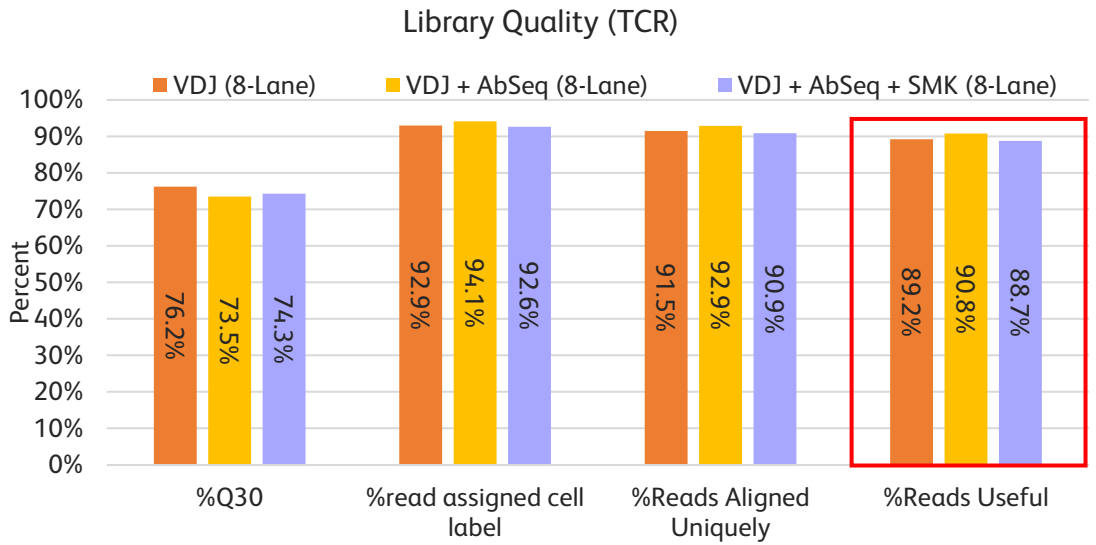


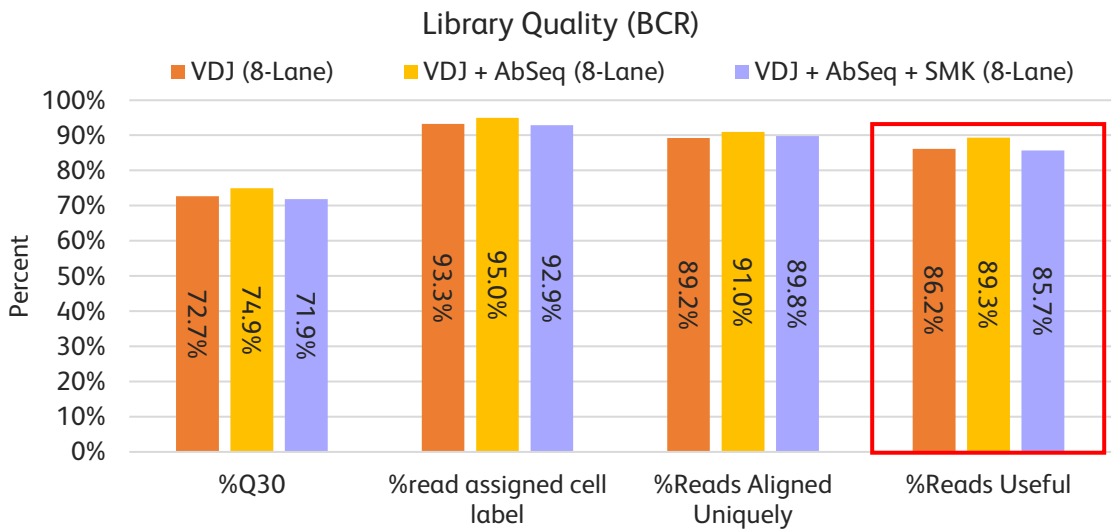
Performance metrics—library mapping

TCR and BCR library quality in three different assays (using a BD Rhapsody™ 8-Lane Cartridge) showing similarly high mapping (>85%) and highly specific VDJ analyses across different assay combinations.

BD Rhapsody™ TCR/BCR Next Assay configurations:

1. WTA + VDJ (PBMCs)
2. WTA + VDJ + AbSeq (PBMCs)
3. WTA + VDJ + AbSeq + SMK (Enriched T + B cells)





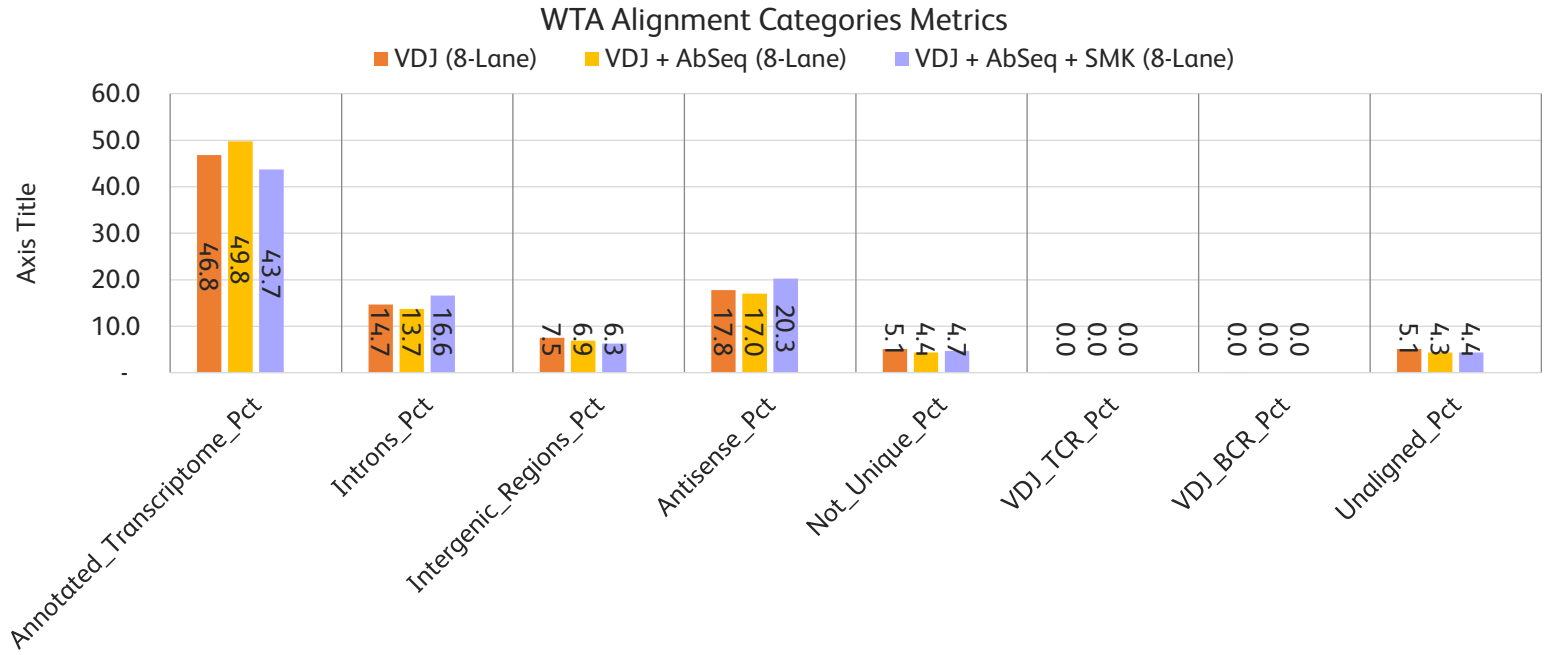
Mapping represented by "% Reads Useful" is a metric reported by the BD Rhapsody™ Sequence Analysis Pipeline as the percentage of read pairs containing a valid cell label and UMI (Read1) that aligned (Read2) uniquely to a valid bioproduct (mRNA exon/intron or other bioproduct sequence).

Performance Metrics—alignment categories

WTA alignment in three different assays (experiments using a BD Rhapsody™ 8-Lane Cartridge) showing similar alignment across different assay combinations.

