

# NextSeq™ 1000 and NextSeq 2000 Sequencing Systems

Expansive application breadth and operational simplicity with proven performance



Broad platform utility with the flexibility and scalability to expand benchtop applications

Streamlined workflow powered by XLEAP-SBS™ chemistry and onboard DRAGENT™ secondary analysis

Robust performance and high-quality data from a proven global leader

**illumina®**

## Introduction

Next-generation sequencing (NGS) has revolutionized biological research, expanding the range and depth of sequencing applications that labs can perform. The research community can ask, and answer, increasingly complex scientific questions. Across the spectrum of oncology and microbiome research, and other emerging applications, more scientists are looking to harness the power of NGS technology.

Illumina is committed to upholding a proven track record of providing solutions that empower scientists to advance and accelerate their sequencing goals. For decades, Illumina has led the charge to improve sequencing capabilities by expanding benchtop applications, streamlining workflows, and maximizing accuracy. With the NextSeq 1000 and NextSeq 2000 Sequencing Systems, Illumina has continued this commitment to innovation ([Figure 1, Table 1](#)). These market-leading benchtop platforms are powered by high-performance Illumina sequencing by synthesis (SBS) chemistry and onboard integrated informatics. Users can obtain the throughput, data quality, and cost required to meet their needs, from small to large batch sizes and simple to data-intensive applications, all on a benchtop system. Add in unwavering expert support and labs are set for today's workload and future emerging applications.

## Robust performance and streamlined workflow

### High-performance SBS chemistry

The NextSeq 1000 and NextSeq 2000 Systems are powered by XLEAP-SBS chemistry, a faster, higher quality, and more robust SBS chemistry built on the proven foundation of standard Illumina SBS chemistry. XLEAP-SBS nucleotides use state-of-the-art dyes and novel linkers and blocks that are more resistant to heat, show a 50× reduction in hydrolysis, and 2.5× faster block cleavage to reduce phasing and prephasing. The XLEAP-SBS polymerase is engineered to incorporate nucleotides faster and with higher fidelity than ever before. Faster turnaround times with XLEAP-SBS chemistry can result in full-day time savings\* compared to run times with standard SBS.

Figure 1: The NextSeq 2000 Sequencing System



The NextSeq 2000 System offers innovative design features, advanced chemistry, simplified bioinformatics, and an intuitive workflow that enable the widest range of applications and flexibility of scale on a benchtop sequencing system.

### Innovations enabling productivity gains

The NextSeq 1000 and NextSeq 2000 Systems leverage advances in optics, instrument design, and reagent chemistry to miniaturize the volume of the sequencing reaction while increasing output and reducing the cost per run. This miniaturization provides scalability for various output quantities while maintaining the same high standards of data quality as experienced with other Illumina platforms. Additional innovations include high cluster-density patterned flow cells and a super-resolution optics system that yields highly accurate imaging data with greater resolution and higher sensitivity than traditional benchtop systems.

The NextSeq 1000 and NextSeq 2000 Systems offer a simplified workflow with load-and-go ease ([Figure 2](#)). Included onboard DRAGEN hardware powers fast secondary analysis and data compression. The instrument includes onboard cluster generation and prepares FASTQ files as part of the sequencing run. No extra equipment or steps are required.

\* For example, times for a P3 flow cell 2 × 150 bp run are eight hours faster with XLEAP-SBS reagents vs standard SBS.

