

# NextSeq™ 550 Sequencing System

Fast, flexible, high-throughput benchtop sequencer enables transcriptome and targeted resequencing applications with the accuracy of Illumina sequencing technology.

## Highlights

- **Highly flexible to fit research demands**  
Supports a broad range of sequencing applications and offers tunable read length with multiple output configurations
- **Fast turnaround time**  
Rapidly generate data for time-critical studies and accelerate research studies
- **Exceptional data accuracy**  
Proven sequencing by synthesis (SBS) chemistry delivers high-quality results even in challenging homopolymer regions
- **Push-button operation and easy data analysis**  
Walk-away DNA-to-data solution with streamlined informatics performed onsite or in the cloud
- **Ability to scan select Illumina BeadChips**  
Determine differentially methylated regions using the Infinium™ MethylationEPIC array or measure copy number variants using select CytoSNP arrays



**Figure 1: NextSeq 550 System**—By leveraging the latest advances in SBS chemistry and user-friendly workflows, the NextSeq 550 System delivers high-quality results for exome, transcriptome, and targeted resequencing applications.

## Introduction

A transformative addition to the proven Illumina next-generation sequencing (NGS) system portfolio, the NextSeq 550 Sequencing System delivers the power of high-throughput sequencing combined with highly complementary microarray scanning (Figure 1). The integrated sequencing and microarray scanning system fits seamlessly into research laboratories, reducing the need for multiple instruments. Its fast, DNA-to-data workflow enables rapid sequencing of exomes, targeted panels, and transcriptomes in a single run, with the flexibility to switch to low- or high-throughput sequencing as needed. Illumina scientists are available at every point along the way with support and guidance, enabling researchers to focus on making the next breakthrough discovery.

## Enabling new discoveries

The NextSeq 550 System enables researchers to keep pace with technology, switching quickly from one application to another, and configuring output based on sample volume and coverage needs. This robust, scalable system turns a broad range of high-throughput applications into affordable everyday research tools. Now, even the smallest laboratory can access a wide range of NGS and microarray applications to advance their studies and research goals.

## Fast and easy sequencing workflow

The NextSeq 550 System is part of a fully integrated workflow from library preparation to data analysis, report generation, and data



**Figure 2: NextSeq 550 System sequencing workflow**—The NextSeq 550 System offers a simple, integrated sequencing workflow from sample preparation to data analysis. Workflow times will vary by experiment and assay type. Details shown are for an mRNA expression profiling study assuming 2 × 75 bp on instrument. Analysis results include differential expression and identification of alternative transcripts.

sharing (Figure 2). The intuitive user interface and load-and-go design allow researchers to perform various sequencing applications with minimal user training or instrument set-up time. It takes less than 10 minutes to load and set up a NextSeq 550 System. While other platforms require additional, specialized equipment, the NextSeq 550 System integrates cluster generation and sequencing into a single instrument, and offers a seamless transition for onsite or cloud-based data analysis.

After library preparation, libraries are loaded into the NextSeq 550 System where sequencing is automated and fast. Data are generated in as little as 12 hours for a 75-cycle sequencing run, and less than 30 hours for paired 150-cycle run. By employing Illumina SBS chemistry and file format conventions, the NextSeq 550 System offers a broad ecosystem of established protocols, workflows, data sets, and data analysis tools.

Table 1: Ultimate flexibility for multiple applications

Application	High-output flow cells		Mid-output flow cells	
	No. of samples	Time	No. of samples	Time
Gene expression profiling				
> 10 M reads	40	11 hours	13	11 hours
1 × 75 bp				
mRNA-Seq				
> 25 M reads	16	18 hours	5	15 hours
2 × 75 bp				
Enrichment panel				
12 Mb region	36	29 hours	12	26 hours
> 20× coverage at > 95% targets				
Whole-exome sequencing				
50× mean coverage	12	18 hours	3	15 hours
Small whole-genome sequencing				
130 Mb genome	30	29 hours	10	26 hours
> 30× coverage				
2 × 150 bp				

## Versatile and flexible to support a wide range of sequencing applications

The NextSeq 550 System offers the right sequencer for various project sizes and sequencing throughputs, providing users with optimal operational efficiency. The NextSeq 550 System also delivers a one-day turnaround for numerous popular sequencing applications. With this instrument, researchers can sequence a broad range of samples per run:

- 1-12 exomes
- 1-16 transcriptomes
- 6-96 targeted panels
- 12-40 gene expression profiling samples

The NextSeq 550 System is easily configured, providing researchers with scalability to handle various project sizes. Based on sample volume and coverage needs, researchers can choose between two flow cell configurations (High-output and Mid-output), easily shifting from low- to high-throughput with each sequencing run (Table 1). The NextSeq 550 System provides integrated support for paired-end sequencing, offering user-defined read lengths up to 2 × 150 bp. The system is supported by the full suite of Illumina library preparation and target enrichment solutions, offering library compatibility across the Illumina sequencing portfolio. This allows researchers to easily scale up studies to the higher-throughput HiSeq™ and NovaSeq™ Systems, or perform follow-up studies on the MiSeq™ System (Figure 3).

