

# iSeq™ 100 Sequencing System

Smallest, most affordable  
Illumina sequencing system

- Cost-effective system for independent operations
- Rapid data generation with fast turnaround times
- Exceptional data accuracy for high analytical sensitivity
- Convenient library quality evaluation and proof-of-principle testing



## Introduction

Next-generation sequencing (NGS) is easier and more affordable with the compact Illumina iSeq 100 System (Figure 1). The iSeq 100 System 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 turn-around times. The iSeq 100 System generates a minimum output of 1.2 Gb of data per run in 19 hours and delivers the high resolution and analytical sensitivity needed for detection of rare variants and transcripts.<sup>1,2</sup>

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

## 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 1: iSeq 100 System—Harness the power of NGS in the most affordable, compact benchtop sequencing system in the Illumina portfolio.

## Fast library preparation

The iSeq 100 System is compatible with the full suite of Illumina library preparation kits. Using the Nextera™ XT and Illumina DNA Prep Library Kits, researchers can prepare multiplexed libraries in 3–4 hours for small genome and direct long-range amplicon sequencing. In addition, the AmpliSeq™ for Illumina Targeted Resequencing Solution offers expertly designed content in ready-to-use fixed panels, community-designed panels, or custom panels 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.

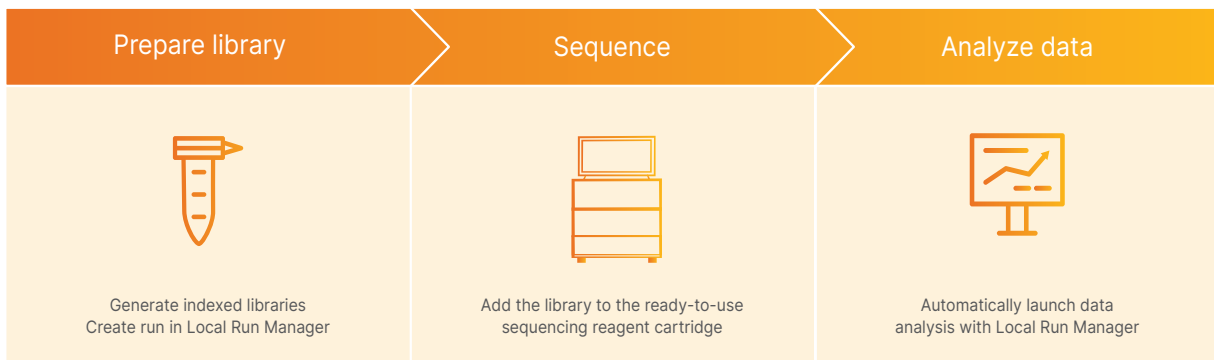


Figure 2: The iSeq 100 System is part of a streamlined DNA-to-data workflow

## Sequencing on the iSeq 100 System

After library preparation, double-stranded libraries are loaded into a thawed, prefilled reagent cartridge and loaded into the iSeq 100 System. Starting a run is as easy as thaw, load, and go and just takes 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 setup time.

### High analytical sensitivity and accuracy with SBS technology

The iSeq 100 System employs proven Illumina SBS technology to deliver high-quality data with > 80% of bases at or above Q30 (Table 1, Figure 3). 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.<sup>4</sup> 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.

