Flexible and high-throughput microwell-based single-cell capture for multiomic ATAC-seq and RNA-seq profiling

Poster# 334

Rosary Nguyen[1], Hongduan Huang[1], Quyen Bao[1], Punya Narayan[1], Hye-Won Song[2], Elham Hatami[2], Zhiqi Zhang[2], Thomas McCarthy[1], Youngsook Kim[1], Ruifang Li[1], Chelsea Gordon[1], Larry Wang[1], and Aruna Ayer[1].

[1]BD Biosciences, 2350 Qume Drive, San Jose, CA 95131; [2]BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121

Abstract

The simultaneous profiling of chromatin accessibility and gene expression is an active area of interest for researchers studying how epigenetic changes regulate gene expression at the single-cell level. Here, we demonstrate the feasibility of single nuclei assay for transposase-accessible chromatin (snATAC) seq either with or without simultaneous gene expression profiling using the microwell based BD Rhapsody™ HT Xpress System. Taking advantage of an eight-lane microwell cartridge, the BD Rhapsody™ HT Xpress System can support the snATAC seq assay with nuclei input ranging from 1,000 to 400,000 nuclei in a single experiment.

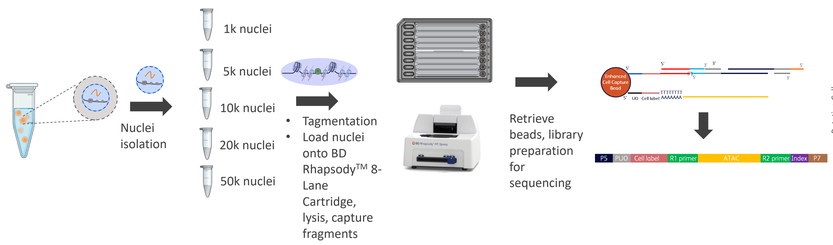
To demonstrate the flexible throughput capabilities, 1,000 to 50,000 nuclei from cryopreserved peripheral blood mononuclear cells (PBMCs) were tagmented and loaded onto a single lane of the BD Rhapsody™ HT Xpress System. The snATAC assay libraries were prepared and sequenced, revealing multiple high-quality ATAC-seq metrics: high counts of unique ATAC fragments, high fraction of reads in peak regions (FRiP), and high transcriptional start site (TSS) enrichment score. The data were obtained consistently across the wide range of input nuclei with use of the gentle and robust nuclei loading capabilities of the BD Rhapsody™ HT Xpress System. Moreover, after capturing ATAC fragments, the BD Rhapsody™ Enhanced Cell Capture Beads are stable and can be stored at 4 °C for up to 3 months, allowing researchers the flexibility in sequencing subsets of nuclei or lanes for QC purposes before sequencing the entire library and returning to samples for complete sequencing.

Furthermore, to demonstrate the consistency of both open chromatin profiling and mRNA gene expression as a combination (multiomic snATAC + snRNAseq), firstly, we compared snRNAseq data from a whole transcriptome assay (WTA) with snRNAseq from an ATAC+mRNA multiomic assay using the same sample. This strategy enabled us to show that there is negligible gene expression differences between snRNAseq from the BD whole transcriptome assay and snRNAseq from the multiomic scATAC and scRNA assay. Likewise, the snATACseq assay and snATACseq assay with simultaneous whole transcriptome profiling shows that chromatin accessibility profiling is equivalent with and without gene expression profiling.

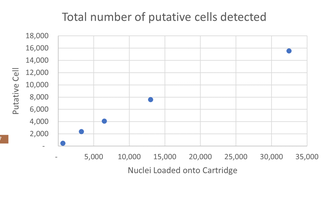
Enabling this new scATAC assay on the BD Rhapsody™ HT Xpress System can advance the study of epigenetic regulation of gene expression with a robust and flexible workflow. This will allow researchers to analyze both open chromatin and mRNA at varying nuclei inputs with single-cell resolution to understand the underlying molecular mechanisms in gene expression regulatory pathways. This assay can be readily extended to broader samples to gain greater understanding of disease states in immunology and immuno-oncology studies.

Robust nuclei loading capabilities on the BD Rhapsody™ 8-Lane Cartridge

2A snATAC Seq assay with nuclei input from 1,000 to 50,000 nuclei



2B Putative cells detected



2C Similar ATAC-seq sequencing metrics and sensitivity seen across different tagmentation nuclei input

