Streamlined transfer of high-parameter flow cytometry panel from cell analyzer to cell sorter prior to in-depth single-cell multiomic analysis of murine lymphocyte subpopulations

A complete solution for high-parameter flow cytometry, cell sorting and single-cell multiomics research

Features

- Transfer of high-parameter flow cytometry panel across BD FACSymphony™ platforms–cell analyzer to cell sorter–without the need for panel re-design and optimization
- BD FACSymphony S6 Cell Sorter enables deep cell identification and sort through detection of more than 18 fluorescent parameters
- Downstream multiomic analysis with BD® AbSeq Antibodies and BD Rhapsody™ Single-Cell Analysis System further reveals distinct signatures across different lymphocyte subpopulations

With recent advancements in single-cell technologies enabling higher parameter analysis, it is possible to achieve a more comprehensive characterization of individual cell populations. In this context, immunophenotypic characterization of a low-frequency cell population and its enrichment prior to downstream multiomic application becomes an essential step. We developed an application for simultaneous sorting of six different lymphocyte subpopulations using a 20-color panel that is compatible across both the BD FACSymphony A5 Cell Analyzer and BD FACSymphony S6 Cell Sorter. This high-parameter flow cytometry panel was used for immunophenotypic analysis and cell sorting that ultimately enabled

purification of six cell populations for downstream single-cell multiomic studies (Figure 1). The ability to combine high-parameter cell sorting with BD AbSeq Antibodies and targeted RNA analysis offered an in-depth approach for interrogating lymphocyte heterogeneity and complexity at the single-cell level.

