Element Biosciences

10x GENOMICS

APPLICATION NOTE

Element AVITI™ System, a Member of the 10x Compatibility Partner Program for Chromium Single Cell 3′ Assays

Highlights

- **AVITI delivers high sequencing quality using the 10x Chromium Single Cell 3′ assay**
- **Unique, random access two-flow cell system provides flexibility of experimental design**

Introduction

New technologies that enable scientists to genetically profile thousands of cells individually using next-generation sequencing (NGS) have transformed our understanding of developmental biology and disease pathology[1]. The 10x Chromium Single Cell 3′ assay (Chromium Single Cell Gene Expression) enables such investigations at scale[2]. Because the assay uses NGS as readout, novel sequencing solutions that offer high quality, low cost, and flexibility of design are needed.

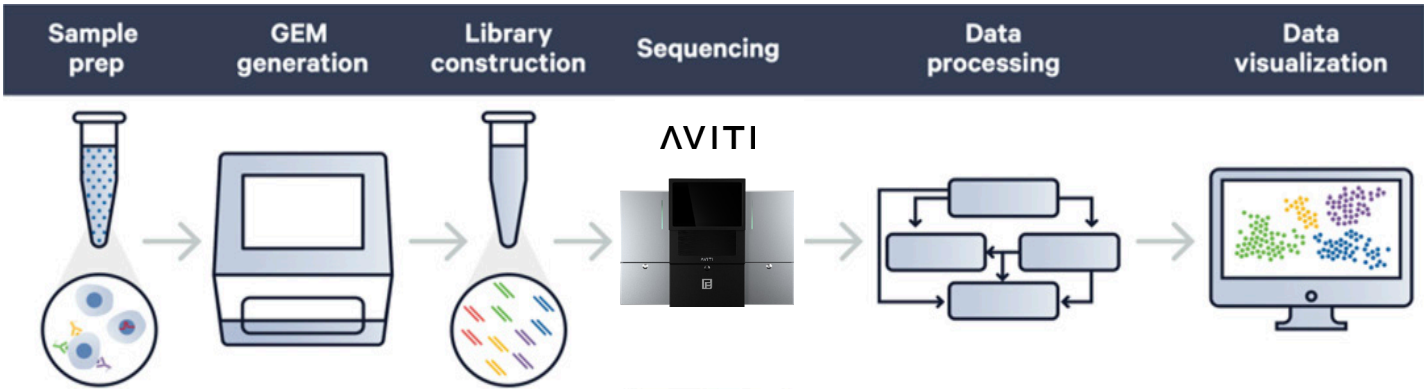
The AVITI System provides such a solution, with performance features that make NGS more accessible to labs than ever before. At its core the AVITI System uses a novel Avidite chemistry that enables a combination of exceptional accuracy, low cost, and operational efficiency. The instrument features two independent flow cells designed to work seamlessly in any NGS workflow.

This application note describes a study that pairs the 10x Genomics Chromium Single Cell 3′ assay with the Element AVITI System to provide a comprehensive solution for single cell sequencing. Two libraries created from human peripheral blood mononuclear cells (PBMCs), which query different cell counts, are used for the demonstration.

Methods

The 10x Chromium Single Cell 3′ assay is a droplet-based system that enables 3′ messenger RNA (mRNA) digital counting of thousands of individual single cells. The end-to-end workflow using the Element AVITI System as the sequencing readout seamlessly fits into the single cell workflow summarized below.

Figure 1: Summary of the 10x Chromium Single Cell workflow using the Element AVITI System



Compatible and streamlined workflow for scRNA-Seq assays. Starting with a single cell suspension, the 10x GEM system generates a single cell 3′ gene expression library. The Element Adept™ Library Compatibility Workflow processes the library to sequence on Element AVITI System. Generated data is processed with Cell Ranger and single cell expression profile is visualized with Loupe Browser.

