BD™ Single-Cell Multiplexing Kits

Increase sample throughput and identify multiplets

Overview

The BD™ Single-Cell Multiplexing Kits (Human and Mouse Immune) utilize oligonucleotide-conjugated antibodies to provide a high sample throughput for single cell 3' RNA-seq assays. A set of 12 antibodies in the kits target the same universally expressed cell-surface antigen for human and CD45 for mouse. Each antibody is conjugated with a Sample Tag, a unique 45-nucleotide barcode sequence (**Figure 1**). Adjacent to the Sample Tag barcode, a universal PCR handle and poly(A) tail allow each **Sample Tag** to be captured by oligo-dT beads, such as the BD Rhapsody™ Cell-Capture Beads, and then amplified by PCR using two Sample-Tag specific primers targeting the PCR handle region, along with BD Rhapsody reagents.

Following a simple antibody staining of Sample Tags to single cell suspension, multiple samples can be combined to perform a single-sample library preparation (**Figure 2**, also see *Single Cell Labelling with the BD™ Single-Cell Multiplexing Kit Protocol Doc ID: 210970 Rev 2.0*). Sample origin is identified after sequencing using the sample determination algorithm (see *BD Single-Cell Genomics Analysis Setup User Guide Doc ID: 47383 Rev 2.0* and *BD Single-Cell Genomics Bioinformatics Handbook Doc ID: 54169 Rev 2.0*). The ability to tag and pool multiple samples into a single cartridge greatly enhances sample throughput and flexibility in experimental design.

Figure 1

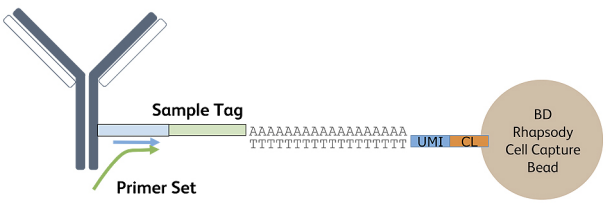

Figure 1
Structure of a Sample Tag and its compatibility for capture by BD Rhapsody Cell Capture Beads. UMI – unique molecular identifier; CL – cell-label barcode.

Figure 2

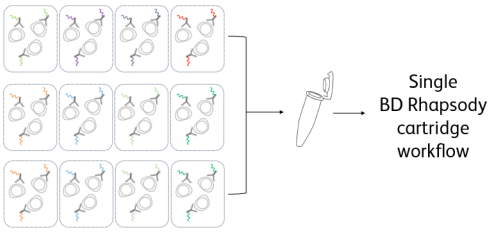

Figure 2
BD Single-Cell Multiplexing Kits allow up to 12 samples to be pooled together in a single cartridge workflow for BD Rhapsody.

BD

Detection of cross-sample multiplets with the BD Single-Cell Multiplexing Kits

In addition to high sample throughput and low library preparation costs, the BD Single-Cell Multiplexing Kits enable users to detect cross-sample multiplets. Multiplets are derived from events where more than one cell is captured by BD Rhapsody Cell-Capture Beads (**Figure 3**). The occurrence of multiplets increases as more cells are loaded into the BD Rhapsody Cartridge, following Poisson distribution calculations (**Table 1**). Even though BD Rhapsody microwell technology provides low multiplet occurrence compared to conventional droplet-based technologies, the user still makes a decision to limit the number of cells loaded into a cartridge to maintain a low multiplet rate. In some cases, multiplets are not easily identifiable, and can be misinterpreted as biologically meaningful.

Figure 3

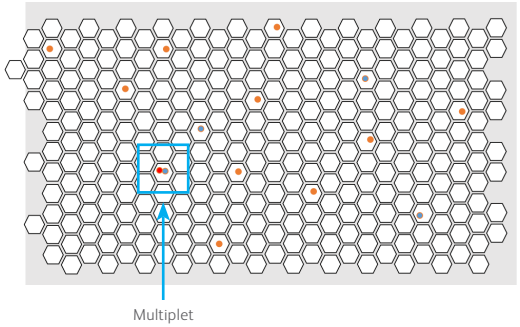

Figure 3
Multiplets are derived from >1 cell entering a microwell and may skew single cell gene expression analysis.

