SPECIFICATION SHEET

NovaSeq™ 6000 Sequencing System

Immense discovery power for deeper insights

- Match data output, time to results, and cost per sample to study needs
- Configure sequencing method, flow cell type, and read length for a broad range of applications
- Increase lab efficiency with a simplified workflow and reduced hands-on time

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Introduction

The NovaSeq 6000 System (Figure 1) unleashes a new era in sequencing with groundbreaking innovations, providing users with the throughput, speed, and flexibility to complete projects faster and more economically than ever before. Leveraging proven Illumina next-generation sequencing (NGS) technology, multiple flow cell types, two library loading workflows, and various read length combinations, the NovaSeq 6000 System enables effective throughput scaling to suit virtually any study needs.

Applications requiring large amounts of data, such as human whole-genome sequencing (WGS), ultradeep whole-exome sequencing (WES), and whole-transcriptome sequencing can now be completed in a more cost-effective manner. For additional flexibility, the NovaSeq Xp workflow supports individual lane loading for sequencing different library pools in each flow cell lane. When combined with lower output flow cells, the same instrument can be used for less data intensive methods. Every project, regardless of the size or goal, will benefit from easy operation and integration with data storage and analysis tools that help streamline the overall experimental workflow. Whether running a single NGS system or a large fleet, the NovaSeq 6000 System opens new possibilities across a range of sample types, sequencing methods, and applications.

Figure 1: NovaSeq 6000 System—Transforming sequencing by combining throughput, flexibility, and ease of use for virtually any method, genome, and scale.

Scalable platform

The NovaSeq 6000 System provides access to a powerful, high-throughout genomics solution that empowers users to perform studies at the throughput and cost per sample that meets their research objectives.

Match data output to project needs

The NovaSeq 6000 System offers output up to 6 Tb and 20B reads per dual S4 run in < 2 days. Multiple flow cell types and read length combinations offer flexible output and run time configurations based on project needs (Table 1). NovaSeq S Prime (SP), S1, and S2 flow cells provide quick and powerful sequencing for most high-throughput applications. With fewer reads than a HiSeq™ 2500 or HiSeq 4000 flow cell and the flexibility of individual lane loading, the NovaSeq SP and S1 flow cells allows existing HiSeq System users to transition easily without the need to batch additional samples per run (comparison reads for the HiSeq 2500 System based on specifications for the HiSeq SBS Kit v4). The NovaSeq S4 flow cell enables high-throughput cost-effective sequencing across a range of applications, making in-house WGS or WES studies an attractive and affordable option for more labs.

Flexible performance

The NovaSeq 6000 System offers tremendous flexibility in sequencing options, supporting an extensive output range (Figure 2). Users can mix and match between four flow cell types (SP, S1, S2, or S4), run one or two flow cells at a time, and choose between multiple read lengths to easily adjust output and sample throughput for each sequencing run (Table 1).

Table 1: NovaSeq 6000 System flow cell specifications

Flow cell type	SP	S1	S2	S4
Lanes per flow cell	2	2	2	4
Output per flow cell[a]				
1 × 35 bp	N/A	N/A	N/A	280-350 Gb
2 × 50 bp	65-80 Gb	134-167 Gb	333-417 Gb	N/A
2 × 100 bp	134-167 Gb	266-333 Gb	667-833 Gb	1600-2000 Gb
2 × 150 bp	200-250 Gb	400-500 Gb	1000-1250 Gb	2400-3000 Gb
2 × 250 bp	325-400 Gb	N/A	N/A	N/A
Single reads CPF	0.65-0.8B	1.3-1.6B	3.3-4.1B	8-10B
PE reads CPF	1.3-1.6B	2.6-3.2B	6.6-8.2B	16-20B
Quality scores[b]				
1 × 35 bp	Q30 ≥ 90%			
2 × 50 bp	Q30 ≥ 90%			
2 × 100 bp	Q30 ≥ 85%			
2 × 150 bp	Q30 ≥ 85%			
2 × 250 bp	Q30 ≥ 75%			
Run time[c]				
1 × 35 bp	N/A	N/A	N/A	~ 14 hr
2 × 50 bp	~ 13 hr	~ 13 hr	~ 16 hr	N/A
2 × 100 bp	~ 19 hr	~ 19 hr	~ 25 hr	~ 36 hr
2 × 150 bp	~ 25 hr	~ 25 hr	~ 36 hr	~ 44 hr
2 × 250 bp	~ 38 hr	N/A	N/A	N/A

