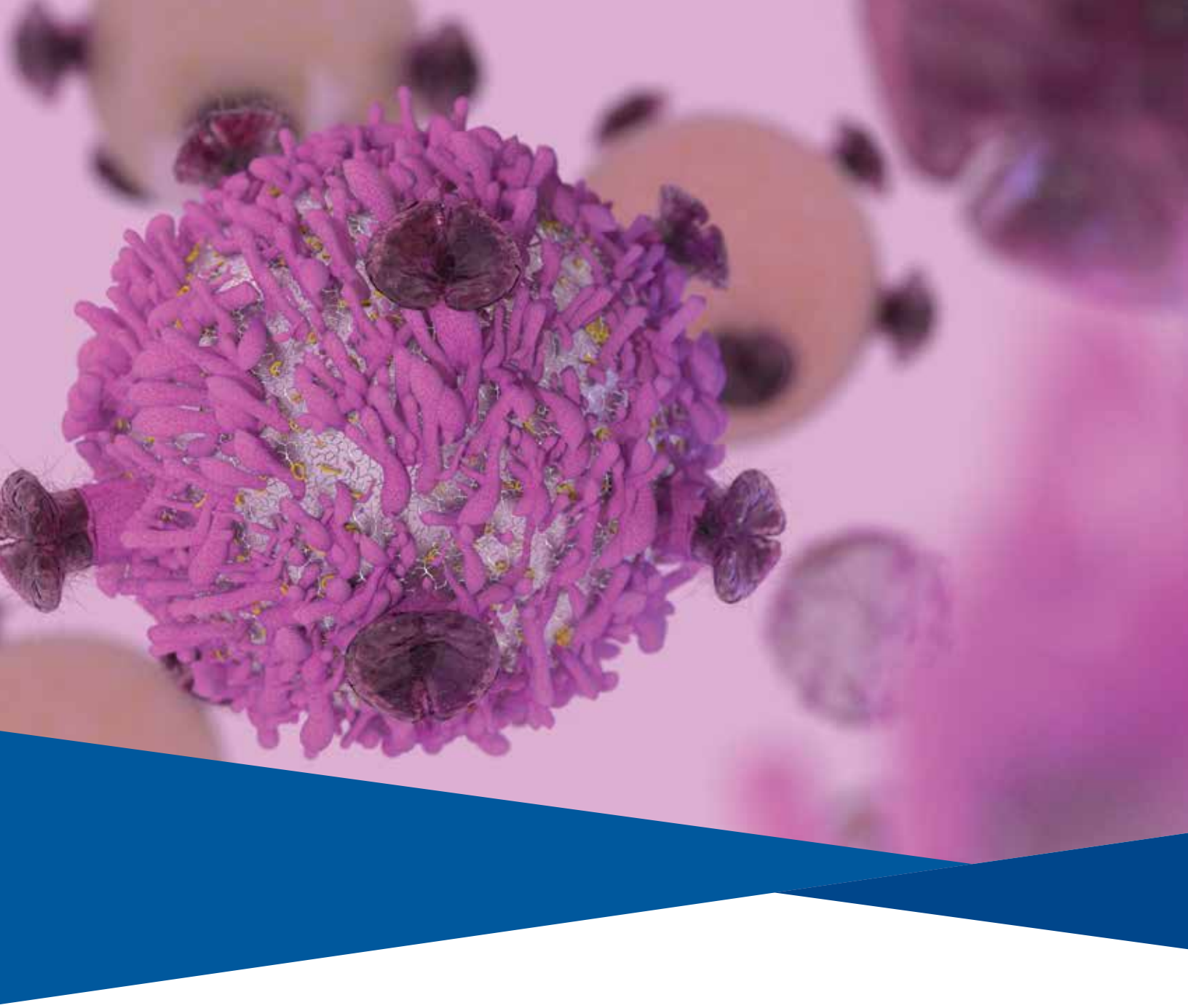


BD Rhapsody™ Single-Cell Analysis System

Analyze hundreds of genes across tens of thousands of single cells in parallel

BD

Analyze hundreds of genes across tens of thousands of single cells in parallel

Single-cell RNAseq is changing how we understand cells. The BD Rhapsody™ Single-Cell Analysis System meets your experimental need to understand cellular form and function on an individual basis with BD's 40 years of expertise in cell biology.