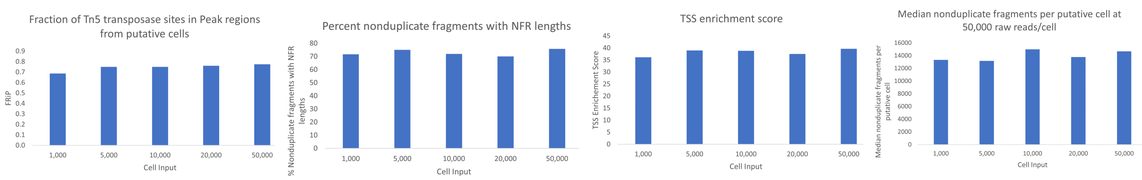
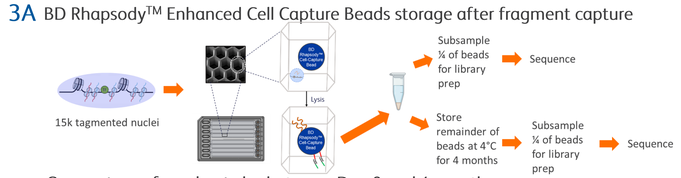


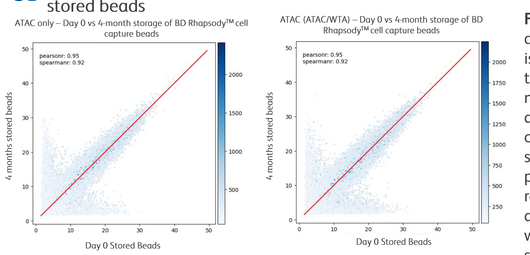
Figure 2 (A) Experimental design where nuclei were isolated from PBMCs, and 1,000 to 50,000 nuclei were tagmented separately. After tagmentation, all tagmented nuclei were loaded into the micro-well cartridge in separate lanes. After lysis, BD Rhapsody™ cell capture beads were retrieved, and library preparation was performed to be sequenced. (B) The expected number of cells were detected in sequencing from starting with 1,000 to 50,000 nuclei showing robustness of the BD Rhapsody™ 8-Lane Cartridge to handle low to high cell input. (C) After sequencing, all libraries were downsampled to 50,000 raw reads per cell. Across different cell inputs, the fraction of Tn5 transposase in peak regions from putative cells (FRiP score) and percent nonduplicate fragments with NFR regions are similar. Likewise, for TSS (Transcription Start Site) enrichment score – a calculation for signal to noise is similar for all cell inputs. Lastly, median nonduplicate fragments per putative cell is within 10% for all nuclei input with use of the micro-well cartridge BD Rhapsody™ 8-Lane Cartridge.

Stored subsampled BD Rhapsody™ Enhanced Cell Capture Beads are stable for 4 months

3A BD Rhapsody™ Enhanced Cell Capture Beads storage after fragment capture



3B Comparison of overlap in bp between Day 0 and 4-month stored beads



3C Similar ATAC-seq sensitivity after 4-month bead storage

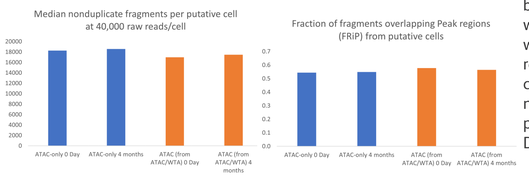
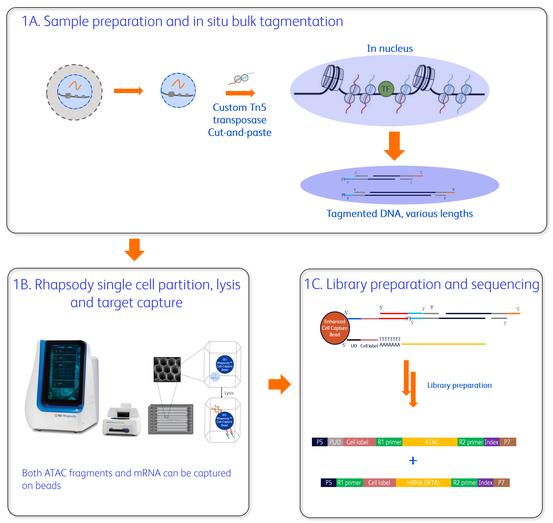


Figure 3 (A) Experimental design where nuclei were isolated from K562 cell lines, tagmented and loaded into the micro-well cartridge. After lysis and bead retrieval, beads with captured ATAC fragments were subsampled and libraries prepped for sequencing. The remaining beads were stored at 4°C for 4 months, after which additional beads were subsampled and library preparation was done. (B) Comparing Day 0 vs 4-month beads, similar overlap in bp was observed. (C) All libraries were downsampled to 40,000 raw reads per cell, where comparable median nonduplicate fragments per putative cell observed between Day 0 and 4-month beads.

Methods



Workflow of scATAC-seq on BD Rhapsody™ HT Xpress System

Figure 1. **(A)** After nuclei isolation from cell, nuclei are tagmented with custom Tn5 transposase. During this tagmentation, the genomic regions accessible are cut and adapter sequences (loaded onto the Tn5) are attached at the end of each of these cut fragments. **(B)** Tagmented nuclei are loaded into a BD Rhapsody™ 8-Lane Cartridge to be partition into individual wells with BD Rhapsody™ Enhanced Cell Capture Beads. Both ATAC and mRNA fragments are captured on beads. **(C)** Separate WTA and ATAC libraries are generated from the retrieved beads and are sequenced.

