# Flexible and high-throughput approach for simultaneously capturing a large number of single cells using microwell-based technology

**Poster#**
P1028

Rosary Nguyen, Zorine Hlathu, Chelsea Gordon, Xueying Zhao, Jamie Moskwa, Devon Jensen, Ricelle A. Acob, Larry Wang, Aruna Ayer
*BD Biosciences, 2350 Qume Drive, San Jose, CA 95131*

## Abstract

Single-cell RNA-seq is a powerful method for studying complex cell populations. As opportunities grow for single-cell multiomics methods in translational research, there is an increased need for high-throughput approaches capable of efficiently capturing and analyzing large numbers of cells simultaneously. Current assays on scRNA-seq platforms have limited throughput due to the low cell recovery and high multiplet rates with high cell inputs.
Here we demonstrate the feasibility of profiling the single-cell gene expression of hundreds of thousands of cells in a single experiment using an eight-lane microwell-based cartridge. We also increased the cell label diversity of cell capture beads to enable high cell loading with a lower rate of barcode collisions. To show the range of cell capture capabilities of the eight-lane microwell cartridge, we loaded different numbers of cells per lane ranging from 100 to over 40,000 cells. To evaluate the multiplet rates and sensitivity, we labeled two different cell types with antibody-based sample multiplexing prior to cell capture. Cell multiplet rates were also assessed through Sample Tag analysis. Detection of Sample Tags used showed no contamination of Sample Tag between samples. In addition, less than 0.5% barcode collision rate was detected from 360,000 viable cells captured with a bead. Targeted mRNA profiling had high correlation ($R^2 > 0.95$) of gene expression across cell capture lanes. This study demonstrates that the eight-lane microwell-based cartridge technology provides flexibility and high-throughput cell capture of thousands of cells and could be readily extended to other existing single-cell technologies such as surface protein marker detection and immune profiling.

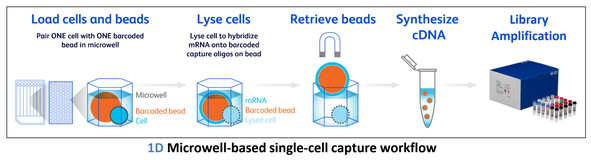
## Methods

**Sample Tag cell staining, capture in microwells and mRNA Targeted and Sample Tag Workflow**

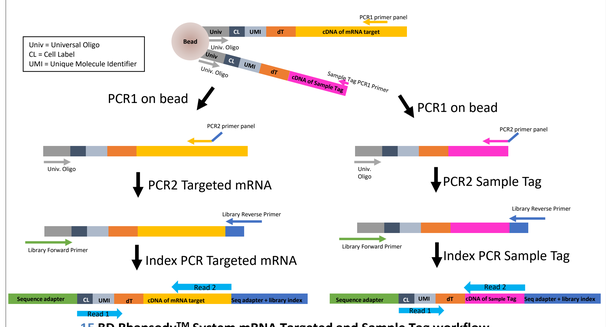

1A BD® Single-Cell Multiplexing Kit (SMK)

1B BD Rhapsody™ Scanner and BD Rhapsody™ HT Xpress System

1C BD Rhapsody™ 8-Lane Cartridge




1D Microwell-based single-cell capture workflow

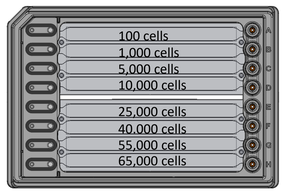



1E BD Rhapsody™ System mRNA Targeted and Sample Tag workflow

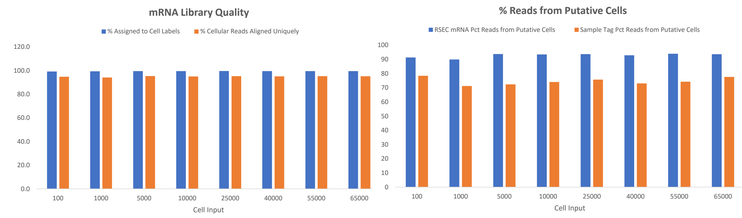
**Figure 1.** (A) BD® Single-Cell Multiplexing Kit (SMK) can be used to multiplex different cell samples to provide higher sample throughput for a single-cell library preparation. After sequencing, samples can be demultiplexed bioinformatically. Cell samples are stained with Sample Tag prior to loading into the microwell cartridge. (B) The BD Rhapsody™ Scanner and BD Rhapsody™ HT Xpress System are used together for single-cell capture. The BD Rhapsody™ Scanner informs users about the quality of their cell capture experiment prior to library preparation. The BD Rhapsody™ HT Xpress System can be used independently of the BD Rhapsody™ Scanner and is a platform for the microwell-cartridge workflow. (C) The BD Rhapsody™ 8-Lane Cartridge is used for single-cell capture. (D) Sample Tag stained Jurkat and Ramos cells are loaded into the 8-lane cartridge on the BD Rhapsody™ HT Xpress System, where one cell is paired with one bead. The cells are lysed, where the cellular mRNA is captured onto the beads and the beads retrieved from the cartridge. cDNA is synthesized, followed by library preparation, sequencing and data analysis. (E) BD Rhapsody™ System mRNA Targeted and Sample Tag protocol workflow to prepare libraries for sequencing using the BD Rhapsody™ Human Immune Response Panel (399 gene panel) and Sample Tag for library amplification.

