Element Biosciences

Fluent BioSciences

APPLICATION NOTE

PIPseq Compatibility on Element Biosciences AVITI™ Sequencer

Introduction

Single-cell RNA sequencing (scRNA-seq) has made profound impacts in the study of cellular and molecular diversity in complex tissues. However, specialized capital investment, high reagent costs, lock of accessibility and scalability are key factors limiting the wide-scale adoption and use of single cell technologies. Fluent BioSciences' breakthrough single-cell analysis technology that relies on Particle-templated Instant Partitions sequencing (PIPseq) offers a low barrier to entry without the need for complex instrumentation or expensive consumables, enabling single cell studies to be easily implemented in any laboratory.

Because the PIPseq assay uses NGS as readout, novel sequencing solutions that offer high quality, low cost, and flexibility of design can add significant convenience and cost advantages to the end to end workflow. 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 and a variety of read outputs which offers ideal mix and match options with the PIPseq single cell suite of kits. This application note describes a study that pairs Fluent Biosciences's PIPseq single-cell RNA assays with the Element AVITI System to provide a workflow solution at advantageous capital and operating costs without performance trade-offs. Several different library preparations across a variety of cell types and cell numbers are used for the demonstration.

Methods

In this technical demonstration, five complete PIPseq libraries were converted for Element AVITI sequencing using Element's Adept™ library compatibility Rapid Circularization Protocol (Element Adept Library Compatibility Kit v1.1 #830-00007). The samples analyzed included a diversity of cell types and sample input scales across Fluent's portfolio of PIPseq 3' Single Cell RNA Kit v4.0 kits. Samples included:

Table 1: Sample Preparation Details

PIPSEQ CATALOG NO.	SAMPLE TYPE	CELL INPUT	REPLICATES
FBS-SCR-T2-8-V4	Cultured cell mix (HEK293, NIH 3T3)	5,000	1
FBS-SCR-T2-8-V4	Viably frozen PBMCs	5,000	2
FBS-SCR-T20-4-V4	Viably frozen PBMCs	40,000	1
FBS-SCR-T100-2-V4	Mouse liver nuclei from frozen tissue	200,000	1

Libraries were prepared according to the respective PIPseq V4.0 user guides and sequenced on Illumina Nextseq 2000 or Novaseq at ~20k RPCC. Complete PIPseq libraries were subsequently converted to AVITI compatible format using the Adept conversion kit and sequenced. T2 and T20 libraries were pooled and sequenced on a single AVITI cartridge, resulting in 969,856,305 reads. The T100 liver nuclei library was sequenced independently on a single AVITI cartridge, resulting in 940,369,433 reads (10,429 reads per cell).

Bioinformatics Methods

Demultiplexed FASTQs from Element Biosciences or Illumina were processed with PIPseeker v3.00.01, using either the human, mouse, or combined human+mouse PIPseeker mapping references. Matched Element and Illumina samples were normalized to 20K reads per cell or the maximum common read depth using the `--downsample-to` option in PIPseeker. Illumina samples were processed first to determine the number of called cells per sample. These cell numbers were then assigned to Element samples using the --force-cells option in PIPseeker, to ensure that the analysis was matched as closely as possible. Cell type annotation was also performed for PBMCs and liver nuclei samples using the respective human PBMC and mouse liver PIPseeker annotation references. Barnyard analysis was performed with PIPseeker for HEK/3T3 samples using the `--run-barnyard` flag.

To quantify sequencing fidelity, we examined whether Element and Illumina differed in the detection of multiplets or species mixing. In PIPseeker, a multiplet is classified as any cell having >85% of transcript counts derived from a single species (human or mouse). Species mixing is calculated as follows: 1) Sum all transcript counts individually for human or mouse cells. 2) For the human and mouse cell populations, sum the total counts belonging to the opposite species. 3) Divide the counts detected in the opposite species (ex. mouse counts detected in human cells) by the total transcript counts for the human or mouse populations, respectively.

Results + Discussion

Key performance metrics for PIPseq were very comparable between Element and Illumina **(Tables 2-3)**. The most notable performance improvement for Element was observed in median transcripts/cell (+12%) and genes/cell (+7%) for the T2 PBMC samples that were sequenced at <100 million reads with Illumina **(Table 3)**. This effect was not observed for the T20 or T100 samples sequenced at much higher read depths on an Illumina NovaSeq. Element's absence of optical duplicates may underlie this phenomenon.

Extremely similar clustering and cell type annotation results were obtained between Element and Illumina sequencing, demonstrating that Element sequencing does not alter the biological interpretation of PIPseq single cell results **(Figures 1-2)**.

