BD Rhapsody™ Whole Transcriptome Analysis Amplification Kit

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Uncover the whole transcriptome of a single cell

The BD Rhapsody™ Whole Transcriptome Analysis (WTA) Amplification kit enables unbiased 3'-based capture, amplification and detection of transcriptomes at a single cell level. The BD Rhapsody WTA Amplification kit has been thoroughly tested and validated to generate consistent, high-quality whole transcriptome data across different workflows, different users and a wide range of cell inputs.

The BD Rhapsody WTA Amplification kit allows you to identify genes of interest that can be used for building your targeted panels. Customizable targeted panel creation makes it easy to focus on the genes that really matter to your research. With the BD® Single-Cell Multiplexing kit (SMK) and the BD Rhapsody WTA Amplification kit working seamlessly together, you can save time and money by running multiple samples at once, giving you the option of multiplexing without compromising the quality of your data. With the BD Rhapsody WTA Amplification kit the power to uncover the whole transcriptome of a single cell is at your fingertips.

Figure 1

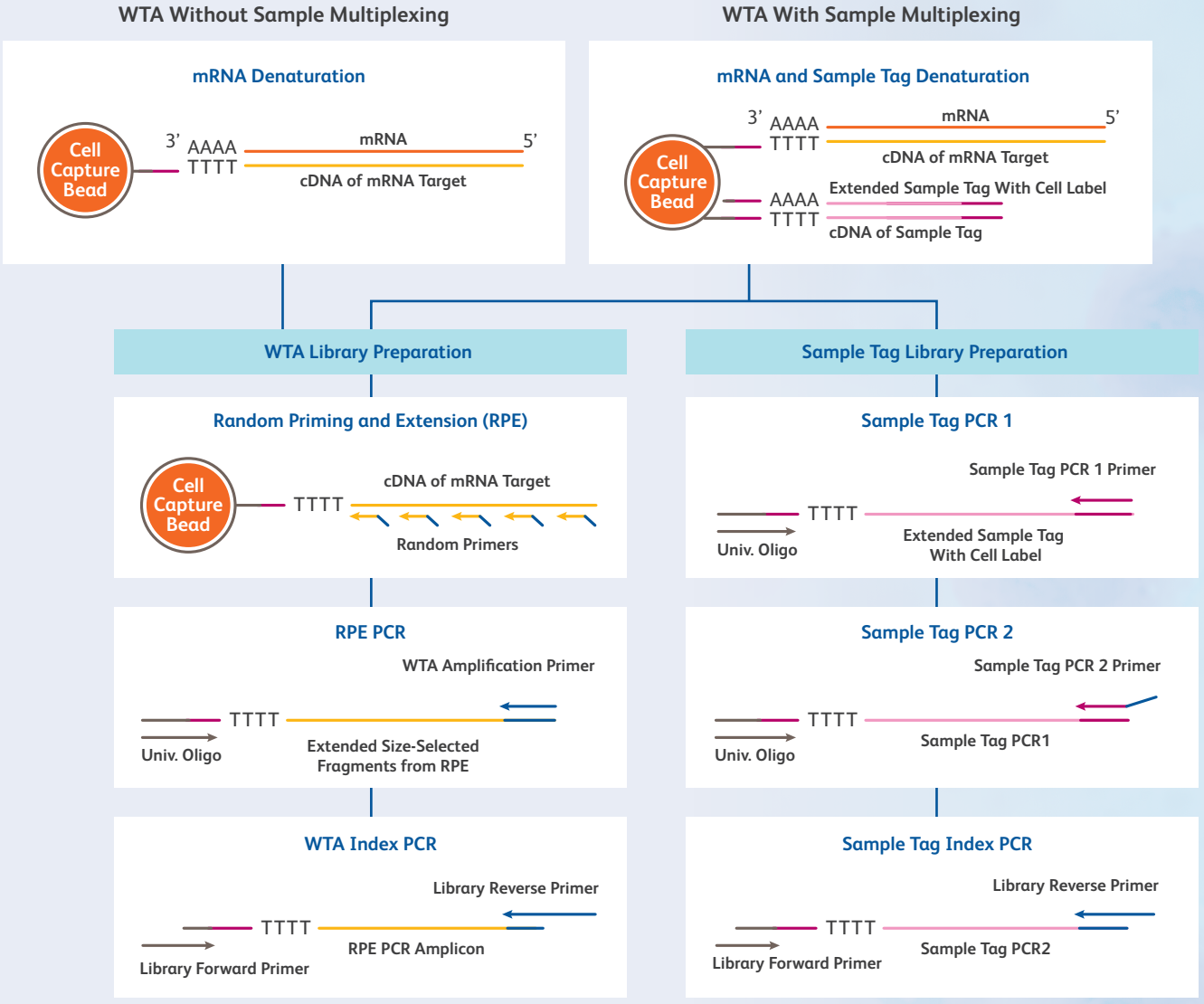


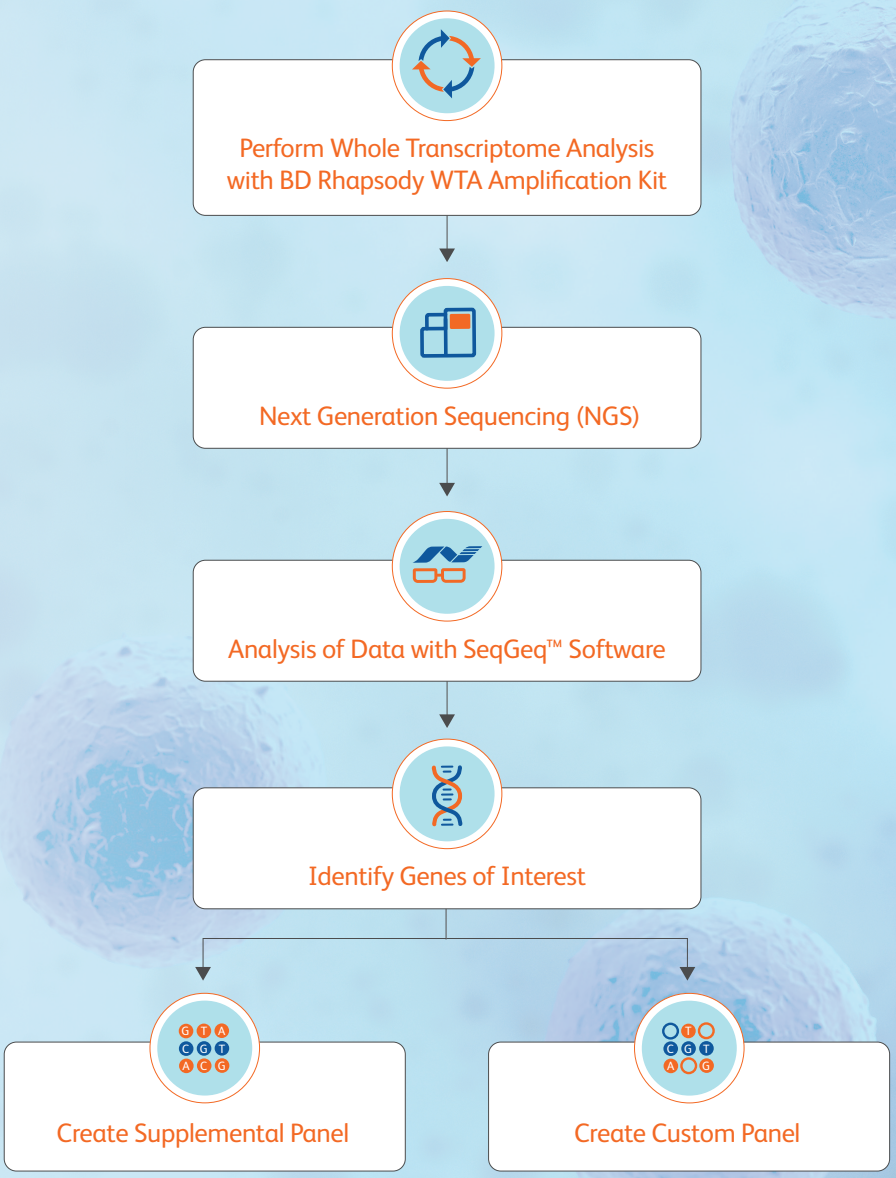
Figure 1. Overview of BD Rhapsody WTA Amplification kit library preparation workflow (with and without SMK)
This workflow is performed following optional Sample Tag staining, single cell isolation, lysis and barcoding using the BD Rhapsody Single-Cell Analysis System. mRNA and Sample Tag oligos are separated from Cell Capture Beads through heat denaturation. Afterwards, whole transcriptome libraries are generated using random primers, followed by amplification and addition of sequencing adapters. If Sample Multiplexing was performed, Sample Tag libraries are generated in parallel with the WTA library from Sample Tag oligos isolated during heat denaturation, using a series of Sample Tag-specific PCRs.