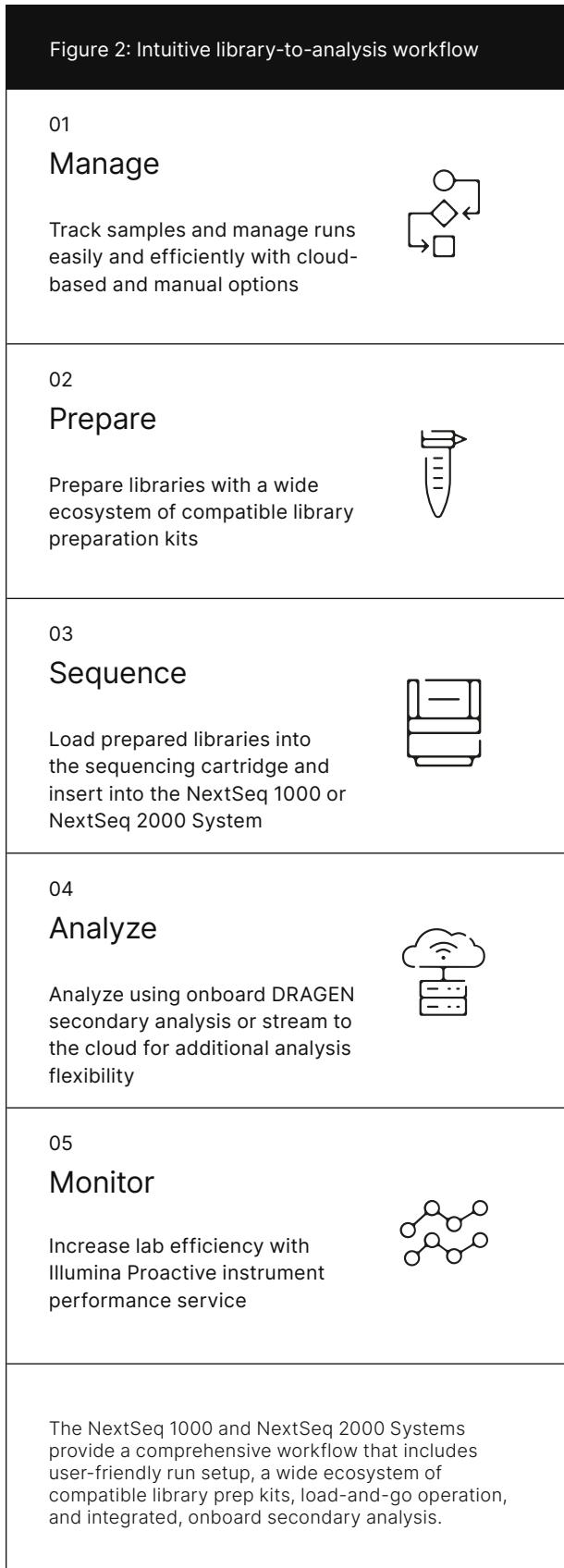
Table 1: Performance parameters for the NextSeq 1000 and NextSeq 2000 Systems

Read length	NextSeq 1000/2000 P1 XLEAP-SBS Reagents	NextSeq 1000/2000 P2 XLEAP-SBS Reagents	NextSeq 2000 P3 XLEAP-SBS Reagents <sup>a</sup>	NextSeq 2000 P4 XLEAP-SBS Reagents <sup>a</sup>
Output per flow cell <sup>b</sup>				
Single-end reads	100M	400M	1.2B	1.8B
1 × 50 bp	–	–	–	90 Gb
2 × 50 bp	10 Gb	40 Gb	120 Gb	180 Gb
2 × 100 bp	–	80 Gb	240 Gb	360 Gb
2 × 150 bp	30 Gb	120 Gb	360 Gb	540 Gb
2 × 300 bp	60 Gb	240 Gb	–	–
Quality scores <sup>c</sup>				
1 × 50 bp, 2 × 50 bp, 2 × 100 bp, 2 × 150 bp	≥ 90% of bases higher than Q30			
2 × 300 bp	≥ 85% of bases higher than Q30			
Run time				
1 × 50 bp	–	–	–	12 hr
2 × 50 bp	8 hr	12 hr	18 hr	20 hr
2 × 100 bp	–	19 hr	31 hr	34 hr
2 × 150 bp	17 hr	22 hr	40 hr	44 hr
2 × 300 bp	34 hr	42 hr	–	–

a. P3 and P4 flow cells only available on the NextSeq 2000 System.

b. Output specifications based on a single flow cell using an Illumina PhiX control library at supported cluster densities.

c. Quality scores are based on an Illumina PhiX control library; performance may vary based on library type and quality, insert size, loading concentration, and other experimental factors.