Figure 1A

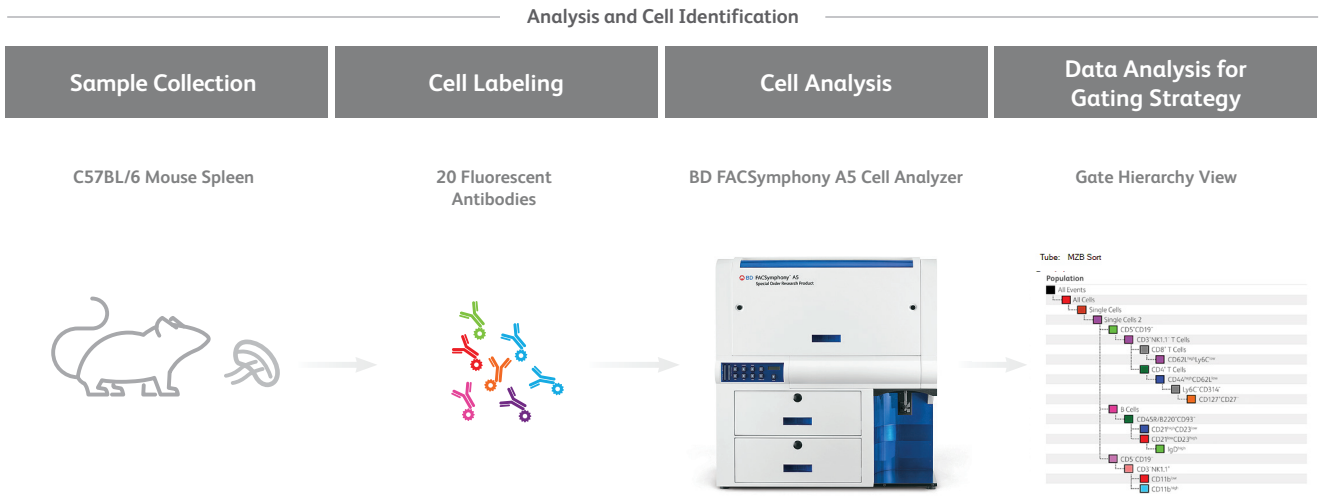

Figure 1B

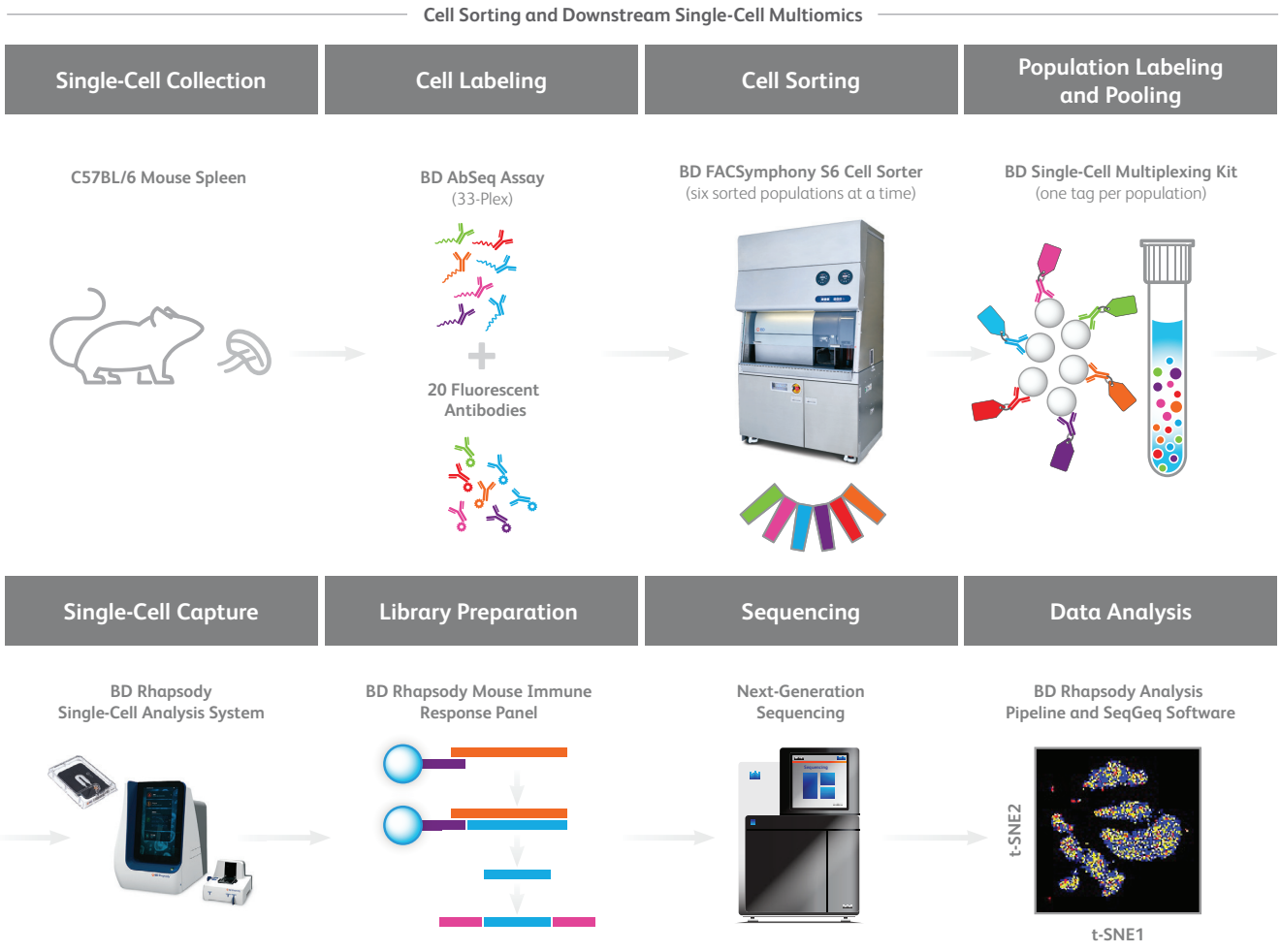

Figure 1. Overview of the workflow for cell sorting followed by single-cell multiomic analysis

