Stability and performance of a partially used 8-lane microwell cartridge on single-cell capture and immune response profiling

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Abstract

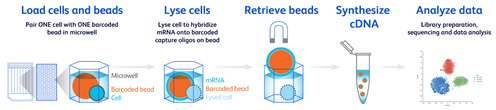
In this study, we introduce an innovative microwell-based approach, offering the capability to capture up to 500,000 single cells and show stability of a partially used 8-lane microwell cartridge up to 6 months after first use. Stability of microwell cartridges used for single-cell capture and the integrity of the individual lanes are crucial factors in genomics research to ensure the accuracy and reliability of generated data. Stability is paramount to ensure consistent results over multiple experiments. High-quality materials and manufacturing processes are essential to prevent wear and tear, ensuring that the microwell cartridges function optimally for an extended period. Integrity between the individual lanes must also be intact to prevent cross-contamination between samples.

To demonstrate partial use stability of the 8-lane microwell cartridge, multiple assay combinations were performed on different lanes of the cartridge, collected at different time points. Whole transcriptome amplification (WTA) together with AbSeq Antibody-Oligonucleotide Conjugates for cellular indexing of transcriptomes and epitopes (CITE-Seq) was performed on peripheral blood mononuclear cells (PBMC), loaded and captured in Lanes 1 and 2, on Day 1; sample multiplexing was used with targeted mRNA assay on Jurkat and Ramos cells, loaded and captured in Lanes 3 and 4, after four months; and WTA together with the AbSeq Assay was performed on PBMC, loaded and captured in Lanes 5 and 6, after six months. The different time points collected were compared to a single-lane microwell cartridge. Our data showed that assay performance was not compromised with the storage of a partially used cartridge, and there was no contamination detected between the lanes. High-throughput cell load and capture using WTA and the AbSeq Assay also showed that each lane of the 8-lane microwell cartridge was able to process up to 65,000 cells with no limit of detection on assay sensitivity.

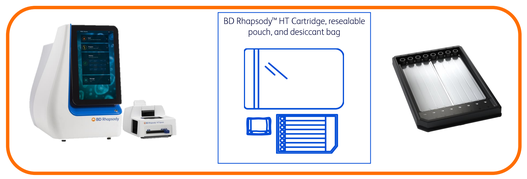
By maintaining cartridge stability and stringent measures to prevent contamination, researchers can ensure the reliability and accuracy of their data. This technological breakthrough revolutionizes our ability to profile cellular immune responses comprehensively and achieve a substantial increase in single-cell throughput on the BD Rhapsody™ System.

Methods

1A



1B



1C

Timepoint	Lanes	Assay	Sample
Day 1	1 and 2	WTA + Abseq	PBMCs
Four months	3 and 4	Targeted + SMK	Jurkat & Ramos
Six months	5 and 6	WTA + Abseq	PBMCs

Figure 1. **(A)** BD Rhapsody™ microwell-based single-cell capture showing the cell capture process and library preparation. **(B)** BD Rhapsody™ HT Single-Cell Analysis System and the BD Rhapsody™ 8-Lane Cartridge showing storage of a partial-used cartridge in a resealable pouch and desiccant bag. **(C)** Lanes 1 and 2 were loaded with 20,000 cells from a single PBMC donor on Day 1, Lanes 3 and 4 were loaded with 20,000 cells consisting of Jurkat and Ramos cells at 1:1 ratio after four months storage, and Lanes 5 and 6 were loaded with 20,000 cells from a single PBMC donor after six months storage of the partially used cartridge. 3,000 cells were subsampled for library preparation using WTA+Abseq assay on Day 1, Targeted and SMK libraries were prepared from four months, and WTA+Abseq libraries were prepared from six months. Performance of the libraries were compared to a BD Rhapsody™ Single Lane Cartridge capture and library preparation. In addition, cell capture up to 100,000 Jurkat and Ramos was performed and Targeted+ST libraries were generated. WTA+Abseq assay was also performed on the 65,000 cell capture to determine the robustness of the BD Rhapsody™ assay conditions.