Table 1

No. of cells loaded in BD Rhapsody Cartridge	% total theoretical multiplets	% theoretical multiplets that remain after identification with N Sample Tags*		
		N=2	N=4	N=8
1,000	0.24%	0.12%	0.06%	0.03%
5,000	1.19%	0.59%	0.30%	0.15%
10,000	2.36%	1.19%	0.59%	0.30%
15,000	3.53%	1.78%	0.89%	0.45%
20,000	4.69%	2.36%	1.19%	0.59%

Theoretical percentage of multiplets increases as more cells are loaded into both a competitor's droplet-based system and the BD Rhapsody Cartridge. Adding Sample Tags, even for the same single cell sample, can identify inter-sample multiplets as distinguished by different Sample Tags. Multiplets that are formed by cells with the same Sample Tag cannot be detected by the pipeline. **Note:** Though it is not validated, users can load more than 20,000 cells per cartridge and continue to remove multiplets in data analysis.

*Calculations are based on equal number of cells per Sample Tag added to the BD Rhapsody Cartridge.

To understand more about multiplets, consider a simple example in which a pool of 1,000 Ramos cells labeled with Sample Tag 3 and 1,000 BT549 cells labeled with Sample Tag 4 were loaded onto a single BD Rhapsody Cartridge. Single cell gene expression profiles were visualized using t-distributed stochastic neighbor embedding (t-SNE) (**Figure 4**). In addition to BT549 and Ramos clusters, a small cluster was observed in between the main clusters (**Figure 4A**) and was identified as cross-sample multiplet by the Sample Determination algorithm, by the presence of both sample tags in the same cell. The identity of the multiplet cluster is further supported by the co-expression of BT549 and Ramos markers, for instance, the BT549 marker LGALS1 and Ramos marker CD79A (**Figure 4B-C**).

Figure 4A

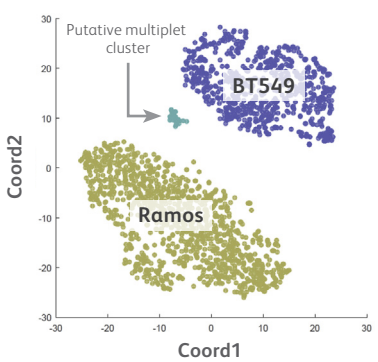

Figure 4B

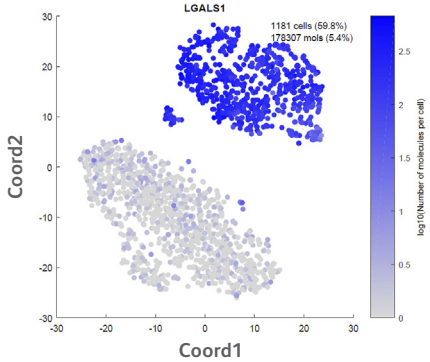

Figure 4C

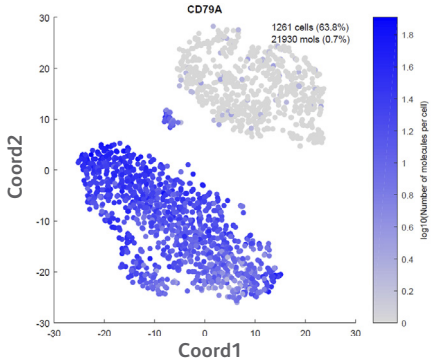

Figure 4
1,000 Ramos cells labeled with Sample Tag 3 and 1,000 BT549 cells labeled with Sample Tag 4 were pooled and analyzed on a single BD Rhapsody Cartridge.