## Range of cell capture capabilities of eight-lane microwell cartridge

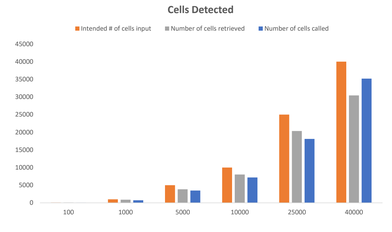
**2A Microwell cartridge loaded with Sample Tag stained Jurkat/Ramos cells**



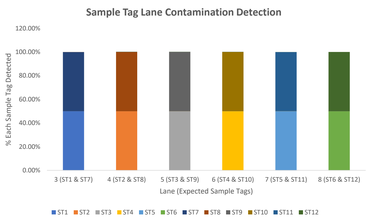

**2B Library quality is similar across cell inputs**



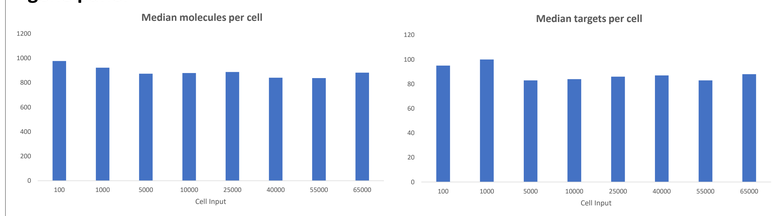

**2C Expected number of cells were recovered**



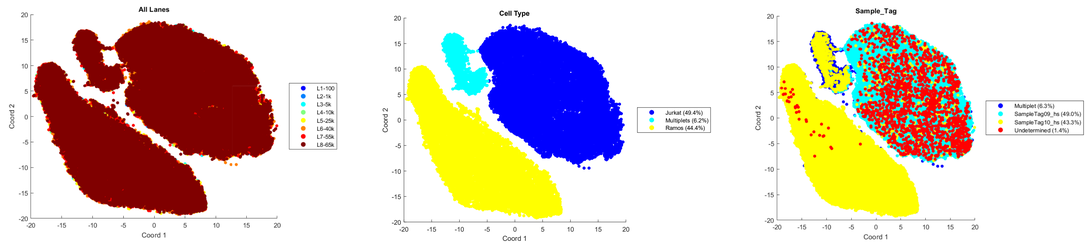

**2D No Sample Tag cross contamination between lanes**




**2E Consistent sensitivity between lanes with Targeted mRNA assay, 399 gene panel**




**2F t-SNE analysis demonstrates strong overlap in cell profiles**




**2G High correlation in gene expression**

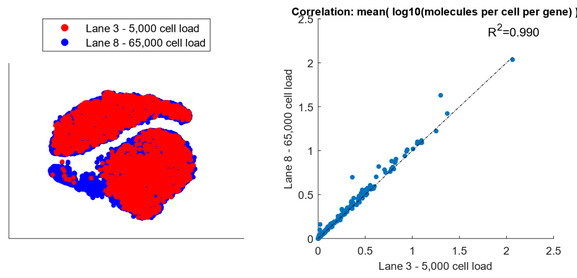
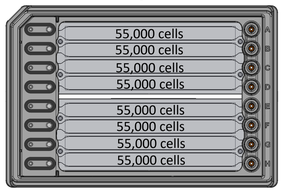


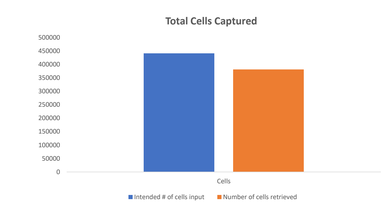

**Figure 2.** (A) Experimental design using eight-lane microwell cartridge loading different number of cells per lane ranging from 100 to over 40,000 cells per lane. Prior to cell sample loading, Jurkat and Ramos cells were stained with Sample Tags. Jurkat and Ramos cells were mixed in a 1:1 ratio prior to loading cells into the microwells. (B) All data from each lane were downsampled to similar total reads per cell. mRNA library quality across all lanes with differing cell input numbers show no significant difference in percent assigned to cell labels and percent cellular reads aligned uniquely to transcriptome. Comparing percent reads from putative cells, it is similar for both mRNA and Sample Tag libraries across different cell inputs. (C) Cell detection is analyzed with the BD Rhapsody™ Scanner to assess cell loss while processing samples. After sequencing, putative cell calling also identifies final yield of cells through library preparation (~80%). (D) Sample Tag cross contamination was found to be less than 1% across all lanes (data taken from similar experiment). (E) Assay sensitivity measured with median molecules and median bioproducts per cell is similar across different cell inputs within 10% for sample inputs greater than 1,000 cells. (F) t-SNE projection of all cell inputs demonstrates strong overlap in the cell profiles identified using mRNA cell calling pipeline. Identification of cell types on t-SNE projection show expected percentages of Jurkat and Ramos cells, in addition to multiplets. t-SNE projection of Sample Tags used to stain Jurkat and Ramos cells are shown with percentages of Sample Tags identified. (G) Comparing 5,000 cell input to 65,000 cell input, there is a high correlation for gene expression. It is expected that with lower cell input the multiplet population will be lower compared to high cell input as demonstrated in the t-SNE projection.