BD Rhapsody™ TCR/BCR Next Assay configurations:

1. WTA + VDJ (PBMCs)
2. WTA + VDJ + AbSeq (PBMCs)
3. WTA + VDJ + AbSeq + SMK (Enriched T + B cells)

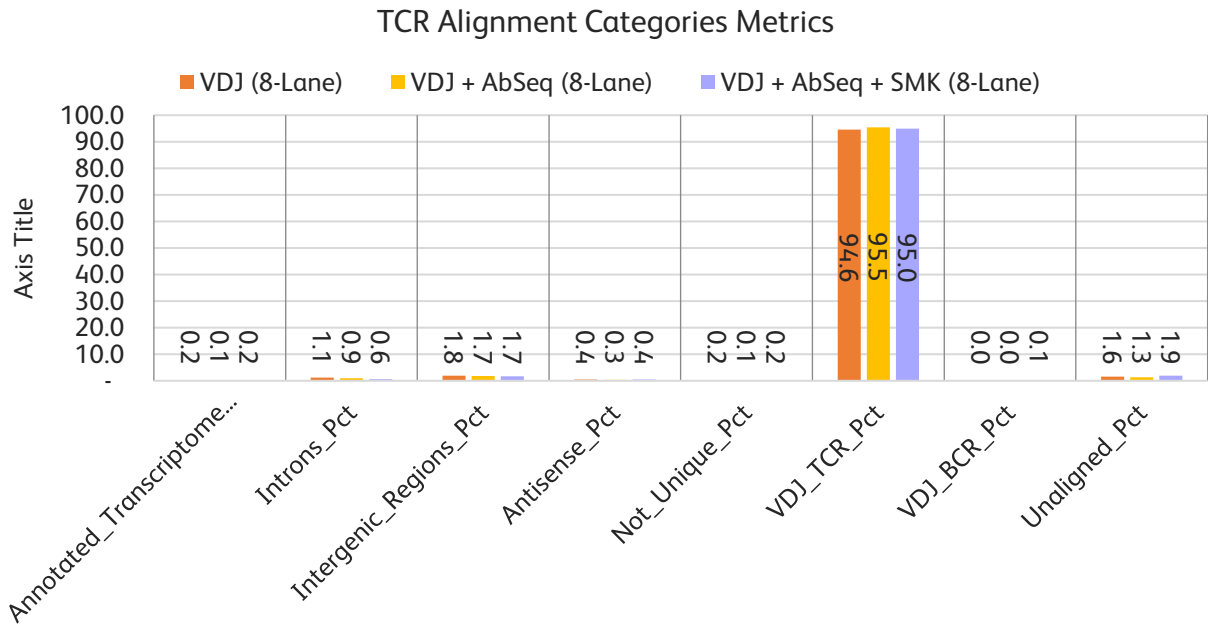


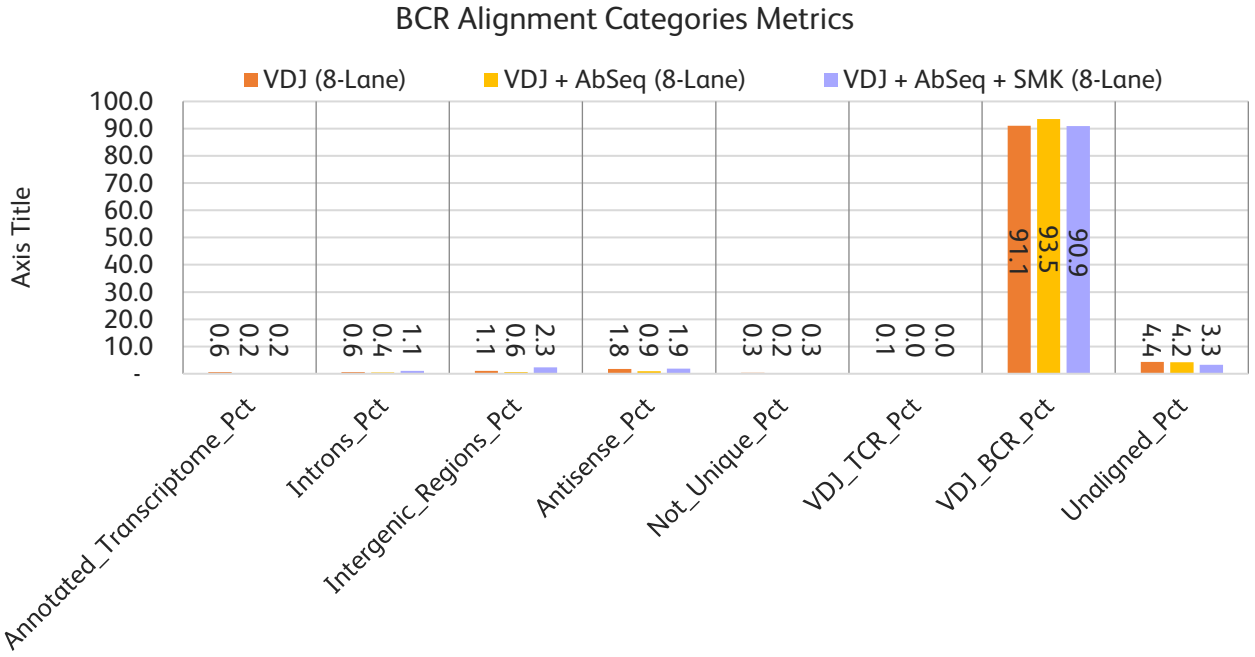
Performance metrics—alignment categories

TCR and BCR alignment in three different assays (using a BD Rhapsody™ 8-Lane Cartridge) showing similar alignment across different assay combinations.

BD Rhapsody™ TCR/BCR Next Assay configurations:

1. WTA + VDJ (PBMCs)
2. WTA + VDJ + AbSeq (PBMCs)
3. WTA + VDJ + AbSeq + SMK (Enriched T + B cells)



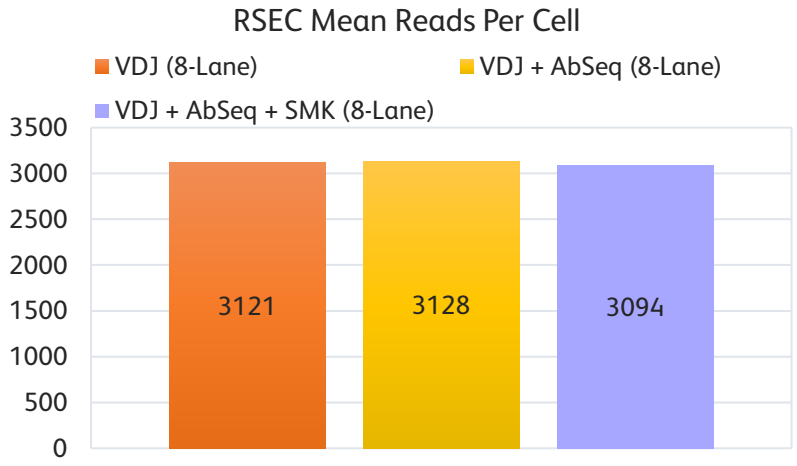


Performance metrics—cell metrics

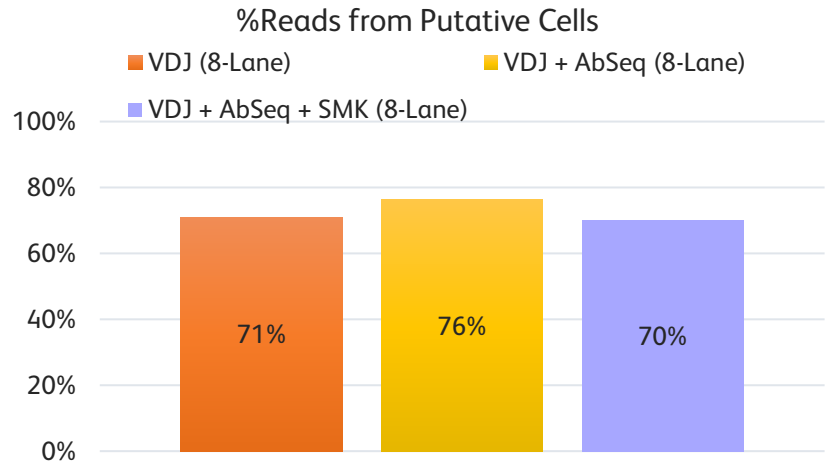
WTA mRNA cell metrics in three different assays (using a BD Rhapsody™ 8-Lane Cartridge) showing similar cell metrics across different assay combinations.

BD Rhapsody™ TCR/BCR Next Assay configurations:

1. WTA + VDJ (PBMCs)
2. WTA + VDJ + AbSeq (PBMCs)
3. WTA + VDJ + AbSeq + SMK (Enriched T + B cells)



WTA RSEC reads were normalized to the same read-depth.



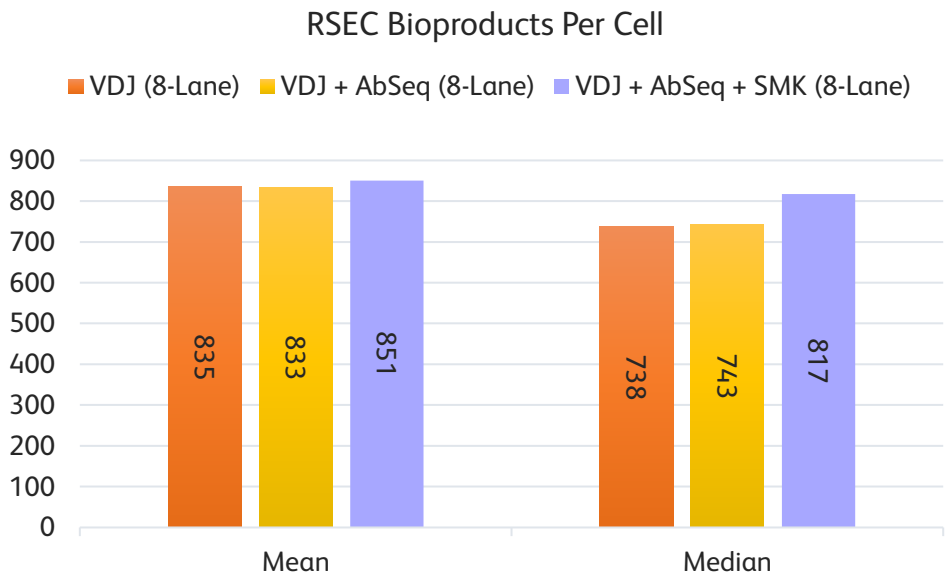
WTA cell metrics represented by % reads from putative cell.
Sequencing data were normalized to the same read-depth.

