Integration of the Element AVITI™ System with BD Rhapsody™ Single-Cell Assays creates a powerful solution for the immuno-oncology researcher

Introduction

Advances in single-cell isolation and barcoding technologies combined with high-quality next-generation sequencing (NGS) offer exceptional opportunities to profile biomolecules at single-cell resolution. The BD Rhapsody™ Single-Cell Analysis System, with its proprietary microwell-based partitioning technology, enables capture and barcoding of thousands of single cells, and the custom library prep protocols enable a multiomic NGS readout with single-cell resolution.

Element Biosciences' benchtop sequencing platform, Element AVITI™ System, is based on its proprietary Avidity Sequencing™ chemistry, which enables a combination of accuracy, low cost and operational efficiency. In this application note, we show compatibility of Element's novel sequencing system with BD Rhapsody™ Single-Cell Assays.

Methods

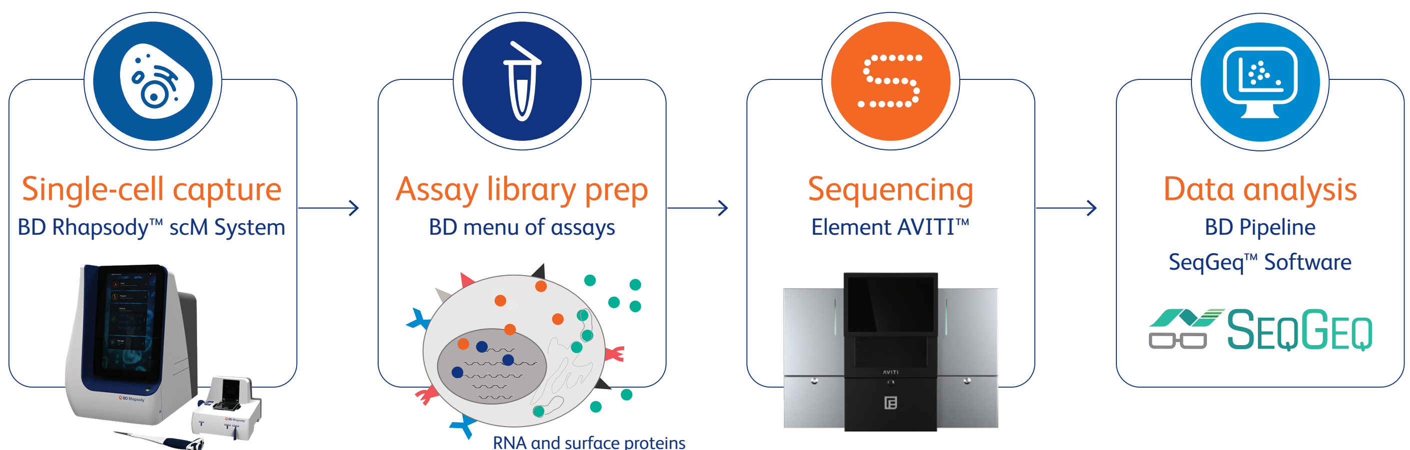


Figure 1. Element AVITI™ sequencing is seamlessly incorporated into the existing BD Rhapsody™ System workflow workflow

To demonstrate platform compatibility, mRNA and surface protein markers were isolated by loading 10,000 and 2,000 AbSeq-stained human PBMCs onto a BD Rhapsody™ Cartridge and using the BD Rhapsody™ System and Enhanced Cartridge Reagent Kit (Cat. No. 664887). Following cDNA synthesis, WTA (Cat. No. 633801), BD® AbSeq Immune Discovery Panel (Cat. No. 625970) and Targeted Human Immune Response Panel (Cat. No. 633774, Cat. No. 633750) libraries were made compatible for sequencing on the AVITI™ System using the Element Adept™ Compatibility Kit (Cat. No. 830-00003), a simple 75-minute protocol in which libraries are circularized (without the use of PCR). After circularization and cleanup, libraries are quantified by qPCR using included standards and loaded at the appropriate concentration onto the AVITI™ System for sequencing.

BD

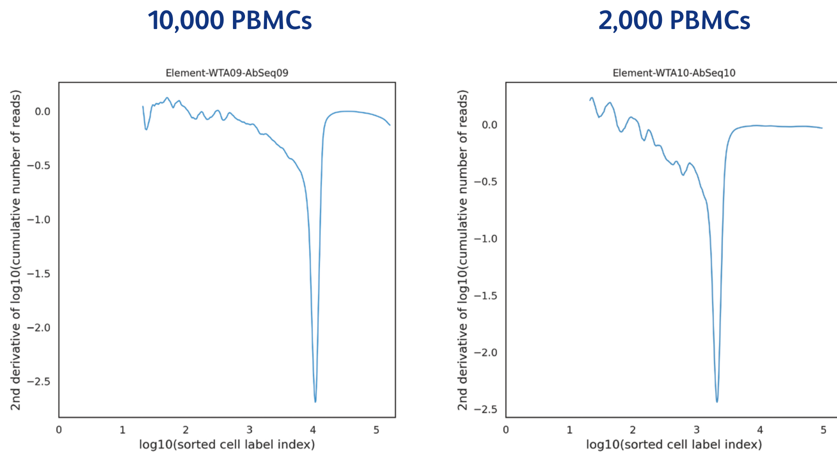


Figure 2. Second derivative from the WTA assay shows well-defined cell calling for high and low cell input

Results

The sequencing results showed compatibility between the two systems and resulted in high-quality, reproducible data from all library combinations tested.