## SBS chemistry delivers exceptional accuracy

At the core of the NextSeq 550 Sequencing System is proven Illumina SBS chemistry—the most widely adopted NGS technology worldwide.<sup>1</sup> This proprietary, reversible, terminator-based method enables the parallel sequencing of millions of DNA fragments, detecting single bases as they are incorporated into growing



**Figure 3: Illumina NGS sequencing systems portfolio**—Illumina NGS systems offer solutions for a broad range of applications, sample types, and sequencing scales. Each delivers high-quality data and high accuracy with flexible throughput and simple, streamlined workflows. Data can be seamlessly compared, exchanged, and analyzed in BaseSpace™ Sequence Hub.

DNA strands. The method virtually eliminates errors and missed calls associated with strings of repeated nucleotides (homopolymers). Furthermore, NextSeq v2.5 Reagent Kits utilize an optimized v2.5 flow cell. The v2.5 flow cell maintains starting fluorescent intensity, resulting in improved performance throughout the run.<sup>2</sup> With NextSeq v2.5 chemistry, the NextSeq 550 System provides exceptional accuracy for a broad range of methods including human genome, targeted panel, exome, or transcriptome data at a wide range of coverage levels.

Table 2: NextSeq 550 System performance parameters

NextSeq 550 System performance parameters <sup>a</sup>					
Flow cell configuration	Read length	Output	Run time	Data quality	Required input
High-output flow cell	2 × 150 bp	100-120 Gb	29 hours	> 75% > Q30	100 ng-1 µg with TruSeq™ Library Prep Kits
Up to 400 M single reads	2 × 75 bp	50-60 Gb	18 hours	> 80% > Q30	
Up to 800 M paired-end reads	1 × 75 bp	25-30 Gb	11 hours	> 80% > Q30	
Mid-output flow cell	2 × 150 bp	32-39 Gb	26 hours	> 75% > Q30	
Up to 130 M single reads	2 × 75 bp	16-19 Gb	15 hours	> 80% > Q30	
Up to 260 M paired-end reads					

a. Total times include cluster generation, sequencing, and base calling on a NextSeq 550 System. Install specifications are based on Illumina PhiX control library at supported cluster densities (between 129 and 165 k/mm<sup>2</sup> clusters passing filter). Actual performance parameters may vary based on sample type, sample quality, and clusters passing filter. The percentage of bases > Q30 is averaged over the entire run.

NextSeq 550 System array scanning parameters			
	BeadChip	Scan time per BeadChip	Scan time per sample
	Infinium MethylationEPIC BeadChip	40 minutes	5 minutes
	Infinium™ CytoSNP-850K BeadChip	40 minutes	5 minutes
	HumanCytoSNP-12 BeadChip	40 minutes	3.3 minutes
	Infinium HumanKaryomap-12 BeadChip	40 minutes	3.3 minutes

## Experimental flexibility with array scanning

The NextSeq 550 System enables experimental flexibility by supporting microarray scanning—a highly complementary technology\* (Table 2). By including microarray scanning on the NextSeq 550 System, researchers have instant access to a powerful technology for further exploration. The ability to scan micorarrays provides a cost-effective, orthogonal method for the rapid confirmation of copy number variants using the CytoSNP BeadChip and the identification of differentially methylated regions using the Infinium MethylationEPIC BeadChip. With the NextSeq 550 System, the menu of cutting-edge research applications is maximized while the instrument costs are simultaneously minimized. The NextSeq 550 System supports flexible options by enabling a broad range of applications in reproductive, genetic health, and oncology research.

## Streamlined bioinformatics

The NextSeq 550 Sequencing System includes several data analysis options. Integrated instrument computers perform base calling and quality scoring. Sequencing run data can be analyzed through a range of open-source or commercial pipelines developed for Illumina data. Alternatively, customers can perform on or off instrument analysis using Local Run Manager. Local Run Manager provides an integrated solution for the creation and analysis of sequencing runs with the ability to link modules from run setup through analysis. A wide array of methods are available to automate analysis such as

amplicon analysis, DNA enrichment analysis, and GenerateFASTQ analysis. A complete list of available modules can be found on our support pages. A cloud based solution is also available through BaseSpace™ Sequence Hub, the Illumina genomics computing environment, where data can be instantly transferred, analyzed, and securely stored. Sequence Hub analysis apps include alignment, variant detection, annotation, visualization, and interpretation. BaseSpace Sequence Hub also includes data analysis apps for exome, transcriptome, whole-genome, and somatic variant calling. With industry-standard data formats, third-party developers have created a rich ecosystem of commercial and open-source tools for more extensive downstream data analysis.