Build targeted panels for analysis

The WTA assay allows you to identify the most differentially-expressed genes in your samples. Once identified, the genes can be used to construct a targeted panel. With the targeted approach you can eliminate unnecessary excessive sequencing reads and obtain increased sensitivity in subsequent sequencing runs on the genes that are important to your research. Using the genes identified with the BD Rhapsody WTA Amplification kit you can create a fully custom panel with up to 500 targets or add up to 100 additional targets to one of BD's pre-designed panels to create a supplemental panel that fits the needs of your experiment. BD's commercial targeted panels include:

- Onco-BC panel *(human)* for breast cancer oncology research
- Immune response panels *(human or mouse)*
- T-cell panel *(human)*

Figure 2



Together, with BD, you can get the panels you need to drive your research forward.

Figure 2. Workflow for building targeted panels
From the demultiplexed sequencing data, discover differentially expressed genes of interest in your sample using SeqGeq v1.5+. To validate the genes you identified from the WTA analyses, use the targeted panels (supplemental or custom) available from BD. Targeted panels help lower sequencing costs and enable you to validate your genes of interest in a cost-effective manner.

Trust your WTA data

The ability of an assay to generate reproducible results across multiple users is critical for generating reliable, high-quality scientific data. The BD Rhapsody WTA Amplification kit generates consistent data across multiple users and between individual runs from a single user. With the BD Rhapsody WTA Amplification kit you can have high confidence in your results.