Performance metrics—WTA sensitivity

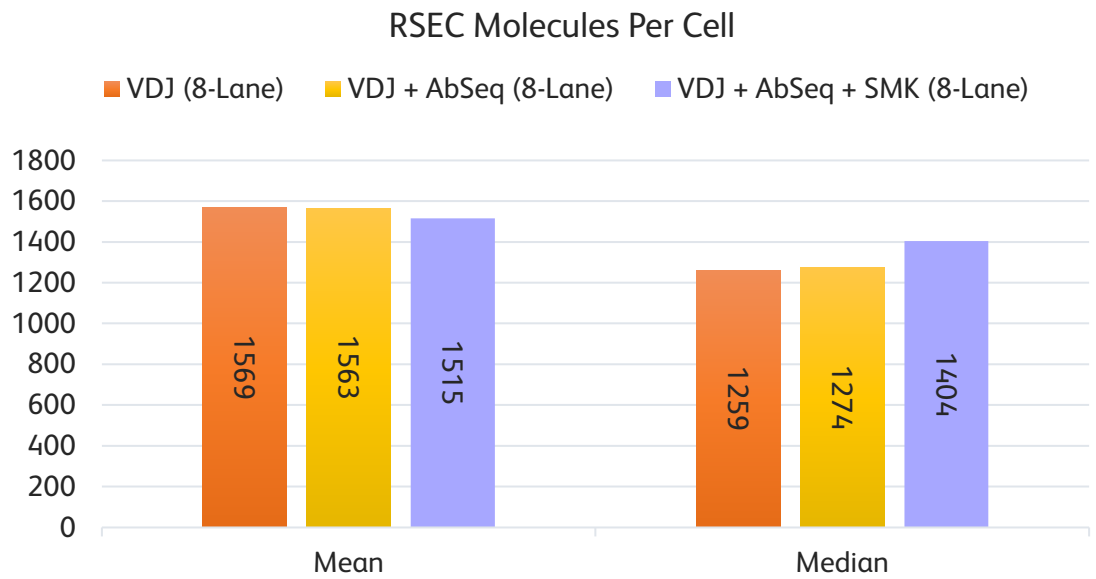
WTA sensitivity represented by median bioproduct and molecules per cell in three different assays (using a BD Rhapsody™ 8-Lane Cartridge) showing similar sensitivity across different assay combinations.

BD Rhapsody™ TCR/BCR Next Assay configurations:

1. WTA + VDJ (PBMCs)
2. WTA + VDJ + AbSeq (PBMCs)
3. WTA + VDJ + AbSeq + SMK (Enriched T + B cells)



WTA sensitivity represented by median RSEC bioproducts. Sequencing data were normalized to the same read-depth.



WTA sensitivity represented by median RSEC molecules. Sequencing data were normalized to the same read-depth.

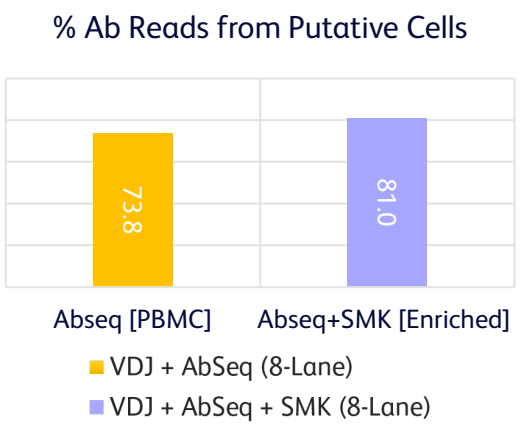
Performance metrics—AbSeq sensitivity

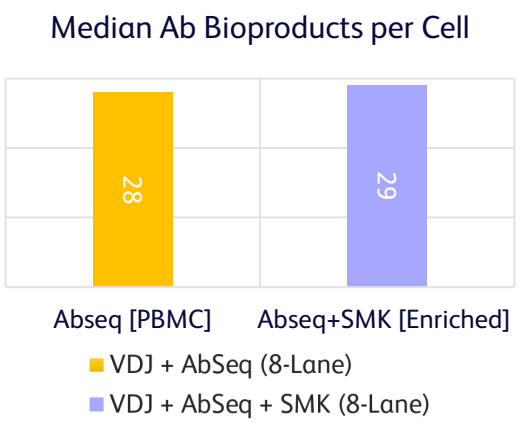
AbSeq sensitivity in two different assays (using a BD Rhapsody™ 8-Lane Cartridge) showing similar sensitivity across different assay combinations.

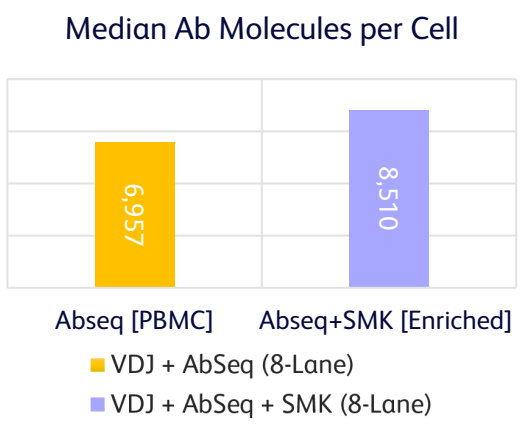
BD Rhapsody™ TCR/BCR Next Assay configurations:

1. WTA + VDJ + AbSeq (PBMCs)
2. WTA + VDJ + AbSeq + SMK (Enriched T + B cells)

AbSeq sensitivity, represented by % AbSeq Reads from Putative Cells, Median AbSeq Bioproducts per Cell, and Median AbSeq Molecules per Cell, is shown below with experiments done using PBMCs and enriched T and B cells.





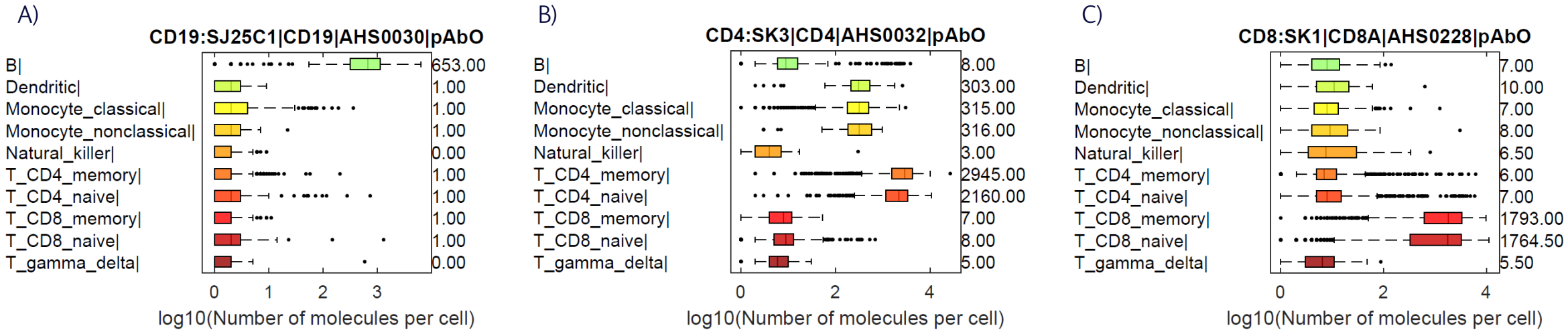


Performance metrics—AbSeq specificity

AbSeq specificity in one assay (using a BD Rhapsody™ 8-Lane Cartridge) showing high expression levels for cell-type specific AbSeq markers in the multiomic assay.

BD Rhapsody™ TCR/BCR Next Assay configuration:

WTA + VDJ + AbSeq (PBMCs)



Box plots showing high levels of molecule expression for (**A**) CD19 (B-cell marker), (**B**) CD4 (T-cell marker) and (**C**) CD8 (T-cell marker) in a BD Rhapsody™ TCR/BCR Next Multiomic Assay experiment with PBMCs, indicating high specificity for these markers in their expected respective cell types.

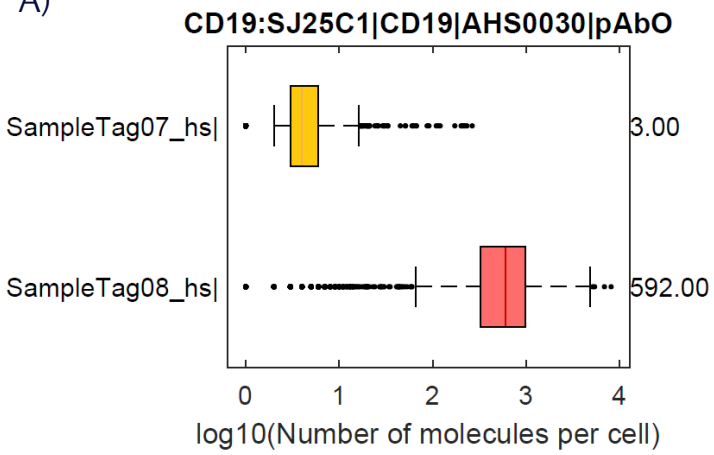
Performance metrics—AbSeq specificity

AbSeq specificity in one assay (using a BD Rhapsody™ 8-Lane Cartridge) showing high expression levels for cell-type specific AbSeq markers in the multiomic assay.