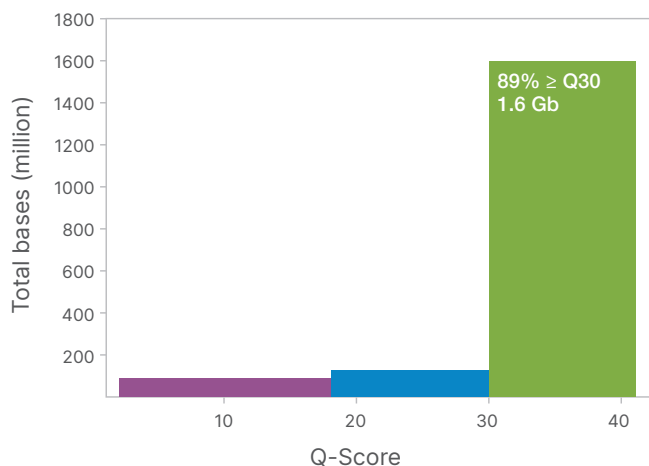


Figure 3: iSeq 100 System quality scores—A quality score (Q-Score) is a prediction of the probability of an error in base calling. A Q-Score of 30 (Q30) is widely considered a benchmark for high-quality data.<sup>3</sup> A microbial pool run on the iSeq 100 System at 2 × 151 bp delivers over 89% bases at ≥ Q30.

### Highly innovative, one-channel SBS chemistry

The iSeq 100 System uses proven Illumina SBS chemistry on a patterned flow cell with nanowells fabricated over a CMOS chip to deliver one-channel sequencing chemistry. 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.

Table 1: iSeq 100 System performance parameters<sup>a</sup>

Run configuration <sup>b</sup>	Reads PF/run	Output	Quality scores <sup>c</sup>	Run time <sup>d</sup>
1 × 36 bp	4M	144 Mb	> 85%	~9.5 hr
1 × 50 bp	4M	200 Mb	> 85%	~10 hr
1 × 75 bp	4M	300 Mb	> 80%	~11 hr
2 × 75 bp	4M	600 Mb	> 80%	~14 hr
2 × 150 bp	4M	1.2 Gb	> 80%	~19 hr

a. Performance parameters may vary based on sample type, sample quality, and clusters passing filter (PF).  
 b. These are common configurations of the iSeq 100 i1 Reagents v2 (300 cycle) kit.  
 c. The percentage of bases > Q30 is averaged over the entire run.  
 d. Times include cluster generation, sequencing, base calling, and quality scoring.

One-channel SBS chemistry uses one dye, two chemistry steps, and two images per sequencing cycle (Figure 4). Nucleotides are identified by analysis of the different emission patterns for each base across the two images. 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).



Read more about one-channel SBS chemistry in the [Illumina CMOS Chip and One-Channel SBS Chemistry technical note](#).

### Easy, flexible data analysis

The iSeq 100 System offers several data analysis options, including onboard and cloud-based solutions. Local Run Manager, a fully integrated onboard analysis software, features modular architecture to support current and future assays. Local Run Manager supports sequencing run planning, library and run tracking with audit trails, and onboard data analysis module integration. While Local Run Manager runs on the instrument computer, users can monitor run progress and view analysis results from remote 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 and 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 cloud-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 more.

