APPLICATION NOTE

NextSeq™ 1000 and 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 XLEAP-SBS chemistry to streamline sequencing workflows.

A flexible scRNA-Seq solution

The NextSeq 1000 and NextSeq 2000 Sequencing Systems (Figure 1) harness Illumina sequencing by synthesis (SBS) XLEAP-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).

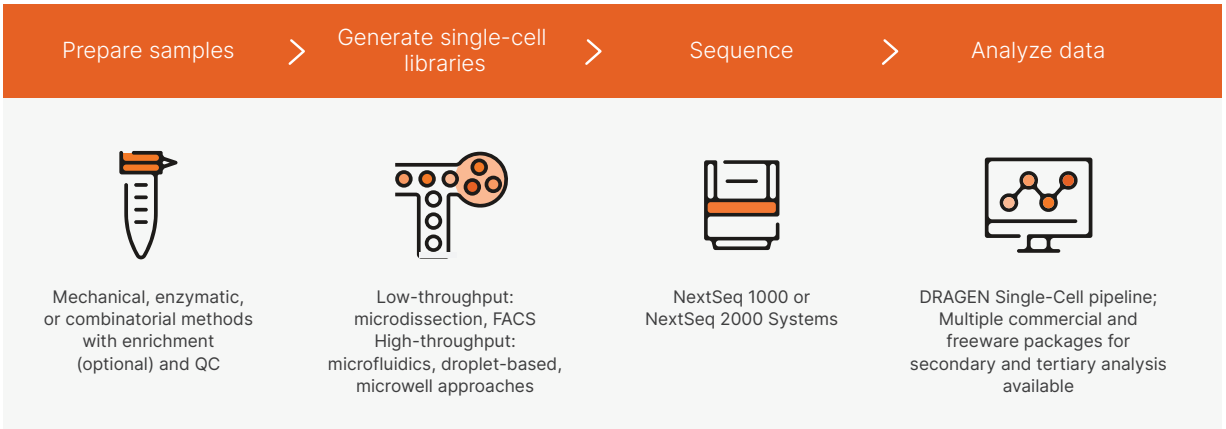

Figure 2: NextSeq 1000 and NextSeq 2000 scRNA-Seq workflow—The NextSeq 1000 and NextSeq 2000 Systems are part of an integrated NGS workflow that proceeds from initial tissue preparation through single-cell isolation and library preparation, sequencing and primary analysis, and data visualization and 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 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 Systems offer a cost-effective solution for sequencing and primary analysis in a benchtop sequencing system. With four available flow cell types, researchers have flexibility to use multiple methods of NGS analysis and accommodate a variety of experimental designs. If even higher sample throughput is needed, studies can be scaled up using the NovaSeq™ X Series (Table 2).

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	10x Genomics Cell Ranger DRAGEN Single-Cell RNA
RNA probe-based capture	10x Genomics Chromium	10x Genomics Single Cell Gene Expression Flex	5K	10K	10x Genomics Cell Ranger 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.

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

Sequencing system	Sequencing reagents	No. of samples per flow cell[a]
NextSeq 1000 and NextSeq 2000 Systems	P1 100 cycles	1[b]
	P2 100 cycles	4
	P3[c] 100 cycles	11–12
	P4[c] 100 cycles	18
NovaSeq X Series[d]	1.5B 100 cycles	15
	10B 100 cycles	96
	25B 300 cycles	250

a. Calculated for 5K cells per sample and 20K read pairs per cell; includes 38 extra cycles to support unique molecular identifiers (UMIs). Number of cells per sample and read pairs per cell depends on your desired application and sample type.
b. P1 Reagents are a good option for quality control experiments.
c. P3 and P4 Reagents are available for the NextSeq 2000 System only.
d. The NovaSeq X Plus System is capable of single flow cell runs or dual flow cell runs. The NovaSeq X System is capable of single flow cell runs.

Discover more with XLEAP-SBS chemistry

The NextSeq 1000 and NextSeq 2000 Systems are powered by XLEAP-SBS chemistry, the fastest, highest quality, and most robust Illumina SBS chemistry to date. Built upon the proven foundation of standard Illumina SBS chemistry, XLEAP-SBS chemistry delivers improved reagent stability with two-fold faster incorporation times. For scRNA-Seq, this 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 million reads of any Illumina benchtop sequencing system, the NextSeq 1000 and NextSeq 2000 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 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 shift between Illumina bulk RNA-Seq, scRNA-Seq, exome sequencing, and other applications. Multiomic insights are more accessible with the higher output from P4 flow cells. For example, researchers can pair scRNA-Seq with exome sequencing on the NextSeq 2000 System 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 Cell Ranger (10x Genomics) and SeqGeq (FlowJo/BD Biosciences), and open source tools such as Seurat[1] and Monocle.[2]

scRNA-Seq data analysis can be performed using tools Illumina DRAGEN™ secondary analysis,† a suite of accurate, comprehensive, and efficient data analysis pipelines. The NextSeq 1000 and NextSeq 2000 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. The DRAGEN Single-Cell pipeline can also be launched on the cloud in BaseSpace™ Sequence Hub or Illumina Connected Analytics. 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.

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

† DRAGEN hardware is included onboard the NextSeq 1000 and NextSeq 2000 Systems. A DRAGEN license is included with the instrument and does not need to be purchased separately.

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Comprehensive Illumina technical support

Illumina provides a world-class support team consisting 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 efficacy of their NextSeq 1000 and NextSeq 2000 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 and enabling 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 Systems with an expansive ecosystem of 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.

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 XLEAP-SBS Reagent Kit (100 cycles)[a,b]	20100983
NextSeq 1000/2000 P1 XLEAP-SBS Reagent Kit (300 cycles)[a]	20100982
NextSeq 1000/2000 P1 XLEAP-SBS Reagent Kit (600 cycles)[a]	20100981
NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (100 cycles)[a]	20100987
NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (200 cycles)[a]	20100986
NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (300 cycles)[a]	20100985
NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (600 cycles)[a]	20100984
NextSeq 2000 P3 XLEAP-SBS Reagent Kit (100 cycles)[a]	20100990
NextSeq 2000 P3 XLEAP-SBS Reagent Kit (200 cycles)[a]	20100989
NextSeq 2000 P3 XLEAP-SBS Reagent Kit (300 cycles)[a]	20100988
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (50 cycles)[a]	20100995
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (100 cycles)[a]	20100994
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (200 cycles)[a]	20100993
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (300 cycles)[a]	20100992
NextSeq 1000/2000 XLEAP-SBS Read and Index Primers[a]	20112856
NextSeq 1000/2000 XLEAP-SBS Index Primer Kit[a]	20112858
NextSeq 1000/2000 XLEAP-SBS Read Primer Kit[a]	20112859

a. XLEAP-SBS reagent kits for the NextSeq 1000 and NextSeq 2000 instruments are shipped and stored at the same temperature as standard SBS reagent kits.
b. For QC analysis.

Learn more

Single-cell RNA sequencing

NextSeq 1000 and NextSeq 2000 Sequencing Systems

DRAGEN secondary analysis

Demo data on BaseSpace Sequence Hub

References

1. 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
2. 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

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