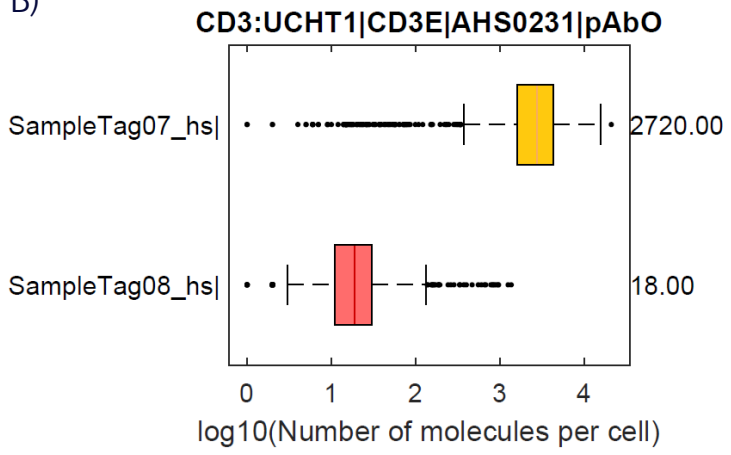
BD Rhapsody™ TCR/BCR Next Assay configuration:

WTA + VDJ + AbSeq + SMK (Enriched T + B cells).
T cell stained were with Sample Tag 7 and B cell with Sample Tag 8.

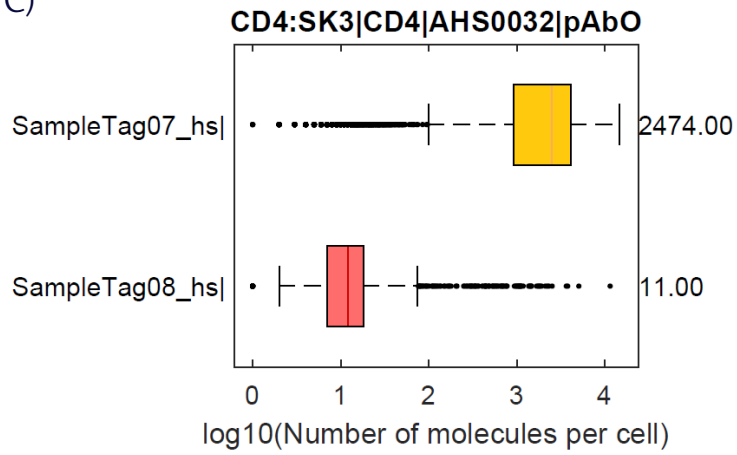
A)



B)



C)



Box plots showing high levels of molecule expression for (**A**) CD19 (B-cell marker), (**B**) CD3 (T-cell marker) and (**C**) CD4 (T-cell marker) in a BD Rhapsody™ TCR/BCR Next Multiomic Assay experiment with enriched T and B cells, indicating high specificity for these markers in their expected respective cell types.

Performance metrics—VDJ sensitivity

VDJ sensitivity represented by TCR/BCR pairing efficiency in three different assays (using a BD Rhapsody™ 8-Lane Cartridge) showing consistently high levels of TCR and BCR pairing across different assay combinations.

BD Rhapsody™ TCR/BCR Next Assay configurations:

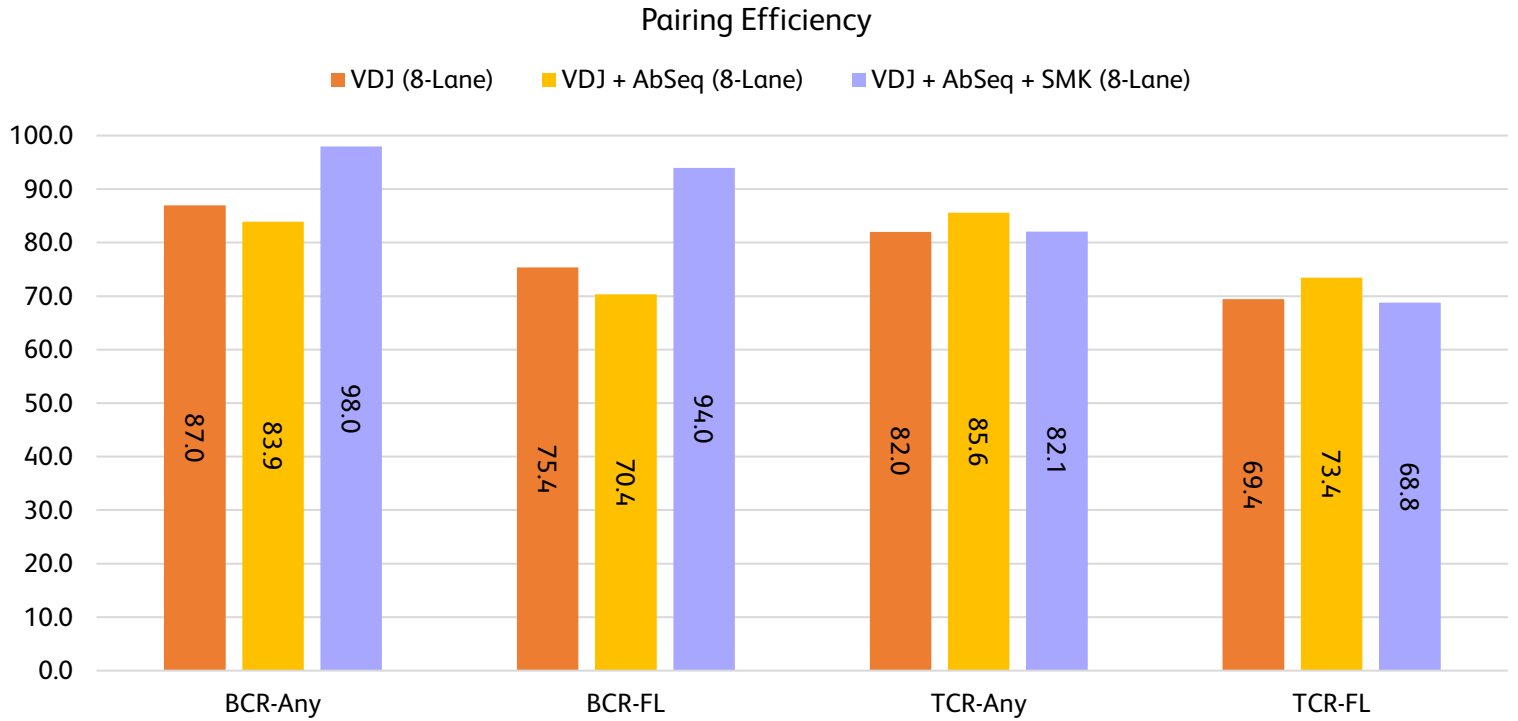
1. WTA + VDJ (PBMCs)
2. WTA + VDJ + AbSeq (PBMCs)
3. WTA + VDJ + AbSeq + SMK (Enriched T + B cells)

BCR-Any: Percentage of cells of each type that had both a BCR heavy chain and BCR light chain (Kappa or Lambda).

BCR-FL: Percentage of cells of each type that had both full-length BCR heavy chain and BCR light chain (Kappa or Lambda).

TCR-Any: Percentage of cells of each type that had either TCR Alpha and TCR Beta, or TCR Gamma and TCR Delta

TCR-FL: Percentage of cells of each type that had either full-length TCR Alpha and TCR Beta, or TCR Gamma and TCR Delta

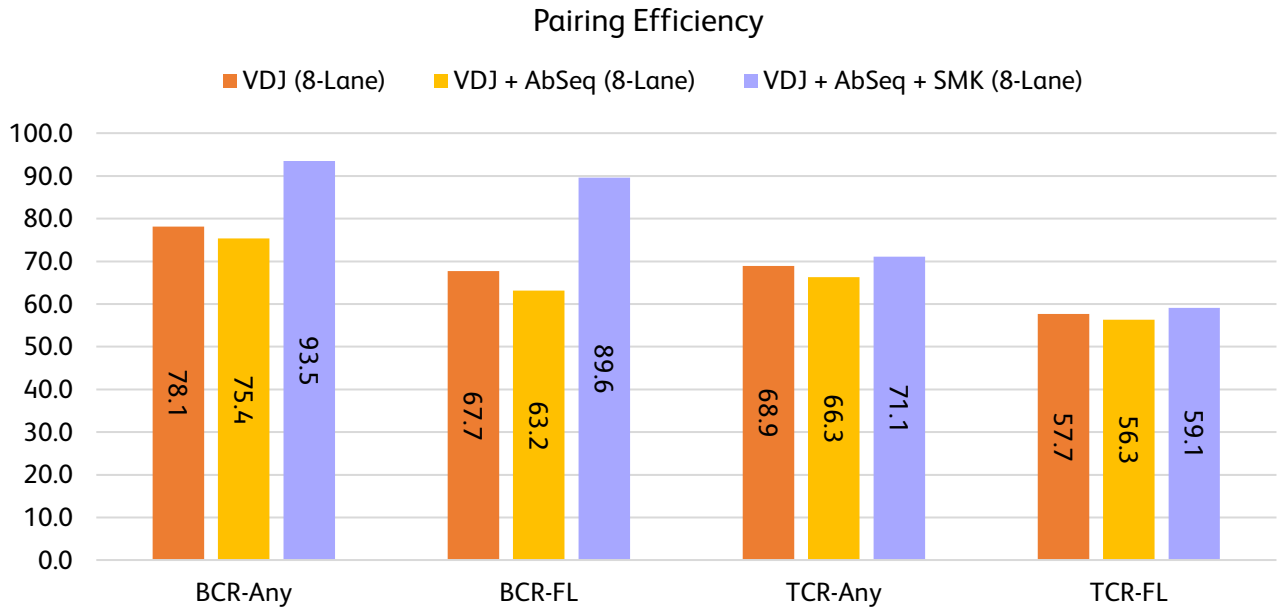


Performance metrics—VDJ sensitivity (not filtered)

VDJ sensitivity represented by TCR/BCR pairing efficiency before applying the high-quality filtering on the BD Rhapsody™ Sequence Analysis Pipeline in three different assays (using a BD Rhapsody™ 8-Lane Cartridge) showing consistently high levels of TCR and BCR pairing across different assay combinations.