Increase cell number analyzed while confidently identifying multiplets

An advantage of using the BD Single-Cell Multiplexing Kits is that one can load a higher number of cells while maintaining a low rate of unidentified multiplets. To illustrate this example, a dataset of 4 sample types—peripheral blood mononuclear cells (PBMCs), Ramos B cells, Jurkat T cells, and T47D breast cancer cells—split across 12 Sample Tags (**Figure 5**) was used to demonstrate the assay's ability to identify multiplets between cell type (**Figure 6**) and of the same cell type (**Figure 7**).

Figure 5

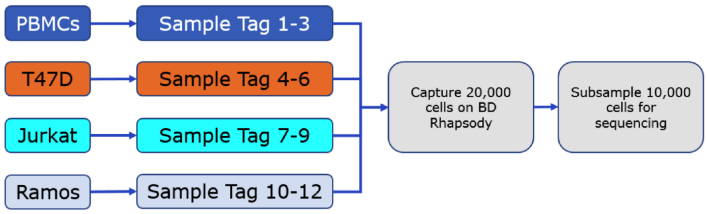

Figure 5
Schematic of the 12-Sample-Tag experiment with 20,000 cells of mixed sample types loaded on BD Rhapsody.

In this 20,000 cell-load experiment, the theoretical multiplet occurrence was ~4.7%; using Sample Tags, 4.3% of the putative cells were identified as multiplets (**Figure 6A-B, Table 1**). When gene-expression profiles were projected using t-SNE and overlaid with the Sample Tag identity, many of the multiplets identified by the Sample Tag determination algorithm resided in small clusters between the major cell populations (**Figure 6A**). These small clusters expressed gene markers from more than one cell type (not shown in this document), thereby validating the use of Sample Tags for multiplet identification.

The BD Single-Cell Multiplexing Kits also can be incorporated into a single cell suspension sample to identify multiplets. In this use case, instead of staining Sample Tags per sample, a single sample can be split into multiplet Sample Tags. An example is shown in the 12-Sample-Tag experiment where each sample type was split between 3 Sample Tags and pooled at an even ratio (**Figure 6**).