This new analysis system overcomes the limitations of traditional assays, such as microarrays and bulk RNAseq, which rely upon averaging measurements across multiple cells by enabling observation of highly subtle differences between individual cells. Users can identify and characterize novel and rare cell types, helping further the understanding of biological processes in fields ranging from immunology to oncology and beyond.

The BD Rhapsody Single-Cell Analysis System enables digital quantitation of hundreds of expressed genes across tens of thousands of single cells, provides customized assays that are flexible enough to meet any experimental need, and comprises an efficient system that reduces experimentation time and sequencing costs.

System components include:

- BD Rhapsody scanner
- BD Rhapsody sample loading station
- BD Rhapsody cartridge
- Reagents for Molecular Indexing and library preparation
- Application-specific targeted panels

How does the BD Rhapsody Single-Cell Analysis System work?

Figure 1 shows how the system enables digital, quantitation of gene expression levels of hundreds of genes. It uses a novel planar array of microwells for cell capture and a bead-based 3' RNAseq assay.

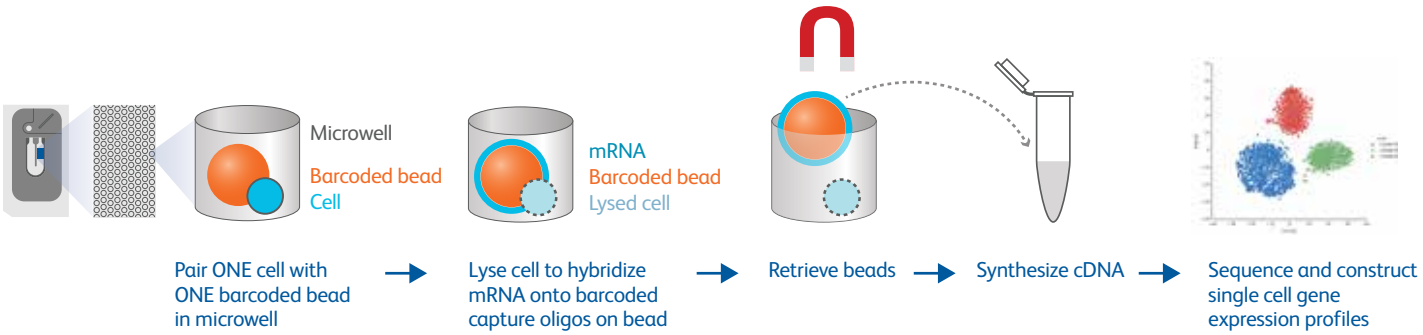


Figure 1. BD Rhapsody system workflow.

Beginnings—cell capture and isolation steps

The BD Rhapsody cartridge enables single-cell capture and molecular indexing of mRNA transcripts on magnetic oligonucleotide barcoded beads. These beads are then pooled into a single tube for cDNA amplification and library construction.