BD Rhapsody™ TCR/BCR Next Assay configurations:

1. WTA + VDJ (PBMCs)
2. WTA + VDJ + AbSeq (PBMCs)
3. WTA + VDJ + AbSeq + SMK (Enriched T + B cells)

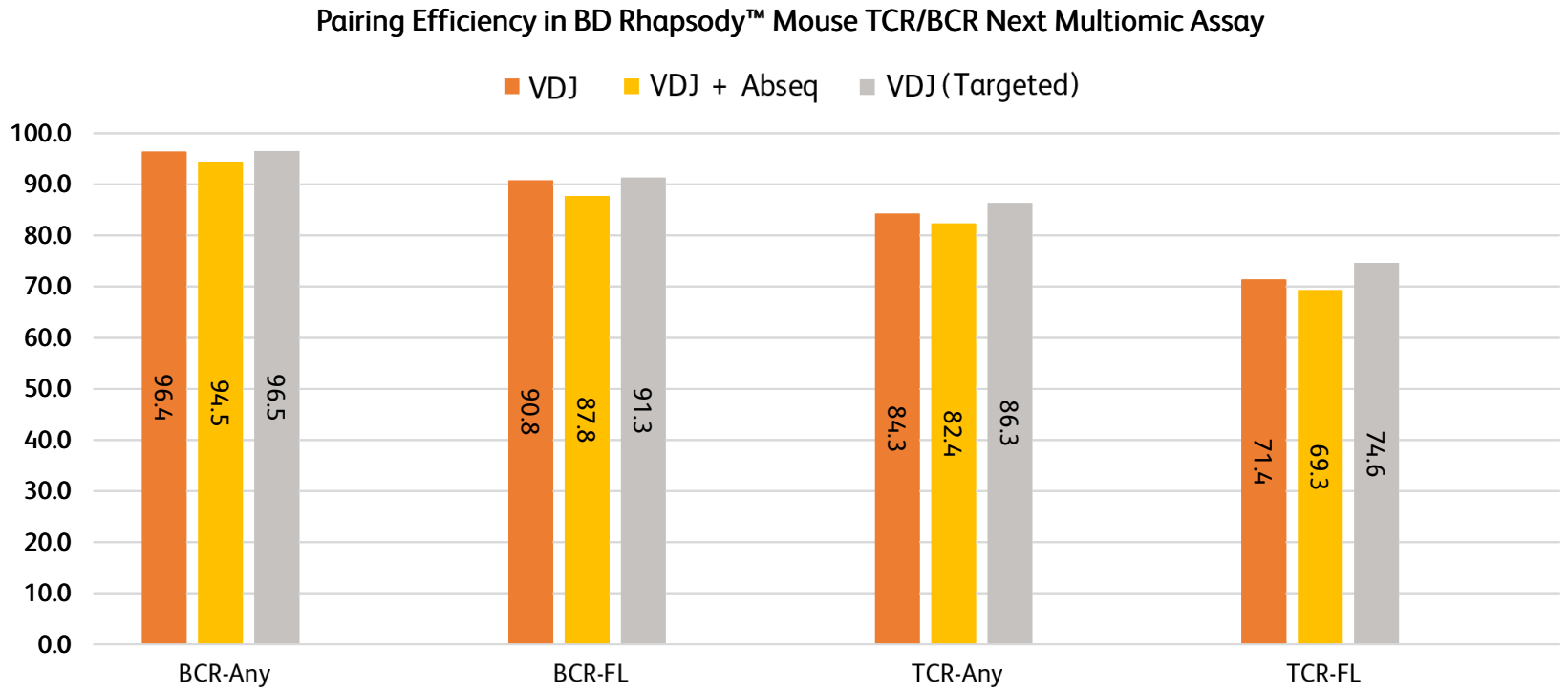


Performance metrics—VDJ sensitivity (mouse assay)

VDJ sensitivity in the BD Rhapsody™ Mouse TCR/BCR Next Multiomic Assay represented by TCR/BCR pairing efficiency in three different assays (using a BD Rhapsody™ 8-Lane Cartridge) showing consistently high levels of TCR and BCR pairing across different assay combinations.

BD Rhapsody™ Mouse TCR/BCR Next Assay configurations:

1. WTA + VDJ (mouse splenocytes)
2. WTA + VDJ + AbSeq (mouse splenocytes)
3. Targeted mRNA + VDJ (mouse splenocytes)

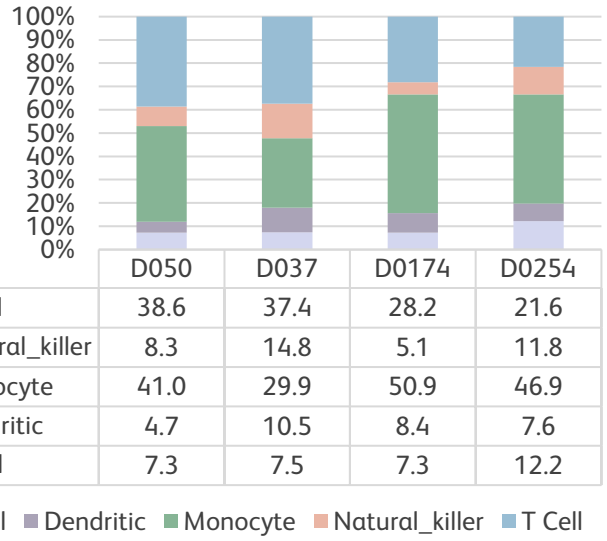


Performance metrics—different donors

VDJ sensitivity, represented by TCR/BCR pairing efficiency, in a BD Rhapsody™ TCR/BCR Next Multiomic Assay experiment (using a BD Rhapsody™ 8-Lane Cartridge) showing consistently high levels of TCR and BCR pairing across different PBMC donors.

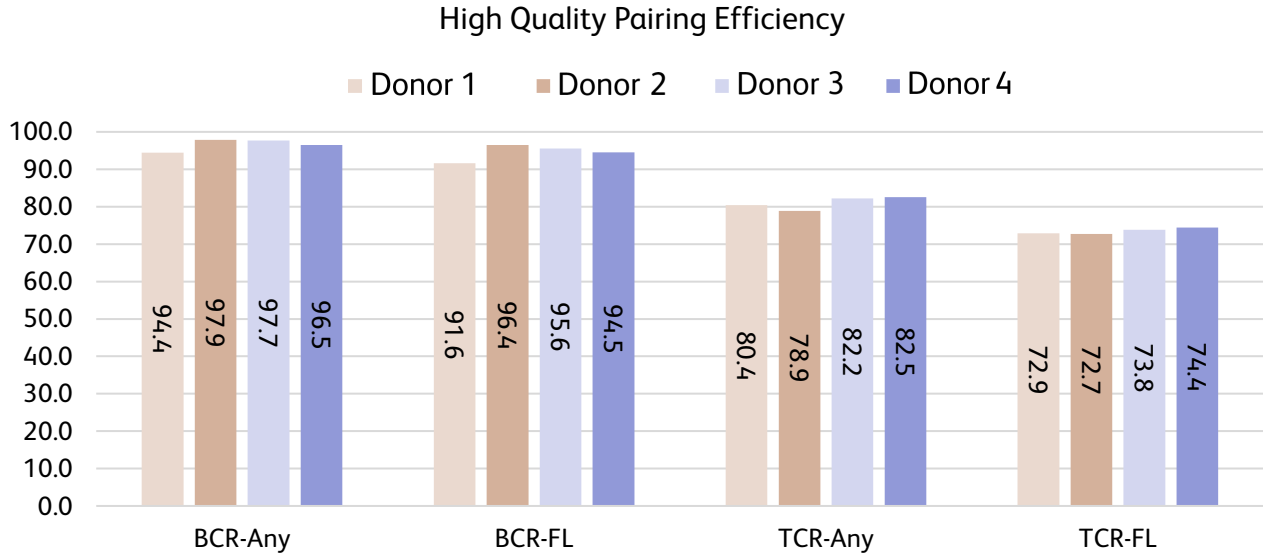
BD Rhapsody™ TCR/BCR Next Assay configurations:

1. WTA + VDJ (PBMCs from four donors)



	D050	D037	D0174	D0254
T Cell	38.6	37.4	28.2	21.6
Natural_killer	8.3	14.8	5.1	11.8
Monocyte	41.0	29.9	50.9	46.9
Dendritic	4.7	10.5	8.4	7.6
B Cell	7.3	7.5	7.3	12.2

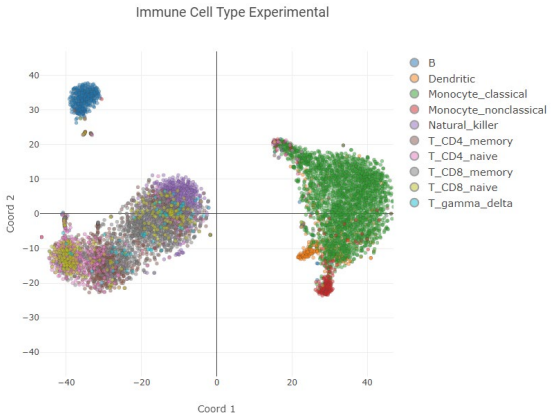
Cell type distributions of four different primary PBMC donors (frozen PBMCs) with WTA + VDJ assay. The four donors show distinct sub-population differences.