A. C57BL/6 mouse spleen was dissociated with BD Horizon™ Dri Tumor & Tissue Dissociation Reagent, and red blood cells were lysed with BD Pharm Lyse™ Lysing Buffer. Cell samples were stained with a 20-color panel and then analyzed on the BD FACSymphony A5 Cell Analyzer to identify populations of interest. **B.** In a different experiment, samples were stained with the same 20-antigen cell surface panel along with 33 BD AbSeq Antibodies for isolation of six subpopulations on the BD FACSymphony S6 Cell Sorter. Each sorted population was tagged with the BD Mouse Immune Single-Cell Multiplexing Kit, pooled and loaded onto the BD Rhapsody Single-Cell Analysis System for single-cell capture. BD AbSeq Antibodies, mRNA (BD Rhapsody Mouse Immune Response Panel) and sample tag libraries were prepared for sequencing. The sequencing results were analyzed using the BD Rhapsody Analysis Pipeline and SeqGeq™ Software v1.6.

The first step in the showcased workflow was to determine the immune cell composition in mouse spleens using the 20-color panel (Table 1) on the BD FACSymphony A5 Cell Analyzer. We identified multiple cell lineages including B cells (Figure 2A), T cells and NK cells (data not shown). The complementary configurations of BD FACSymphony A5 Cell Analyzer and BD FACSymphony S6 Cell Sorter enabled the use of the same panel for cell sorting. Furthermore, transfer of CD4 target median fluorescence values ensured consistent results across the two platforms (Figures 2B and 2C). Before sorting, the splenocytes were also co-stained with 33 BD AbSeq Antibodies (Table 2) for downstream single-cell multiomic analysis on the BD Rhapsody System.

Table 1. Multicolor flow cytometry panel used for either cell analysis or sort

20-color multicolor flow cytometry panel		
Fluorochrome*	Marker	Clone
BUV805	CD45R/B220	RA3-6B2
BUV737	Ly-6C	AL-21
BUV661	CD21/CD35	7G6
BUV615	CD314 (NKG2D)	CX5
BUV496	IgD	11-26C.2A
BUV395	CD8a	53-6.7
BV711	CD27	LG.3A10
BV650	CD23	B3B4
BV570	CD4	GK1.5
BV480	I-A/I-E	M5/114.15.2
BV421	CD44	IM7
BB790	NK1.1	PK136
BB660	CD127	A7R34
BB630	CD11c	HL-3
BB515	CD62L	MEL-14
PE-CF594	CD93	AA4.1
PE	CD5	53-7.3
APC-H7	CD19	1D3
APC-R700	CD11b	M1/70
APC	CD3e	145-2C11

*BB = BD Horizon Brilliant™ Blue Reagent; BUV = BD Horizon Brilliant™ UV Reagent; BV = BD Horizon Brilliant Violet™ Reagent

Table 2. BD AbSeq Panel

33-plex BD AbSeq Cocktail	
Marker	Clone
CD103	M290
CD122	TM-β1
CD138	281-2
CD14	rmC5-3
CD16-2	9E9
CD172a (SIRPα)	P84
CD182 (CXCR2)	V48-2310
CD184 (CXCR4)	2B11/CXCR4
CD197 (CCR7)	4B12
CD1d	1B1
CD24	M1/69
CD25	PC61
CD273 (BTLA)	TY25
CD274 (B7-H1)	MIH5
CD28	37.51
CD335	29A1.4
CD357 (GITR)	DTA-1
CD370 (Clec9A)	10B4
CD40	43913
CD43	S7
CD49a	Ha31/8
CD49b	HMA2
CD49d	9C10 (MFR4.B)
CD49f	GoH3
CD54 (ICAM-1)	3E2
CD80	16-10A1
CD86 (B7-2)	GL1
CD93	493
CD95 (Fas)	JO2
Ly-6A/E	D7
Ly-6G	1A8
Siglec-F	E50-2440
TIGIT	TX99

The BD FACSymphony S6 Cell Sorter supported the simultaneous isolation of six individual subsets, as represented in Figure 2D. The sorted subsets were individually tagged with the BD Mouse Immune Single-Cell Multiplexing Kit (SMK) sample tags prior to being loaded on the BD Rhapsody Platform. Since sample tags present unique DNA barcodes, the use of sample multiplexing allowed multiple populations to be tagged, mixed and processed simultaneously. The tagged populations were then bioinformatically deconvoluted in SeqGeq Software during analysis. As shown in Figure 3A, application of t-distributed stochastic neighbor embedding (t-SNE) for unsupervised analysis of the sorted subpopulations identified six major cell clusters, which were initially depicted based on sample tag labeling.

