Cell sorting enables in-depth deconvolution of cell subsets and identification of rare cell populations using single-cell multiomics

Comparison of unsupervised clustering between unsorted and sorted samples highlights the benefits of cell sorting for downstream single-cell multiomic analysis

Features

- Simultaneous enrichment of six immune populations from the tumor microenvironment using the BD FACSymphony™ S6 Cell Sorter
- Cell sorting upstream facilitates detection of rare immune cell populations in single-cell multiomics
- Cell sorting upstream reveals more cellular subsets and permits deep characterization of the sorted populations in single-cell multiomic analysis

Single-cell multiomics has been increasingly used to reveal cellular heterogeneity and evolution trajectories in both basic and clinical research. However, profiling rare cell populations using single-cell multiomics could require interrogating a large number of cells in order to detect the rare cell types, leading to high reagent and sequencing costs. The ability to isolate rare cell types from heterogeneous and complex biological samples using a cell sorter eliminates unwanted cells and increases the number of cells of interest for downstream single-cell multiomics.

In this study, we designed a comprehensive workflow that included 6-way cell sorting using the BD FACSymphony™ S6 Cell Sorter followed by single-cell multiomic analysis using the BD Rhapsody™ Single-Cell Analysis System. With the comparison of cell types identified in unsorted and sorted samples, we highlight that upstream cell sorting helps enrich rare cell populations and distinguish cell subsets in greater detail when combined with single-cell multiomic analysis.

BD

Figure 1

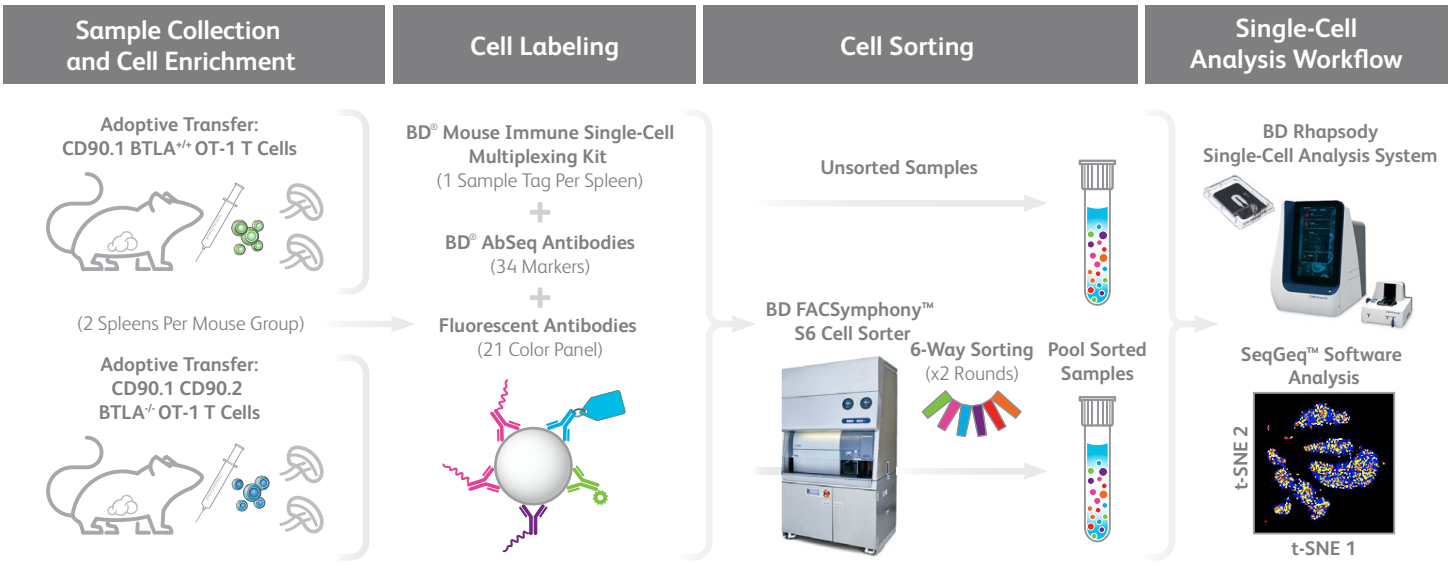

Figure 1. Experimental overview of cell sorting using BD FACSymphony™ S6 Cell Sorter and single-cell capture workflow using BD Rhapsody™ Single-Cell Analysis System

