



**Table 1: Unprecedented Flexibility for Multiple Applications**

Application	High Output Flow Cell Configuration		Mid Output Flow Cell Configuration	
	No. of Samples	Time	No. of Samples	Time
Gene Expression Profiling > 10 M Reads 1 x 75 bp	40	11 hours	N/A	N/A
mRNA-Seq > 40 M Reads 2 x 75 bp	10	18 hours	3	15 hours
Enrichment Panel 12 Mb Region > 20x coverage at > 95% targets	N/A	N/A	20	26 hours
Whole-Exome Sequencing > 90% at > 10x coverage	9	18 hours	3	15 hours
Human Whole Genome Sequencing 3 GB Genome > 30x coverage 2 x 150 bp	1	29 hours	N/A	N/A

By employing the Illumina industry-leading sequencing by synthesis (SBS) chemistry and file format conventions, the NextSeq 500 System offers customers access to the broadest ecosystem of established protocols, workflows, data sets, and data analysis tools.

### Industry-Leading SBS Chemistry Delivers Highest Accuracy

At the core of the NextSeq 500 System is proven Illumina SBS chemistry, the most widely adopted NGS technology. This proprietary reversible terminator-based method enables the massively parallel sequencing of millions of DNA fragments, detecting single bases as they are incorporated into growing DNA strands. The method virtually eliminates errors and missed calls associated with strings of repeated nucleotides (homopolymers).

Illumina sequencing delivers the most accurate human genome, exome, or transcriptome at any coverage, the highest yield of error-free reads, and the highest percentage of base calls above Q30<sup>1</sup> in the industry. Such high data quality results in low false positive and false negative rates, reducing the need for extensive downstream validation and giving researchers full confidence in the data generated.

The NextSeq 500 System leverages the latest evolution of SBS chemistry, reducing cycle and data processing times, while delivering the same high quality, accuracy, and industry-standard file structure that sets Illumina systems apart.

### Versatile and Flexible to Support the Widest Range of Applications

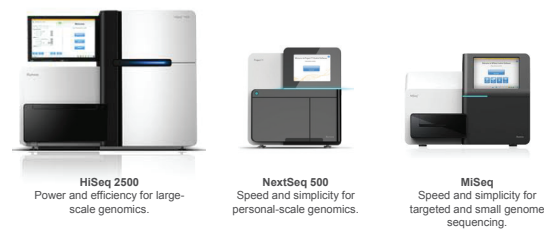
The NextSeq 500 System is the right sequencer for any project size and sequencing throughput, providing users with optimal operational efficiency. It is the only desktop sequencing system capable of sequencing a high-coverage (30x) whole human genome in one run.

The NextSeq 500 System also delivers a one-day turnaround for a number of popular sequencing applications. With this instrument, researchers can sequence:

- 1–16 exomes/run
- 1–20 transcriptomes/run
- 6–96 targeted panels/run
- 12–40 gene expression profiling samples/run

The NextSeq 500 System is easily configured providing researchers with scalability to handle low to high-throughput project sizes for maximum operational efficiency. 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 higher throughput processing with each sequencing run (Table 1).

**Figure 3: Illumina NGS Portfolio Makes Sequencing Accessible to All Researchers**



Illumina NGS systems offer solutions for every application, sample type, and sequencing scale. Each delivers high data quality and accuracy with flexible throughput and simple, streamlined workflows. Data can be seamlessly compared, exchanged, and analyzed in BaseSpace.

<sup>1</sup>Q30 = 1 error in 1,000 base calls or an accuracy of 99.9%

The NextSeq 500 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 sample preparation and target enrichment solutions, offering library compatibility across the Illumina sequencing portfolio. This allows researchers to scale-up studies easily to the higher throughput HiSeq® System or perform follow-up studies on the MiSeq® System (Figure 3).

**Streamlined Bioinformatics**

The NextSeq 500 System supports a number of data analysis options. Primary data analysis, including base calling and quality scoring, is performed by integrated instrument computers. Sequencing run data can be run through a wide range of open-source or commercial pipelines developed for Illumina data, or instantly transferred, analyzed, and stored securely in BaseSpace® (Cloud or Onsite), the Illumina genomics computing environment. BaseSpace downstream data analysis includes alignment and variant detection, annotation, visualization, and interpretation.

BaseSpace also includes Illumina-developed data analysis apps for exome, transcriptome, whole-genome, and somatic variant calling. Thanks to Illumina’s industry-standard data formats, third-party developers have created a rich ecosystem of commercial and open-source tools for more extensive downstream data analysis.

**Summary**

The NextSeq 500 System is a transformative sequencer that enables NGS to become an everyday tool in laboratories worldwide. Incorporating the latest advancements in SBS chemistry, the flexible NextSeq 500 System’s push-button operation and streamlined sample-to-results workflow allow researchers to perform the most popular high-throughput applications in less than a day. Its multiple flow cell and reagent configurations enable low-throughput sequencing as needed, providing researchers with the operating efficiency to handle a range of project sizes.

**Learn More**

Go to [www.illumina.com/systems/nextseq-sequencer.ilmn](http://www.illumina.com/systems/nextseq-sequencer.ilmn) to learn more about the next revolution in sequencing.

**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 Performance Parameters<sup>s</sup>**

Flow Cell Configuration	Read Length (bp)	Output (Gb)	Run Time	Required Input	Data Quality
High Output Flow Cell	2 × 150	100–120	29 hours	100 ng–1 µg with TruSeq® Sample Prep Kits	> 75% higher than Q30 at 2 × 150 bp
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		
Mid Output Flow Cell	2 × 150	32–39	26 hours		
Up to 130 M single reads	2 × 75	16–19	15 hours		
Up to 260 M paired-end reads					

<sup>s</sup>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/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.