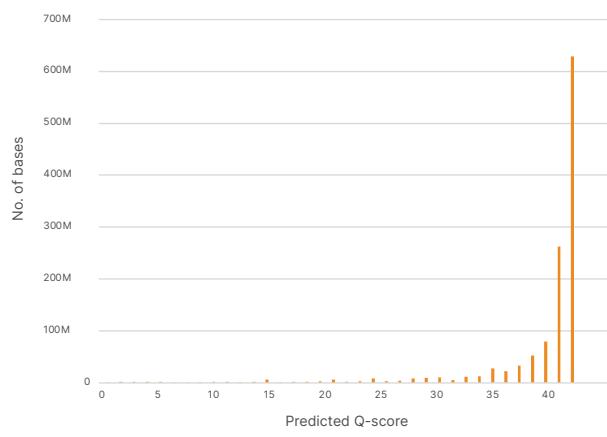


## Exceptional real-world performance

In addition to system specifications, it is important to consider real-world performance when evaluating which Illumina sequencing system is best suited for specific experimental needs. Illumina product specifications are traditionally set to guarantee performance at or above specification. When output exceeds specifications, the real-world cost per sample decreases.

Labs using both standard SBS and XLEAP-SBS kits consistently achieve data far above specifications for both yield and percentage of bases at or above Q30 ( $\% \geq Q30$ ) on their NextSeq 1000 and NextSeq 2000 Systems. A review of historical performance to specifications for standard SBS kits over thousands of runs demonstrates that more than 89% of real-world runs achieved output and  $\% \geq Q30$  above specifications (Table 2). Similarly, a review of hundreds of real-world runs shows that data generated using XLEAP-SBS kits consistently perform above specifications (Table 3). Additionally, internal Illumina studies demonstrate that high-quality  $80\% \geq Q40$  data are achieved with P4 XLEAP-SBS reagents (Figure 3).

**Figure 3: High-quality data generated on NextSeq 1000 and NextSeq 2000 Systems using XLEAP-SBS chemistry**



This filtered Q-score histogram generated by Illumina demonstrates that P4 XLEAP-SBS reagents have 80% or more bases at or above Q40. Q40 was measured using an internal real-time analysis software that provides unbinned Q scores.

Table 2: Examples of real-world data generated using standard SBS reagents

NextSeq 1000/2000 Reagents	Parameter	Specification	Average across recent runs	Average across top 10% of runs
NextSeq 1000/2000 P2 Reagent kits (200 and 300 cycles)	Clusters passing filter	400M	498M	558M
	Q30 score	85%	90%	96%
NextSeq 1000/2000 P3 Reagent kits (200 and 300 cycles)	Clusters passing filter	1.2B	1.3B	1.4B
	Q30 score	85%	90%	94%

Table 3: Examples of real-world data generated using XLEAP-SBS reagents

NextSeq 1000/2000 Reagents	Parameter	Specification	Average across runs
NextSeq 1000/2000 P2 XLEAP-SBS Reagent kits	Clusters passing filter	400M	523M
	Q30 score	90%	93%
NextSeq 2000 P3 XLEAP-SBS Reagent kits	Clusters passing filter	1.2B	1.3B
	Q30 score	90%	94%
NextSeq 2000 P4 XLEAP-SBS Reagent kits	Clusters passing filter	1.8B	1.9B
	Q30 score	90%	93%

## Easy-to-use integrated platform

The NextSeq 1000 and NextSeq 2000 Systems take advantage of an integrated reagent cartridge that includes fluidics and the waste holder, simplifying library loading and instrument use ([Figure 4](#)). Thaw and prepare the reagent cartridge, load the flow cell and prepared libraries into the cartridge, and then insert the assembled cartridge into the instrument. Denaturation and dilution steps occur onboard automatically.

In addition to ease of use, the fully integrated cartridge design improves efficiency throughout the sequencing run. By miniaturizing many of the sequencing reactions, the unique design:

- Lowers operating costs
- Improves recyclability
- Minimizes waste volume

Reagents never leave the cartridge, resulting in a dry instrument design that does not require washing, enables streamlined instrument maintenance, and optimizes instrument efficiency.

## Flexibility to do more, scalability to grow more

The NextSeq 1000 and NextSeq 2000 Systems offer broad platform utility with the flexibility to expand applications and scale efficiently. With fast, accurate results, outputs from 10 Gb up to 540 Gb, and integrated informatics options, the NextSeq 1000 and NextSeq 2000 Systems are ideal for a wide range of applications ([Table 4](#), [Table 5](#)) across metagenomics, spatial transcriptomics, single-cell studies, and more.