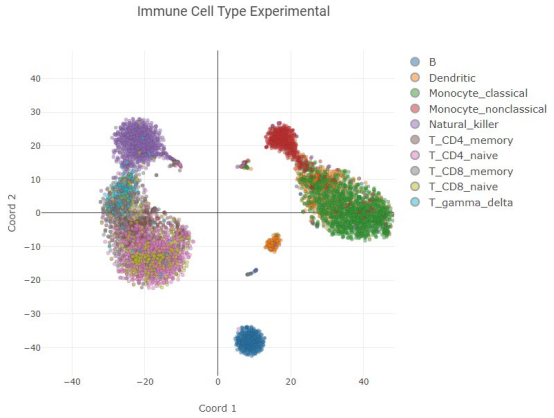
Pairing efficiency of BD Rhapsody™ TCR/BCR Next Assay with four different primary PBMC donors (frozen PBMCs) in a WTA + VDJ experiment. The four donors show similarly high pairing efficiency metrics.

Performance metrics—different donors

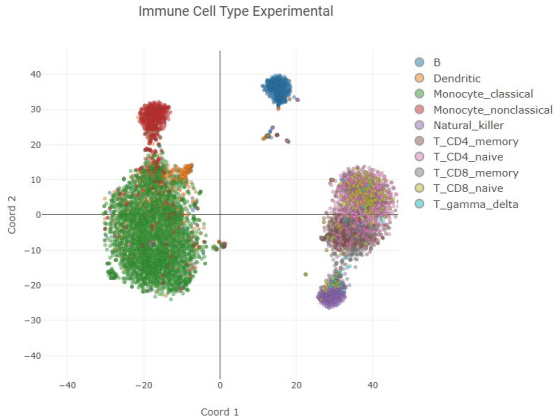
Cell type clustering in WTA + VDJ experiments with four donors. Clear cell type identification for all donors, with no extra clusters observed.



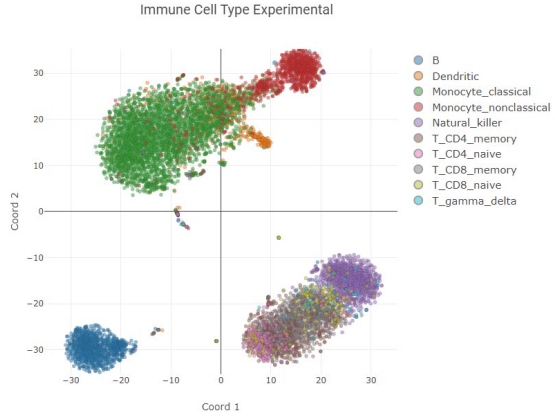
Donor 1



Donor 2



Donor 3



Donor 4

Chapter II

- BD Rhapsody™ TCR/BCR Next Multiomic Assays performance
- **Reproducibility and repeatability data:**
- Compatibility with BD® OMICS-Guard Sample Preservation Buffer

Reproducibility and repeatability with WTA + VDJ + AbSeq experiments across:

1. Operator
2. Repeat
3. Day

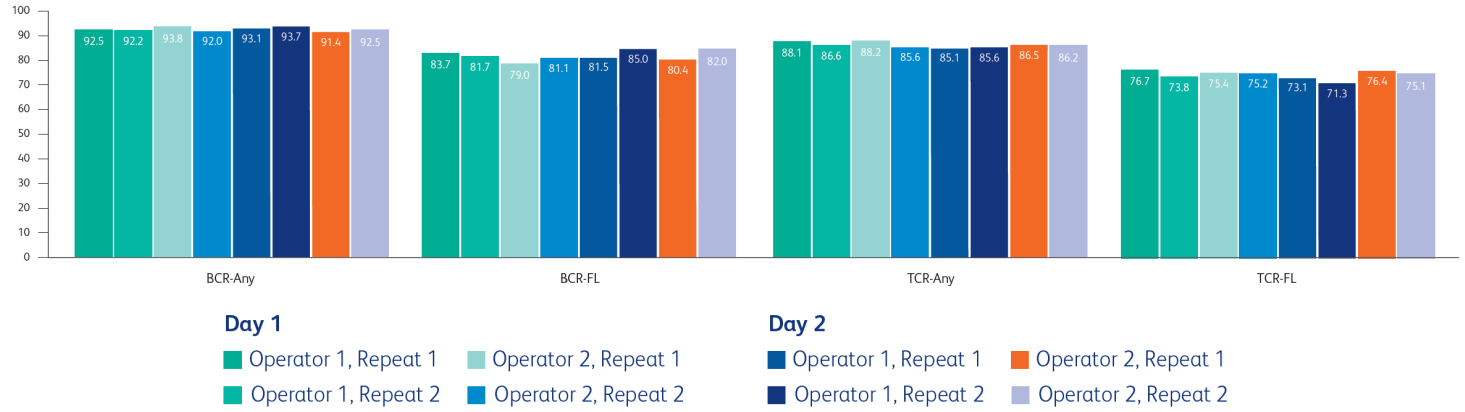
BD Rhapsody™ TCR/BCR Next Multiomic Assay on BD Rhapsody™ 8-Lane and Single-Lane Cartridges.

Performance metrics—reproducibility and repeatability

VDJ sensitivity, represented by TCR/BCR pairing efficiency, in BD Rhapsody™ TCR/BCR Next Multiomic Assay experiments (using a BD Rhapsody™ 8-Lane Cartridge) showing consistently high levels of TCR and BCR pairing across different operators, repeats and days.

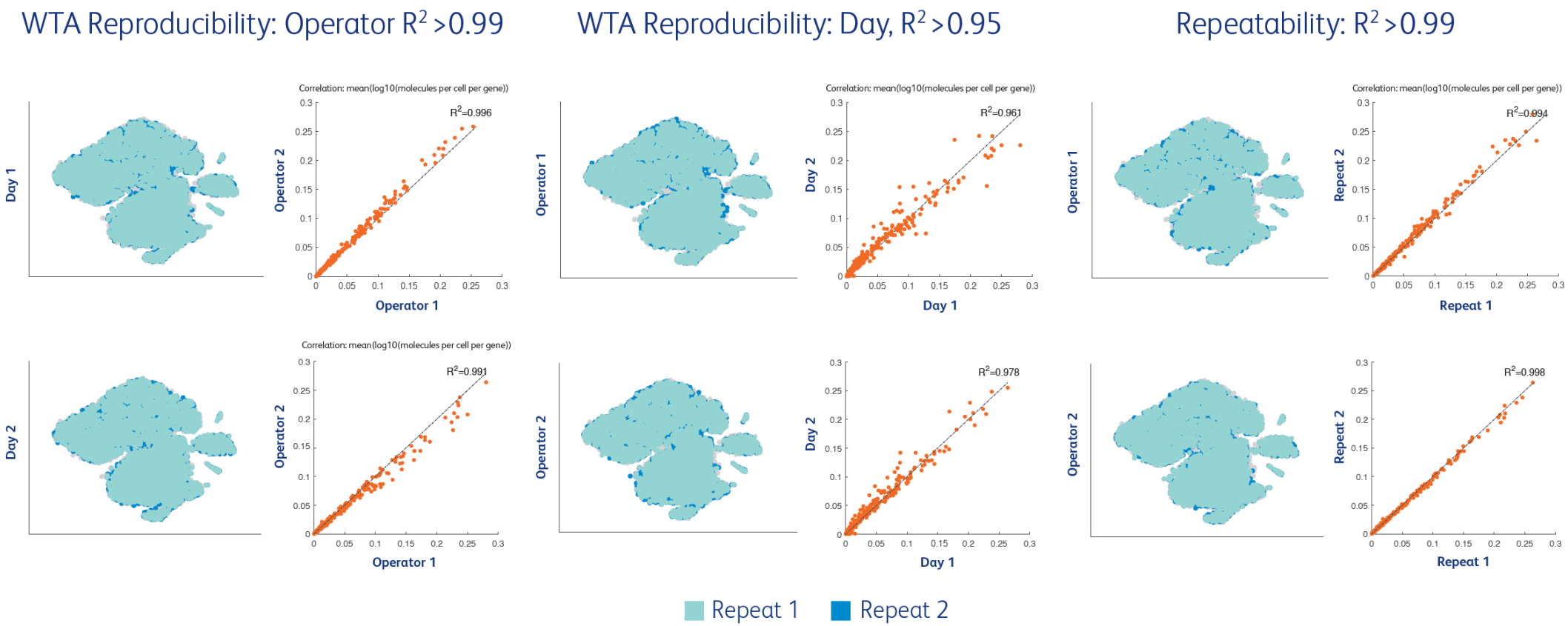
BD Rhapsody™ TCR/BCR Next Assay configuration:

WTA + VDJ + AbSeq (PBMCs)



High reproducibility and repeatability with BD Rhapsody™ TCR/BCR Next Multiomic Assays. Consistent TCR/BCR full-length pairing efficiency across different operators, repeats and days, with 75% (σ = 1.8%) for TCR and 82% (σ = 1.8%) for BCR full-length pairing efficiency on average from VDJ + WTA + AbSeq experiments on human PBMC samples. The data represent values obtained after applying the high-quality filtering function on the BD Rhapsody™ Sequence Analysis Pipeline. BCR-Any indicates the percentage of cells of each type that had both a BCR heavy chain and BCR light chain (Kappa or Lambda) and TCR-Any indicates the percentage of cells of each type that had either TCR Alpha and TCR Beta or TCR Gamma and TCR Delta. BCR-FL indicates the percentage of cells of each type that had full-length contigs for both BCR heavy chain and BCR light chain (Kappa or Lambda) and TCR-FL indicates the percentages of cells of each type that had full length contigs for either TCR Alpha and TCR Beta or TCR Gamma and TCR Delta.