Figure 3A

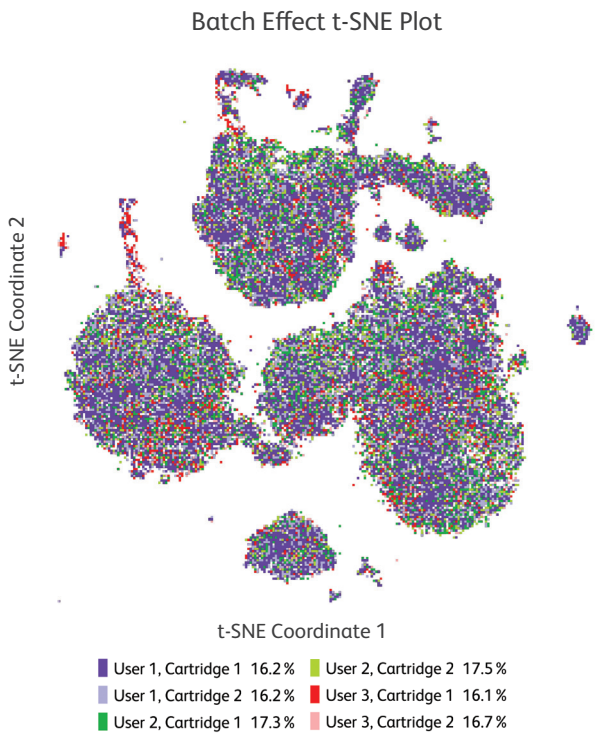


Figure 3B

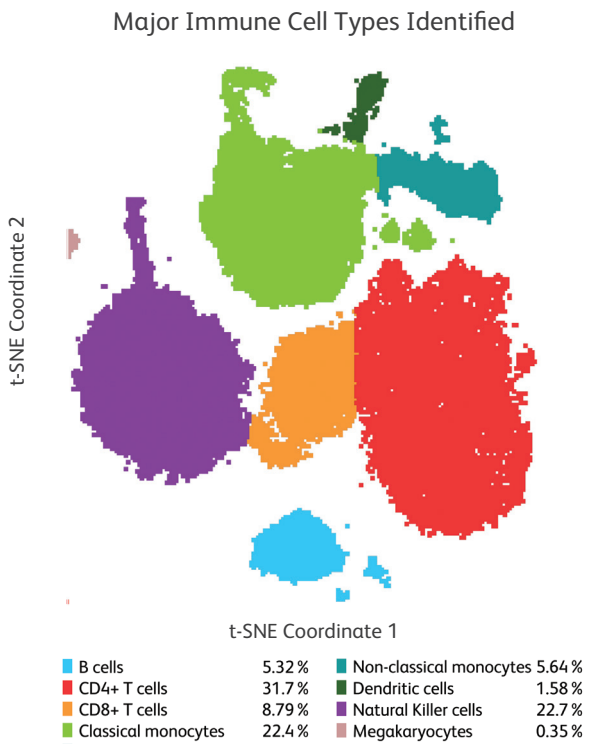


Figure 3C

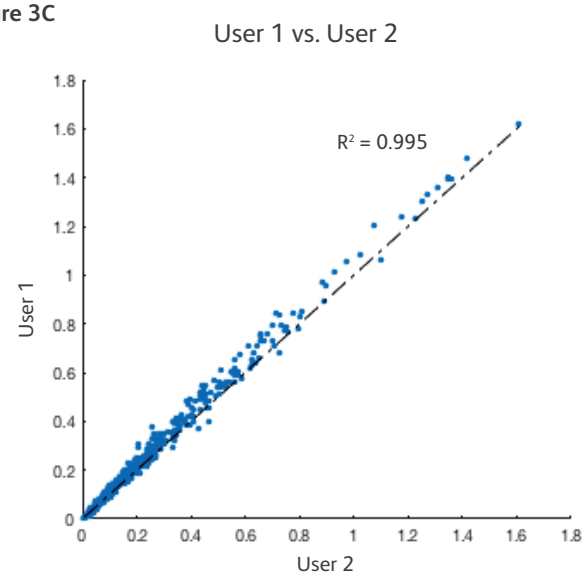


Figure 3D

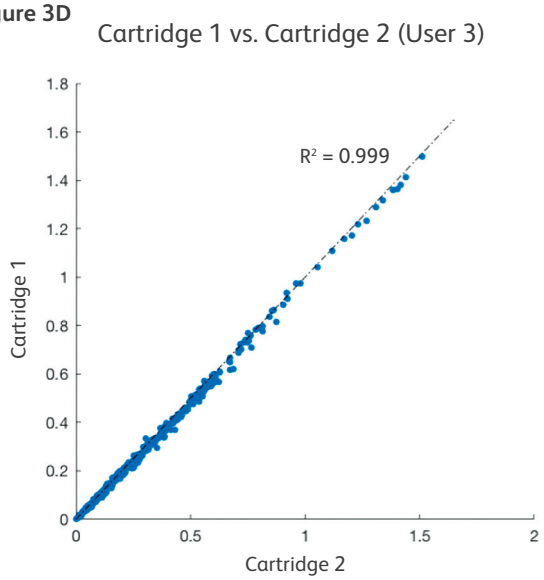


Figure 3. Sequencing of peripheral blood mononuclear cells by 3 users

Three different users processed peripheral blood mononuclear cells (PBMCs) from the same donor on two separate cartridges. Each user ran ~10,000 PBMCs per cartridge on the same day. WTA libraries were prepared from the individual cartridges and sequenced at an equivalent depth. **A.** t-Distributed Stochastic Neighbor Embedding (t-SNE) plot demonstrating an absence of batch effects between different replicates run by different users, suggesting high reproducibility of the WTA assay. **B.** t-SNE plot showing the major immune cell types identified with the BD Rhapsody WTA Amplification kit. **C.** Scatter plot showing high correlation of average gene expression levels across all genes between replicates run by different users on a log10 scale. **D.** Scatter plot showing high correlation of average gene expression levels across all genes between replicates run by the same user on a log10 scale.