Consistent chromatin profiling and mRNA gene expression across different assay combinations

4A Experimental workflow to compare ATAC only and WTA only to WTA/ATAC combination

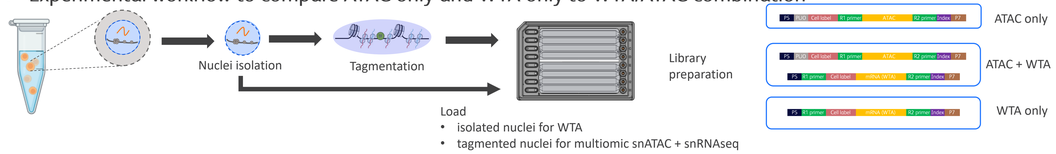
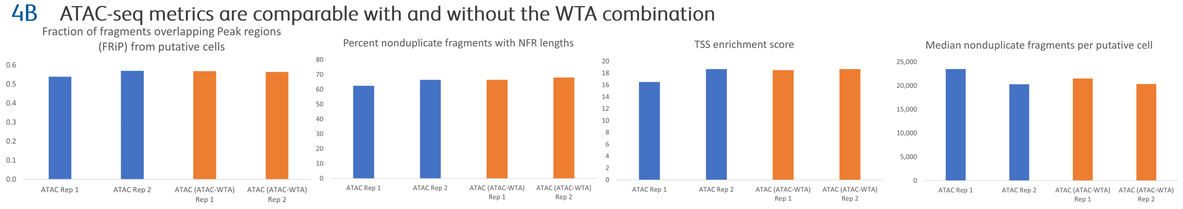
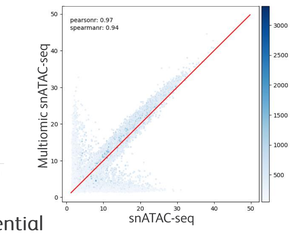


Figure 4 (A) Experimental design where K562 nuclei is isolated and tagmented then loaded into the microwell cartridge along with isolated nuclei (without tagmentation) in separate lanes. Respective library preparations were done and all ATAC libraries and WTA libraries were sequenced separately. Replicates were conducted for ATAC samples.

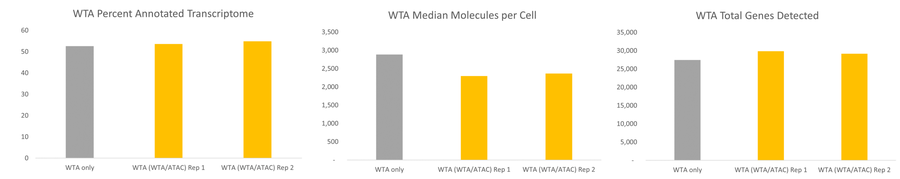
4B ATAC-seq metrics are comparable with and without the WTA combination



4C Overlap bp between snATAC-seq and multiomic ATAC-seq



4D Whole Transcriptome Assay (WTA) metrics are comparable with and without ATAC-seq combination



4E WTA differential expression R^2>0.8

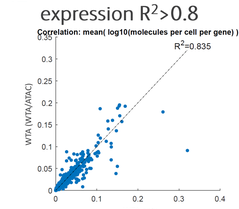


Figure 4 (B) ATAC-seq metrics from ATAC only and ATAC from ATAC/WTA are comparable for Fraction of Tn5 transposase sites in Peak regions from putative cells, percent nonduplicate fragments with NFR lengths, TSS enrichment and Median nonduplicate fragments per putative cell at 50,000 raw reads per cell. (C) Comparing snATAC-seq vs multiomic snATAC-seq show similar overlap in bp. (D) WTA metrics from WTA only and WTA from ATAC/WTA are comparable for percent annotated transcriptome, median molecules per cell and total bioproducts detected. (E) The differential expression R^2 between WTA only and WTA from ATAC/WTA is >0.8.

Conclusions

- The BD Rhapsody™ 8-Lane Cartridge can capture 1,000 to 50,000 tagmented nuclei per lane, allowing users flexibility for nuclei input and effective nuclei recovery, which can be useful for low abundance samples. Furthermore, similar ATAC-seq sequencing metrics are observed across different nuclei inputs.
- The storage of BD Rhapsody™ Enhanced Cell Capture Beads containing ATAC fragments are shown to be stable for up to 4 months without deterioration of ATAC fragments or sequencing outputs, allowing users flexibility for library preparation for sequencing subsets of nuclei for QC checks prior to complete sequencing of all samples.
- Comparison of library combinations for ATAC-seq, snRNAseq (WTA) and WTA/ATAC show negligible gene expression differences for whole transcriptome assay, likewise snATACseq assay from multiomic scATAC and scRNA assay are comparable without compromise of chromatin accessibility profiling with and without gene expression profiling.