a. Output and read number specifications based on a single flow cell using Illumina PhiX control library at supported cluster densities; the NovaSeq 6000 System can run one or two flow cells simultaneously
b. Quality scores are based on NovaSeq 6000 SP, S2, and S4 Reagent Kits v1.5 run on the NovaSeq 6000 System using an Illumina PhiX control library; performance may vary based on library type and quality, insert size, loading concentration, and other experimental factors
c. Run times are based on running two flow cells of the same type; starting two different flow cells will impact run time

N/A, not applicable; CPF, clusters passing filter; PE, paired-end

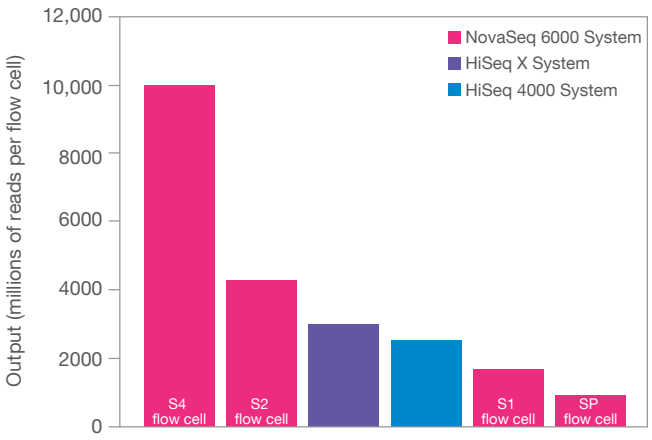

Figure 2: The NovaSeq 6000 System offers the broadest output range—The NovaSeq 6000 System generates from 80 Gb and 800M reads to 3 Tb and 10B reads of data in single flow cell mode. In dual flow cell mode, output can be up to 6 Tb and 20B reads. The tunable output makes the NovaSeq 6000 System accessible for a wide range of applications.

Maximize library loading configurations

The NovaSeq 6000 System offers two methods for flow cell loading: the NovaSeq Xp and standard workflows.

NovaSeq Xp workflow

With the optional NovaSeq Xp workflow, available separately, users can load each flow cell lane individually to separate different projects or methods between lanes. The NovaSeq Xp workflow also allows users to multiplex samples within a lane to maximize the total number of samples per flow cell (eg, 96-plex per lane in each of the four lanes on a NovaSeq S4 flow cell for a total of 384 samples). As an added benefit, this multiplexing reduces the amount of DNA input required compared to the standard workflow.

The NovaSeq Xp workflow offers an alternative to standard onboard cluster generation. It consists of the NovaSeq Xp Kit, containing reagents and a disposable manifold for sample loading, and requires the NovaSeq Xp Flow Cell Dock that holds flow cells for loading. The NovaSeq Xp workflow is compatible with automation.

Standard workflow

For faster sample loading, the NovaSeq 6000 System offers the standard workflow featuring fully automated onboard cluster generation for ease of use and reduced hands-on time. Prepared libraries are loaded directly into a sample tube that sits in a preconfigured reagent cartridge, which is loaded directly onto the system for automated cluster generation.

Wide range of sequencing methods

The NovaSeq 6000 System is compatible with various Illumina library preparation kits, supporting a wide range of methods, from expression profiling to WGS and beyond (Table 2).

