

iSeq™ 100 Sequencing System

The smallest, most affordable Illumina sequencing system delivers fast and efficient low-throughput sequencing for virtually any lab.

Highlights

- Rapid Data Generation**
 Run smaller projects on a dedicated, low-throughput instrument with fast turnaround times
- Convenient Library Quality and Proof-of-Principle Testing**
 Evaluate library quality before large runs, perform pilot studies, or generate grant submission data
- Independent, Convenient Operations**
 Control the sequencing process from beginning to end and maintain an independent sequencing schedule instead of outsourcing
- High Analytical Sensitivity and Exceptional Data Accuracy**
 Leverage higher analytical sensitivity for the detection of rare variants and transcripts compared to qPCR or Sanger sequencing^{1,2}



Figure 1: The iSeq 100 System—Measuring just over one cubic foot, the iSeq 100 System delivers the power of NGS in the most compact benchtop sequencing system in the Illumina portfolio.

Introduction

The latest innovation in next-generation sequencing (NGS) is here. The compact Illumina iSeq 100 System (Figure 1) combines complementary metal-oxide-semiconductor (CMOS) technology with the proven accuracy of Illumina sequencing by synthesis (SBS) chemistry to deliver high-accuracy data with fast turnaround times. The iSeq 100 System generates 1.2 Gb of data per run in 17.5 hours and delivers the high resolution and analytical sensitivity needed for detection of rare variants and transcripts.^{1,2}

While the iSeq 100 System has a small footprint, it offers big advantages. It provides fast and cost-effective, small-scale runs without the need for larger systems. With a iSeq 100 System in the

lab, researchers can perform runs at their own convenience, without having to wait for optimal batch sizes on higher throughput systems or outsourcing. Additionally, researchers can maintain control of the sequencing process from beginning to end, providing higher confidence in sample integrity and data analysis results. With a list price that falls within the reach of virtually any lab, the iSeq 100 System delivers a cost-effective solution for independent, small-scale, next-generation sequencing.

Streamlined Three-Step Workflow

The iSeq 100 System is part of a streamlined three-step workflow that includes library preparation, sequencing, and data analysis (Figure 2).

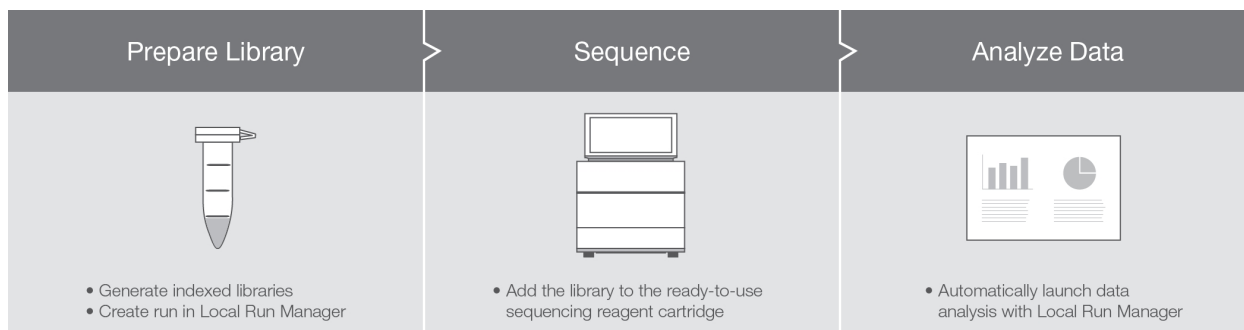


Figure 2: The iSeq 100 System Workflow—The iSeq 100 System is part of a streamlined DNA-to-Data workflow.

Table 1: iSeq 100 System Performance Parameters^a

Run Configuration	Reads (Passing Filter)/Run	Output	Quality Scores ^b	Run Time ^c
1 × 36 bp	4 M	144 Mb	> 85%	9 hrs
1 × 50 bp	4 M	200 Mb	> 85%	9 hours
1 × 75 bp	4 M	300 Mb	> 80%	10 hours
2 × 75 bp	4 M	600 Mb	> 80%	13 hours
2 × 150 bp	4 M	1.2 Gb	> 80%	17.5 hours

a. Performance parameters may vary based on sample type, sample quality, and clusters passing filter.

b. The percentage of bases > Q30 is averaged over the entire run.

c. Times include cluster generation, sequencing, base calling, and quality scoring.