Single Cell 3′ Dual-Index Gene Expression Library and AVITI Sequencing

Using the Chromium Next GEM Single Cell 3′ Kit v3.1 (catalog # 1000268)[3], scRNA-Seq libraries were prepared from two lots of PBMC cell suspensions (10,000 cells and 1,000 cells), with two replicates of each suspension.

Each library was quantified and individually processed for sequencing on the AVITI System using the Element Adept Library Compatibility Kit (catalog # 830-00003)[4]. The entire protocol required 75 minutes of total time, with fewer than 30 minutes hands-on time. The processed libraries were pooled and sequenced on the AVITI System with 28 cycles for Read 1, 90 cycles for Read 2, and index reads (catalog # 880-00001)[5].

Following sequencing, the Bases2Fastq Software was used to generate FASTQ files for compatible upload into 10x Cloud and subsequent analysis with the 10x Genomics Cell Ranger analysis package. Data visualization of single cell gene expression profiling was generated using 10x Genomics Loupe Browser.

Results

Table 1 summarizes resulting single cell gene expression profiles of the PBMC samples prepared in this study (targeting 10,000 and 1,000 cells). Both samples and their respective replicates surpassed the 10x Genomics Compatibility Product Program (CPP) qualifying metrics across every category. AVITI System run-to-run reproducibility was also high. At each scale the results were consistent in replicate sequencing runs, exhibiting high quality of both barcode Unique Molecular Identifer reads and RNA reads.

Table 1: 10x Chromium 3′ Single Cell Gene Expression profile sequencing metrics from the AVITI System

	10K cells	1K cells
Valid barcodes	97.3%	97.4%
Reads mapped confidently to exonic regions	52.1%	53.7%
Reads mapped confidently to transcriptome	48.9%	51.0%
Fraction reads in cells	94.9%	91.5%
Q30 bases in barcode	96.5%	97.3%
Q30 bases in RNA read	89.2%	92.2%
Mean reads per cell	72,573	73,430

Table 1 (Continued): 10x Chromium 3' Single Cell Gene Expression profile sequencing metrics from the AVITI System

	10K cells	1K cells
Median genes per cell	1,821	1,718
Estimated number of cells	8,501	907
Sequencing saturation	76.8%	76.5%

The data output from the Element AVITI System is suitable for a variety of read depth and sample numbers, providing researchers a wide range of flexibility to study greater cell numbers at greater read depth. The flexibility stems from the fact that different libraries can be loaded on the two flow cells and sequencing start times can be staggered.

Table 2: Sequencing capacity using the AVITI System

# Reads/Cell* (PE)	# Minimum Cells/Flow Cell	Run Time (hrs) – Up to two flow cells per run
5,000	160,000	< 24
20,000	40,000	< 24
50,000	16,000	< 24

* Listed as example of read depth. Choose the cell numbers and reads/cell numbers based on study needs and calculate the number of samples can be loaded on to each flow cell.

All datasets showed high-quality cell barcode data and good separation of the cell-associated barcodes from the empty barcodes as demonstrated in the barcode rank plota (Figure 2).

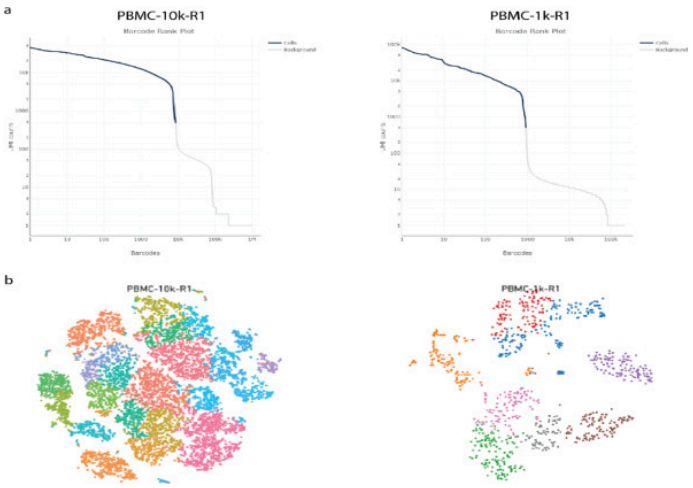


Figure 2: (a) Left, barcode rank plot for the PBMC 10,000 cell, replicate one dataset. Here, all 10x barcodes detected during sequencing are plotted in decreasing order of the number of UMIs associated with that particular barcode. The number of UMIs detected in each GEM is used by Cell Ranger to determine which GEMs likely contain a cell (to the left of the "knee")