Figure 2A

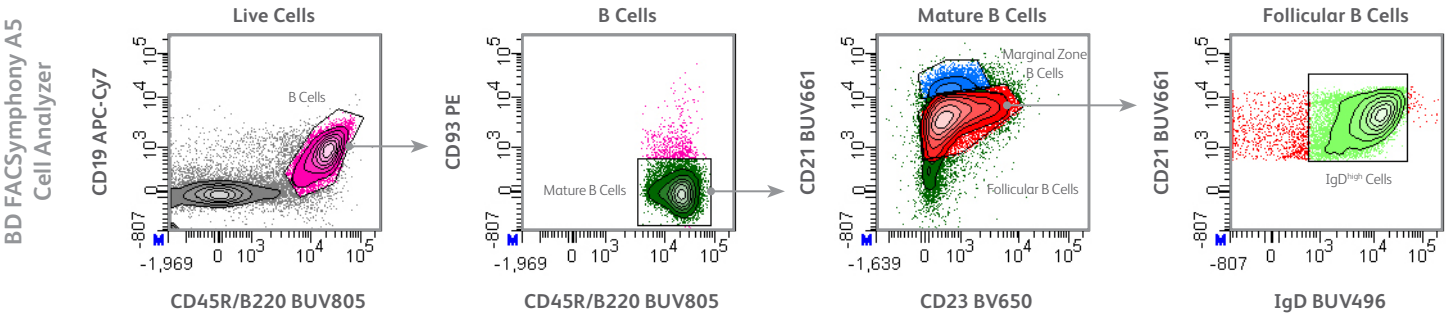

Figure 2B

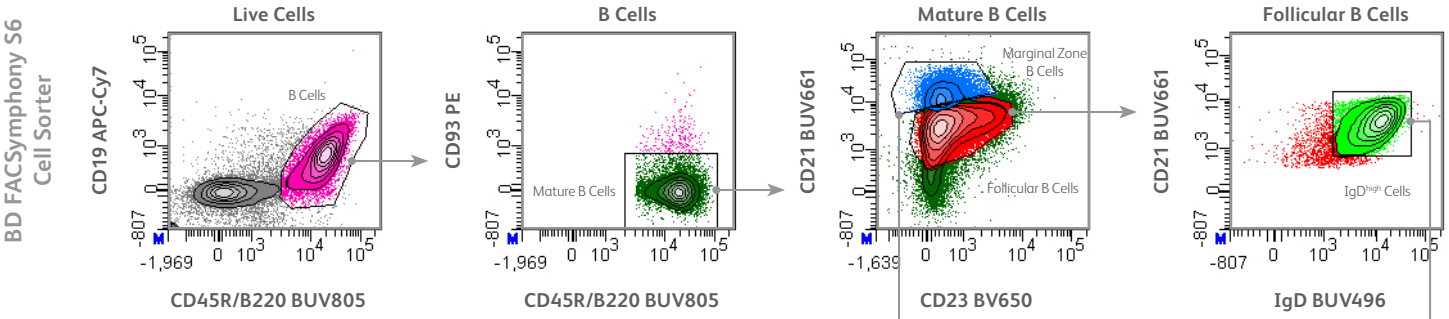

Figure 2C

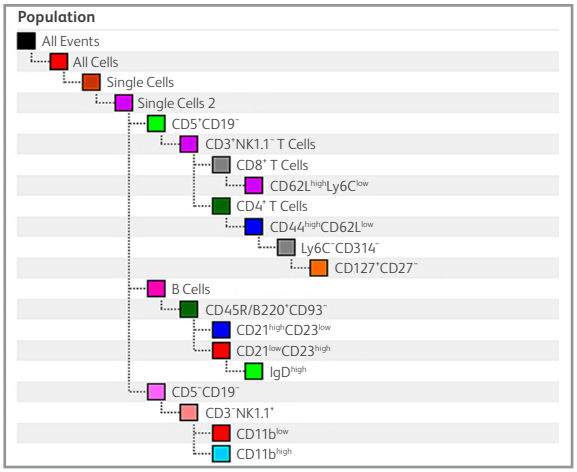

Figure 2D

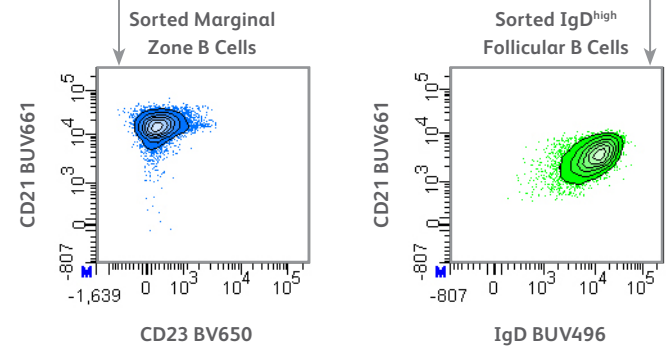

Figure 2. Representative flow cytometry plots show gating strategies to identify and sort lymphocyte subpopulations in the mouse spleen

C57BL/6 mouse splenocytes were processed and stained as described in Figure 1. **A.** Representative analysis of B cell subsets on the BD FACSymphony A5 Cell Analyzer. **B.** Representative analysis of the same B cell subsets on the BD FACSymphony S6 Cell Sorter prior to cell sorting. **C.** Gating hierarchy used for sorting of the following six lymphocyte subpopulations: naïve CD8$^+$CD62^{high}Ly6C^{low} T cells, memory CD4$^+$CD127$^+$CD27$^-$ T cells, CD21^{low}CD23^{high}IgD^{high} follicular B cells, CD21^{high}CD23^{low} marginal zone B cells, CD11b^{low} and CD11b^{high} NK cells. **D.** Representative post-sort analysis showing purity of marginal zone and IgD^{high} follicular B cells sorted subsets.

Successful separation of distinct subpopulations such as marginal zone B cells and follicular B cells was further confirmed based on differential expression patterns of selected markers known to distinguish these two cell types (Figure 3B). Additionally, we performed differential expression analysis of all 33 proteins and 397 mRNA transcripts between these two cell subsets (Figure 3C). As expected, marginal zone B cells expressed higher levels of *Cd1d1* mRNA and corresponding protein CD1d, Ly6A/Ly6E and *Cr2*, as compared to follicular B cells. Consistent with literature findings, the majority of the marginal zone B cells also showed higher levels of CD274 (PD-L1) and CD172a (Sirpα).

