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NextSeq™ 2000 Single-Cell RNA-Seq Solution

Cost-effective generation of RNA sequencing libraries from single cells.

Highlights

- **Reliable protocols for generating single-cell data**
  Robust transcriptome analysis of high-quality single cells using a large ecosystem of sequencing solutions
- **Flexible sequencing throughput**
  Two output modes and multiple read lengths support a broad range of project needs
- **Discover more**
  High-resolution analysis enables discovery of cellular differences usually masked by bulk sampling methods
- **Complete Illumina technical support**
  Illumina scientists and engineers provide installation, training, and support for customers through the entire workflow from library preparation through data analysis

Introduction

Single-cell sequencing is a next-generation sequencing (NGS) method that examines the transcriptomes of individual cells, providing a high-resolution view of cell-to-cell variation. In contrast to traditional RNA sequencing (RNA-Seq), where populations of cells are sampled in bulk, highly sensitive single-cell RNA sequencing (scRNA-Seq) methods enable researchers to explore the distinct biology of individual cells in complex tissues and understand cellular subpopulation responses to environmental cues. These assays enhance the study of cellular function and heterogeneity in time-dependent processes, such as differentiation, proliferation, and tumorigenesis.

Figure 1: NextSeq 2000 Sequencing System—The NextSeq 2000 Sequencing System harnesses the latest advances in SBS chemistry and streamlines sequencing workflows.

scRNA-Seq offers several advantages over bulk sampling methods, which include enabling researchers to:

- Identify cell types and attribute transcriptional activities to specific cell types
- Discover new cell types that may serve novel functions in complex systems
- Detect transcriptional patterns in lower-frequency cell types that would be masked in bulk sampling methods
- Resolve transcriptional changes down to individual cell types to inform mechanistic and pathway models

A flexible scRNA-Seq solution

The NextSeq 2000 Sequencing System (Figure 1) harnesses industry-leading Illumina sequencing by synthesis (SBS) chemistry as part of a comprehensive scRNA-Seq workflow. scRNA-Seq includes initial tissue preparation, a broad range of single-cell isolation and library preparation options, sequencing and primary analysis, and data visualization and interpretation (Figure 2).

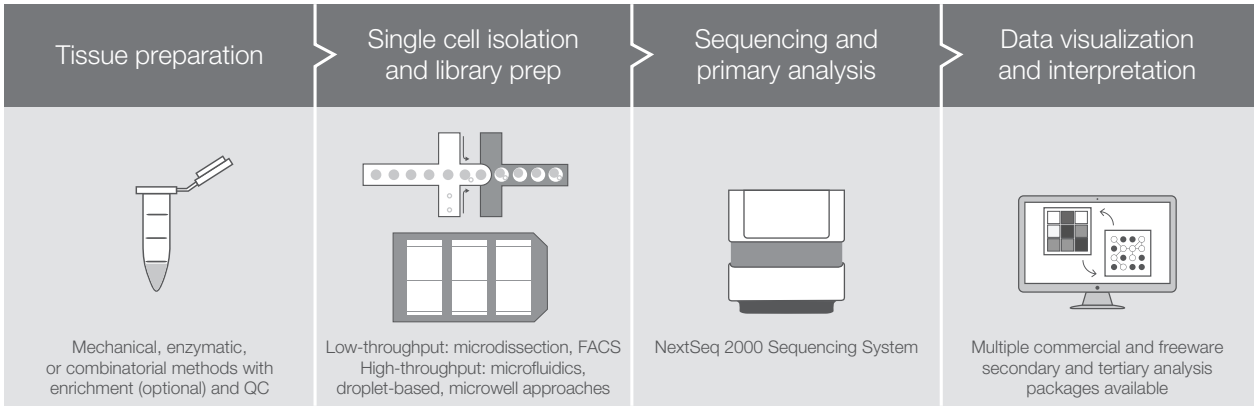

Figure 2: NextSeq 2000 scRNA-Seq workflow—The NextSeq 2000 Sequencing System is part of an integrated NGS workflow that proceeds from initial tissue preparation through single-cell isolation and library preparation, sequencing and primary analysis, and data visualization and interpretation.