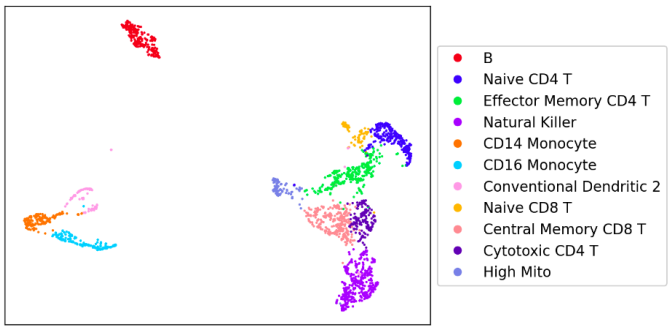
As an additional means of quantifying sequencing fidelity, we examined species mixing between platforms **(Table 4)**. A small amount of species mixing in single-cell experiments results from ambient RNA that naturally forms during cell preparation. However, a portion of species mixing can occur artifactually during library preparation and from sequencing artifacts, such as barcode hopping. Interestingly, we noted that Element had an average reduction of 17% of human transcripts observed in mouse cells and a 12% reduction in mouse transcripts observed in human cells. Observed multiplets were comparable, with 1 less multiplet observed on average with Element (0.73% reduction). These results provide direct evidence of higher-fidelity sequencing results on the Element platform.

Overall, these results indicate that PIPseq libraries are fully compatible with Element Biosciences sequencing and that sequencing quality is very comparable to Illumina NextSeq 2000 and NovaSeq instruments.

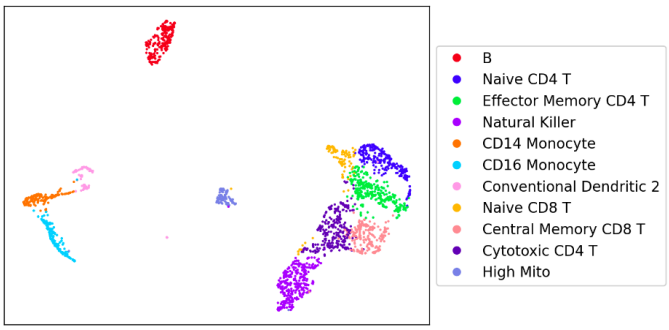
Figure 1: Cell type clustering for matched samples sequenced with Element vs Illumina

Human PBMCs (T2)

Element

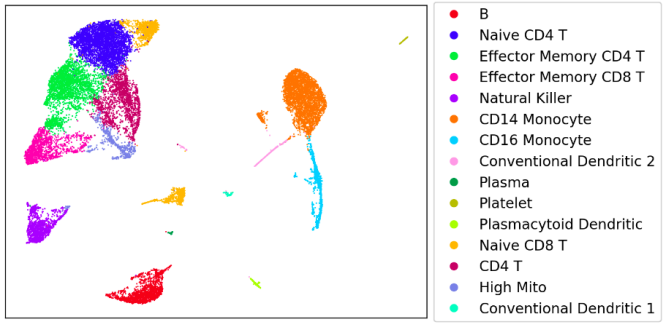


Illumina

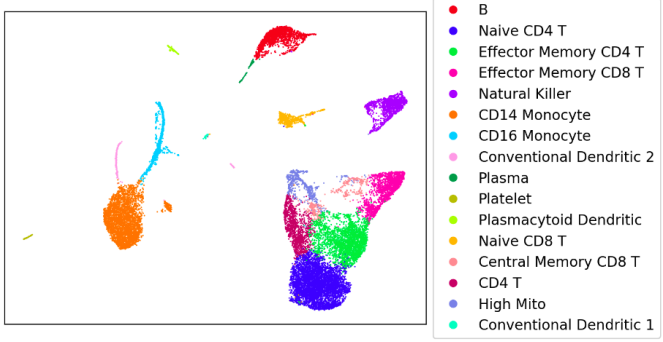


Human PBMCs (T20)

Element



Illumina



Liver nuclei (T100)

Element

Illumina

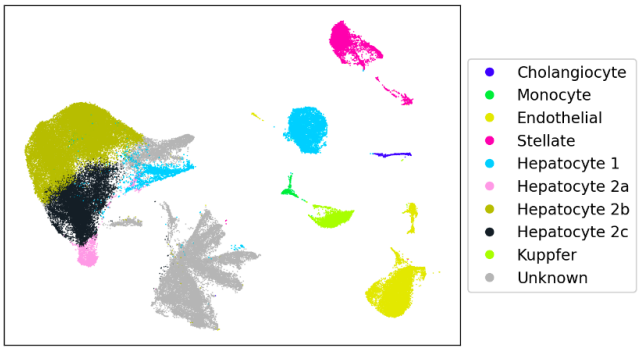


Figure 2: Mixed Cell Type Plots (HEK/3T3, T2 kit)

