illumina®

Single-Cell RNA Sequencing on the NovaSeq™ 6000 System

Gain comprehensive insight into gene expression with a scalable and cost-effective solution with the NovaSeq 6000 System.

Introduction

The NovaSeq 6000 system is a powerful, flexible, and highly scalable platform, built to enable cost-effective sequencing across a wide range of applications and study sizes.[1] With its high data quality, streamlined workflow, and increased output, the NovaSeq 6000 System redefines the power and possibility of high-throughput single-cell RNA sequencing (RNA-Seq).

Single-cell RNA-Seq enables gene expression analysis of cell function, cellular heterogeneity, and delineation of cell states to address questions that can only be answered by interrogating individual cells.[2] However, generating thousands of sequencing libraries for single cells in an affordable, high-throughput, and user-friendly manner remains challenging.[3]

The Illumina Bio-Rad Single-Cell Sequencing Solution[4] combines the highly innovative Bio-Rad Droplet Digital technology with Illumina library preparation, next-generation sequencing (NGS), and data analysis technologies. This new platform accelerates biological discovery by enabling transcriptome analysis of hundreds to tens of thousands of single cells in a single experiment. When combined with the NovaSeq 6000 System and proven Illumina support for the entire NGS workflow, this scalable, robust, single-cell NGS methodology enables routine transcriptome profiling at single-cell resolution. This application note demonstrates the exceptional performance of the NovaSeq 6000 System as part of the Illumina Bio-Rad Single-Cell Sequencing Solution.

Methods

To demonstrate the high-quality single-cell RNA-Seq data achieved with the Illumina Bio-Rad Single-Cell Sequencing Solution, experiments were performed with human embryonic kidney 293 (HEK 293) cells.

Cell Isolation and Library Prep

Cells were processed for single-cell sequencing using the Illumina Bio-Rad SureCell WTA 3′ Library Prep Kit for the ddSEQ System (Illumina, Catalog No. 20014280). Twenty-four technical replicate libraries were generated by loading HEK 293 cells across four sample chambers of six cartridges onto the Bio-Rad ddSEQ Single-Cell Isolator (Bio-Rad, Catalog No. 12004336).

Sequencing and Data Analysis

Libraries were combined into a 24-plex pool and run at the read length of 68 × 75 bp using the NovaSeq 6000 S2 Reagent Kit (300 cycles) (Illumina, Catalog No. 20012860) on the NovaSeq 6000 System with PhiX spike in of 1%. Sequencing results were analyzed using the SureCell RNA Single-Cell App in BaseSpace™ Sequence Hub.

Results

High-Quality RNA-Seq Data

The NovaSeq 6000 System generated high-quality whole-transcriptome 3′ RNA sequencing data from a total of 12,308 single cells. A single S2 flow cell yielded over 1.9 billion reads passing filter (PF) with quality scores (Q30) > 90% for both Read 1 and Read 2 (Table 1). On average, each of the 24 uniquely indexed libraries yielded 156 million PF reads and captured 513 cells, resulting in an average sequencing read depth of 304 K reads/cell. At this read depth, a high sensitivity of single-cell gene expression was achieved, with the median number of genes detected per cell at > 5500 across libraries. Analysis of RNA-Seq metrics revealed that 89% of Read 1 sequences aligned to cell barcodes and 93% of Read 2 sequences aligned to transcripts across libraries (Table 2).

Table 1: NovaSeq 6000 Sequencing Run Metrics

Metric	Overall	
Total Reads (M)	2880.7	
Reads Passing Filter (M)	1909.3	
Metric	**Read 1**	**Read 2**
Number of Cycles	68	75
Quality Score (Q30)	96.1%	92.2%
Error Rate	0.36%	0.39%

Table 2: NovaSeq 6000 Single-Cell RNA-Seq Metrics

Metric	Average (n = 24)	CV (n = 24)
No. of Cells per Sample	513	11%
Median No. of Genes per Cell	6383	5%
Percent Reads with Valid Barcode	89%	0.5%
Percent Reads Aligned to hg19	93%	0.5%
No. of Reads Passing Filter per Library	156 M	15%
No. of Reads Passing Filter per Cell	307,487	17%

Highly-Concordant RNA-Seq Data

The reproducibility of gene expression quantification in single-cell libraries was compared between the NovaSeq 6000 System and other Illumina sequencing platforms (Figure 1). The same single-cell library was sequenced on the NovaSeq 6000, HiSeq™ 4000, and NextSeq™ 500 Systems. Sequencing data were analyzed using the SureCell RNA Single-Cell App with the data set normalized to equivalent read depth by subsampling to 100K reads per cell.