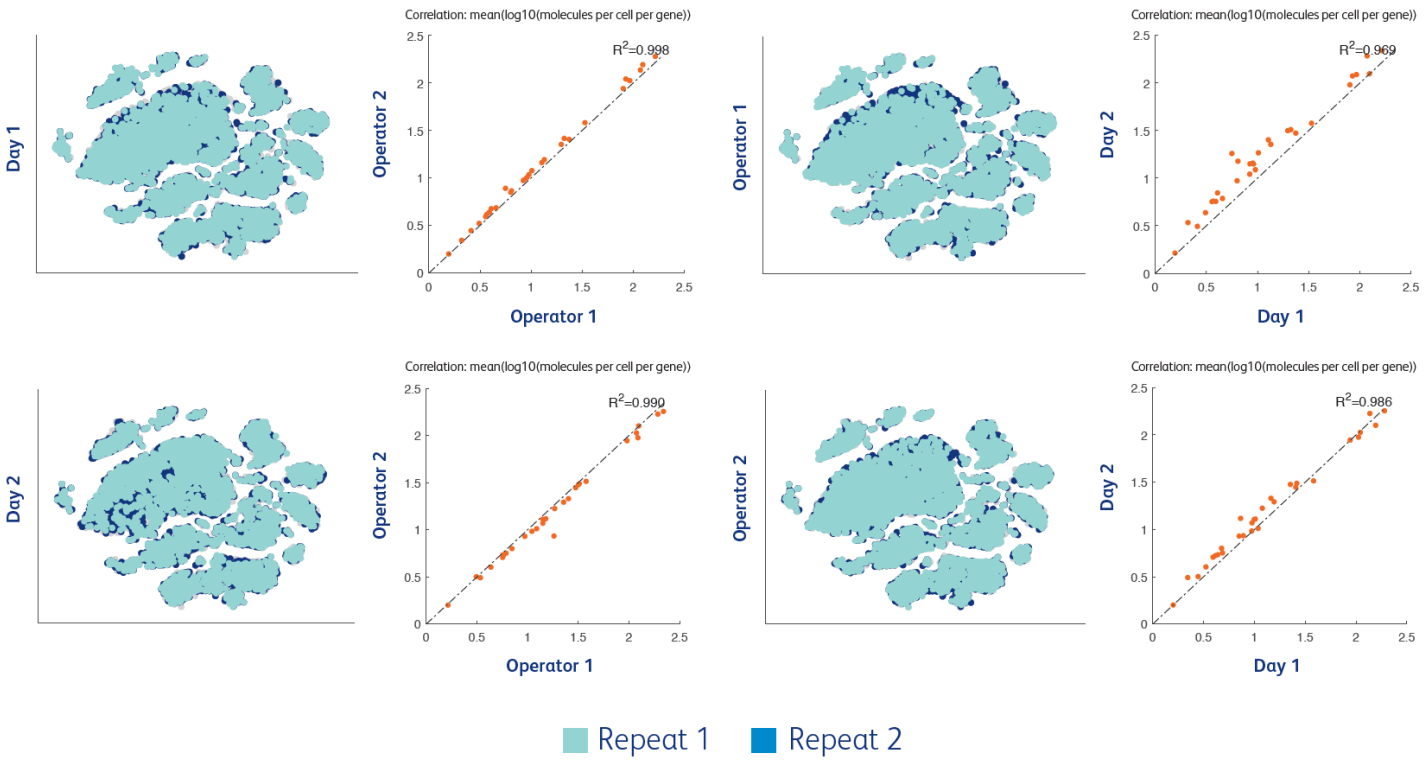
Performance metrics—reproducibility and repeatability



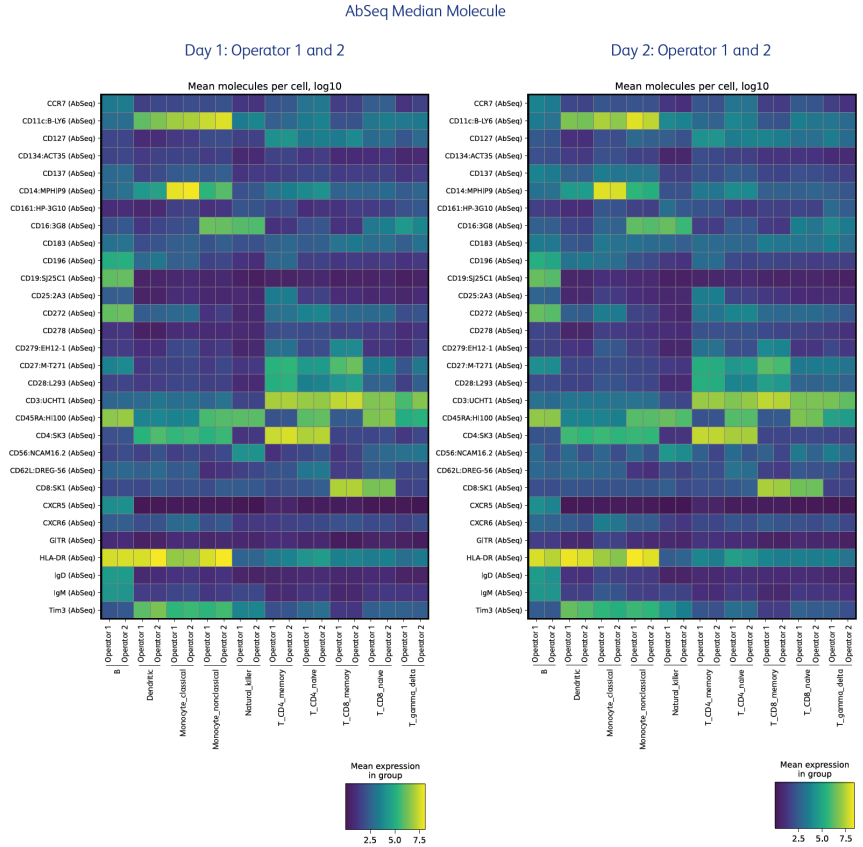
High reproducibility and repeatability with BD Rhapsody™ TCR/BCR Next Multiomic Assays. Clustering and gene expression correlation analysis for the BD Rhapsody™ Whole Transcriptome Analysis (WTA) Assay, indicating no batch effect in libraries made across different operators, repeats and days.

Performance metrics—reproducibility and repeatability

A)



B)



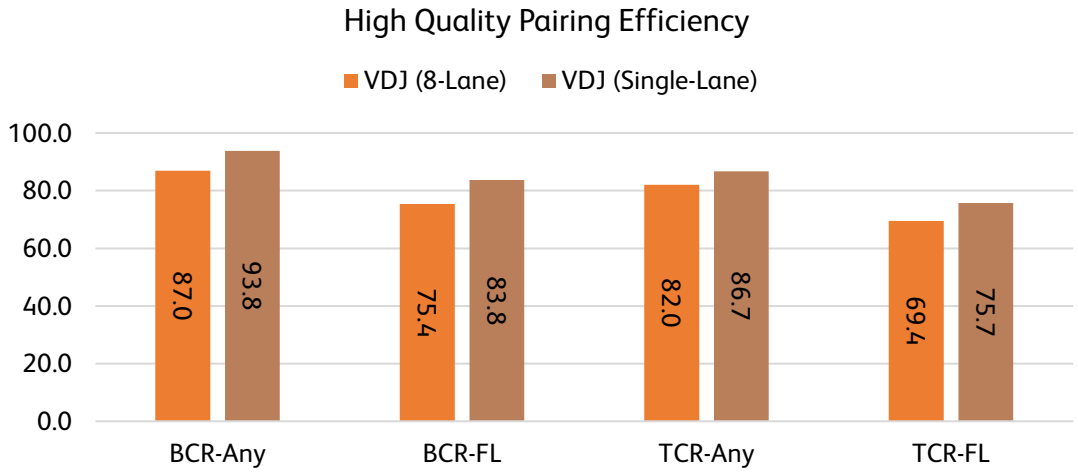
High reproducibility and repeatability with BD Rhapsody™ TCR/BCR Next Multiomic Assays. (**A**) Clustering and expression correlation analysis for the AbSeq assay, indicating no batch effect in libraries made across different operators and days. (**B**) Median molecules for the AbSeq assay across different operators and days. Overall, the high full-length pairing efficiency and robust gene and protein expression correlations demonstrated in these multiomic experiments underscore the ability of the assay to reproducibly support integrated, highly consistent multiomic workflows. All experiments were performed on BD Rhapsody™ 8-Lane Cartridges.