Figure 4A

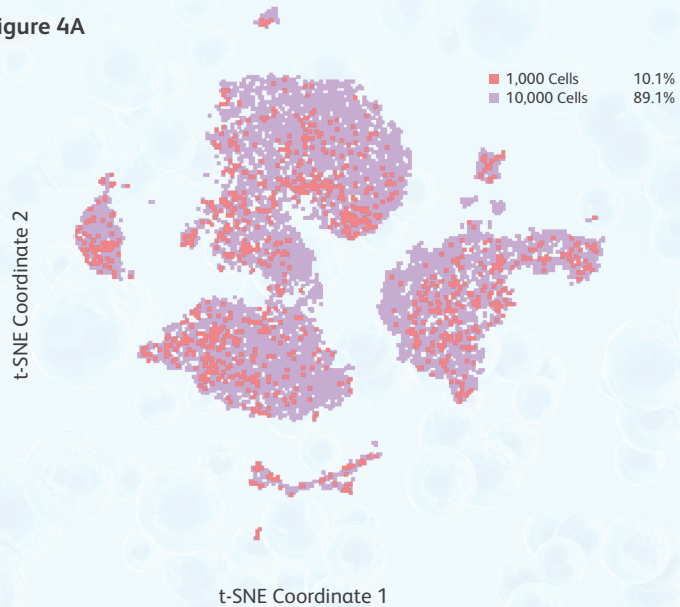


Figure 4B

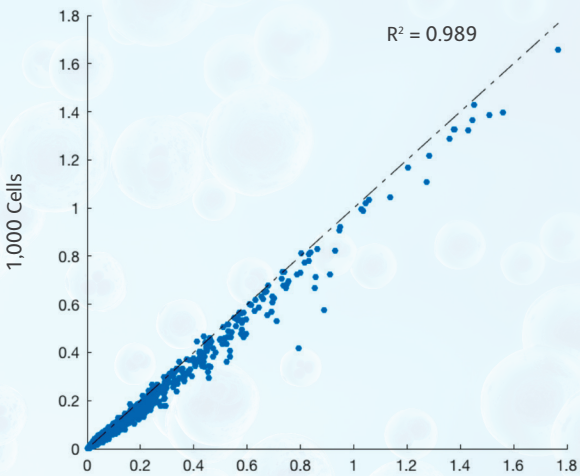


Figure 4C

Cell Input	Sequencing Reads Per Cell	Sequencing Saturation	Median Transcripts Per Cell	Median Genes Per Cell	Q30
1,000	7,801.98	77.92%	1393	667	85.66%
10,000	7,693.64	65.18%	1718	791	87.96%

Figure 4. Comparison of 1,000 cell and 10,000 cell inputs
1,000 and 10,000 PBMCs from the same donor were run on separate BD Rhapsody cartridges in parallel. Single cell WTA RNA sequencing libraries were prepared using the BD Rhapsody WTA Amplification kit and sequenced at an equivalent depth. **A.** t-SNE analysis reveals similar alignment of the different cell groups identified between the 1,000 and 10,000 cell inputs. **B.** Correlating the results between 1,000 and 10,000 PBMCs yielded almost identical results with R2 = 0.989, further establishing the consistency of the assay across different cell input levels. **C.** The table includes different metrics analyzed for the WTA assay between the 1,000 and 10,000 cell input experiments.

Generate consistent results across different cell inputs

The ability of an assay to generate consistent results across different cell inputs or different sample types that have varying cell numbers is critical. The BD Rhapsody WTA Amplification kit produces consistent data whether you're testing 1,000 cells or 10,000 cells.