Table 2: NovaSeq 6000 System applications

Whole-genome sequencing
Illumina DNA PCR-Free Prep
Illumina DNA Prep
Targeted resequencing
Illumina DNA Prep with Enrichment + Illumina Exome Panel
Illumina RNA Prep with Enrichment + Illumina Exome Panel
RNA sequencing
Illumina Stranded Total RNA Prep with Ribo-Zero Plus
Illumina Stranded mRNA Library Prep
Epigenetic sequencing
TruSeq Methyl Capture EPIC
Unique dual indexing
IDT for Illumina DNA/RNA UD Indexes, Tagmentation (384 indexes across Sets A-D)

Library prep methods listed are only examples of those available for use with the NovaSeq 6000 System. For a complete list, visit www.illumina.com.

Streamlined operation

The NovaSeq 6000 System increases lab efficiency through several advanced features (Figure 3):

- Load-and-go reagent cartridges represent an 80% reduction in consumables compared to the HiSeq Series of Systems
- Ready-to-use reagents require no preparation other than thawing and inversion, eliminating the need for user intervention, minimizing human error, and drastically reducing run setup time
- Radio-frequency identification (RFID)–encoded consumables enable automated reagent traceability and ensure compatibility of all sequencing reagents and flow cells
- Automated flow cell loading and onboard cluster generation minimize hands-on time
- Extended reagent shelf life supports efficient planning for future projects
- A 35-cycle kit increases workflow options, including COVIDSeq™ Test and counting applications, while lowering the cost per read
- Enhanced reagent chemistry streamlines the workflow, increases the number of unique molecular identifiers (UMIs) to accommodate more complex indexing strategies, and supports the wide range of Illumina library preparation solutions (Table 2)

Cutting-edge technology

The NovaSeq 6000 System provides powerful, simple, scalable, and reliable high-throughput sequencing, producing outstanding data quality. The instrument relies on proven Illumina sequencing by synthesis (SBS) chemistry. This proprietary reversible terminator–based method enables the massively parallel sequencing of billions of DNA fragments, detecting single bases as they are incorporated into growing DNA strands. The method significantly reduces errors and missed calls associated with strings of repeated nucleotides (homopolymers).

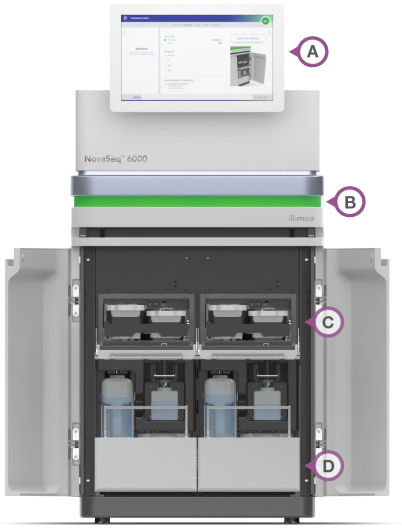

Figure 3: Straightforward operation—Many features of the NovaSeq 6000 System are designed to simplify genomic studies, including (A) intuitive touch screen interface, (B) lighted LED display that indicates flow cell status, (C) snap-in cartridges containing ready-to-use reagents, and (D) waste containers that can be easily removed for disposal.

Ingenious design

The NovaSeq 6000 System unites high-performance imaging with patterned flow cell technology to deliver massive increases in throughput. Superior optics offer high-resolution, high-speed scanning, contributing to making the NovaSeq 6000 System the highest throughput Illumina sequencing platform yet. Each NovaSeq flow cell contains billions of nanowells at fixed locations for even cluster spacing and uniform feature size. NovaSeq flow cells reduce spacing between nanowells, significantly increasing cluster density. Combining the higher cluster density with proprietary exclusion amplification clustering maximizes the number of nanowells occupied by DNA clusters originating from a single DNA template for a substantial increase in data output.

