# HiSeq® 2500 Sequencing System

Unsurpassed power and efficiency for production scale sequencing.

### HiSeq System Highlights

- **Incredible Speed and Throughput**
  Complete projects in record time with the highest daily throughput of any individually sold sequencing system
- **Unprecedented Flexibility**
  Choose between rapid-run and high-output modes, offering scalable output from 10 Gb to 1 Tb for a broad range of applications and sample sizes
- **Exceptional Data Quality**
  Build confidence in your results with the most widely adopted and proven Illumina SBS chemistry
- **Complete End-to-End Sequencing Solutions**
  Enjoy integrated streamlined workflows from library prep through data analysis

## Introduction

The HiSeq 2500 has become the undisputed powerhouse of Illumina sequencing systems for highthroughput sequencing applications (Figure 1). To date, millions of samples have been sequenced and thousands of papers have been published utilizing Illumina sequencing technology. With unrivaled data quality, flexible run configurations, and a 10 gigabase (Gb) to 1 terabase (Tb) per run capacity, the HiSeq 2500 is the production platform of choice for all major genome centers and leading institutions around the world.[1]

## Incredible Speed and Throughput

The HiSeq 2500 System delivers the highest daily throughput and total yield of any individually sold sequencer on the market today. With a massive daily throughput of 160 Gb per day or 1 Tb per run, more samples can be simultaneously sequenced at a greater depth than ever before—producing richer, more meaningful data. Large studies can be completed with reduced hands-on time and reagent cost. In high output mode, the HiSeq 2500 System can process 8 human genomes at 30× coverage or 150 human exomes, assuming 4 Gb per exome at 2 × 75 bp, per run. The HiSeq 2500 System, in high-output mode, is perfect for production scale projects with large genome sizes or studies with hundreds of samples.

## Unprecedented Flexibility

While the HiSeq 2500 System can generate massive data, it is also highly flexible. The HiSeq 2500 is the first Illumina sequencing platform to feature 2 run modes—rapid run and high-output run mode. Furthermore, the system can be configured to run 1 flow cell alone or 2 flow cells simultaneously—enabling a scalable solution tailored to your specific research needs (Figure 2). Rapid-run mode leverages on-board cluster generation and reduced cycle times to significantly accelerate run times. In rapid-run mode, fully automated clustering and sequencing for short read applications can be completed in 7 hours and a single human genome at 30× coverage can be completed in 27 hours. With rapid or high-output modes and single or dual flow cell options, the HiSeq 2500 can be tuned to meet output needs ranging from 10 Gb to 1 Tb or 300 million to 4 billion reads per run, supporting the broadest range of applications.

## Exceptional Data Quality

With the HiSeq 2500 System, high data quality is ensured by leveraging the most widely adopted chemistry in the industry: sequencing by synthesis (SBS). SBS technology on the HiSeq 2500 utilizes a proprietary reversible terminator-based method that detects single bases as they are incorporated into DNA template strands. As all 4 reversible, terminator-bound dNTPs are present during each sequencing cycle, natural competition minimizes incorporation bias and greatly reduces raw error rates compared to other technologies.[2,3] The result is highly accurate base-by-base sequencing that virtually eliminates sequence-context-specific errors, even within repetitive sequence regions and homopolymers. Illumina sequencing delivers the most accurate human genome, the highest yield of error-free reads, and the highest percentage of base calls above Q30 in the industry (Figure 3).

**Figure 1: The HiSeq 2500 System**

The HiSeq 2500 System is the only high-throughput sequencing system that combines scalable output from 10 Gb – 1 Tb with flexible run modes to support multiple applications and superior turnaround times.