## Sample multiplexing across 8 lanes for high cell input on eight lane microwell cartridge

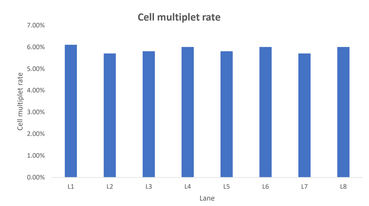
**3A Microwell cartridge loaded with Sample Tag stained Jurkat/Ramos cells**



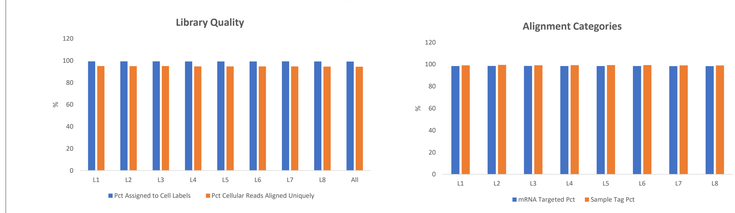

**3B >85% of cells captured on cartridge**



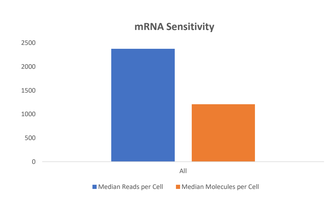

**3C <10% Multiplet rates within each lane**



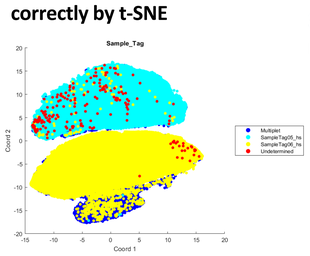

**3D Similar library quality for all eight lanes**




**3E Targeted mRNA sensitivity**




**3F Cell types identified correctly by t-SNE**




**3G Low cell barcode collision rate with 250,000 cells**

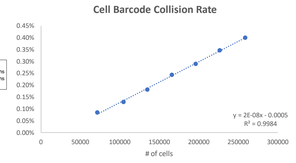



**Figure 3.** (A) Experimental design using eight-lane microwell cartridge loading the same number of cells across all lanes aiming for 440,000 of 1:1 mixture of Sample Tag stained Jurkat and Ramos cells. (B) 440,000 cells were intended for this experiment, after cell capture and retrieval on the BD Rhapsody™ HT Xpress System, approximately 380,000 cells were collected. Eighty-six percent of the intended cells were retained. (C) Cell multiplet rates (on average 6%) within lanes were determined from the BD Rhapsody™ Scanner detection of cell multiplets in microwells. (D) Data analysis was performed looking at individual lanes in addition to bioinformatically combining all FASTQ files to analyze all cells together. Library quality is similar across all eight individual lanes and combined lanes for both % assigned to cell labels and % cellular reads aligned uniquely to transcriptome. Alignment to mRNA and Sample Tag libraries are similar for all individual lanes and combined lanes. (E) mRNA sensitivity for combined lanes analysis is comparable to what is seen for individual lanes (see Figure 2E) . (F) Identification of Sample Tags on t-SNE plots demonstrate the expected Sample Tags and populations. (G) Cell barcode collision contributed less than 0.5% to the overall multiplet rate.

## Conclusions

- Eight-lane microwell-based cartridge technology (BD Rhapsody™ HT Xpress System) used for this set of experiments shows feasibility for flexibility and high-throughput cell capture ranging from 100 to over 40,000 cells per lane with capability to combine all library FASTQs into one bioinformatic analysis for higher cell throughput. The cell recovery at varying cell inputs is effective, which can be useful for low abundance samples.
- Cell multiplets were detected with the BD Rhapsody™ Scanner showing less than 6% multiplets when assessing individual wells. The barcode collision multiplet rate for combined FASTQs for 250,000 cells is <0.5%.
- Sensitivity was similar across all individual lanes with no contamination of Sample Tags between samples or lanes. Targeted mRNA profiling showed high correlation $R^2 > 0.95$ of gene expression across all capture lanes.
- This eight-lane microwell-based cartridge can be readily extended to other existing single-cell technologies such as protein marker surface detection and other immune profiling to efficiently capture and analyze varying cell inputs and types.