Comprehensive NGS workflow

The NovaSeq 6000 System forms the cornerstone of a sequencing ecosystem encompassing workflow management, manual or automated library preparation, sequencing, data analysis and interpretation, service, and support (Figure 4).

Laboratory information management system (LIMS)

Out of the box, the NovaSeq 6000 System is fully compatible with BaseSpace™ Clarity LIMS. Using a LIMS, labs can improve operational efficiency with comprehensive sample and reagent tracking, automated workflows, and integrated instrument operation. BaseSpace Clarity LIMS offers an intuitive user interface and preconfigured workflows, enabling rapid adoption for immediate process tracking and scalability. The NovaSeq 6000 System can also be integrated with user-developed and other third-party LIMS.

Automated library preparation

Illumina has partnered with leading automated liquid-handling suppliers to develop several “Illumina Qualified” methods (Table 2). The Illumina Qualified designation means that libraries prepared with these methods perform comparably to those prepared manually. Illumina Qualified methods can be rapidly installed for immediate use in any lab, minimizing time-consuming, costly development efforts. Automating library preparation increases uniformity across experiments, minimizes errors, reduces hands-on time, and enables higher throughput, allowing users to harness the unmatched productivity of the NovaSeq 6000 System.

Data analysis and interpretation

Data from the NovaSeq 6000 System can be streamed into BaseSpace Sequence Hub, a user-friendly genomics cloud computing platform optimized for processing large data volumes. BaseSpace Sequence Hub offers simplified data management, analysis, and storage. There, users can access the DRAGEN™ (Dynamic Read Analysis for GENomics) Bio-IT Platform for accurate, ultrarapid secondary analysis of NGS data or a number of BaseSpace apps for alignment and variant detection, annotation,

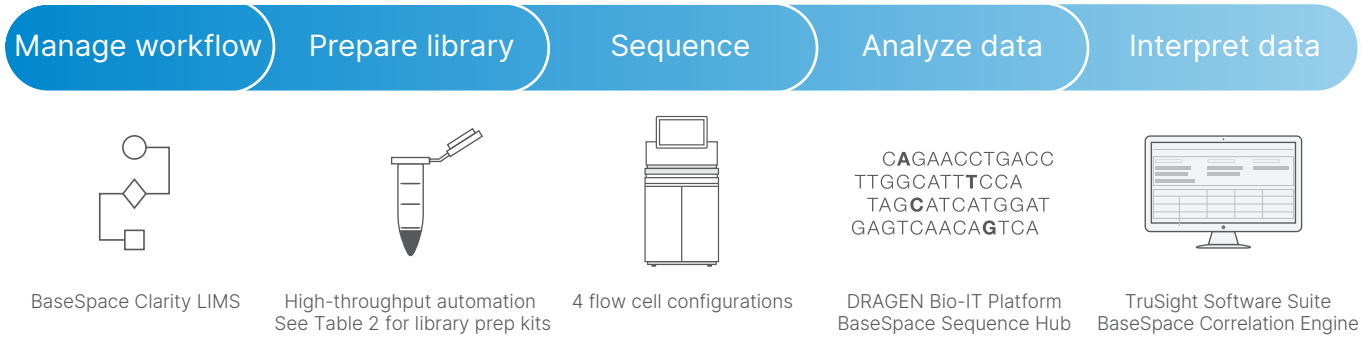

Figure 4: NovaSeq 6000 System NGS workflow—The NovaSeq 6000 System is compatible with BaseSpace Clarity LIMS, the Illumina library preparation kit portfolio, Illumina Qualified Methods support, data analysis solutions such as the DRAGEN Bio-IT Platform and BaseSpace Sequence Hub, and downstream data interpretation tools such as TruSight Software Suite and BaseSpace Correlation Engine.

visualization, and more. For other analysis options, including in-house pipelines, the NovaSeq System software generates base calls and quality scores that are translated into FASTQ files for downstream analysis.

