APPLICATION NOTE

NextSeq™ 1000 & NextSeq 2000 single-cell RNA sequencing solution

Cost-effective, flexible workflow for measuring gene expression in single cells

- Reliable protocols for generating single-cell data using a large ecosystem of Illumina and third-party sequencing solutions
- Scalable sequencing throughput to support a broad range of single-cell experiments
- High-resolution analysis to discover cellular differences usually masked by bulk sampling methods

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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.

scRNA-Seq offers several advantages over bulk sampling methods. These advantages enable 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

Figure 1: NextSeq 1000 and NextSeq 2000 Sequencing Systems—The NextSeq 1000 and NextSeq 2000 systems harness the latest advances in SBS chemistry and streamline sequencing workflows.

A flexible scRNA-Seq solution

The NextSeq 1000 and NextSeq 2000 Sequencing Systems (Figure 1) harness 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).

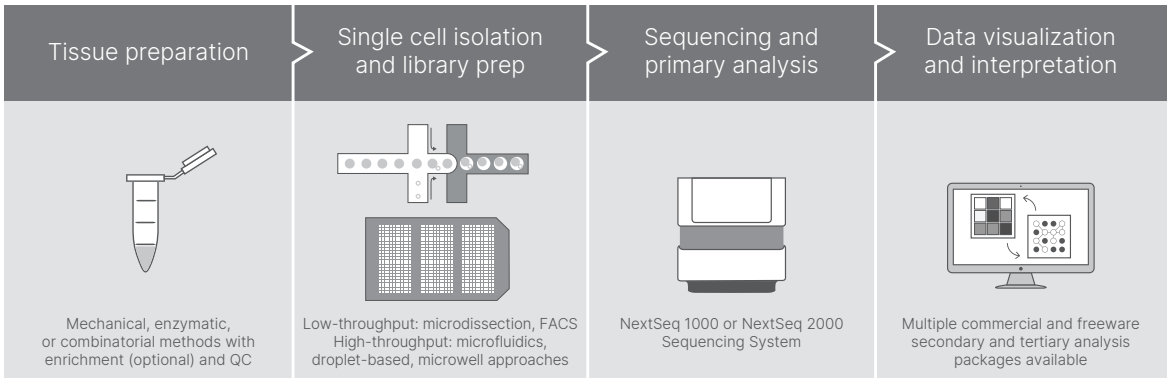

Figure 2: NextSeq 1000 and NextSeq 2000 scRNA-Seq workflow—The NextSeq 1000 and NextSeq 2000 Sequencing Systems are part of an integrated NGS workflow that proceeds from initial tissue preparation through single-cell isolation and library preparation, sequencing and primary analysis, and data visualization and interpretation.

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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 availability of high-throughput, microfluidic-based methods for cell isolation now 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 (Table 1).

The NextSeq 1000 and NextSeq 2000 Sequencing Systems

The NextSeq 1000 and NextSeq 2000 Sequencing Systems offer the power and flexibility to accommodate a wide range of project needs, allowing adjustment of cells per sample, reads per cell, and samples per experiment. Whether researchers want to sequence deeper to access lower-abundance transcripts, or sequence more cells or samples, the NextSeq 1000 and NextSeq 2000 Sequencing Systems offer a cost-effective solution for sequencing and primary analysis in a benchtop sequencer. Three available sequencing flow cell configurations on the NextSeq 1000 and NextSeq 2000 Sequencing Systems 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 using the NovaSeq™ 6000 System (Table 2).

Table 2: Single-cell RNA-Seq sample throughput on Illumina sequencing systems

Sequencing system	Sequencing reagents	No. of samples per run[a]
NextSeq 1000 and NextSeq 2000 Systems[b]	P2 100 cycles	4
	P3[c] 100 cycles	11
NovaSeq 6000 System	SP 100 cycles	8
	S1 100 cycles	16
	S2 100 cycles	40
	S4 200 cycles	96

a. Calculated for 5K cells per sample and 20K read pairs per cell; number of cells per sample and read pairs per cell depends on your particular application
b. P1 Reagents are not recommended for single-cell applications
c. P3 Reagents are available for the NextSeq 2000 System only

Table 1: Example study designs for scRNA-Seq[a]