Right, same plot as in (a) repeated for the PBMC 1,000 cell, replicate one dataset. (b) Respectively shows a tSNE projection of the 10,000 (left) and 1,000 (right) cells

The AVITI System also demonstrated high run-to-run reproducibility when comparing gene expression counts between the two replicates (Figure 3a). Two sequencing runs using the same library from 10,000 PBMC cells were compared in several contexts. Average gene expression values for 21,261 unique features found in replicate sequencing runs of the 10,000 PBMC sample library were analyzed. Points representing features are colored to represent data density. Feature counts were log normalized using Seurat (scale.factor=100,000,000) and average expression values were calculated.

Figure 3: (3a) UMI gene count R^2; t-SNE plot of cell expression profiles of 10,000 cells, (3b) split view of cell expression profiles of the two repeated sequencing run data, and (3c) merged view. (3d) Selected gene expression profiles (e.g., CD4+ T cells, B cells, NK cells) to compare two repeated sequencing run data

Figure 3a

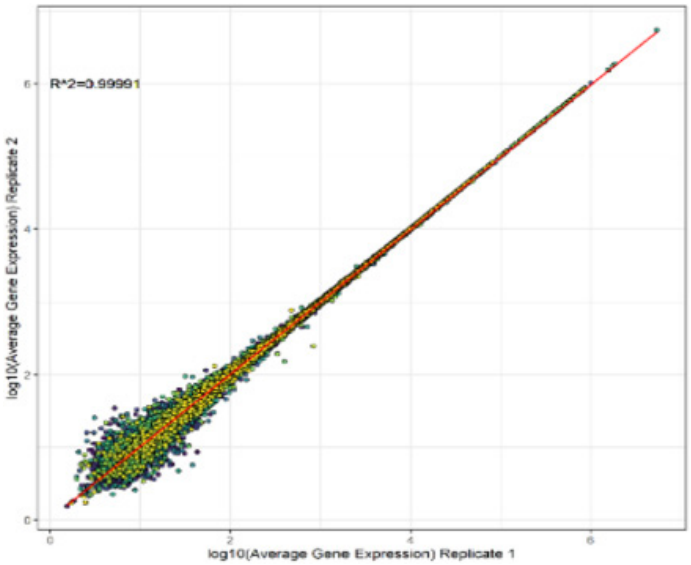


Figure 3b, 3c, 3d

Conclusions

For high-throughput, droplet-based gene expression profiling, the 10x Genomics Chromium Single Cell 3′ assay and the Element AVITI System proved to be an effective combination. Sequencing metrics from the assay at both the small cell population scale (1,000) and large cell population scale (10,000) exceeded all required CPP performance criteria. The additional flexibility of the AVITI System enabled by the two-flow cell design accommodates a wide range of experimental designs and throughput requirements.

References

1. Serena Liu and Cole Trapnell, Single-cell transciptome sequencing: recent advances and remaining challenges, F1000 Research, Vol. 5 (2016)
2. Grace X.Y. Zheng et al., Massively parallel digital transcriptomal profiling of single cells, Nature Communications, 8, Article number: 14049 (2017)
3. Chromium Single Cell 3' Reagent Kits User Guide (v3.1) (CG000204)
4. Element Adept Library Compatibility Workflow Guide (MA-00001)
5. Element AVITI System Workflow Guide (MA-00008)

Additional Information

For additional information, visit:

Applications: go.elembio.link/apps

Resources: go.elembio.link/resources

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