## HiSeq 2500 System Information

**Figure 2: Unprecedented Flexibility for Multiple Applications**

| | Rapid-Run Mode | | High-Output Mode | |
|---|---|---|---|---|
| **Transcription Factor**<br>ChIP-Seq<br>15 M Reads<br>1 × 36 bp | 40 Samples | 7 Hours | 260 Samples | 29 Hours |
| **mRNA-Seq**<br>50 M Reads<br>2 × 50 bp | 12 Samples | 16 Hours | 80 Samples | 2.5 Days |
| **Nextera Rapid Capture Exome**<br>37 Mb Region<br>2 × 75 bp | 20 Samples | 27 Hours | 150 Samples | 5 Days |
| **Human Whole Genome**<br>> 30× Coverage<br>2 × 100 bp | 1 Sample | 27 Hours | 8 Samples | 6 Days |
| **De Novo Sequencing**<br>2.5 Gb Genome<br>100× Coverage<br>2 × 250 bp | 1 Sample | 60 Hours | | |

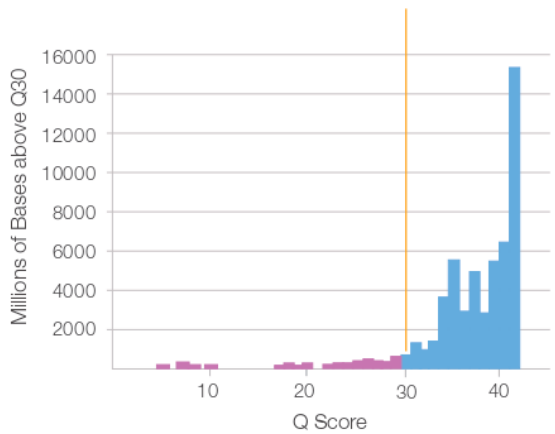
With 2 run modes and the ability to process 1 or 2 flow cells simultaneously, the HiSeq 2500 supports a broad range of applications and study sizes. Values here are examples based on system performance capabilities using 2 flow cells. Variation can be expected due to cluster density, sample quality, and other experimental factors. Run times are approximate and include on-board cluster generation and sequencing for rapid-run mode, and sequencing only for high-output mode. Indexing run times are not included.

## Complete End-to-End Sequencing Solutions

The comprehensive Illumina portfolio provides a solution for every step of the sequencing process from library preparation to data analysis and data storage (Figure 4). The Nextera® and TruSeq® library preparation kit families support a broad range of sequencing applications including whole genome, exome, transcriptome, epigenome sequencing, and more. For final data analysis and storage, onsite IlluminaCompute™ or cloud-based Illumina BaseSpace® provide simple and scalable solutions for complete end-to-end sequencing workflows.

BaseSpace, the Illumina genomics cloud computing environment, is directly integrated with all HiSeq instruments. BaseSpace includes data analysis tools and provides access to the App Store: an online store offering third-party vendor applications for downstream analysis. BaseSpace features real-time data upload, alignment and variant calling, run monitoring, and options to share data instantly with anyone in the world. For those who prefer on-site solutions, Illumina offers IlluminaCompute: a plug-and-play data analysis and storage solution. With BaseSpace or IlluminaCompute, labs can become fully operational in weeks instead of months—without preexisting IT infrastructure.

**Figure 3: Reads vs. Q-score in Rapid-Run Mode**




Quality scores for a human genome 2 × 101 base pair run on a HiSeq 2500 System in rapid-run mode. This example shows 92% of bases sequenced above Q30.

## HiSeq System Performance Parameters

| High-Output Run Mode | | | |
|---|---|---|---|
| **Read Length** | **Dual Flow Cell** | **Single Flow Cell** | **Dual Flow Cell Run Time** |
| **1 × 36** | 128–144 Gb | 64–72 Gb | 29 hours |
| **2 × 50** | 360–400 Gb | 180–200 Gb | 2.5 days |
| **2 × 100** | 720–800 Gb | 360–400 Gb | 5 days |
| **2 × 125*** | 900 Gb–1 Tb | 450–500 Gb | 6 days |
| | | | |
| **Reads Passing Filter†** | Up to 4 billion | Up to 2 billion | |
| **Quality** | ≥ 85% of bases above Q30 at 2 × 50 bp<br>≥ 80% of bases above Q30 at 2 × 100 bp<br>≥ 80% of bases above Q30 at 2 × 125 bp | | |