Figure 4: NextSeq 1000 and NextSeq 2000 reagent cartridge



The integrated cartridge includes reagents, fluidics, and the waste holder. Simply thaw and prepare the reagent cartridge, insert the flow cell, load the library, and place into the instrument.

## Breadth of applications

The NextSeq 2000 System offers four flow cell types (P1, P2, P3, and P4) and 14 kit configurations, while the NextSeq 1000 System offers<sup>†</sup> two flow cell types (P1 and P2) and seven kit configurations ([Table 1](#)). Read lengths from 1 × 50 bp to 2 × 300 bp support a wide variety of applications and study sizes ([Table 4](#)):

- 600-cycle kits for read length-dependent applications such as *de novo* assembly of small whole genomes, targeted metagenomics, shotgun metagenomics, metatranscriptomics, and immune repertoire profiling
- 300-cycle or 200-cycle kits for sequencing of targeted panels, exomes and large panels, transcriptomes, circulating tumor DNA, and large whole genomes
- 100-cycle or 50-cycle kits for counting-focused applications such as gene expression, single-cell, and spatial profiling

The NextSeq 2000 P4 flow cell delivers up to 1.8 billion single-end reads per run, maximizing batching and powering data-rich projects like exome sequencing, multiomics, single-cell, and spatial analysis.

<sup>†</sup>To ensure future scalability, customers who purchase a NextSeq 1000 System can easily upgrade to the NextSeq 2000 System.

## Streamlined analysis options

The NextSeq 1000 and NextSeq 2000 Systems offer access to onboard, local, and cloud-based analysis software, giving users the flexibility to analyze data in a manner that meets their needs ([Figure 5](#)).

Runs can be set up locally or in the cloud. For local setup, users can create their own sample sheet or take advantage of a convenient, preset Illumina template. Cloud-based setup uses the Run Planner app in BaseSpace™ Sequence Hub. After the run setup information is ready, it is imported into the NextSeq 1000 and NextSeq 2000 Systems. Users then select and start the run of interest.

Optimized instrument software provides a cleaner interface and improved visualization of instrument and run status compared to earlier benchtop systems. The NextSeq 1000 and NextSeq 2000 Systems output industry-standard file formats used by various laboratory information management systems (LIMS) for secure, automated sample tracking and information management. Secondary analysis can be configured as part of run setup, reducing the number of user touchpoints required.

## Accurate and efficient analysis with onboard DRAGEN secondary analysis

Onboard DRAGEN (Dynamic Read Analysis for GENomics) secondary analysis offers an accurate, efficient solution for variant calling. The DRAGEN platform uses optimized, hardware-accelerated algorithms for a wide variety of genomic analysis solutions, including base call (BCL) file conversion, compression, mapping, alignment, sorting, duplicate marking, and variant calling.

The onboard solution provides access to select DRAGEN informatics pipelines ([Table 5](#)), enabling users to generate results in as little as two hours. DRAGEN informatics use best-in-class pipeline algorithms to help users overcome bottlenecks in data analysis and reduce reliance on external informatics experts. Onboard DRAGEN analysis is included in the instrument cost and does not require the purchase of an additional license.

Table 4: Some of the broader applications available on the NextSeq 1000 and NextSeq 2000 Systems

Application <sup>a</sup>	P1 XLEAP-SBS		P2 XLEAP-SBS		P3 XLEAP-SBS		P4 XLEAP-SBS	
	No. samples	Time	No. samples	Time	No. samples	Time	No. samples	Time
Small whole-genome sequencing (300 cycles) 130 Mb genome, > 30x coverage	7	17 hr	30	22 hr	92	40 hr	138	44 hr
Whole-exome sequencing (200 cycles) ~8 Gb per exome, 100x mean coverage	~2 <sup>b</sup>	17 hr <sup>b</sup>	10	19 hr	30	31 hr	45	34 hr
Solid tumor FFPE comprehensive genomic profiling (200 cycles)	–	–	8	19 hr	24	31 hr	36	34 hr
Total RNA-Seq (200 cycles) 50M read pairs per sample	2 <sup>b,c</sup>	17 hr <sup>b</sup>	8	19 hr	24	31 hr	36	34 hr
mRNA-Seq (200 cycles) 25M read pairs per sample	4 <sup>b,c</sup>	17 hr <sup>b</sup>	16	19 hr	48	31 hr	72	34 hr
Single-cell RNA-Seq (100 cycles) 5K cells, 20K reads per cell	1 <sup>d</sup>	8 hr	4	12 hr	12	18 hr	18	20 hr
miRNA-Seq or small RNA analysis (50 cycles) 11M reads per sample	9 <sup>e</sup>	8 hr <sup>e</sup>	36 <sup>e</sup>	12 hr <sup>e</sup>	108 <sup>e</sup>	18 hr <sup>e</sup>	163	12 hr
16S RNA sequencing (600 cycles)	384 <sup>f</sup>	34 hr	384 <sup>f</sup>	42 hr	–	–	–	–