Flexibility with partial use of the BD Rhapsody™ 8-Lane Cartridge

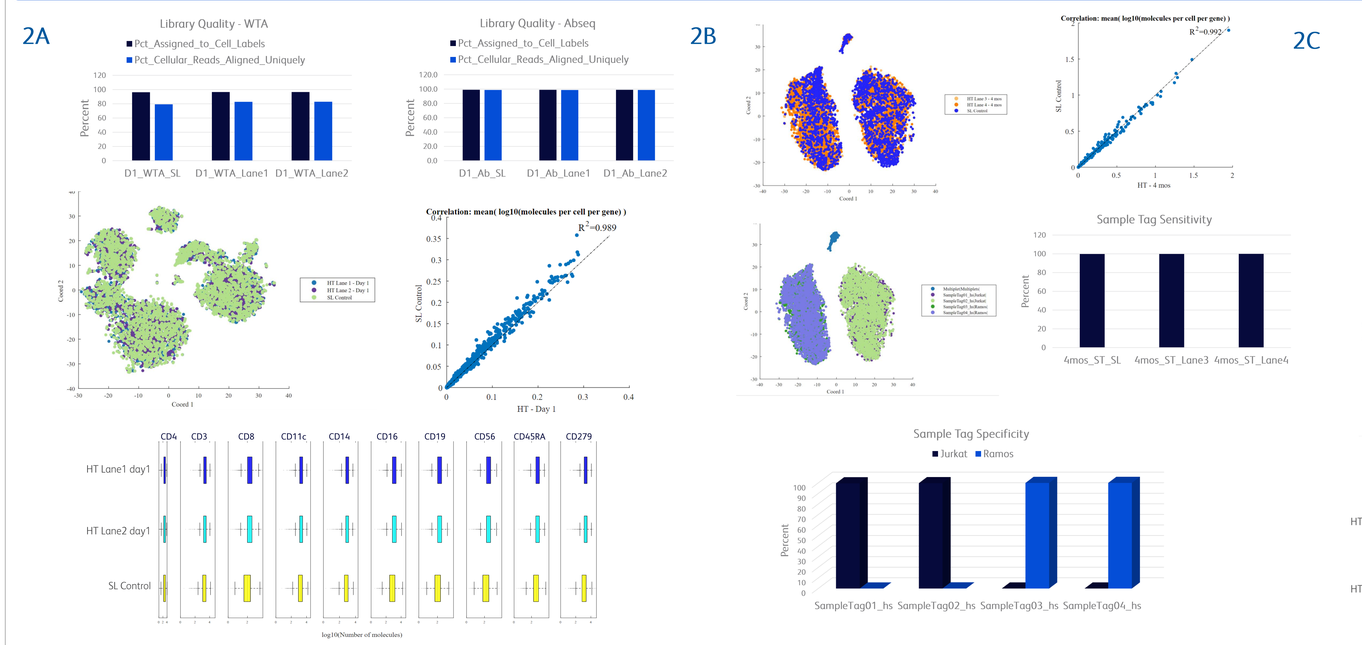


Figure 2. **(A)** WTA and Abseq (10-plex) libraries had similar library qualities between Lanes 1 and 2 from the 8-lane cartridge compared to the Single-Lane cartridge. No batch effect from the t-SNE plot was observed and correlation of gene expression was R^2=0.989. Abseq marker detection was also comparable. **(B)** No batch effect from t-SNE from Targeted mRNA and Sample Tag libraries was also observed from samples captured in Lanes 3 and 4 after 4-months partial-use storage. Correlation of gene expression compared to the Single-Lane cartridge was R^2=0.992 and Sample Tag sensitivity and specificity were >95%. **(C)** Library performance after 6-months storage was also comparable to the Single-Lane cartridge. WTA library quality and putative cell detection was not affected by the long-term storage. No batch effect from the t-SNE plot, with correlation of gene expression at R^2=0.999. Abseq library performance was also not affected by partial-use storage.