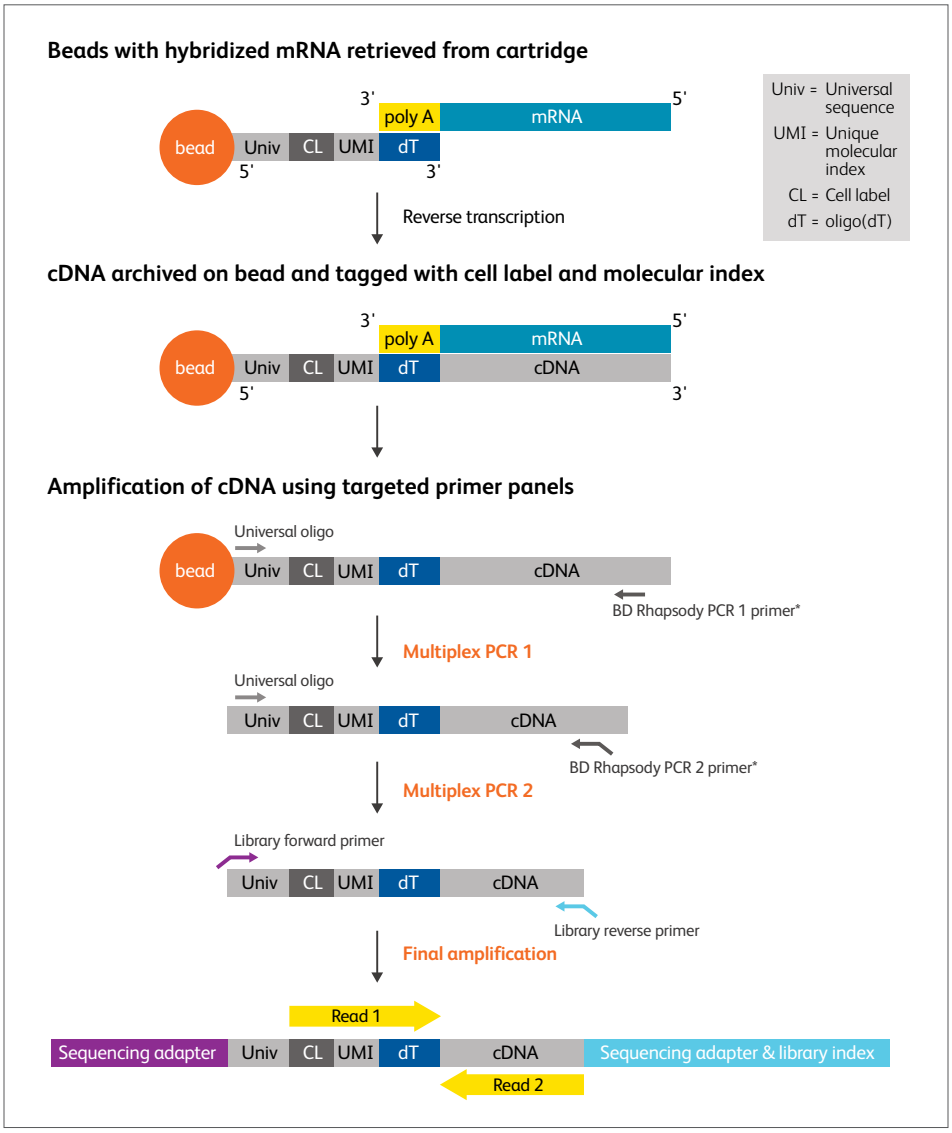


Figure 2. mRNA capture and library preparation steps.

Bypassing PCR bias – BD Molecular Indexing

PCR amplification bias can result in inaccurate quantitation of genes when sequence reads are counted. BD patented Molecular Indexing technology assigns unique molecular indices (UMI) for counting individual genes in individual cells, also known as "molecular barcoding." This allows the experimenter to overcome amplification bias for more accurate enumeration of transcript levels.

Customized workflows tailored for your experiments

Single-cell experimental workflows often require varying degrees of sample manipulation, quality, and throughput between users and experiments. BD instrument options can be configured for specific needs. The BD FACS sorter (like BD FACSMelody™), is a valuable option for the BD Rhapsody System for upstream sample enrichment. The BD Rhapsody Scanner provides automated cell counting and viability of cell samples to help users prepare samples at optimal concentrations for single-cell capture on the cartridge. The scanner provides counts of cells captured with beads on the cartridge. The BD Rhapsody Sample Loading Station is light, portable, and easily moved within a lab. Bioinformatics pipeline and visualization tools are also provided. These tools consist of UMI analysis algorithms and visualization tools enabling even inexperienced users to analyze and understand single-cell data.

