NextSeq® 500 System WGS Solution

An accessible, high-quality whole-genome sequencing solution for any species.

Highlights

- High-Quality, High-Coverage Genome Illumina chemistry offers highest read quality and alignability, with best coverage of GC-rich regions
- Unrestricted View of the Genome Now and in the Future
 Rich data set can be mined repeatedly as new

discoveries are made

- Scalable, Affordable WGS Rapid and cost-effective sequencing of complex genomes in your laboratory
- Easy, Accessible Data Analysis and Storage Solutions

Rich ecosystem of user-friendly informatics tools to analyze and interpret data

• End-to-End Illumina Support Illumina scientists and engineers are there every step of the way, providing installation, training, applications, and data analysis support

Introduction

The NextSeq 500 System Whole-Genome Sequencing (WGS) Solution enables researchers and clinicians to explore the entire genome of any species cost-effectively for a deeper understanding of biology. It leverages industry-standard Illumina next-generation sequencing (NGS) technology responsible for most global WGS, delivering the best data quality and highest coverage to identify variants in coding and noncoding regions of the genome. High-quality library preparation kits are optimized for low-input, unbiased coverage, and rapid workflow. With push-button sequencing, simple data analysis, and minimal hands-on time, the NextSeq 500 System WGS Solution enables researchers to interrogate simple prokaryotic and complex eukaryotic genomes quickly and efficiently.



Figure 1: NextSeq 500 System Sample-to-Answer WGS Sequencing Workflow. The NextSeq 500 System's simple workflow delivers highly accurate sequencing data. Data analysis includes alignment and variant calling.

A Fast, Efficient WGS Workflow

The NextSeq 500 System WGS workflow offers a simple, fast, end-to-end solution for characterization of any genome (Figure 1). It begins with streamlined library preparation using high-quality TruSeq[®] library preparation kits. The NextSeq 500 System's dual density flow cell configurations and accompanying reagent kits deliver 20 Gb to 120 Gb, enabling researchers to match sequencer output with sequencing of prokaryotic and eukaryotic genomes. New v2 reagent kits are optimized to improve base calling and data quality even further.

Data analysis, from alignment and variant calling to annotation and beyond, can be performed in BaseSpace[®], the Illumina genomics computing environment. Utilizing the Illumina industry-leading NGS workflow, the NextSeq 500 System provides access to the world's largest collection of commercial and opensource data analysis software tools (Figure 2).



Figure 2: NextSeq 500 Sequencing System. The NextSeq 500 System leverages the latest advances in SBS chemistry and the industry's simplest workflow.

The NextSeq 500 System also offers cross-application flexibility, enabling researchers to easily transition from one sequencing project to another (Figure 3). The system is fully compatible with the industry's widest range of library preparation kits from Illumina and third parties, enabling an easy transition between Illumina WGS, exome, and RNA-Seq. For example, researchers can follow up WGS with exome sequencing to obtain much deeper coverage and increased ability to call rare variants in coding portions of the genome. They can also pair WGS with RNA-Seq to assess whether the coding and noncoding variants they just identified interfere with transcript expression. A wide range of Illumina targeted resequencing solutions are also available to validate variants discovered from any sequencing application.



Figure 3: NextSeq 500 System Sequencing Applications. The flexible NextSeq 500 System enables researchers to seamlessly transition from one sequencing application to another to advance their research.

Simple, Rapid Library Preparation

Illumina offers a variety of library preparation kits to accommodate a range of sample types and genome sizes. These kits have been developed and tested for Illumina systems and include everything needed to prepare samples. TruSeq DNA library preparation kits deliver unsurpassed data quality with fast, streamlined workflows that require minimal hands-on time (Table 1).