- a. Recommended sequencing depth will largely depend on sample type and experimental objective and will need to be optimized for each study.
- b. 200-cycle kits not available for P1 flow cells. Use P1 300-cycle kit.
- c. Recommended read lengths are 2 × 75 bp for Illumina Stranded Total RNA Prep and Illumina Stranded mRNA Prep and 2 × 100 bp for Illumina RNA Prep with Enrichment.
- d. P1 reagents are a good option for single-cell quality control experiments.
- e. 50-cycle kits not available for P1, P2, or P3 XLEAP-SBS flow cells. Use 100-cycle kits.
- f. A maximum of 384 unique dual indexes are available.

Figure 5: Flexible informatics suite

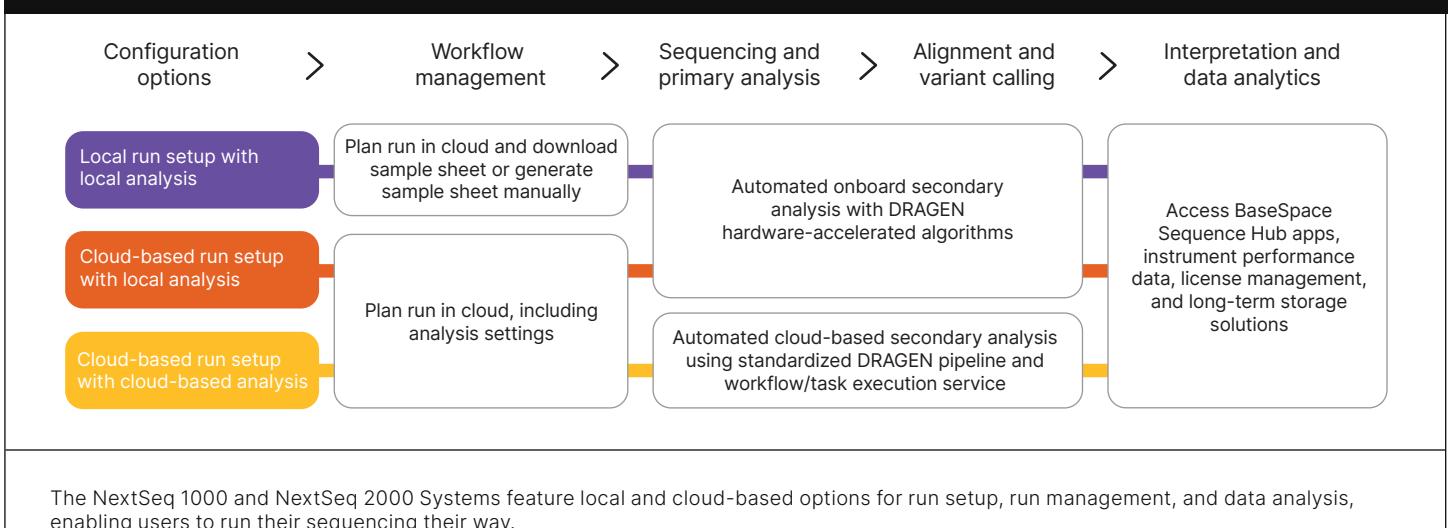


Table 5: DRAGEN informatics pipelines integrated into the NextSeq 1000 and NextSeq 2000 Systems