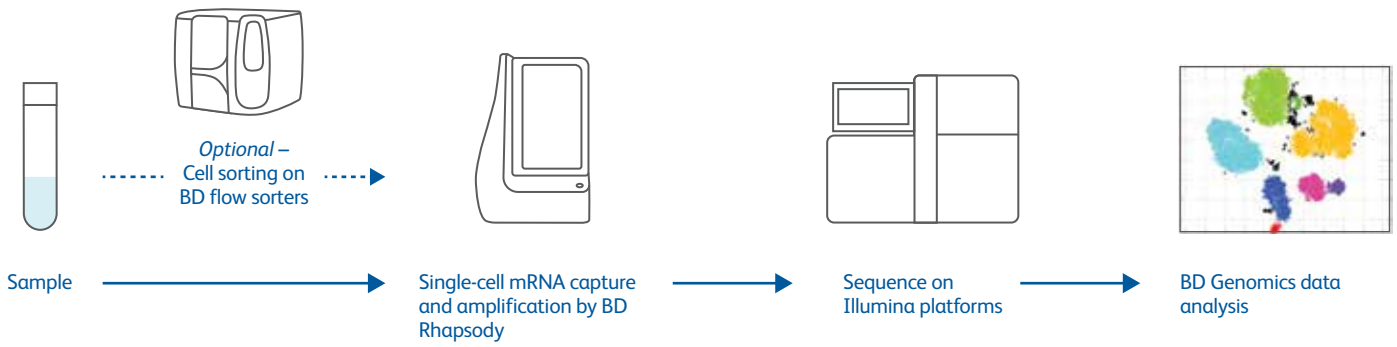


Figure 3. Example BD Rhapsody workflow including cell isolation (FACS optional), cell capture and molecular indexing, sequencing and data analysis.

More efficient sequencing yields higher throughput at lower costs

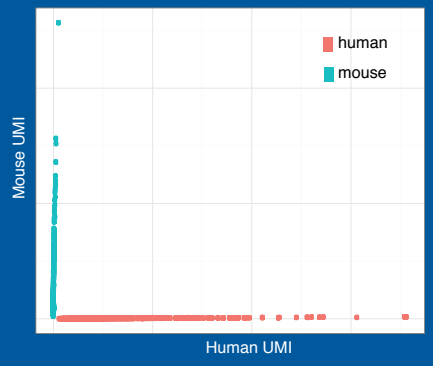
Single-cell experiments are very expensive due to sequencing costs associated with high-throughput experimental methods. The BD Rhapsody Single-Cell Analysis System was designed with multiple features that help reduce experimental costs by utilizing sequencing reads more efficiently.

- Targeted assays—a specific gene panel approach can help generate similar results as a Whole Transcriptome Analysis (WTA) assay at a fraction of time and cost, due to improved detection sensitivity. This method also improves UMI counting efficiency, yielding dramatic experimental time and cost savings.
- Archiving—Storing magnetic beads stably with intact cDNA generated from your sample allows storage of precious sample for up to 12 weeks. This gives users flexibility to sequence samples when time is available, and run multiple assays on the same sample by aliquoting or subsampling a pool of stored beads.
- Sub-sampling—cDNA archived on magnetic beads may be split into one or more aliquots and amplified using custom or pre-designed panels. Sub-sampling allows users to reduce cost by providing the option to sequence a portion of the sample and the flexibility to test different panels on the sample.

Evaluating BD Rhapsody performance

To determine the technical performance of the BD Rhapsody Single Cell Analysis System, a 1:1 mixture of human 293T and mouse NIH/3T3 cells was loaded onto the BD Rhapsody cartridge at different concentrations. Archived cDNA molecules were universally amplified and sequenced at ~ 8,700 reads per cell. At the loading condition in which 1,109 cells were detected in sequenced data, an average of 99.4% of molecules from each cell were detected in each cell as only human or only mouse, indicating minimal crosstalk between microwells.

Multiplet incidence was close to zero at a cell loading density yielding 1,000 or fewer cells detected by sequencing, and was less than 5% at 15,000 cells.