Tissue preparation and cell-isolation

Early methods for single-cell isolation were low throughput, able to process only dozens to a few thousand cells per experiment. The emerging availability of high-throughput, microfluidic-based methods for cell isolation permits researchers to examine hundreds to tens of thousands of cells per experiment in a cost-effective manner. Researchers can choose from a large ecosystem of tissue preparation, single-cell isolation, and library preparation providers, enabling scRNA-Seq studies to be tailored to a wide variety of tissues, species, cell types, and methods.

The NextSeq 2000 Sequencing System

The NextSeq 2000 Sequencing System offers the power and flexibility to accommodate a broad range of project needs, allowing adjustment of cells per sample, reads per cell, and samples per experiment (Table 1). Whether researchers want to sequence deeper to access lower-abundance transcripts, or sequence more cells or samples, the NextSeq 2000 Sequencing System offers a cost-effective solution for sequencing and primary analysis in a desktop sequencer. Two available sequencing output modes on the NextSeq 2000 Sequencing System add flexibility for researchers using multiple methods of NGS analysis, and for accommodating a variety of experimental designs. If even higher sample throughput is needed, studies can be scaled up to hundreds of samples per run using the NovaSeq™ 6000 System (Table 1).

Discover more with industry-leading SBS read quality

Illumina NGS technology powered by SBS chemistry is responsible for generating more than 90% of the world's sequencing data.[1] SBS chemistry delivers high yields of error-free reads,[2] enabling robust base calling for scRNA-Seq that results in more accurate fold-change estimates and superior sensitivity when detecting genes, transcripts, and single-cell barcodes. With the highest read output and lowest price per read of any Illumina desktop sequencer, the NextSeq 2000 Sequencing System enables researchers to access:

- More reads per cell to capture information about lower-abundance transcripts
- More cells and samples to empower experimental designs within a given research budget
- Additional conditions, time points, or methods to investigate more complex facets of biology

Cross-application flexibility

Beyond scRNA-Seq, the NextSeq 2000 Sequencing System offers extensive cross-application flexibility, enabling researchers to transition easily between sequencing projects (Figure 3). The system is compatible with a wide range of library preparation kits from Illumina and third parties, enabling an easy transition between Illumina bulk RNA-Seq, scRNA-Seq, exome sequencing, and other applications. For example, researchers can pair scRNA-Seq with exome sequencing on the NextSeq 2000 Sequencing System to assess whether coding variants impact transcript expression, or perform ATAC-Seq to analyze chromatin accessibility and better characterize functional regulation.

Figure 3: NextSeq 2000 sequencing applications—The NextSeq 2000 Sequencing System enables researchers to transition seamlessly between applications to advance research.

Streamline single-cell data analysis

Sequencing data generated with the NextSeq 2000 System is available in standardized formats compatible with an extensive ecosystem of commercial and open source single-cell sequencing software tools, including commercial software like CellRanger (10x Genomics) and SeqGeq (FlowJo/BD Biosciences), and open source tools such as Seurat[3] and Monocle.[4]

scRNA-Seq data analysis can be performed using tools from the Illumina DRAGEN™ Bio-IT Platform, a suite of fast and accurate data analysis pipelines, and a broad ecosystem of commercial and open-source data analysis software tools. Coming in late 2020, the NextSeq 2000 Sequencing System will offer the DRAGEN Single-Cell Pipeline as an onboard data analysis pipeline, capable of accelerating data analysis, improving accuracy with error correction, and supporting various custom barcoding designs to enable new single-cell methods

Table 1: Example study designs for scRNA-Seq

Method	Example cell isolation method	Example library prep method	Cells per sample	Reads per cell	Samples per run: NextSeq 2000 System P2	NextSeq 2000 System P3	NovaSeq 6000 System SP	NovaSeq 6000 System S1	Data analysis
Full-length RNA-Seq	FACS	Takura SMARTer cDNA Synthesis Kits	100	1 M	4	10	8	16	DRAGEN RNA RNA Express (STAR)
mRNA end-tag amplification (3′ WTA or 5′ WTA)	10x Genomics Chromium	10x Genomics Chromium Single Cell Gene Expression Solution (3′ WTA)	5000	20,000	4	10	8	16	CellRanger
Targeted panel	BD Rhapsody Single-Cell Analysis System	BD Rhapsody Single-Cell Analysis	1000 5000	20,000 2000	20 40	50 100	40 80	80 160	SeqGeq

Stated cell, read, and sample numbers are for illustrative purposes only. Cell, read, and sample numbers should be carefully selected based on study design requirements. Refer to the Single-Cell Sequencing eBook for more information. WTA = whole-transcriptome amplification; BD = Becton Dickinson.

in this rapidly evolving research area. Beyond scRNA-Seq, Illumina offers an extensive collection of commercial and open source data analysis software tools to support additional methods needed to complete a research project.