No sample contamination between lanes

3

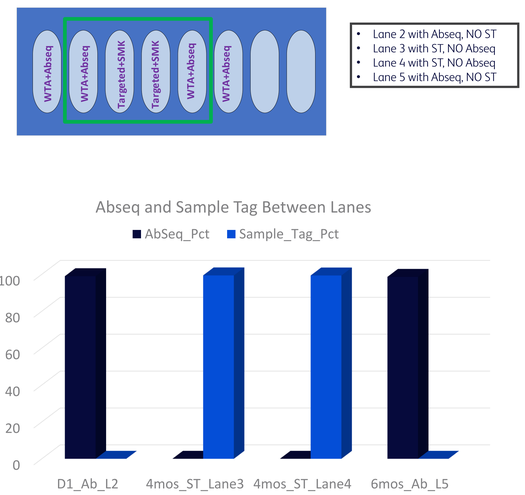


Figure 3. Absence of Abseq or Sample Tag signal in lanes where the assay was not performed showed that the performance of a partially-stored BD Rhapsody™ 8-Lane Cartridge was not compromised up to six months. Percent reads aligned from raw sequencing reads was used to determine presence or absence of signal.

Cell capture and assay performance

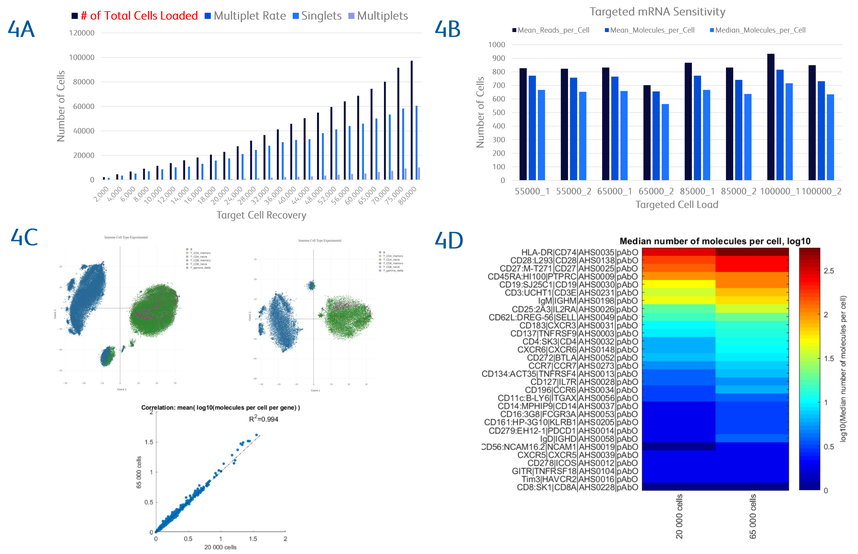


Figure 4. **(A)** Jurkat and Ramos cells were captured in increasing number of cells showing a multiplet rate of ~15% with 80,000 target capture. **(B)** Targeted assay performance was comparable up to 100,000 cell capture. Dataset was represented from two lanes on the BD Rhapsody™ loaded with the same number of cells. **(C)** No batch effect was observed between 20,000 and 65,000 cells where WTA+Abseq libraries were generated. Correlation of gene expression was R^2=0.994. **(D)** Abseq assay performance and marker detection was comparable up to 65,000 cell capture target showing the robustness of the BD Rhapsody™ assay reaction conditions.

Conclusions

- Cells such as Jurkat, Ramos and PBMCs were successfully captured using a partially-used BD Rhapsody™ 8-Lane cartridge up to six months of storage.
- The number of cells loaded and recovered were comparable between the partially-used and stored BD Rhapsody™ 8-Lane and Single-Lane cartridges.
- Similar number of molecules and targets per cell from the WTA assay were detected using a partially-used and stored BD Rhapsody™ 8-Lane and Single-Lane cartridges.
- No batch effect was observed on the different assay combinations tested, and correlation of gene expression was R^2 = >0.98.
- Detection of expressed genes from cells captured showed dependable capture and profiling of cells on the BD Rhapsody™ HT Single-Cell Analysis System.
- Minimal lane-to-lane variability was observed on the 8-lane cartridge and performance was not impacted by partial-use storage.