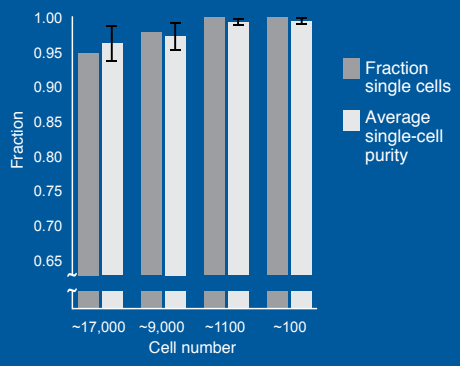


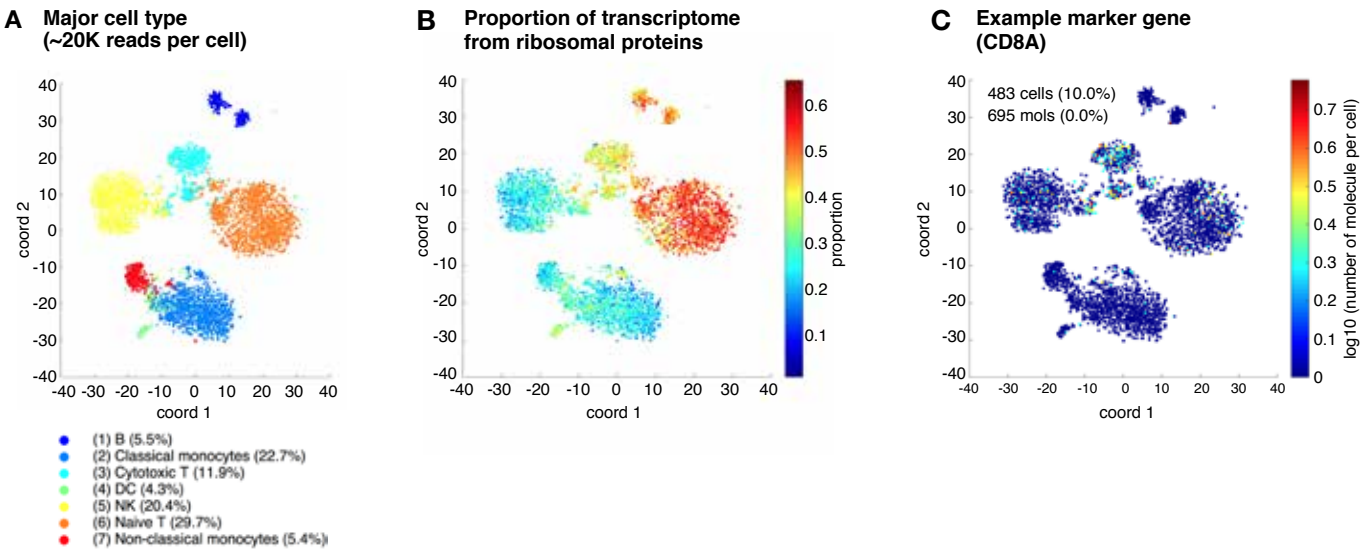
Figure 4. Minimal cross talk and low multiplet rate between microwells using BD Rhapsody

Making single-cell sequencing more efficient

The benefits of a targeted approach to single-cell analysis are demonstrated by profiling peripheral blood mononuclear cells (PBMCs) using a competitor WTA assay and the BD Rhapsody Immune Response Panel (Human) at a similar sequencing depth. While t-SNE profiling from both assays showed clustering results of known PMBC cell types, targeted panels showed higher sensitivity (reads/cell) and UMI counting efficiency than WTA. In a PBMC profiling experiment (Figure 5), the BD Rhapsody assay achieved similiar clustering performance with nearly 10x fewer reads than a competitor WTA 3'RNA-seq assay (2k reads per cell vs. 20k reads per cell). Unlike using a WTA approach, targeted panels avoid sequencing of many genes with high expression or low expression variability (e.g. ribosomal proteins), thereby driving improved assay efficiency and sensitivity. In comparison to competitor WTA assay data (C), the greater sensitivity of the BD Rhapsody targeted approach enabled detection of the low abundant but important marker CD8A, yielding greater resolution of interesting T-cell subpopulations (B).

To test archiving of pooled beads (see Figure 6), two subsamples of 1,000 cells obtained from a lymph node containing sparse metastatic breast cancer cells were profiled with the BD Rhapsody Onco-BC Targeted Panel. Subsamples were profiled at 0, 6 and 12 weeks after bead storage at 4°C. Strong correlations of gene expression between subsamples indicated minimal batch effect and high stability of archived cDNA.