Complete Illumina technical support

With a NextSeq 2000 Sequencing System in their laboratory, researchers join a worldwide community of thousands of scientists using Illumina technology for their research studies. Illumina schedules community events throughout the year, bringing researchers together to share ideas. User group meetings, scientific symposiums, and blog forums provide venues to discuss new research methods and breakthrough studies.

An integral part of the Illumina community is our dedicated service and support team, consisting of hundreds of people worldwide, 75% of whom have advanced degrees. Illumina technical support begins when the NextSeq 2000 System is delivered, with Illumina scientists and engineers assisting with system installation and setup, and the training of laboratory personnel. They are there 24/7 globally to answer questions every step of the way, giving researchers the peace of mind to focus on their next research study.

As researchers' needs change, new systems are brought into the laboratory, or new methods undertaken, the Illumina Support and Training teams are there to provide assistance. In addition to on-site support, training courses (via webinar or at an Illumina facility) are available to bring laboratory personnel quickly up to speed.

Summary

The NextSeq 2000 Sequencing System offers a flexible solution for scRNA-Seq, providing a clear, comprehensive view of the single-cell transcriptome. Two output modes allow researchers to adjust cells per experiment, and reads per cell to fit their needs. The NextSeq 2000 Sequencing System delivers the highest output of any Illumina desktop sequencer, allowing labs to expand the set of tools at their disposal. Combining the power, speed, and flexibility of the NextSeq 2000 Sequencing System with an expansive ecosystem of library preparation and software solutions, scRNA-Seq on the NextSeq 2000 System enables researchers to access a deeper understanding of the transcriptome at single-cell resolution.

Learn more

To learn more about single-cell sequencing, visit www.illumina.com/single-cell-rna-sequencing to download the Illumina Single-Cell Sequencing eBook.

Refer to the Illumina Single-Cell Sequencing eBook for an overview of single-cell technologies and third-party product recommendations.

NextSeq 2000 Sequencing System specifications

Instrument configuration
Self-contained, dry instrument with integrated DRAGEN Bio-IT field-programmable gate array technology (FPGA) secondary analysis
Instrument control computer
Base Unit: 2U Microserver located inside the instrument Memory: 288 GB Hard Drive: 3.8 TB SSD Operating System: Linux CentOS 7.6
Operating environment
Temperature: 15°C-30°C Humidity: 20%-80% relative humidity, non-condensing Altitude: 0-2000 meters For Indoor Use Only
Laser
Wavelengths: 449 nm, 523 nm, 820 nm Safety: Class 1 Laser Product
Dimensions
W×D×H: 60 cm × 65 cm × 60 cm Weight: 141 kg **Crated Dimensions** Crated W×D×H: 92 cm × 120 cm × 118 cm Crated Weight: 232 kg
Power requirements
Instrument Input Voltage: 100 VAC to 240 VAC Instrument Input Frequency: 50/60 Hz
Bandwidth for network connection
200 Mb/s/instrument for internal network uploads 200 Mb/s/instrument for BaseSpace Sequence Hub uploads 5 Mb/s/instrument for instrument operational data uploads
Product safety and compliance
NRTL certified IEC 61010-1 CE marked FCC/IC approved

Ordering information

System	Catalog no.
NextSeq 2000 Sequencing System	20038897
Sequencing reagents	**Catalog no.**
NextSeq 1000/2000 P2 Reagents (100 cycles)	20038899
NextSeq 1000/2000 P2 Reagents (200 cycles)	20040557
NextSeq 1000/2000 P2 Reagents (300 cycles)	20040558

References

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2. Bentley DR, Balasubramanian S, Swerdlow HP, et al. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature*. 2008;456:53-59.
3. Satija R, Farrell JA, Gennert D, Schier AF, Regev A. Spatial reconstruction of single-cell gene expression. *Nat Biotechnol*. 2015;33(5):495-502.
4. Trapnell C, Cacchiarelli D, Grimsby J, et al. The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nat Biotechnol*. 2014;32(4):361-386.

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