Figure 3: The NGS Sequencing Systems Portfolio—Illumina NGS systems offer solutions for a broad range of applications, sample types, and throughput needs. Each delivers high-accuracy data with flexible throughput and simple, streamlined workflows.

Fast Library Preparation

The iSeq 100 System is compatible with the full suite of Illumina library preparation kits. With the Nextera™ XT and Nextera DNA Flex Library Prep Kits, researchers can prepare multiplexed libraries in 3–4 hours for small genome and direct long-range amplicon sequencing. In addition, the new AmpliSeq™ for Illumina Targeted Resequencing Solution offers expertly designed content. AmpliSeq Targeted Panels are available in ready-to-use fixed panels, community-designed panels, or they can be customized to meet specific research needs. Depending on the kit, Illumina library prep kits require as little as 1 ng of input DNA or RNA (cDNA), and have the flexibility to accommodate DNA extracted from formalin-fixed, paraffin-embedded (FFPE) samples, such as preserved tumor tissue.

The iSeq 100 System is compatible with all Illumina library preparation kits, which allows cross-compatibility across all Illumina instruments. This cross-compatibility allows researchers to easily compare data between systems or scale to larger systems, such as the MiniSeq™, MiSeq™, or NextSeq™ Systems (Figure 3).

Sequencing on the iSeq 100 System

After library preparation, libraries are loaded into a prefilled reagent cartridge for the iSeq 100 System. With a prefilled cartridge, starting a run on the iSeq 100 System is as easy as thaw, load, and go in five minutes (total hands-on time). The iSeq 100 System integrates library denaturing steps, clonal amplification, sequencing, and data analysis into a single instrument, eliminating the need to purchase ancillary equipment. The intuitive user interface provides guidance through

every step of the run setup and run initiation processes, allowing researchers to perform various sequencing applications with minimal user training and minimal set-up time.

High Analytical Sensitivity and Accuracy with SBS Chemistry

During the sequencing run, the iSeq 100 System employs proven Illumina SBS chemistry—the most widely adopted NGS chemistry in the world.³ The iSeq 100 System delivers high-quality data with >80% of bases at or above Q30* (Table 1, Figure 4).

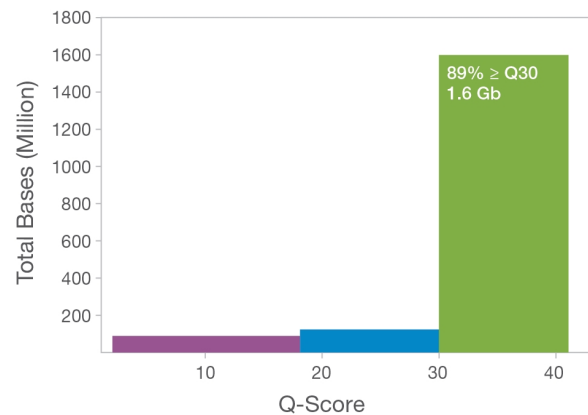


Figure 4: The iSeq 100 System Quality Scores—A quality score (Q-Score) is a prediction of the probability of an error in base calling. A Q-Score of 30 (Q30) is widely considered a benchmark for high-quality data.⁴ A microbial pool run on the iSeq 100 System configured at 2 × 151 bp delivers over >89% of bases ≥ Q30.