Figure 3A

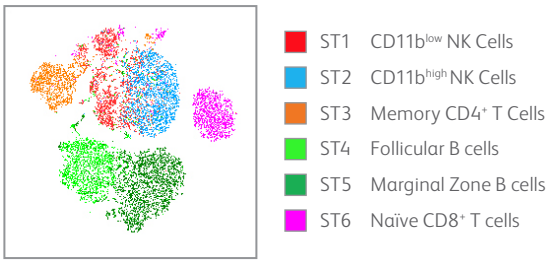

Figure 3B

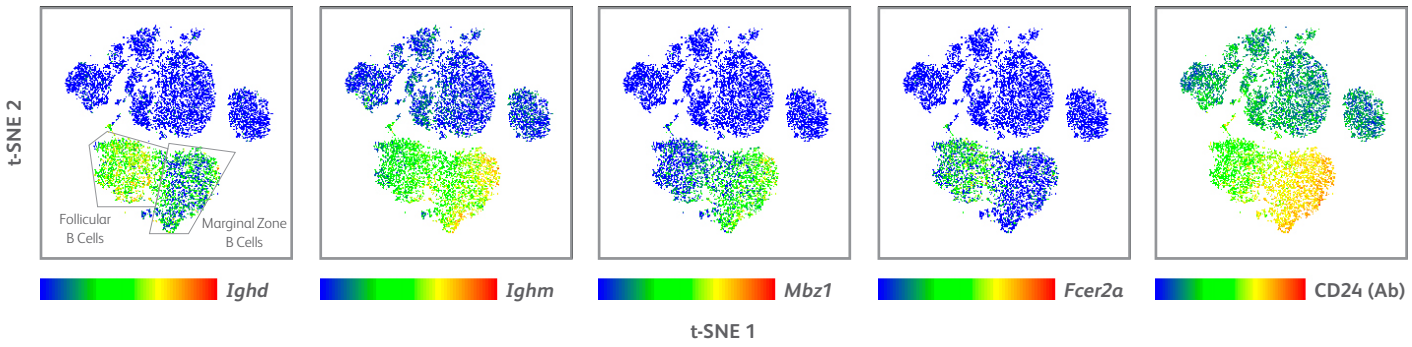

Figure 3C

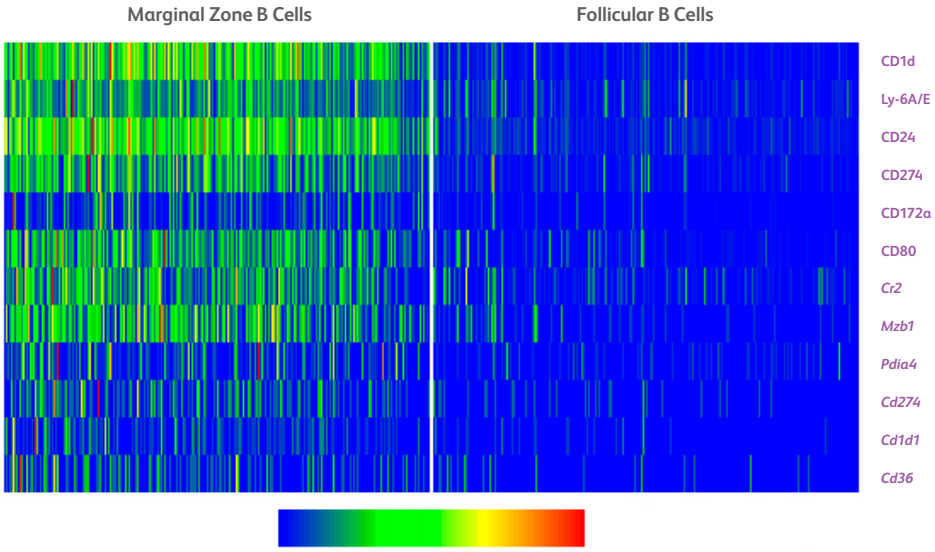

Figure 3. Simultaneous measurement of protein and mRNA expression through single-cell multiomic analysis

C57BL/6 mouse splenocytes were processed and stained as described in Figure 1B. **A.** Identification of the six sorted cell subpopulations based on sample tags (ST1-ST6) on a t-SNE plot generated using SeqGeq Software. **B.** Confirmation of cell identity through analysis of markers known to be upregulated in either follicular B cells or marginal zone B cells. **C.** Single-cell heatmap showing representative proteins and mRNA transcripts that are highly expressed in marginal zone B cells compared to follicular B cells.

In summary, our data demonstrate, as a proof-of-principle, the ability to characterize immune cell populations with a high-parameter flow cytometry panel on a BD FACSymphony A5 Cell Analyzer and the ease of transfer of the same panel to a BD FACSymphony S6 Cell Sorter for isolation of cell populations of interest. Furthermore, these pure sorted cell populations can be analyzed on a BD Rhapsody Single-Cell Analysis System for additional high-plex, simultaneous protein and mRNA expression analysis. This comprehensive workflow for cell characterization reveals greater detail on cellular heterogeneity and may lead to the discovery of new markers defining populations of interest.

Ordering information

Systems and software
Description
BD FACSymphony™ S6 Cell Sorter
BD FACSymphony™ A5 Cell Analyzer
BD Rhapsody™ Single-Cell Analysis System
SeqGeq™ Software

Class 1 Laser Product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

23-22582-01

BD Life Sciences, San Jose, CA, 95131, USA

bdbiosciences.com