Performance metrics—BD Rhapsody™ 8-Lane vs Single-Lane Cartridges

Assay sensitivity, represented by TCR and BCR pairing efficiency, in WTA + VDJ experiments using the BD Rhapsody™ 8-Lane Cartridge and BD Rhapsody™ Single-Lane Cartridge, showing similar performance across two different cartridges.

BD Rhapsody™ TCR/BCR Next Assay configurations:

WTA + VDJ (PBMCs)



Pairing efficiency in WTA + VDJ experiments using the BD Rhapsody™ 8-Lane Cartridge and BD Rhapsody™ Single-Lane Cartridge

Chapter III

- BD Rhapsody™ TCR/BCR Next Multiomic Assays performance
- Reproducibility and repeatability data
- **Compatibility with BD® OMICS-Guard Sample Preservation Buffer:**

WTA +VDJ + AbSeq experiments with BD Rhapsody™ TCR/BCR Next Multiomic Assay with:

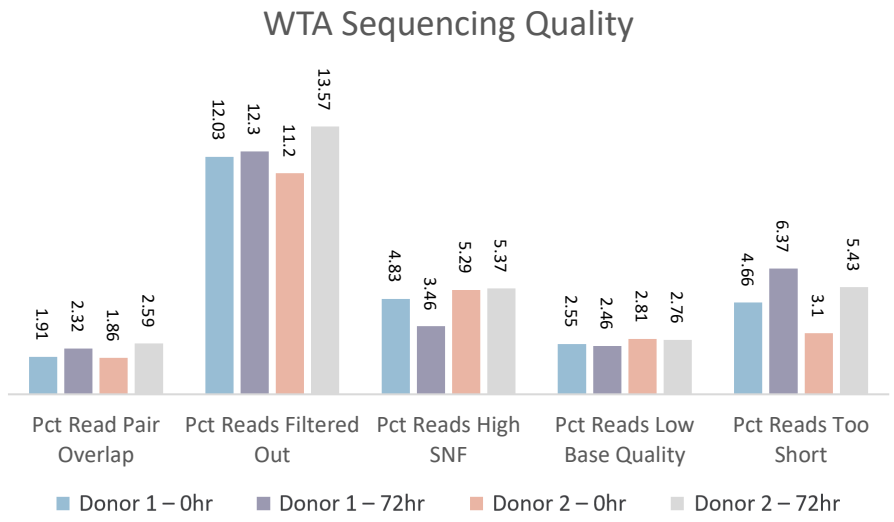
1. Fresh sample
2. Sample preserved in BD® OMICS-Guard Sample Preservation Buffer for up to 72 hours

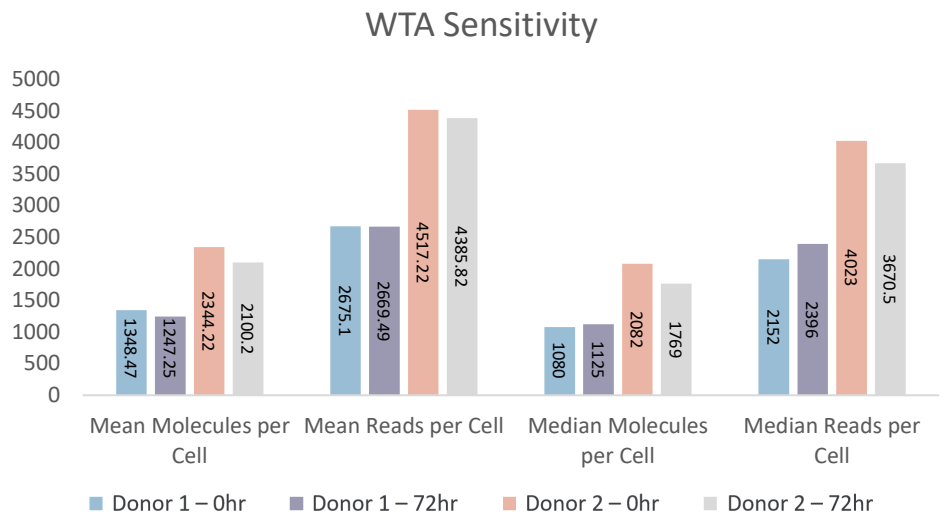
Performance metrics—BD® OMICS-Guard Sample Preservation Buffer

WTA sensitivity in experiments with the BD Rhapsody™ TCR/BCR Next Multiomic Assay (using a BD Rhapsody™ 8-Lane Cartridge) showing consistent performance across samples from two donors used in fresh conditions and after being preserved in BD® OMICS-Guard Sample Preservation Buffer for 72 hours.

BD Rhapsody™ TCR/BCR Next Assay configuration:

WTA + VDJ + AbSeq (PBMCs)



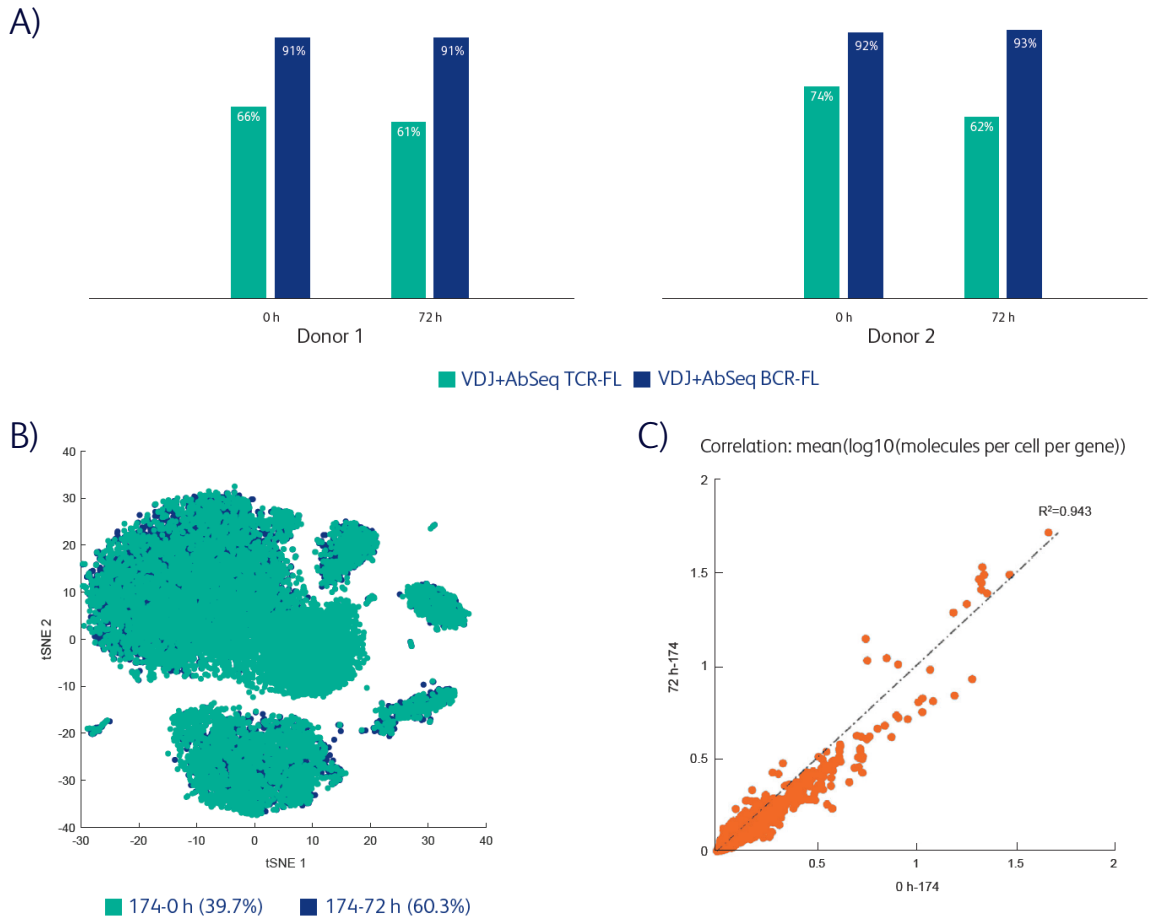


Performance metrics—BD® OMICS-Guard Sample Preservation Buffer

TCR and BCR sensitivity in experiments with the BD Rhapsody™ TCR/BCR Next Multiomic Assay (using a BD Rhapsody™ 8-Lane Cartridge) showing consistently high pairing efficiency across samples from two donors used in fresh conditions and after being preserved in BD® OMICS-Guard Sample Preservation Buffer for 72 hours.

BD Rhapsody™ TCR/BCR Next Assay configuration:

WTA + VDJ + AbSeq (PBMCs)



Cell preservation upstream of the BD Rhapsody™ TCR/BCR Next Multiomic Assay workflow. The data are from a VDJ + WTA + AbSeq experiment using two different PBMC donors with control samples at 0 hour vs samples from 72 hours of storage. **A**) Consistent TCR/BCR pairing efficiency across different duration and samples. **B and C**) Representative clustering and gene expression correlation analysis for the WTA assay indicates no batch effect between control and in libraries made from cells preserved for 72 hours.

Thank you