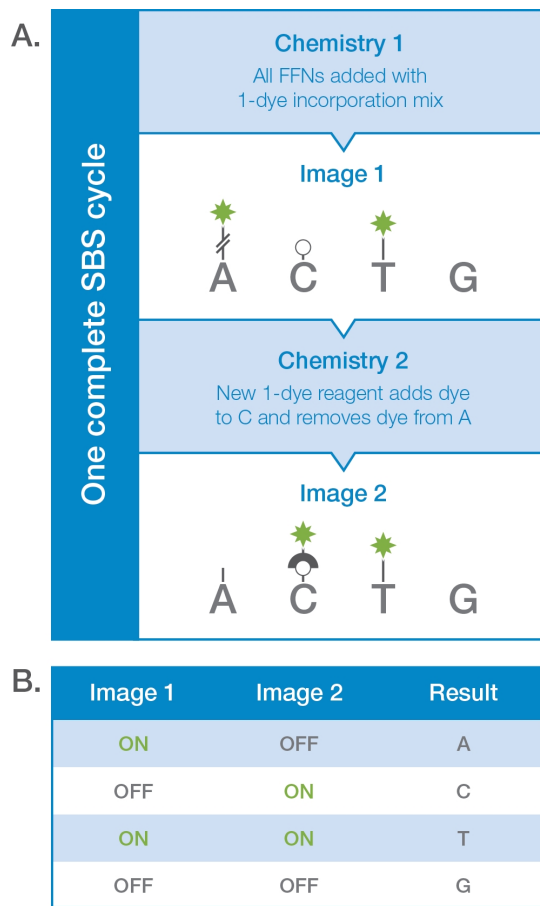


Figure 5: One-Channel SBS Chemistry—(A) One-channel SBS chemistry features two chemistry steps and two imaging steps per sequencing cycle using nucleotides that can be labeled or unlabeled depending on the chemistry step. (B) The base call is determined by the signal pattern across both images.

This reversible terminator-based method detects single bases as they are incorporated into growing DNA strands and enables the parallel sequencing of millions of DNA fragments. Illumina SBS chemistry employs natural competition among all four labeled nucleotides, which reduces incorporation bias and allows more accurate sequencing of repetitive regions and homopolymers.⁵ Compared to capillary electrophoresis-based Sanger sequencing, NGS can detect a broader range of DNA variants, including low-frequency variants and adjacent phased variants, with a faster time to results and fewer hands-on steps.^{1,2}

Highly Innovative, One-Channel SBS Chemistry

The iSeq 100 System combines proven Illumina SBS with CMOS technology to deliver one-channel sequencing chemistry. The iSeq 100 System uses a patterned flow cell with nanowells fabricated over a CMOS chip. Clustering and sequencing occur in the nanowells, which are aligned directly over each CMOS photodiode (pixel). Proprietary ExAmp chemistry ensures that only one cluster forms in each nanowell. Using a CMOS sensor embedded in the consumable is a simple and fast detection method.

Unlike four-channel SBS chemistry, where sequencers use four different dyes for each nucleotide, one chemistry step, and four images per sequencing cycle, the iSeq 100 System uses one dye, two chemistry steps, and two images per sequencing cycle (Figure 5). In one-channel chemistry, adenine has a removable label and is labeled in the first image only. Cytosine has a linker group that can bind a label and is labeled in the second image only. Thymine has a permanent fluorescent label and is therefore labeled in both images, and guanine is permanently dark (unlabeled). Nucleotides are identified by analysis of the different emission patterns for each base across the two images.

Easy, Flexible Data Analysis

The iSeq 100 System offers several data analysis options, including onboard and cloud-based data analysis. The Local Run Manager software, a fully integrated onboard analysis software, features modular architecture to support current and future assays. Local Run Manager software supports planning sequencing runs, tracking libraries and runs with audit trails, and integration with onboard data analysis modules. While Local Run Manager runs on the instrument computer, users can monitor run progress and view analysis results from other computers connected to the same network. After a sequencing run is complete, Local Run Manager automatically launches data analysis using one of the application-specific analysis modules. The modules can produce alignment data, identify single-nucleotide variants (SNVs), structural variants, perform expression analysis, small RNA analysis, and more (Table 2).

Alternatively, sequence data can be instantly transferred, analyzed, and stored in BaseSpace™ Sequence Hub, the Illumina genomics computing environment. Due to industry-standard data formats, third-party developers have created a rich ecosystem of commercial and open-source apps in BaseSpace Sequence Hub for downstream data analysis. These apps provide automated algorithms for whole-genome, exome, transcriptome, and targeted resequencing data for alignment, variant detection, annotation, visualization, and beyond.