WTA + AbSeq

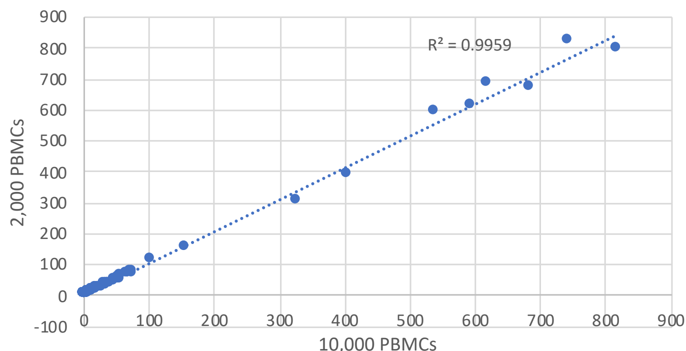
The Whole Transcriptome Analysis libraries resulted in high detection of cells and cell labels (Table 1), high gene correlation between the high and low cell input samples (Figure 2) and tSNE plots with cell clustering by type.

WTA + AbSeq profile and quality metrics

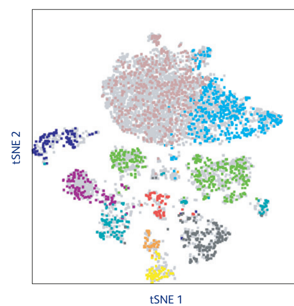
Library type intended cell input	WTA 10,000	WTA 2,000	AbSeq 10,000	AbSeq 2,000
Putative cell count	11,150	2,169	11,150	2,168
Raw reads per cell	25,303	25,977	32,518	32,515
% Sequencing saturation	83	88	96	96
Median molecules per cell	4,573	3,765	5,912	5,325
Median bioproducts per cell	1,523	1,295	27	26
% Reads assigned to cell labels	97	96	100	100
% Reads filtered out	4	5.3	1	0.8
% Q30 bases in filtered R2	93	93	95	94
% Aligned to transcriptome/AbSeq	69	71	98	98

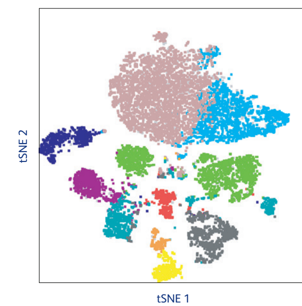
Table 1. Exhibits the high sequence quality of the Element AVITI™ System, indicated by the high Q30, cell label detection and low percent reads filtered out.

A. **WTA + AbSeq 2,000 vs 10,000 PBMCs**



B. **2,000 PBMCs** **10,000 PBMCs**





	Subset Name	Count	Freq. of Parent
	kmeans_k10 subset-9	169	8.22
	kmeans_k10 subset-8	66.0	3.21
	kmeans_k10 subset-7	116	5.64
	kmeans_k10 subset-6	123	5.99
	kmeans_k10 subset-5	609	29.6
	kmeans_k10 subset-4	118	5.74
	kmeans_k10 subset-3	309	15.0
	kmeans_k10 subset-2	42.0	2.04
	kmeans_k10 subset-1	62.0	3.02
	kmeans_k10 subset	441	21.5
	QualityCells	12865	96.6

	Subset Name	Count	Freq. of Parent
	kmeans_k10 subset-9	848	7.84
	kmeans_k10 subset-8	372	3.44
	kmeans_k10 subset-7	762	7.05
	kmeans_k10 subset-6	689	6.37
	kmeans_k10 subset-5	3343	30.9
	kmeans_k10 subset-4	626	5.79
	kmeans_k10 subset-3	1822	16.9
	kmeans_k10 subset-2	181	1.67
	kmeans_k10 subset-1	373	3.45
	kmeans_k10 subset	1794	16.6
	QualityCells	12865	96.6