CD90.2 C57BL/6 mice were inoculated with lymphoma cells and then received an adoptive cell transfer of either CD90.1 BTLA$^{+/+}$ or CD90.1 CD90.2 BTLA$^{-/-}$ OT-1 T cells. Splenocytes were labeled with BD Pharmingen™ Biotin Rat Anti-Mouse CD19, allowing both B cells and CD19^{+} lymphoma cells to be depleted by BD IMag™ Streptavidin Particles Plus-DM. Enriched cells were then co-stained with BD® AbSeq Antibody-Oligonucleotide Conjugates and fluorescent antibodies in the presence of BD Pharmingen™ Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™ Reagent) and BD Horizon™ Brilliant Stain Buffer Plus. Each spleen was also stained with a unique DNA-barcoded antibody from the BD® Mouse Immune Single-Cell Multiplexing Kit, allowing the pooling of spleens from different mice. Pooled samples from the same mice group were sorted using the BD FACSymphony™ S6 Cell Sorter to acquire six different immune populations (M-MDSC, G-MDSC, exogenous and endogenous CD8 T cells, effector CD4 T cells and NK cells). Concurrently, a fraction of the pooled samples was left unsorted. The sorted populations from the two groups of mice were pooled and captured using the BD Rhapsody™ Single-Cell Analysis System. The unsorted cell fraction was also captured for single-cell sequencing. Equivalent sequencing reads/cell and comparable sequencing saturation were achieved between the unsorted and sorted samples. The sequencing data were analyzed using the BD Rhapsody™ Analysis Pipeline and SeqGeq™ v1.6 Software.

We performed t-Distributed Stochastic Neighbor Embedding (t-SNE) analysis using combined mRNA and AbSeq protein single-cell sequencing data and applied cell type specific markers to identify the immune populations for both unsorted and sorted samples (Figure 2). In the unsorted sample, we were unable to identify the six immune populations selected for sorting such as the rare NK cell population. We also detected a low number of CD4 T cells in the unsorted sample (Figure 2A, white box), making it hard for a thorough characterization of the different CD4 subsets. As expected, in the sorted sample, all six sorted populations including NK cells were observed and formed distinct clusters (Figure 2B). High-parameter cell sorting enabled the detection of effector CD4 T cells in the t-SNE plot and allowed characterization of this rare population within the tumor microenvironment using single-cell multiomics. By analyzing mRNA and proteins (BD® AbSeq Antibodies), we can clearly deconvolute CD90.1 CD90.2 BTLA$^{-/-}$ transferred OT-1 cells (Figure 2B, yellow), which were not resolved in the t-SNE plot of the unsorted sample. Interestingly, three distinct clusters within the monocytic myeloid-derived suppressor cell (M-MDSC) were uncovered in the sorted sample, suggesting that cell sorting upstream of single-cell multiomic analysis can reveal cellular heterogeneity within the sorted population that would not be evident in the unsorted sample with the same cell number.

Figure 2A Unsorted

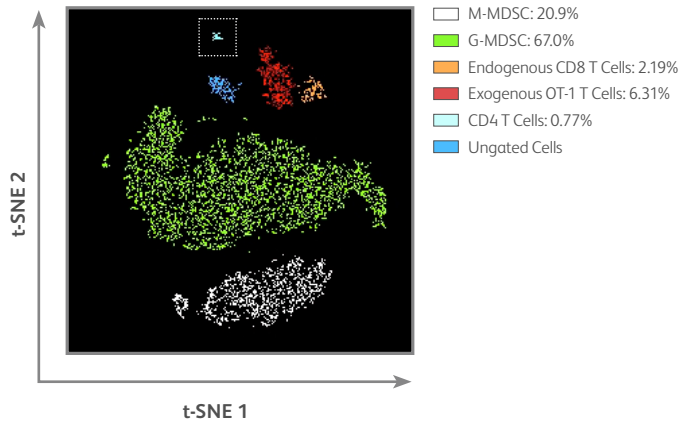

Figure 2B Sorted

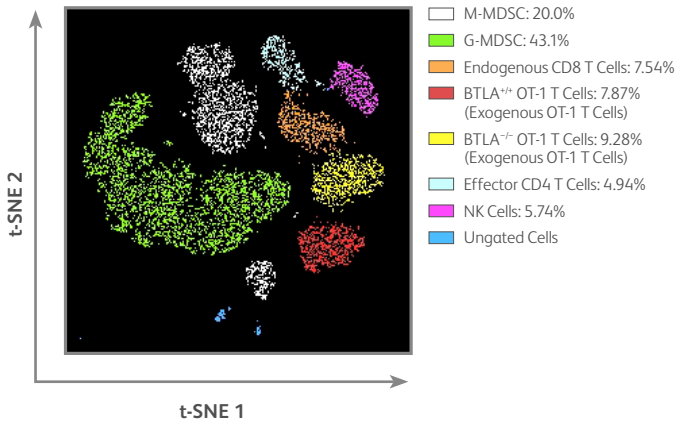

Figure 2. Dimensionality reduction by t-SNE analysis of unsorted (Figure A) and sorted samples (Figure B)

t-SNE analysis was performed using the same parameters and iterations for the two samples. Immune cell populations were identified using cell type specific mRNA and proteins such as Ly6G/C, CD115, CD90.1, CD90.2, CD4 and NCR1 and overlaid onto t-SNE plots. The frequency of each cell type within total live cells is listed.

Ordering information

Systems and software
Description
BD FACSymphony™ S6 Cell Sorter
BD Rhapsody™ Single-Cell Analysis System
SeqGeq™ v1.6 Software

For a more in-depth analysis of this study, please refer to our datasheet "Leveraging the power of high-parameter cell sorting and single-cell multiomics to profile intratumoral immune cells in a model of B-cell lymphoma."

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