Versatile to Support a Wide Range of Applications

With a maximum output of 1.2 Gb, the iSeq 100 System offers rapid, multiplexed sequencing for a range of applications:

- Small whole-genome sequencing
- Targeted resequencing
 - AmpliSeq for Illumina Targeted Resequencing
 - Long-range PCR
- *De novo* sequencing
- Gene editing validation
- Metagenomics (16S rRNA-sequencing)
- Targeted mRNA sequencing
- Small RNA sequencing
- Multiple genome assessment
- Human leukocyte antigen (HLA) sequence-based typing

Table 2: Example iSeq 100 System Applications and Run Configurations

Application	Samples/Run	Run Time
Small Genome Sequencing		
5-10 Mb Genomes, 30× Coverage 2 × 150 bp	1–8	17.5 hrs
Targeted Gene Expression Profiling		
Up to 500 targets 1 × 50 bp	1–48	9 hrs
Targeted Amplicon Sequencing		
Up to 3000 amplicons 2 × 150 bp	1–48	17.5 hrs

Ordering Information

System	Catalog No.
iSeq 100 Sequencing System	20021532
Sequencing Reagent Kits	Catalog No.
iSeq 100 i1 Reagent (300-cycle single kit)	20021533
iSeq 100 i1 Reagent 4 Pack (300-cycle quad kit)	20021534

Summary

While the iSeq 100 System is the smallest instrument in the Illumina portfolio, it delivers big advantages. Compared to larger sequencing systems or outsourcing, the iSeq 100 System provides faster and more cost-effective small-scale runs, independence from outsourcing, and control of the sequencing process from beginning to end. In addition, as part of a comprehensive solution that encompasses a range of library prep kits, sequencing, and user-friendly data analysis, the iSeq 100 System delivers a fully supported and integrated workflow. With an affordable price point and small footprint, the iSeq 100 System brings the power of NGS to virtually any laboratory, with virtually any budget.

References

1. Precone V, Monaco VD, Esposito MV, et al. *Cracking the Code of Human Diseases Using Next-Generation Sequencing: Applications, Challenges, and Perspectives*. *Biomed Res Int*. 2015;161648.
2. Shokralla S, Porter TM, Gibson JF, et al. *Massively parallel multiplex DNA sequencing for specimen identification using an Illumina MiSeq platform*. *Sci Rep*. 2015;5:9687.
3. Data calculations on file. Illumina, Inc., 2015.
4. Illumina (2011) *Quality Scores for Next-Generation Sequencing*. Accessed December 1, 2017.
5. Bentley DR, Balasubramanian S, Swerdlow HP, et al. *Accurate Whole Human Genome Sequencing using Reversible Terminator Chemistry*. *Nature*. 2008;456(7218):53-59.

iSeq 100 System Specifications

Parameter	Specifications
Instrument Configuration	RFID tracking for consumables
Instrument Control Computer (Internal)^a	Base Unit: Celeron J1900, 2 GHz, Quad Core CPU Memory: 8 GB RAM Hard Drive: 240 GB SSD Operating System: Windows 10 IoT Enterprise
Operating Environment	Temperature: 15°C to 30°C (22.5°C ± 7.5°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, 1.5 W/cm ² at image plane
Dimensions	W×D×H (raised monitor): 30.5 cm × 33 cm × 42.5 cm (12.0 in × 13.0 in × 16.7.0 in) Weight: 16 kg (35 lbs) Crated Weight: 21 kg (47 lbs)
Power Requirements	90–264 VAC, 47–63 Hz 80 W
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.

Learn More

To learn more about the iSeq 100 System, visit www.illumina.com/iseq

For more information on CMOS technology and one-channel SBS, download the [Illumina CMOS Chip and One-Channel SBS Chemistry Technical Note](#)

To learn more about microbial or mitochondrial sequencing on the iSeq 100 System, read the [Microbial WGS with the iSeq 100 System](#) or the [Mitochondrial DNA Sequencing on the iSeq 100 System](#) Application Notes

For a review of Frequently Asked Questions, visit the [iSeq 100 System FAQs Page](#)