HiSeq® 3000/HiSeq 4000 Sequencing Systems

Setting a new standard for cost-effective, high-throughput, production-scale sequencing across multiple applications.

Highlights

- **Dramatically Increased Data Output and Throughput**
  Maximize efficiency through innovative patterned flow cell technology
- **Exceptional Data Quality**
  Leverage proven, industry-leading Illumina sequencing by synthesis chemistry
- **Unsurpassed Capacity**
  Sequence 12 genomes, 100 transcriptomes, or 96 exomes in fewer than 3.5 days

Figure 1: The HiSeq 4000 System. The HiSeq 4000 System provides cost-effective, efficient sequencing for large-scale studies across multiple applications.

Introduction

Over the last decade, Illumina sequencing by synthesis (SBS) chemistry has revolutionized next-generation sequencing (NGS), establishing the HiSeq Series of sequencing systems as the platform of choice for high-throughput, production-scale sequencing laboratories. Building on the proven performance of the HiSeq 2500 System, and harnessing the patterned flow cell technology originally developed for the HiSeq X™ Ten System, the HiSeq 3000/HiSeq 4000 Systems provide unparalleled speed and performance.

Higher Throughput, Faster Results

Offering ultra-high throughput, the HiSeq 3000/HiSeq 4000 Systems deliver > 750 Gb per flow cell in 3.5 days. With the highest daily throughput and lowest price per sample across multiple applications, the dual-flow cell HiSeq 4000 System (Figure 1) generates > 400 Gb per day and up to 1.5 Tb per run. This power allows sequencing of more samples at greater depth, generating richer, more meaningful data in less time. Large studies can be completed faster, with less hands-on time and at a lower cost. The HiSeq 3000 System provides the same throughput per flow cell as the HiSeq 4000 System, but only processes a single flow cell at a time to yield > 200 Gb per day and 750 Gb per run.

Innovative Patterned Flow Cell

Taking advantage of the innovative patterned flow cell technology first introduced in the HiSeq X Ten System, the HiSeq 3000/HiSeq 4000 Systems offer an exceptional level of throughput. Patterned flow cells contain billions of nanowells at fixed locations (Figure 2). The structured organization of the flow cell provides for even cluster spacing and uniform feature size, enabling accurate resolution of flow cells clustered at extremely high densities. A proprietary clustering method, exclusion amplification, ensures that only a single DNA template binds and forms a cluster within a single well, resulting in high well occupancy and maximum data output. Using this technology, the HiSeq 3000/HiSeq 4000 Systems can sequence up to 6 genomes, 50 whole transcriptome samples, or 48 exomes per flow cell in fewer than 3.5 days.

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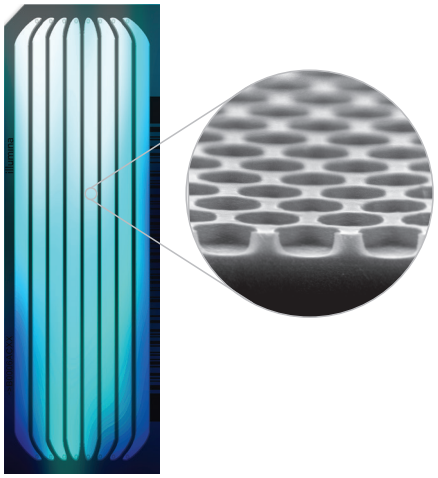

Figure 2: Advanced Patterned Flow Cell Design Enables Ultra-High Throughput. Patterned flow cells contain billions of nanowells at fixed locations, providing even cluster spacing and uniform feature size to deliver extremely high cluster density.

Proven Technology, Exceptional Data Quality

To deliver high data quality, the HiSeq 3000/HiSeq 4000 Systems leverage the proven SBS chemistry used in all Illumina sequencing systems, including the widely adopted HiSeq 2500 System. SBS chemistry relies on a proprietary reversible terminator–based method that detects single bases as they are incorporated into DNA template strands. All 4 reversible terminator–bound dNTPs are present during each sequencing cycle, minimizing incorporation bias and greatly reducing raw error rates compared to other technologies.[1,2] The result is highly accurate base-by-base sequencing that virtually eliminates sequence-context–specific errors, even within repetitive sequence regions and homopolymeric regions. The HiSeq 3000/HiSeq 4000 Systems generate high-quality data that is comparable to the HiSeq 2500 System with vastly improved total output and significant decreases in run times. Illumina sequencing delivers the most accurate genomes, exomes, and transcriptomes, the highest yield of error-free reads, and the highest percentage of base calls above Q30 in the industry.

Flexible Configurations

The latest HiSeq Sequencing Systems are available as single- or dual-flow cell instruments to meet various throughput needs (Table 1). The HiSeq 4000 System allows processing of 1 or 2 flow cells in parallel, offering maximum output with the highest throughput in the least amount of time. The HiSeq 3000 System offers the same output per flow cell as the HiSeq 4000 System, but only processes 1 flow cell at a time. Even with 1 flow cell, the HiSeq 3000 System still generates > 200 Gb per day. To meet changing sequencing throughput needs, the HiSeq 3000 System can be upgraded quickly and easily on-site to a dual-flow cell HiSeq 4000 System. The upgraded HiSeq 3000 System delivers the same performance, throughput, and price per sample as a factory-built HiSeq 4000 System.