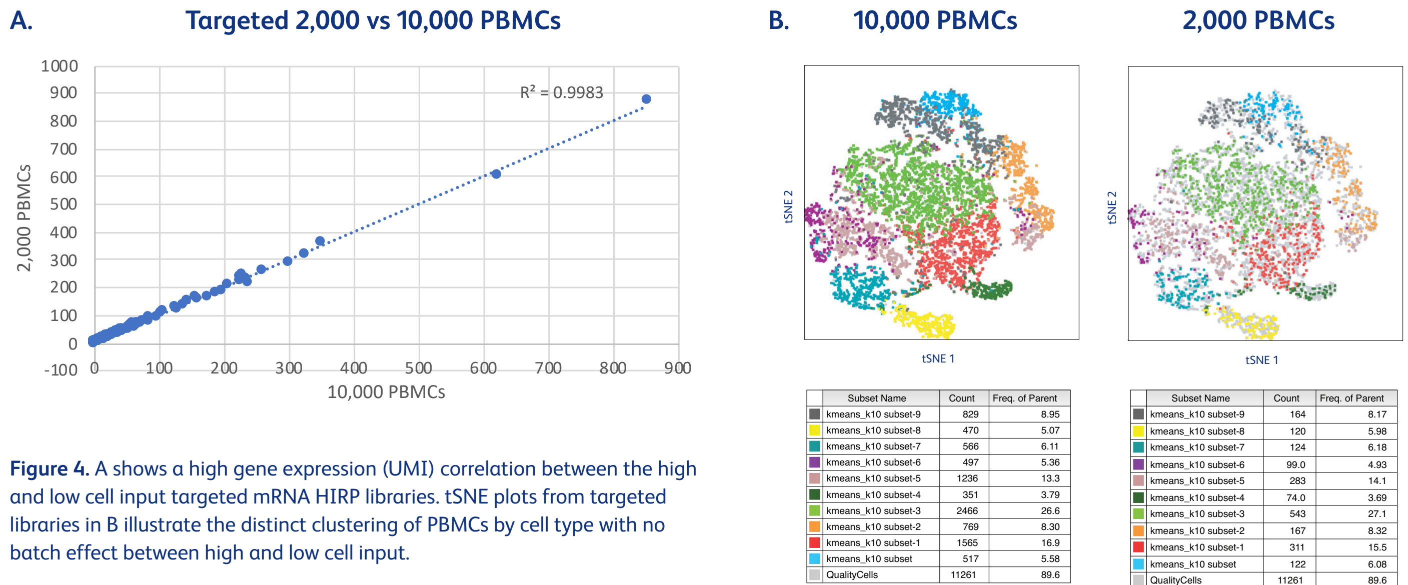
Figure 3. A shows a high gene expression (UMI) correlation between the high and low cell input WTA + AbSeq libraries. tSNE plots from the WTA + AbSeq assay in B illustrate the distinct clustering of PBMCs by cell type with no batch effect between high and low cell input.

Targeted Human Immune Response Panel (HIRP)

The Targeted assay quality and sensitivity metrics mirrored the favorable results achieved by the WTA + AbSeq library sequencing. Table 2 summarizes the targeted library metrics for both the high and low cell input libraries.

Library type intended cell input	Targeted 10,000	Targeted 2,000
Putative cell count	10,458	2,112
Raw reads per cell	5,802	5,561
% Sequencing saturation	97	97
Median molecules per cell	465	471
Median bioproducts per cell	78	76
% Reads assigned to cell labels	99	99
% Reads filtered out	1	1.2
% Q30 bases in filtered R2	94	95

Table 2. Key quality and sensitivity metrics, such as Q30, cell label detection and reads filtered out, as performance indicators for the Element AVITI™ System.



10,000 PBMCs

Subset Name	Count	Freq. of Parent
kmeans_k10 subset-9	829	8.95
kmeans_k10 subset-8	470	5.07
kmeans_k10 subset-7	566	6.11
kmeans_k10 subset-6	497	5.36
kmeans_k10 subset-5	1236	13.3
kmeans_k10 subset-4	351	3.79
kmeans_k10 subset-3	2466	26.6
kmeans_k10 subset-2	769	8.30
kmeans_k10 subset-1	1565	16.9
kmeans_k10 subset	517	5.58
QualityCells	11261	89.6

2,000 PBMCs

Subset Name	Count	Freq. of Parent
kmeans_k10 subset-9	164	8.17
kmeans_k10 subset-8	120	5.98
kmeans_k10 subset-7	124	6.18
kmeans_k10 subset-6	99.0	4.93
kmeans_k10 subset-5	283	14.1
kmeans_k10 subset-4	74.0	3.69
kmeans_k10 subset-3	543	27.1
kmeans_k10 subset-2	167	8.32
kmeans_k10 subset-1	311	15.5
kmeans_k10 subset	122	6.08
QualityCells	11261	89.6

Figure 4. A shows a high gene expression (UMI) correlation between the high and low cell input targeted mRNA HIRP libraries. tSNE plots from targeted libraries in B illustrate the distinct clustering of PBMCs by cell type with no batch effect between high and low cell input.

Conclusions

The results of this study demonstrate clear compatibility between the two systems, as high-quality results were shown across all combinations of BD Rhapsody™ System libraries tested.

This suggests the AVITI™ System offers an alternative NGS readout for the BD Rhapsody™ Single-Cell System portfolio.

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