Method	Example cell isolation method	Example library prep method	Cells per sample	Read pairs per cell	Data analysis
Full-length RNA-Seq	FACS	Takara SMARTer cDNA Synthesis Kits	100	1M	DRAGEN Single-Cell RNA
mRNA end-tag amplification (3' WTA or 5' WTA)	10x Genomics Chromium	10x Genomics Chromium Single Cell Gene Expression	5K	20K	CellRanger DRAGEN Single-Cell RNA
Targeted panel	BD Rhapsody Single-Cell Analysis System	BD Rhapsody Single-Cell Analysis	5K	2K	Seven Bridges Genomics
mRNA end-tag amplification (3' capture)	Dolomite Bio Nadia instrument	Dolomite Bio RNAdia kit	6K	25K	dropSeqPipe Partek Flow

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

Discover more with industry-leading SBS read quality

Illumina NGS technology is powered by sequencing by synthesis (SBS) chemistry, which delivers high yields of error-free reads.[1] This enables 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 benchtop sequencer, the NextSeq 1000 and NextSeq 2000 Sequencing Systems enable 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 1000 and NextSeq 2000 Sequencing Systems offer extensive cross-application flexibility, enabling researchers to transition easily between sequencing projects. The systems are 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 1000 and NextSeq 2000 Sequencing Systems to assess whether coding variants impact transcript expression, or perform ATAC-Seq* to analyze chromatin accessibility and better characterize functional regulation.

Streamline single-cell data analysis

Sequencing data generated with the NextSeq 1000 and NextSeq 2000 Systems 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[2] and Monocle.[3]

scRNA-Seq data analysis can be performed using tools from the Illumina DRAGEN™ Bio-IT Platform, a suite of ultrarapid and accurate data analysis pipelines. The NextSeq 1000 and NextSeq 2000 Sequencing Systems 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 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.

Comprehensive Illumina technical support

Ilumina provides a world-class support team comprised of experienced scientists who are experts in library preparation, sequencing, and analysis. This dedicated team includes highly qualified field service engineers (FSE), technical applications scientists (TAS), field applications scientists (FAS), system support engineers, bioinformaticians, and IT network experts, all deeply familiar with NGS and the applications that Illumina customers perform around the globe. Technical support is available via phone five days a week or via online support 24/7, worldwide and in multiple languages.

With this unmatched service and support, Illumina helps users maximize the efficacy of their NextSeq 1000 or NextSeq 2000 Sequencing Systems, train new employees, and learn the latest techniques and best practices.

Summary

The NextSeq 1000 and NextSeq 2000 Sequencing Systems offer a flexible solution for scRNA-Seq, providing a clear, comprehensive view of the single-cell transcriptome, allowing labs to expand the set of tools at their disposal. Different flow cell configurations allow researchers to adjust cells per experiment, and read pairs per cell to fit their needs. Combining the power, speed, and flexibility of the NextSeq 1000 and NextSeq 2000 Sequencing Systems with an expansive ecosystem of

* ATAC-Seq, assay for transposase-accessible chromatin with sequencing

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library preparation and software solutions, scRNA-Seq on the NextSeq 1000 and NextSeq 2000 Systems enables researchers to access a deeper understanding of the transcriptome at single-cell resolution.

Learn more

Single-cell RNA sequencing, illumina.com/single-cell-rna-sequencing

NextSeq 1000 and NextSeq 2000 Sequencing Systems, illumina.com/systems/sequencing-platforms/nextseq-1000-2000.html

DRAGEN Bio-IT Platform, illumina.com/products/by-type/informatics-products/dragen-bio-it-platform.html

References

1. Bentley DR, Balasubramanian S, Swerdlow HP, et al. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature*. 2008;456:53-59. doi:10.1038/nature07517
2. Satija R, Farrell JA, Gennert D, Schier AF, Regev A. Spatial reconstruction of single-cell gene expression. *Nat Biotechnol*. 2015;33(5):495-502. doi:10.1038/nbt.3192
3. 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. doi:10.1038/nbt.2859

Ordering information

Product	Catalog no.
NextSeq 2000 Sequencing System	20038897
NextSeq 1000 Sequencing System	20038898
NextSeq 1000 to NextSeq 2000 upgrade	20047256
NextSeq 1000/2000 P1 Reagents (300 cycles)	20050264
NextSeq 1000/2000 P2 Reagents (100 cycles)	20046811
NextSeq 1000/2000 P2 Reagents (200 cycles)	20046812
NextSeq 1000/2000 P2 Reagents (300 cycles)	20046813
NextSeq 2000 P3 Reagents (50 cycles)	20046810
NextSeq 2000 P3 Reagents (100 cycles)	20040559
NextSeq 2000 P3 Reagents (200 cycles)	20040560
NextSeq 1000/2000 Read and Index Primers	20046115
NextSeq 1000/2000 Index Primer Kit	20046116
NextSeq 1000/2000 Read Primer Kit	20046117
NextSeq 1000/2000 Recycling Tool	20050631

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