Table 1: Performance Parameters of the HiSeq 3000/4000 Systems.[a]

	HiSeq 3000 System	HiSeq 4000 System
Number of Flow Cells per Run	1	1 or 2
Output[b]		
1 × 50 bp	105–125 Gb	210–250 Gb
2 × 75 bp	325–375 Gb	650–750 Gb
2 × 150 bp	650–750 Gb	1300–1500 Gb
Clusters Passing Filter (Single Reads)	2.1–2.5 billion	4.3–5 billion
Quality Scores		
2 × 50 bp	≥ 85% of bases above Q30	
2 × 75 bp	≥ 80% of bases above Q30	
2 × 150 bp	≥ 75% of bases above Q30	
Daily Throughput	> 200 Gb	> 400 Gb
Run Time	< 1–3.5 days	< 1–3.5 days
Human Genomes per Run[c]	up to 6	up to 12
Exomes per Run[d]	up to 48	up to 96
Transcriptomes per Run[e]	up to 50	up to 100

a. Install specifications based on Illumina PhiX control library at supported cluster densities (1310–1524 K clusters/mm^2 passing filter). Run times correspond to sequencing only. Performance may vary based on sample quality, cluster density, and other experimental factors.
b. Output for the HiSeq 4000 System reflects the use of 2 flow cells per run. For output using 1 flow cell per run, refer to the values listed for the HiSeq 3000 System.
c. Assumes 30× coverage of a human genome.
d. Assumes 100× coverage with 80% on target using 2 × 75 bp reads.
e. Assumes 50 million reads per sample.

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Figure 3: A Complete Sequencing Solution. The HiSeq 3000/HiSeq 4000 Systems are part of a complete sequencing workflow that includes library preparation, sequencing, and data analysis.

Complete Sequencing Solutions

Illumina offers a comprehensive portfolio that crosses the entire sequencing workflow, from library preparation to data analysis (Figure 3). TruSeq® DNA and RNA Library Prep Kits support whole-genome sequencing, exome sequencing, and RNA-Seq on the HiSeq 3000/4000 Systems. Additional library preparation kits such as, Nextera® XT DNA Library Prep Kit, Nextera Mate-Pair Library Prep Kit, TruSeq Synthetic Long-Read DNA Library Prep Kit, ChIP-Seq DNA Library Prep Kit, and the TruSeq Small RNA Library Prep Kit are also compatible with the HiSeq 3000/4000 Systems.

HiSeq 3000/4000 Reagent Kits provide ready-to-use reagents for cluster generation and sequencing, reducing hands-on time and potential errors. Three different HiSeq 3000/4000 SBS Kits are available for use on the HiSeq 3000/HiSeq 4000 Systems. The SBS kits come in 50-cycle, 150-cycle, and 300-cycle kit sizes and can be used with either the single read (SR) or the paired end (PE) flow cells. Multiple kit sizes provide read length flexibility up to 2 × 150 bp and support dual indexing workflows.

HiSeq Systems are integrated with the cloud-based BaseSpace® computing environment that provides simple, scalable data analysis and storage. The BaseSpace environment includes access to third-party vendor applications for downstream analysis through the App Store. Apps currently available include BWA and Isaac™ for WGS and exome enrichment and Express and Tophat/Cufflinks for RNA-Seq. BaseSpace computing features real-time data upload, alignment and variant calling, run monitoring, and options to share data instantly with anyone in the world.

Intuitive System Control Software

The HiSeq 3000/HiSeq 4000 Systems offer easy-to-use, touch screen–operated instrument control software. With optimized step-by-step directions, the control software guides users through each step of the sequencing workflow, including experiment setup, as well as reagent and flow cell loading. The software also generates real-time quality statistics for accurate run monitoring.

Summary

The HiSeq 3000/HiSeq 4000 Systems combine industry-leading SBS chemistry with innovative patterned flow cell technology to offer the highest daily throughput, fastest turnaround times, and lowest price per sample across multiple applications. With the HiSeq 3000/HiSeq 4000 Systems, every laboratory can access the latest sequencing technology and increase their genomics power.

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Learn More

To learn more about the HiSeq 3000/HiSeq 4000 Systems, visit www.illumina.com/hiseq.

Ordering Information

Product	Catalog No.
Systems	
HiSeq 4000 Sequencing System	SY-401-4001
HiSeq 3000 Sequencing System	SY-401-3001
HiSeq 4000 System Upgrade	SY-401-4002
Reagent Kits	
HiSeq 3000/4000 SBS Kit (50 cycles)	FC-410-1001
HiSeq 3000/4000 SBS Kit (150 cycles)	FC-410-1002
HiSeq 3000/4000 SBS Kit (300 cycles)	FC-410-1003
HiSeq 3000/4000 SR Cluster Kit	GD-410-1001
HiSeq 3000/4000 PE Cluster Kit	PE-410-1001

References

1. Ross MG, Russ C, Costello M, et al. Characterizing and measuring bias in sequence data. *Gen Biol*. 2013;14:R51.
2. Liu L, Li Y, Li S, et al. Comparison of next-generation sequencing systems. *J Biomed Biotechnol.* 2012;2012:251364.

HiSeq 3000/HiSeq 4000 System Specifications

Specifications
Instrument Configuration Computer and touch screen display Installation setup and accessories Data collection and analysis software
Instrument Control Computer Base Unit: 2× Intel Xeon E5-2697V2 64 BIT 2.7 GHz CPU 30 MB Cache Memory: 128 GB RAM Hard Drive: 8 × 1.0 TB SATA 7200 RPM 3.5” Constellation ES.3 (2 RAID 0 drives, 6 RAID 5 drives) Solid-State Drive: 5 × 400 GB (5 RAID 0 drives) Operating System: Microsoft Windows 7 Professional Note: Computer specifications will be regularly upgraded. 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: 225.9 kg (498 lbs) Crated Weight: 316.6 kg (698 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

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