To assist with data interpretation, Illumina offers the TruSight™ Software Suite and BaseSpace Correlation Engine. TruSight Software Suite provides intuitive and comprehensive tools for visualizing, triaging, and interpreting variants associated with genetic disease. BaseSpace Correlation Engine integrates data with the world's genomic knowledgebase for comparison across a large, curated repository of public data sets.

Summary

The NovaSeq 6000 System expands NGS possibilities for all researchers. With unmatched scalable throughput, tremendous flexibility for a range of applications, and streamlined operation, the NovaSeq 6000 System is the most powerful high-throughput Illumina sequencing system to date, perfectly positioned to help users uncover more about the genome than ever before.

Learn more

NovaSeq 6000 System, illumina.com/novaseq

Ordering information

System	Catalog no.
NovaSeq 6000 System	20013850
Sequencing reagent kits	**Catalog no.**
NovaSeq 6000 SP Reagent Kit v1.5 (100 cycles)	20028401
NovaSeq 6000 SP Reagent Kit v1.5 (200 cycles)	20040719
NovaSeq 6000 SP Reagent Kit v1.5 (300 cycles)	20028400
NovaSeq 6000 SP Reagent Kit v1.5 (500 cycles)	20028402
NovaSeq 6000 S1 Reagent Kit v1.5 (100 cycles)	20028319
NovaSeq 6000 S1 Reagent Kit v1.5 (200 cycles)	20028318
NovaSeq 6000 S1 Reagent Kit v1.5 (300 cycles)	20028317
NovaSeq 6000 S2 Reagent Kit v1.5 (100 cycles)	20028316
NovaSeq 6000 S2 Reagent Kit v1.5 (200 cycles)	20028315
NovaSeq 6000 S2 Reagent Kit v1.5 (300 cycles)	20028314
NovaSeq 6000 S4 Reagent Kit v1.5 (35 cycles)	20044417
NovaSeq 6000 S4 Reagent Kit v1.5 (200 cycles)	20028313
NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles)	20028312
Sequencing reagent kits	**Catalog no.**
NovaSeq Xp Flow Cell Dock	20021663
NovaSeq Xp 2-Lane Kit v1.5	20043130
NovaSeq Xp 4-Lane Kit v1.5	20043131

NovaSeq 6000 System specifications

Specifications
Instrument configuration Computer and touch screen display Installation setup and accessories Data collection and analysis software
Instrument control computer Base unit: Portwell WADE-8022 with Intel i7 4700EQ CPU Memory: 2 × 8 GB DDR3L SODIMM. Hard drive: None Solid-state drive: 256 GB mSATA Operating System: Windows 10 Note: Computer configurations will be upgraded regularly; contact your local account manager for current configuration.
Operating environment Temperature: 19°C to 25°C (22°C ±3°C), < 2°C change per hour Humidity: noncondensing 20–80% relative humidity Altitude: below 2000 meters (6500 feet) Ventilation: maximum of 8530 BTU/h and average 6000 BTU/h For indoor use only
Laser Class 1 laser product embedded with class IV lasers: 532 nm, 660 nm, 780 nm, 790 nm
Dimensions W × D × H: 80.0 cm (31.5 in) × 94.5 cm (37.2 in) × 165.6 cm (65.2 in) with monitor. Weight: 481 kg (1059 lb), includes 3.5 kg (7.8 lb) for leak tray and 0.9 kg (2 lb) for keyboard and mouse, Crated weight: 628 kg (1385 lb)
Power requirements 200–240 VAC 50/60Hz, 16A, single phase, 2500 W Illumina provides a region-specific uninterruptible power supply
Radio frequency identifier (RFID) Frequency: 13.56 MHz Power: supply 3.3 volts DC ± 5%, current 120 mA, RF output power 200 mW
Network connection Dedicated 1 Gb connection between the instrument and data management system. Connect directly or through network.
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

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1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

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