Figure 6A

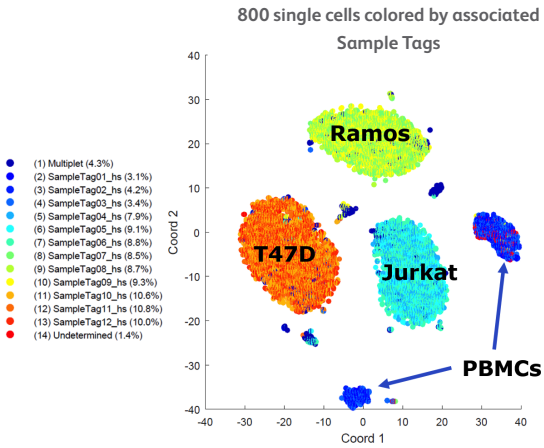

Figure 6B

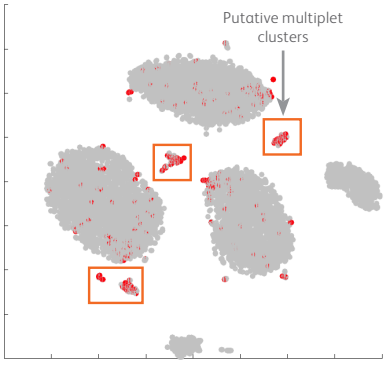

Figure 6C

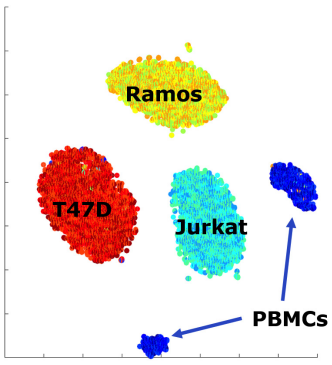

Figure 6
t-SNE visualization of cell clusters in the 12-Sample-Tag experiment with BD Rhapsody system using the Immune Response Panel. **A.** Annotation of major cell types identified by their markers, with putative multiplet clusters in between major clusters, many of which are identified using Sample Tags. **B.** Highlight of Sample Tag-identified multiplets: multiplets can be identified using Sample Tags even within one cell-type cluster. **C.** Removal of multiplets identified by Sample Tags and their associated putative clusters yields cleaner single cell RNA-seq data.

By visualizing a single cell type only (Jurkat cluster in **Figure 7A**), the cross-sample multiplets were identified by the Sample Determination algorithm even within the same cell type. Many of these cross-sample multiplets had higher gene detection (**Figure 7B**) and more mRNA molecules detected (**Figure 7C**), suggesting that these were true multiplets. The BD Single-Cell Multiplexing Kits can be used to identify multiplets even within a single sample.

Figure 7A

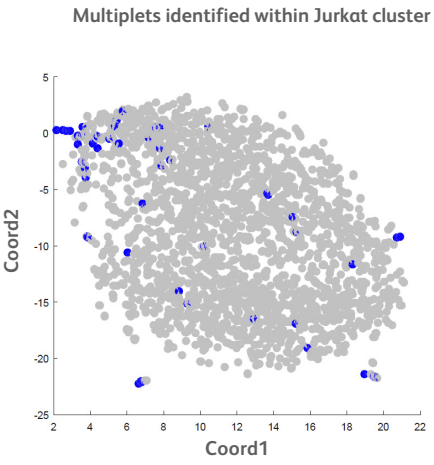

Figure 7B

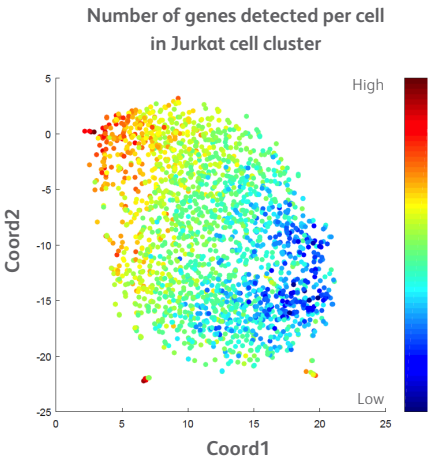

Figure 7C

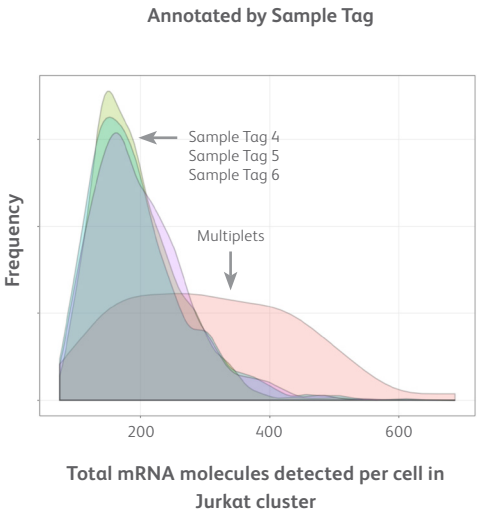

Figure 7

Visualization of Jurkat-only cluster (Sample Tags 4-6) from the 12-Sample-Tag experiment **A.** Multiplets are highlighted as blue points within the Jurkat cluster, notice that many Jurkat multiplets are located on the top-left of the cluster. **B.** Top-left putative cells identified in the Jurkat cluster are associated with higher detection of genes per cell. **C.** Extracting total mRNA molecules detected per putative cell, those that are identified as multiplets by Sample Tags have higher molecule detection, suggesting that they are bona fide multiplets.

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