Table 1: Illumina WGS Library Preparation Kits.			
Specification	TruSeq DNA PCR-Free	TruSeq Nano DNA	
Sample DNA input type	Genomic DNA	Genomic DNA	
WGS applications	Human or other large, complex genomes	Human or other large, complex genomes	
Input DNA	1–2 µg	100–200 ng	
Typical median insert size	350 bp	350 bp	
Read lengths supported	All read lengths	All read lengths	
DNA libraries	Single, paired-end, and indexed sequencing	Single, paired-end, and indexed sequencing	
Assay time	~ 6 hours	~ 6 hours	
Hands-on time	~ 4 hours	~ 5 hours	

Fully Characterized, Highly Accurate Genomes of Any Species

The NextSeq 500 System delivers the power of high-throughput sequencing with the simplicity of a desktop sequencer, enabling researchers to scale their WGS studies from one to hundreds of genomes. Its dual sequencing output modes and accompanying reagents deliver 20 Gb to 120 Gb, enabling researchers to finetune their WGS studies to optimize for sample volume and coverage needs. This desktop system delivers a cost-effective whole human genome in a single run with the high data quality researchers demand. Leveraging Illumina NGS technology, the NextSeq 500 System delivers industry-leading sequencing accuracy of > 75% of sequenced bases over Q30.^{*}

The NextSeq 500 System delivers a rich data set that can be reanalyzed in the future to provide an up-to-date view of the genome as new gene variant discoveries are made. It can successfully sequence even the most difficult regions (GC-rich, homopolymers) delivering the data output and quality necessary to characterize a genome fully. Paired-end sequencing on the NextSeq 500 System provides even greater resolution, enabling the detection of structural variants such as deletions, duplications, and large-scale copy-number variants that play a role in disease.

* Q30 = 1 error in 1,000 base calls or an accuracy of 99.9%

Illumina Sequencing System		Read Length	Output	No. of samples
NovtSog 500 System	Mid Output Flow Cell (130 M)	2 × 150 bp	40 Gb	up to 24 small genomes
NexiSed 500 System	High Output Flow Cell (400 M)	2 × 150 bp	120 Gb	1 x 30× human genome
HiSeq 2500 System	Rapid Run Mode	2 × 100 bp	100–120 Gb	1 x 30× human genome
	High Output Mode	2 × 125 bp	900 Gb-1 Tb	8 x 30× human genome

Table 2: Illumina WGS Solutions.

Based on industry-leading Illumina sequencing by synthesis chemistry, the NextSeq 500 System enables researchers to compare and integrate the data it generates with data from studies performed on other Illumina systems. For example, NextSeq 500 System WGS data can be integrated with data from follow-up studies on the Illumina MiSeq[®] System or large-scale WGS sequencing studies run on an Illumina HiSeq[®] System (Table 2).

Simplified Bioinformatics in BaseSpace

Illumina has removed much of the complexity from the typical informatics workflow. Bases generated on the NextSeq 500 System are instantly and securely transferred, stored, and analyzed in BaseSpace (Cloud or Onsite), delivering annotated variants in as little as 12 hours using Illumina Isaac pipeline¹ or 45 hours using the industry-standard BWA/GATK method. Analytic tools from NextBio (an Illumina company) can be used to annotate and filter variants, as well as integrate and interpret genomic data in the context of other molecular and phenotypic data. Analysis results, including

coverage statistics and annotated SNPs and indels are
presented in intuitive, easy-to-interpret reports.

The BaseSpace environment also includes BaseSpace apps, a growing community of software solutions for visualization, analysis, and sharing. Because Illumina NGS technology is the most established and broadly adopted sequencing solution, researchers can also take advantage of the largest collection of WGS analysis software available. Through its intuitive user interface and a rich ecosystem of commercial and third-party tools and apps, BaseSpace enables researchers to transform raw data into biologically meaningful results (Figure 4).

Flow Cell Configuration	Read Length (bp)	Output (Gb)	Run Time	Required Input	Data Quality
High Output Flow Cell	2 × 150	100–120	29 hours		
Up to 400 M single reads	2 × 75	50-60	18 hours		
Up to 800 M paired-end reads	1 × 75	25–30	11 hours	100 ng–1 µg with TruSeq Library Prep	> 75% higher than 0.30 at 2 x 150 bp
Mid Output Flow Cell	2 × 150	32–39	26 hours	Kits	
Up to 130 M single reads Up to 260 M paired-end reads	2 × 75	16–19	15 hours		

a. Total times include cluster generation, sequencing, and base calling on a NextSeq 500 System. Install specifications are based on Illumina PhiX control library at supported cluster densities (between 129 and 165 K/mm² 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.