Pipeline <sup>a</sup>	Applications
DRAGEN Enrichment	<ul style="list-style-type: none"> <li>Whole-exome sequencing</li> <li>Targeted resequencing</li> </ul>
DRAGEN RNA	<ul style="list-style-type: none"> <li>Whole-transcriptome gene expression</li> <li>Gene fusion detection</li> </ul>
DRAGEN Single-Cell RNA	<ul style="list-style-type: none"> <li>Single-cell whole-transcriptome sequencing</li> </ul>
DRAGEN Germline	<ul style="list-style-type: none"> <li>Whole-genome sequencing</li> </ul>
DRAGEN Amplicon <sup>b</sup>	<ul style="list-style-type: none"> <li>DNA amplicon panels</li> <li>Targeted resequencing</li> </ul>
DRAGEN ORA <sup>c</sup> Compression	<ul style="list-style-type: none"> <li>Compression of FASTQ files</li> </ul>

a. Additional DRAGEN informatics pipelines are available in the cloud; visit [illumina.com/DRAGEN](https://illumina.com/DRAGEN) for a complete list.  
 b. Supported for DNA samples only.  
 c. ORA, original read archive; DRAGEN ORA Compression can be enabled with any of the DRAGEN pipelines.

## Automatic integration with the cloud

For users who prefer a cloud-based analysis solution, the NextSeq 1000 and NextSeq 2000 Systems are integrated with BaseSpace Sequence Hub and Illumina Connected Analytics, the Illumina genomics cloud-computing platforms. DRAGEN analysis on BaseSpace Sequence Hub and Illumina Connected Analytics features advanced results visualization and analysis capabilities packaged in an intuitive interface. In the cloud, users can access a wide selection of bioinformatics tools and share data globally. Data generated using the NextSeq 1000 and NextSeq 2000 Systems are compatible with industry-standard formats for convenient import into the solution of choice.

## Proven global leader

The NextSeq 1000 and NextSeq 2000 Systems offer the certainty of a field-tested solution and a reliable sequencing partner. Since the launch in 2020, over 2000 instruments have been installed and used to generate thousands of publications. With a mature global quality and manufacturing infrastructure, Illumina provides comprehensive support and best-in-class product consistency, setting the standard for NGS solutions.

Joining this community gives access to a large ecosystem of applications, protocols, and informatics that have been built in collaboration with thousands of researchers and industry thought leaders across the globe.

## Confidence in continued innovation

Illumina has a proven track record of developing genomics solutions that empower researchers to perform studies at the throughput, scale, and price meeting their research objectives. Customer experience is at the center of every innovation, making it as easy as possible to prepare samples, sequence, and analyze data.

## Simple, fast support

### Modular construction

The NextSeq 1000 and NextSeq 2000 Systems are built in a modular fashion, simplifying service and support. Onboard sensors monitor system performance and alert users to possible issues. Troubleshooting and repairs are easier for the service engineer to perform, ultimately saving time.

### Illumina Proactive

Illumina Proactive is a secure and remote instrument performance support service designed to detect risk failure preemptively, troubleshoot runs more efficiently, and prevent in-run failures. The complementary service helps minimize unplanned downtime and avoid unnecessary sample loss by anticipating repairs and alerting Illumina field personnel to schedule maintenance visits.

## Commitment to your success

Each system purchase includes a one-year service warranty. Comprehensive maintenance, repair, and qualification solutions are also available. In addition, Illumina offers on-site training, ongoing support, phone consults, webinars, and courses at various Illumina locations globally. We're here with all the resources that you need to accelerate progress.

Our world-class support team is composed of experienced scientists who are experts in library prep, sequencing, and analysis to help you maximize your investment and support peak performance. This dedicated team includes highly qualified field service engineers (FSE), technical applications scientists (TAS), field application 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 access online support 24/7, worldwide and in multiple languages.

## Summary

The NextSeq 1000 and NextSeq 2000 Systems expand what can be accomplished with a benchtop sequencing system. High flexibility and scalability, combined with enhanced performance using XLEAP-SBS chemistry, enable a wide range of applications. The NextSeq 1000 and NextSeq 2000 Systems offer proven NGS technology with operational simplicity, a streamlined workflow including analysis, and comprehensive support.

**Learn more →**

[NextSeq 1000 and NextSeq 2000 Systems](#)

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

## NextSeq 1000 and NextSeq 2000 Systems specifications

Parameter	Specifications
Instrument configuration	Self-contained, dry instrument with integrated DRAGEN field-programmable gate array (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, noncondensing 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: 55 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



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