The total gene counts for each gene were summed across all cells in the sample and compared between platforms. R-squared values were calculated for a linear regression fit to the data. An R-squared value of 0.99 was calculated for data generated on the NovaSeq 6000 System compared to NextSeq data (Figure 1A) or HiSeq 4000 data (Figure 1B) for all genes with greater than 10 counts, which includes over 12,000 genes (Table 3).

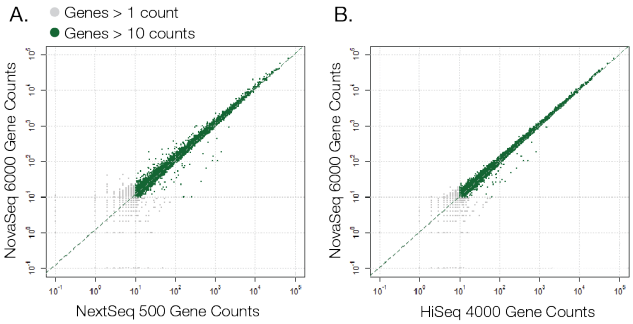

Figure 1: Reproducibility of Gene Expression Data Between Illumina Sequencing Systems—The same single-cell sample (sample 16) was sequenced on multiple platforms. Plots of data generated on the NovaSeq 6000 System versus (A) the NextSeq 500 System or (B) HiSeq 4000 System shows highly concordant gene expression data between platforms.

Table 3: Reproducibility of Gene Expression Data Between Illumina Sequencing Systems

	NovaSeq 6000 System	
Metric	NextSeq 500 System	HiSeq 4000 System
R^2 (genes with > 10 counts)	0.99	0.99
R^2 (genes with > 1 count)	0.98	0.99

Summary

To advance understanding of the transcriptional heterogeneity that drives complex biological systems, researchers need a scalable, high-throughput, and user-friendly method for generating NGS sequencing results from hundreds to tens of thousands of single cells. The Illumina Bio-Rad Single-Cell Sequencing Solution is a comprehensive workflow developed in collaboration by industry experts in droplet-based cell isolation and NGS technologies. Simple yet powerful data analysis options in the SureCell RNA Single-Cell App can resolve heterogeneous cell populations and identify subpopulations of interest using gene expression profiles and data visualization tools.

For high-throughput RNA-Seq studies, the NovaSeq 6000 System can facilitate analysis of tens of thousands of single cells in parallel, including multiplexing of up to 48 samples derived from distinct treatment conditions, time-points, or tissue types. The NovaSeq 6000 System combined with the Illumina Bio-Rad Single-Cell Sequencing Solution enables highly sensitive and reproducible interrogation of single-cell transcriptomes with unmatched scalable throughput.

Learn More

To learn more about the Illumina Bio-Rad Single-Cell Sequencing Solution, visit www.illumina.com/surecell or www.bio-rad.com/ddSEQ.

To learn more about the NovaSeq 6000 System, visit www.illumina.com/novaseq.

To explore the data set used in this application note, access the Single-Cell Sequencing Project on the NovaSeq 6000 System in BaseSpace Sequence Hub.

Ordering Information

Product	Catalog No.
Cell Isolation	
ddSEQ Single-Cell Isolator (from Bio-Rad)	12004336
Library Prep Kit	
SureCell WTA 3′ Library Prep Kit (two cartridge kit)	20014279
SureCell WTA 3′ Library Prep Kit (six cartridge kit)	20014280
Sequencing System	
NovaSeq 6000 System	20012850
Sequencing Reagent Kit	
NovaSeq 6000 S2 Reagent Kit (300 cycles)	20012860
NovaSeq 6000 S2 Reagent Kit (200 cycles)	20012861

References

1. Illumina (2017). NovaSeq 6000 System. www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/novaseq-6000-system-specification-sheet-770-2016-025.pdf. Accessed October 16, 2017.
2. Kolodziejczyk AA, Kim JK, Svensson V, Marioni JC, Teichmann SA. The technology and biology of single-cell RNA sequencing. *Mol Cell*. 2015;58 (4):610–620.
3. Shapiro E, Biezuner T, Linnarsson S. Single-cell sequencing-based technologies will revolutionize whole-organism science. *Nat Rev Genet*. 2013;14(9):618–630.
4. Illumina (2016). Illumina Bio-Rad SureCell WTA 3′ Library Prep Kit for the ddSEQ System. www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/surecell-single-cell-rna-seq-data-sheet-1070-2016-014.pdf. Accessed June 27, 2017.

Illumina, Inc. • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

For Research Use Only. Not for use in diagnostic procedures.

illumina®