For array-based molecular cytogenetics studies or combined array and NGS data from *in vitro* fertilization (IVF) applications, BlueFuse™ Multi Software provides a framework for comprehensive data analysis. The software manages search and display data generated by whole-genome analysis experiments using sophisticated processing algorithms and an intuitive visualization formats. For Infinium MethylationEPIC BeadChip data, analysis is supported by the GenomeStudio™ Methylation Module, enabling researchers to perform differential methylation analysis for small scale studies. For large-scale studies, there are many freely available analysis packages that work in the software framework R for normalization and differential analysis of methylation data.<sup>3,4</sup>

\*Microarray scanning includes support for the Infinium MethylationEPIC, CytoSNP-850K, HumanCytoSNP-12, and HumanKaryomap-12 DNA BeadChips.

## Summary

The NextSeq 550 System is a transformative instrument that enables NGS and microarray scanning to become everyday operations in laboratories worldwide. Incorporating the latest advances in SBS chemistry, the flexible NextSeq 550 System features user-friendly operation and a streamlined DNA-to-data workflow that allows researchers to perform the most popular high-throughput applications in less than a day. The multiple flow cell and reagent configurations also enable low-throughput sequencing as needed, providing researchers with the operational flexibility to handle a range of project sizes and project needs.

## Ordering information

System name	Catalog no.
NextSeq 550 System	SY-415-1002

Output kit name	Catalog no.
NextSeq 500/550 Mid-Output v2.5 Kit (150 cycles)	20024904
NextSeq 500/550 Mid-Output v2.5 Kit (300 cycles)	20024905
NextSeq 500/550 High-Output v2.5 Kit (75 cycles)	20024906
NextSeq 500/550 High-Output v2.5 Kit (150 cycles)	20024907
NextSeq 500/550 High-Output v2.5 Kit (300 cycles)	20024908
TG <sup>a</sup> NextSeq 500/550 Mid-Output Kit v2.5 (150 cycles)	20024909
TG NextSeq 500/550 Mid-Output Kit v2.5 (300 cycles)	20024910
TG NextSeq 500/550 High-Output Kit v2.5 (75 cycles)	20024911
TG NextSeq 500/550 High-Output Kit v2.5 (150 cycles)	20024912
TG NextSeq 500/550 High-Output Kit v2.5 (300 cycles)	20024913

a. TG-labeled consumables have features that help customers reduce the frequency of revalidation. These consumables are available only under supply agreement and require customers to provide a binding forecast. Contact your account manager for more.

## References

1. Data on file. Illumina, Inc. 2015.
2. Data on file. Illumina, Inc. 2018.
3. Morris TJ, Butcher LM, Feber A, et al. [ChAMP: 450K chip analysis methylation pipeline](#). *Bioinformatics*. 2014;30(3):428-430.
4. Assenov Y, Muller F, Lutsik P, et al. [Comprehensive analysis of DNA methylation with RnBeads](#). *Nat Methods*. 2015;11(11):1138-1140.

## NextSeq 550 System specifications

Parameters	Specifications
Instrument configuration	RFID tracking for consumables
Instrument control computer (Internal) <sup>a</sup>	Base Unit: Dual Intel Xeon E5-2448L 1.8 GHz CPU Memory: 96 GB RAM Hard Drive: 750 GB Operating System: Windows 10 embedded standard
Operating environment	Temperature: 19°C to 25°C (22°C ± 3°C) Humidity: Noncondensing 20%-80% relative humidity Altitude: Less than 2000 m (6500 ft) Air Quality: Pollution degree rating of II Ventilation: Up to 2048 BTU/hr @ 600 W For Indoor Use Only
Light emitting diode (LED)	520 nm, 650 nm; Laser diode: 780 nm, Class IIIb
Dimensions	W×D×H: 53.3 cm × 63.5 cm × 58.4 cm (21.0 in × 25.0 in × 23.0 in) Weight: 83 kg (183 lbs) Crated Weight: 151.5 kg (334 lbs)
Power requirements	100-120 VAC 15 A 220-240 VAC 10 A
Radio frequency identifier (RFID)	Frequency: 13.56 MHz Power: Supply current 120 mA, RF output power 200 mW
Product safety and compliance	NRTL certified IEC 61010-1 CE marked FCC/IC approved

a. Computer specifications are subject to change.