| Rapid-Run Mode | | | |
|---|---|---|---|
| **Read Length** | **Dual Flow Cell** | **Single Flow Cell** | **Dual Flow Cell Run Time** |
| **1 × 36** | 18–22 Gb | 9–11 Gb | 7 hours |
| **2 × 50** | 50–60 Gb | 25–30 Gb | 16 hours |
| **2 × 100** | 100–120 Gb | 50–60 Gb | 27 hours |
| **2 × 150** | 150–180 Gb | 75–90 Gb | 40 hours |
| **2 × 250*** | 250–300 Gb | 125–150 Gb | 60 hours |
| **Reads Passing Filter†** | Up to 600 million | Up to 300 million | |
| **Quality** | ≥ 85% of bases above Q30 at 2 × 50 bp<br>≥ 80% of bases above Q30 at 2 × 100 bp<br>≥ 75% of bases above Q30 at 2 × 150 bp | | |

Install specifications based on Illumina PhiX control library at supported cluster densities (between 870–930 k clusters/mm$^2$ passing filter using HiSeq v4 or 700–820 K clusters/mm$^2$ passing filter using HiSeq Rapid v2 kits). Run times for rapid-run mode correspond to on-board cluster generation (1.5 hours) and sequencing; for high-output mode, run times correspond to sequencing only. Performance can vary based on sample quality, cluster density, and other experimental factors. *Maximum supported read length is 2 × 125 bp for high-output mode and 2 × 250 bp for rapid-run mode. †Single-end reads.

## Simple Intuitive Instrument Control Software

The HiSeq 2500 features HiSeq Control Software (HCS): an easy-to-use, touchscreen-operated instrument control interface. With optimized step-by-step workflows, HCS guides you through experimental set-up, loading flow cells and reagents, flow-checks and postrun washes. Quality statistics from 1 or multiple runs can be monitored in real-time either on the instrument, from the lab, or from the comfort of your own home via BaseSpace on any browser.

## Learn More

For more information about the HiSeq 2500 System, visit www.illumina.com/hiseq

To learn more about BaseSpace, visit www.illumina.com/software/basespace.ilmn

To see how Illumina fuels powerful science, visit www.illumina.com/science/publications.ilmn

## References


1. Nakazato T, Ohta T, Bono H. (2013) Experimental design-based functional mining and characterization of high-throughput sequencing data in the sequence read archive. PLoS One 22;8(10): e77910.
2. Ross MG, Russ C, Costello M, Hollinger A, Lennon NJ, et al. (2013) Characterizing and measuring bias in sequence data. Gen Biol 14: R51.
3. Liu L, Li Y, Li S, Hu N, He Y, et al. (2012) Comparison of next-generation sequencing systems. J Biomed Biotechnol 2012: 251364.


## HiSeq 2500 System Specifications

**Instrument Configuration**
Computer and touch screen display
Installation setup and accessories
Data collection and analysis software

**Instrument Control Computer**
Base Unit: Intel® Xeon® CPU E5-2620 @ 2.00 GHz (2 processors)
Memory: 64 GB RAM
Hard Drive: 8 × 1.0 TB 3.5" Enterprise SATA (2 OS - RAID1 drives, 6 data RAID 5 drives)
Operating System: Microsoft Windows 7

Note: Computer specifications will be regularly modified. Contact your local account manager for current configuration.

**Operating Environment**
Temperature: 22°C ± 3°C
Humidity: Noncondensing 20%–80%
Altitude: Less than 2000 m (6500 ft)
Air Quality: Pollution degree rating of II
Ventilation: Maximum of 4000 BTU/h
For Indoor Use Only