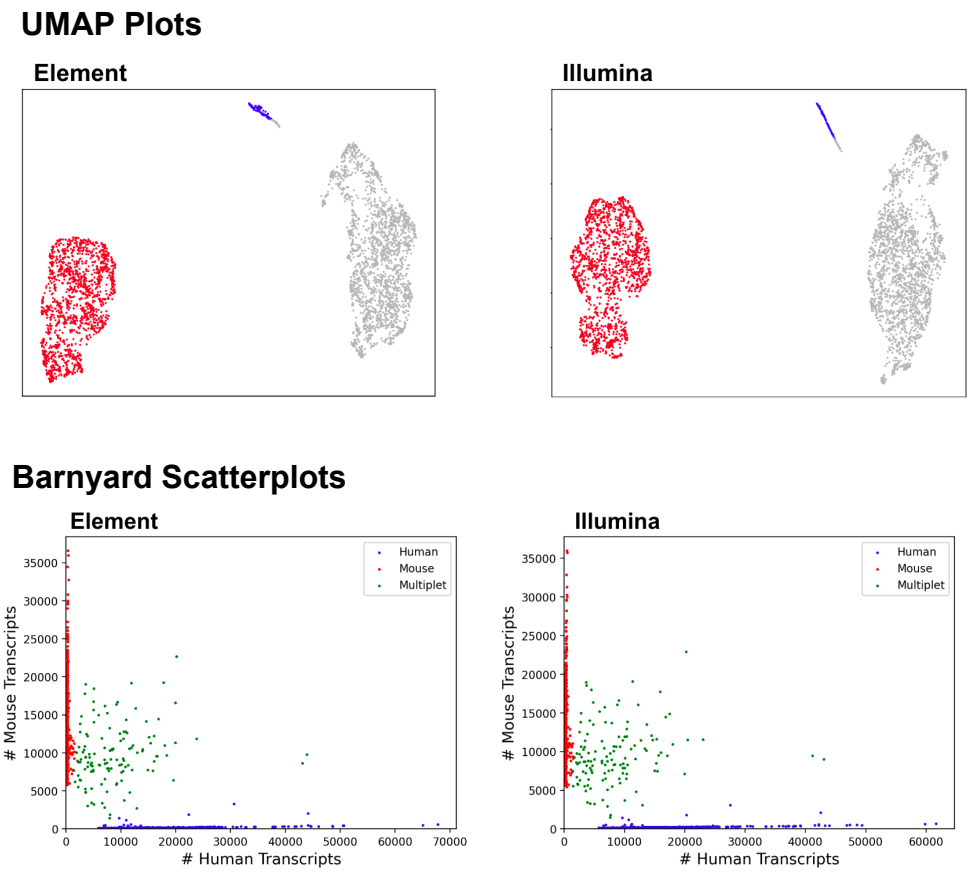


Table 2: Key Metrics from PIPseq samples sequenced with Element AVITI

	HEK/3T3 (T2)	PBMCS (T2)	PBMCS (T20)	LIVER NUCLEI (T100)
READS MAPPED (TRANSCRIPTS)	64,693,731 (±0.6%)	31,099,653 (±1.2%)	358,057,199	693,747,515
MAPPING RATE	95 ± 0%	91 ± 0.24%	94.46%	87.28%
MEDIAN TRANSCRIPTS / CELL	10337 ± 748	3286 ± 455	4100	2324
MEDIAN GENES / CELL	3230 ± 134	1375 ± 268	1396	1332

Table 3: Element vs. Illumina (% Difference)

	HEK/3T3 (T2)	PBMCS (T2)	PBMCS (T20)	LIVER NUCLEI (T100)
READS MAPPED (TRANSCRIPTS)	1.58 ± 0.02%	-3.47 ± 0.42%	-0.32%	-0.83%
MAPPING RATE	0.67 ± 0.36%	0.41 ± 0.01%	0.31%	-1.26%
MEDIAN TRANSCRIPTS / CELL	3.4 ± 0.14%	12.68 ± 0.64%	-0.19%	-0.47%
MEDIAN GENES / CELL	2.49 ± 0.44%	7.91 ± 0.16%	-0.92%	-0.82%

Table 4: Barnyard analysis metrics

	ELEMENT	ILLUMINA	% DIFFERENCE
% HUMAN IN MOUSE CELLS	1.05%	1.26%	-17.04 ± 0.73%
% MOUSE IN HUMAN CELLS	1.62%	1.86%	-12.34 ± 1.91%
MULTIPLETS	136	137	-0.73 ± 0.06%