Competitor WTA 3' RNA-seq



BD Rhapsody Targeted

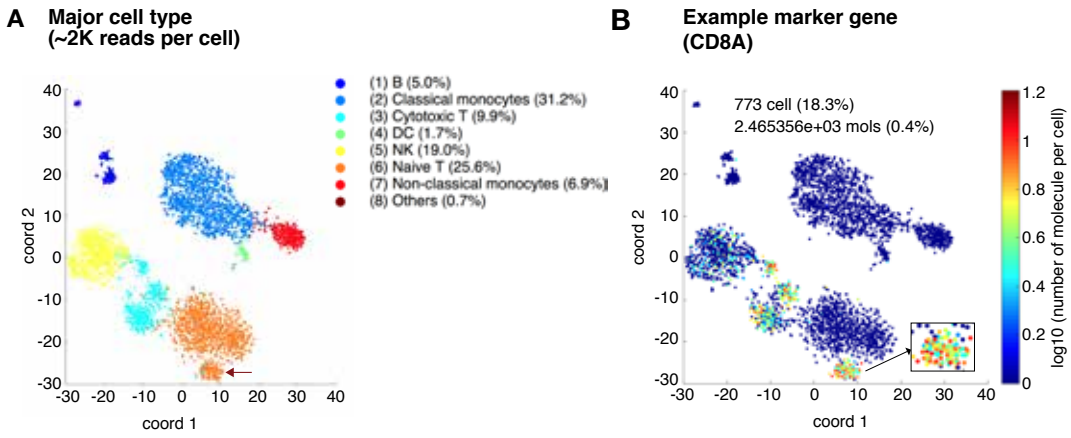


Figure 5. Example results showing assay sensitivity and UMI counting efficiency between competitor WTA and BD Rhapsody targeted assays. By avoiding high expressing proteins with low variance (e.g. ribosomal proteins), targeted assays yield high quality clustering results with dramatically far fewer reads (2K per cell vs. 20K per cell), thus reducing sequencing costs. Targeted sequencing also allows more sensitive detection of low abundant but important surface marker genes, for instance, CD8A. Unlike competitor WTA data, this targeted approach enables the distinction of naïve CD8 T cells from naïve CD4 T cells (plot B magnified cluster).