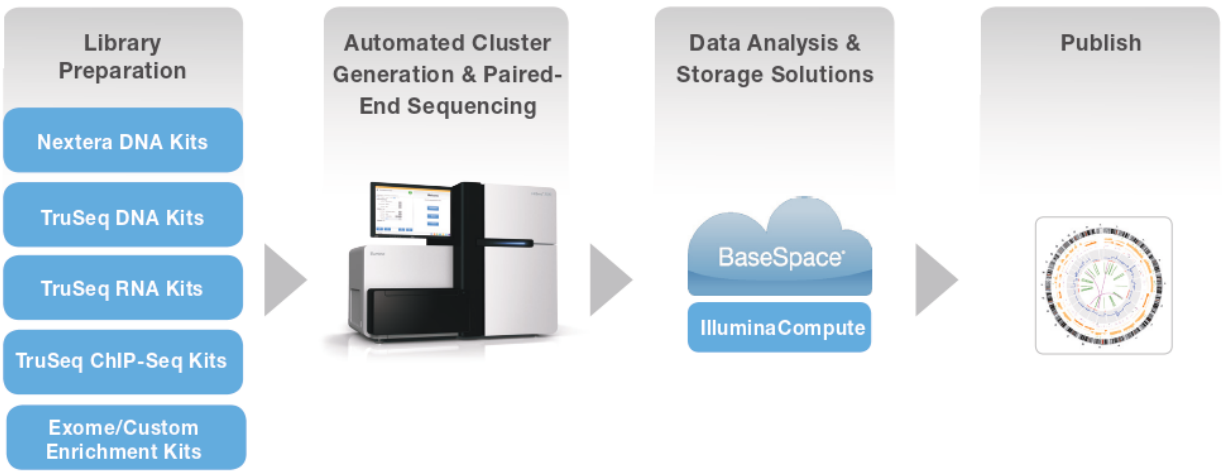
**Laser**
532 nm, 660 nm, 650 nm (barcode reader)

**Dimensions**
W×D×H: 118.6 cm × 76.0 cm × 94.0 cm (46.7 in × 30.0 in × 37.0 in)
Weight: 221.4 kg (488 lbs)
Crated Weight: 312 kg (688 lbs)

**Power Requirements**
100–240V AC 50/60Hz, 20A, 1500 W
Illumina provides a region-specific uninterruptible power supply for all HiSeq instruments

**Product Safety**
CE-marked and ETL-listed instrument

## Figure 4: Your Complete End-to-End Sequencing Solution




The HiSeq system offers a complete workflow with integration from library preparation though data analysis. The incredible speed and throughput of the HiSeq System supports production-scale sequencing and the broadest range of applications.

## Ordering Information

| Systems | Catalog No. |
|---|---|
| HiSeq 2500 Sequencing System | SY–401–2501 |
| cBot Clonal Amplification System | SY–301–2002 |

| High Output Kits | Catalog No. |
|---|---|
| HiSeq SBS Kit v4 (50 Cycle) | FC–401–4002 |
| HiSeq SBS Kit v4 (250 Cycle) | FC–401–4003 |
| HiSeq SR Cluster Kit v4–cBot–HS | GD–401–4001 |
| HiSeq PE Cluster Kit v4–cBot–HS | PE–401–4001 |

| Rapid Run Kits | Catalog No. |
|---|---|
| HiSeq SR Rapid Cluster Kit v2 | GD–402–4002 |
| HiSeq PE Rapid Cluster Kit v2 | PE–402–4002 |
| HiSeq Rapid SBS Kit v2 (50 Cycle) | FC–402–4022 |
| HiSeq Rapid SBS Kit v2 (200 Cycle) | FC–402–4021 |
| HiSeq Rapid SBS Kit v2 (500 Cycle) | FC–402–4023 |

## Join the Illumina Community

When an Illumina platform is installed in their lab, 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 scientists together from around the world. User group meetings, scientific symposiums, community newsletters, and blog forums provide venues to discuss new research methods, establish collaborations, and reveal ground breaking results.

Our dedicated service and support team is an integral part of the Illumina community consisting of over 300 people worldwide—75% of whom have advanced degrees. Illumina support begins when the sequencing system is delivered and continues through system installation, setup, and training of all laboratory personnel. The global support team is available 24/7 to answer questions every step of the way. In addition to on-site assistance, we offer webinars, online training courses, and instructor-led training courses at the state-of-the-art Illumina campus in San Diego to bring laboratory personnel quickly up to speed.

For more about support, webinars, and training courses, visit support.illumina.com/training.ilmn.

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