	L.
Analysis Name:	BWA Whole Genome Sequencing v1 12/19/201:
Save Results To:	Select a Project
Sample:	Select Sample
Reference Genome:	Human (UCSC hg19)
Enable SV/CNV Calling:	
Annotation:	💿 RefSeq 💮 Ensembl 🕔

Figure 4: Storage and Analysis of NextSeq 500 System Data in the BaseSpace Cloud. NextSeq 500 System data can be securely and seamlessly uploaded to the BaseSpace cloud for fast, cost-effective analysis and storage.

Summary

Delivering the highest data quality and accuracy, the NextSeq 500 System WGS Solution enables researchers to characterize and explore whole genomes fully. This high-throughput, desktop sequencer can cost-effectively perform WGS of any species, with a streamlined workflow that minimizes hands-on time. Integrated data analysis in BaseSpace, supplemented with a large collection of commercial and open source software tools, enables researchers to mine the rich NextSeq 500 System WGS data set now and in the future as new discoveries are made.

Learn More

Go to www.illumina.com/applications/sequencing/dna_ sequencing/whole_genome_sequencing.ilmn to learn more about the next revolution in WGS.

Join the Illumina Community

With a NextSeq 500 System in their laboratory, researchers join a worldwide community of over 60,000 scientists using Illumina technology for their research studies. Illumina schedules community events throughout the year, bringing researchers together to share ideas. User group meetings, scientific symposiums, and blog forums provide venues to discuss new research methods and breakthrough studies.

An integral part of the Illumina community is our dedicated service and support team, consisting of more than 300 people worldwide, 75% of whom have advanced degrees. Illumina technical support begins when the NextSeq 500 System is delivered, with Illumina scientists and engineers assisting with system installation and setup, and the training of laboratory personnel. They are there 24/7 globally to answer questions every step of the way, giving researchers the peace of mind to focus on their next research study.

As researchers' needs change, new systems are brought into the laboratory, or new methods are undertaken, the Illumina support and training teams are there to provide assistance. In addition to on-site support, training courses (via webinar or at an Illumina facility) are available to bring laboratory personnel quickly up to speed.

NextSeq 500 System Specifications.

Instrument Configuration

RFID tracking for consumables

Instrument Control Computer (Internal)^a

Base Unit: Dual Intel Xeon ES-2448L 1.8 GHz CPU Memory: 96 GB RAM Hard Drive: 750 GB Operating System: Windows 7 embedded standard a. Computer specifications are subject to change.

Operating Environment

Temperature: 19°C to 25°C ($22°C \pm 3°C$) Humidity: Non-condensing 20%–80% relative humidity Altitude: Less than 2,000 m (6,500 ft) Air Quality: Pollution degree rating of II Ventilation: Up to 2,048 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: 58.5 cm × 53.4 cm × 63.5 cm (23.0 in × 21.0 in × 25 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

Ordering Information.	
System Name	Catalog No.
NextSeq 500 Sequencing System	SY-415-1001
Output Kit Name	Catalog No.
NextSeq 500 Mid Output Kit (150 cycles)	FC-404-1001
NextSeq 500 Mid Output Kit (300 cycles)	FC-404-1003
NextSeq 500 High Output Kit (75 cycles)	FC-404-1005
NextSeq 500 High Output Kit (150 cycles)	FC-404-1002
NextSeq 500 High Output Kit (300 cycles)	FC-404-1004
NextSeq 500 Mid Output v2 Kit (150 cycles)	FC-404-2001
NextSeq 500 High Output v2 Kit (150 cycles)	FC-404-2002
NextSeq 500 Mid Output v2 Kit (300 cycles)	FC-404-2003
NextSeq 500 High Output v2 Kit (300 cycles)	FC-404-2004
NextSeq 500 High Output v2 Kit (75 cycles)	FC-404-2005

References

 Raczy C, Petrovski R, Saunders CT, et al. Isaac: ultra-fast wholegenome secondary analysis on Illumina sequencing platforms. *Bioinformatics*. 2013;29:2041-2043.

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