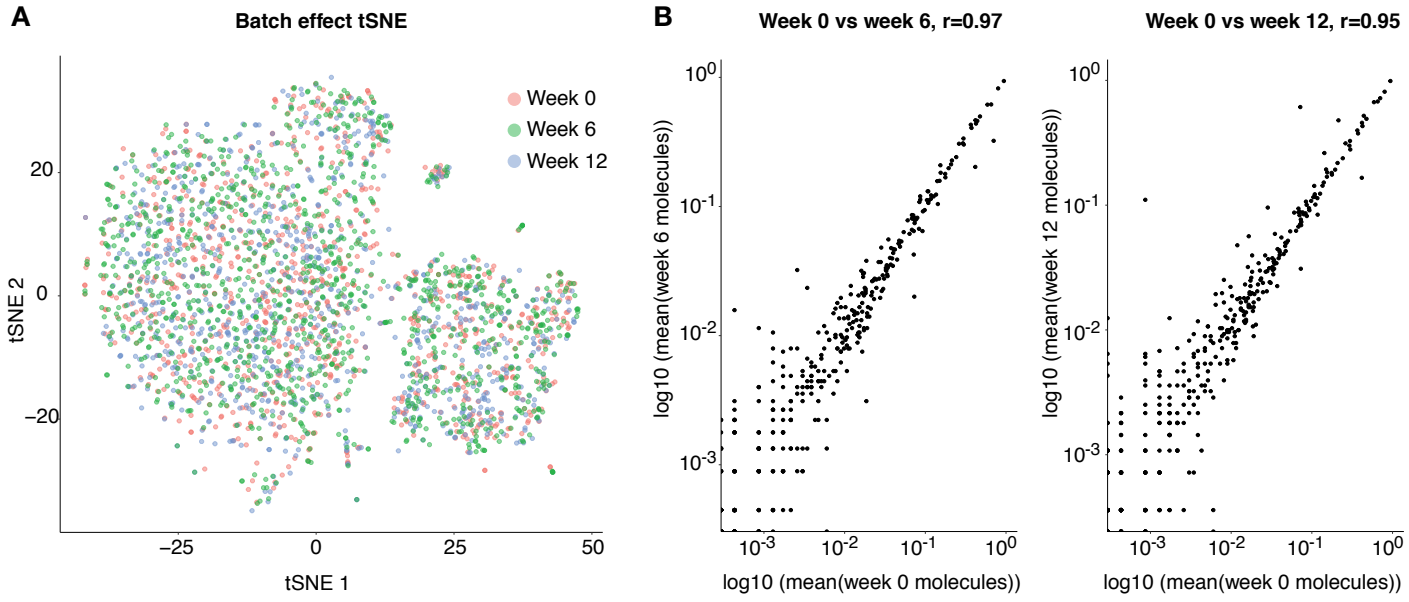


Figure 6. Magnetic beads are stably stored with intact cDNA from one preparation and show little batch effect over time (A). Correlation of gene expression in batches prepared 12 weeks apart shows stability of archived sample (B).

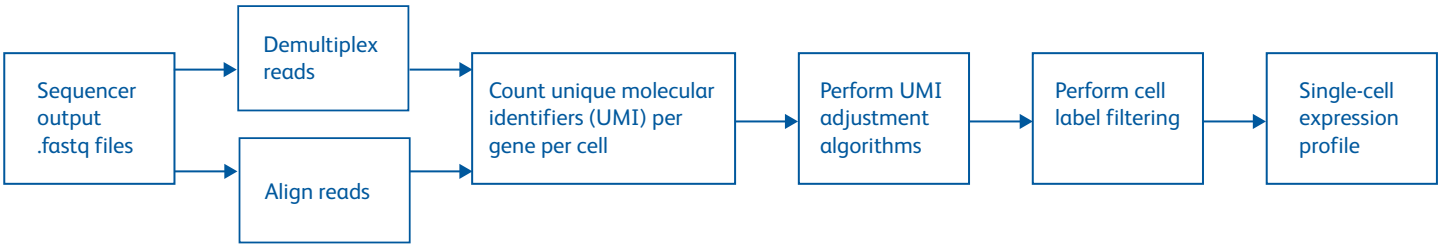
Targeted panels for major applications

Targeted approaches help boost single-cell sequencing efficiency, as well as furthering discoveries in some of the most challenging scientific areas today. Our validated panels show robust and reproducible arrays of cell heterogeneity, and are useful for exploring cell types related to numerous applications. The BD Rhapsody System includes these panels:

- BD Rhapsody Onco-BC Targeted Panel – 400 genes associated with breast cancer and immune cells
- BD Rhapsody Immune Response Panel – profiling for immune cell types
- BD Rhapsody T-Cell Targeted Panel
- BD Rhapsody Supplemental Panel
- BD Rhapsody Custom Panel

Advanced software and visualization adds flex to your results

Bioinformatics pipeline and visualization tools are also provided with the BD Rhapsody system. These tools consist of UMI analysis algorithms and visualization tools enabling even inexperienced users to analyze and understand single cell data.



BD Rhapsody system data sets are available for download at www.sbgenomics.com/bdgenomics

Example data sets:

- Single-cell profiling of PMBCs from a healthy donor
- T-cell phenotyping analysis
- Single-cell profiling of breast cancer tumor biopsies

Total end-to-end system for single-cell research

The BD Rhapsody Single-Cell Analysis system empowers and streamlines your research with a complete system of tools, including reagents and analysis software, that work together to meet your unique single-cell experimental needs. Achieve results while saving time and money with assays that dramatically reduce experimental cost and complexity, improving data and increasing efficiency of sequencing. For 40 years, BD has been a trusted partner in single-cell biology. You can rely on us to help open new frontiers in single-cell analysis.

www.bd.com/genomics

BDGM1012 Rev. 1