Process different samples together in a single WTA experiment

Mixing multiple samples and processing them together in a single experiment is cost effective and saves time, but this requires the ability to mix and process the samples together while also ensuring that the samples can be reliably separated during data analysis. The ability to multiplex samples is extremely useful for assays such as WTA that often involve long processing times. The BD Rhapsody WTA Amplification kit works with the BD Human Single-Cell Multiplexing kit to enable easy tagging and identification of different cell types in a sample.

Figure 5A

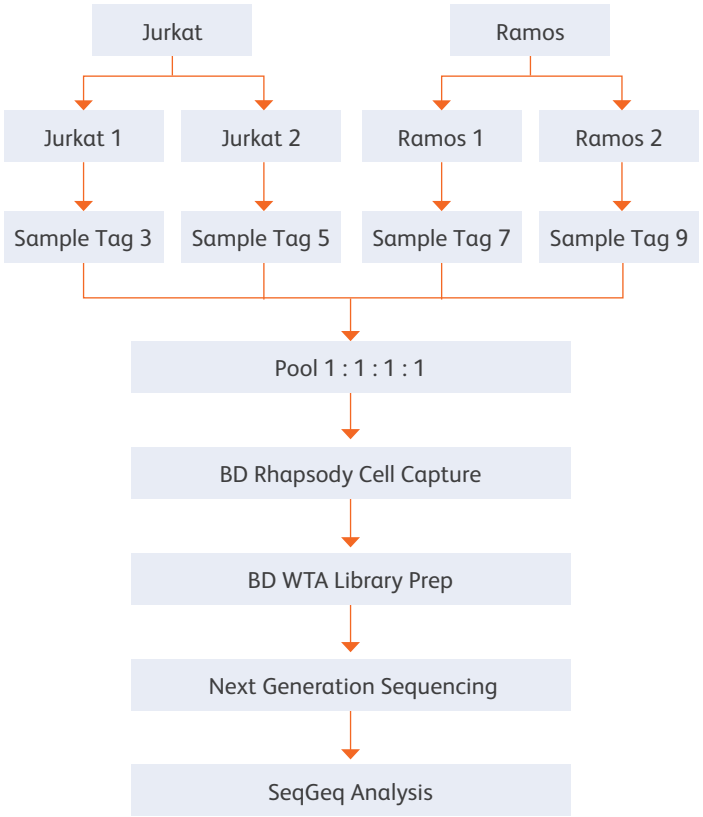


Figure 5B

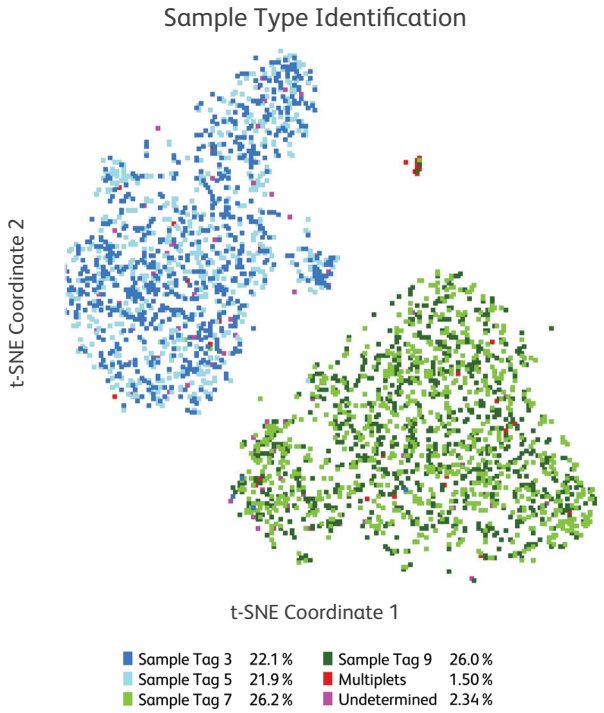
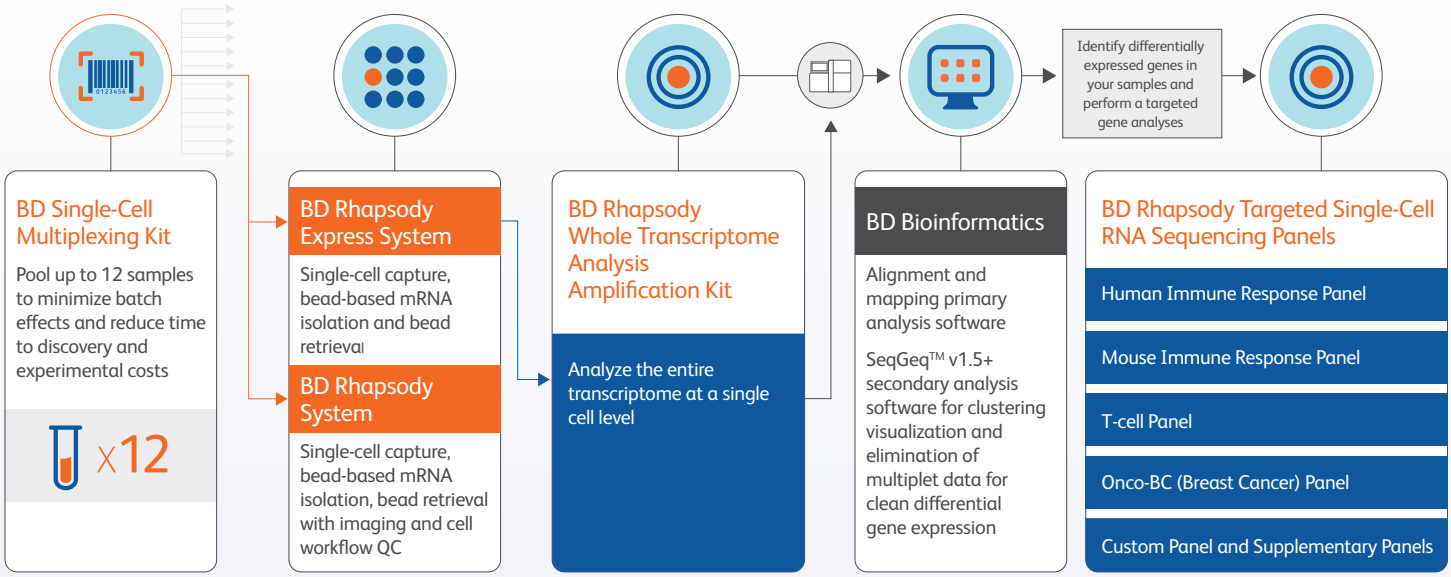


Figure 5. Tagging and processing different cell lines together
A. Human cell lines, Jurkat and Ramos were labeled with four unique Sample Tags from the BD Human Single Cell Multiplexing Kit. The four labeled samples were then mixed at an equal ratio (1: 1: 1: 1) pooled, processed using the BD Rhapsody Single-Cell Analysis System and libraries were generated using the BD Rhapsody WTA Amplification kit. **B.** t-SNE plot analyses of the above experiment revealed that Jurkat cells (blue) with Sample Tags 3, 5 clustered together and distinct from Ramos cells (green) tagged with Sample Tags 7, 9. The sensitivity (measure of cells with correct Sample Tag called) and specificity (measure of cell types with the expected Sample Tag) was >98% in this experiment.

The BD Rhapsody WTA Amplification kit is part of BD's broad portfolio of reagents, instruments and software created to take your research further every step of the way.



Starting with the BD Single-Cell Multiplexing kit, you can pool up to 12 different samples together, then isolate the single cells with precision on the BD Rhapsody Single-Cell Analysis System. You can now extract and amplify the whole transcriptome at a single cell level using the BD Rhapsody WTA Amplification kit, and with BD's bioinformatics you can perform the primary analysis of the sequencing data. Dive deeper into your data by using SeqGeq™ software and see just how easy single cell RNA sequencing can be. With the single cell multiomics workflow from BD, users can multiplex, run, amplify and analyze samples, with the same trusted partner, from start to finish.

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