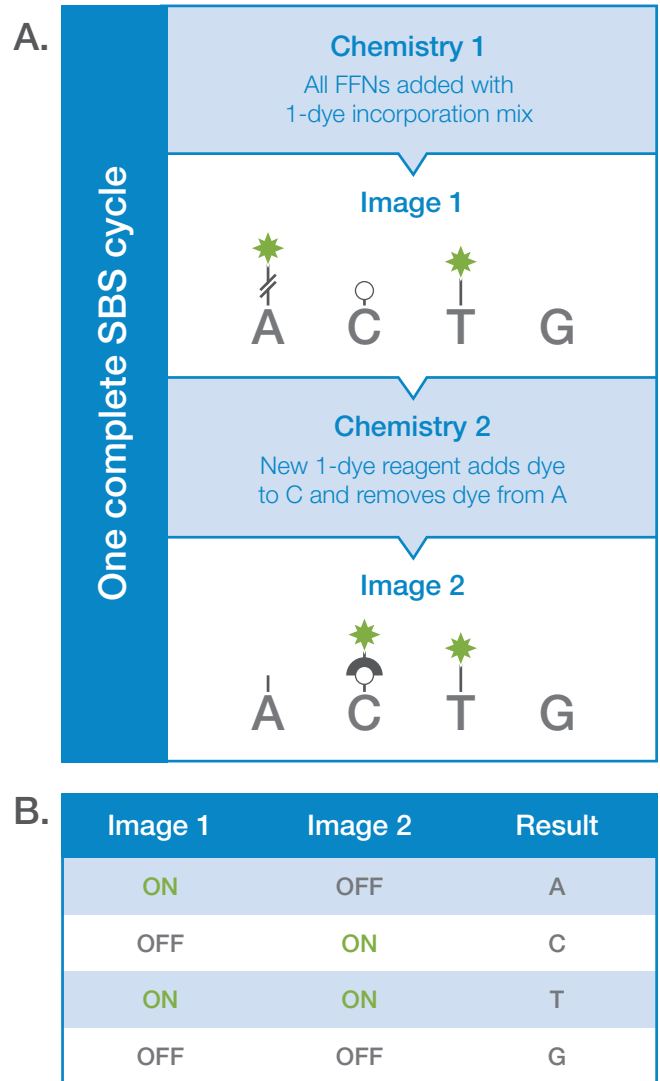


Figure 4: One-channel SBS chemistry—(A) One-channel SBS chemistry features two chemistry steps and two imaging steps per sequencing cycle using nucleotides that can be labeled or unlabeled depending on the chemistry step. (B) The base call is determined by the signal pattern across both images.

## Wide range of applications

With a minimum output of 1.2 Gb for longest supported read length, 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 applications and run configurations

Application	Samples/run	Run time
<b>Small genome sequencing</b> 5–10 Mb genomes, 30× coverage, 2 × 150 bp	1–8	~19 hr
<b>Targeted gene expression profiling</b> Up to 500 targets 1 × 50 bp	1–48	~9.5 hr
<b>Targeted amplicon sequencing</b> Up to 3000 amplicons 2 × 150 bp	1–48	~19 hr

## 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.

## Learn more

iSeq 100 Sequencing System, [illumina.com/iseq](https://illumina.com/iseq)

Applications for iSeq System, [illumina.com/systems/sequencing-platforms/iseq/applications.html](https://illumina.com/systems/sequencing-platforms/iseq/applications.html)

Frequently asked questions, [iSeq 100 System FAQ page](#)

## 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. doi:10.1155/2015/161648
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. doi:10.1038/srep09687
3. Illumina. [Quality Scores for Next-Generation Sequencing](#). [illumina.com/documents/products/technotes/technote\\_Q-Scores.pdf](https://illumina.com/documents/products/technotes/technote_Q-Scores.pdf). Published 2011. Accessed February 2, 2023.
4. Bentley DR, Balasubramanian S, Swerdlow HP, et al. [Accurate whole human genome sequencing using reversible terminator chemistry](#). *Nature*. 2008;456(7218):53–59. doi:10.1038/nature07517

iSeq 100 System specifications

Parameter	Specification
Instrument configuration	RFID tracking for consumables
Instrument control computer (internal)	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 <sup>2</sup> 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 in) Weight: 16 kg (35 lbs) Crated weight: 21 kg (47 lbs)
Power requirements	100–240 VAC at 50/60 Hz 80 W
Radio frequency identifier (RFID)	Frequency: 13.56 MHz Power: suppl current 120 mA, RF output power 200 mW
WLAN specifications	Frequency: 2.4 GHz and 4 GHz standard IEEE 802.11a IEEE 802.11b IEEE 802.11g IEEE 802.11n IEEE 802.11aC Power: 3.3 VDC, supply current 780 mA
Product safety and compliance	NRTL certified IEC 61010-1 CE marked FCC/IC approved

a. Computer specifications are subject to change.

## Ordering Information

System	Catalog no.
iSeq 100 Sequencing System	20021535
Sequencing reagent kit	Catalog no.
iSeq 100 i1 Reagent v2 (300-cycle)	20031371
iSeq 100 i1 Reagent v2 (300-cycle) 4 pack	20031374
iSeq 100 i1 Reagent v2 (300-cycle) 8 pack	20040760



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