# iSeq<sup>™</sup> 100 System

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

### Highlights

- Rapid data generation Run smaller projects on a dedicated 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
- Indendent, convenient operations
  Control the sequencing process from beginning to end and
  maintain an independent schedule instead of outsourcing
- Exceptional data accuracy Leverage higher analytical sensitivity to detect rare variants and transcripts compared to qPCR or Sanger sequencing

### 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-oxidesemiconductor (CMOS) technology with the proven accuracy of Illumina sequencing by synthesis (SBS) chemistry to deliver highaccuracy data with fast turnaround times. The iSeq 100 System generates up to 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



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

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).

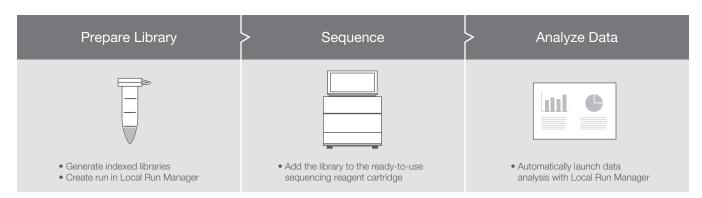


Figure 2: The iSeq 100 System workflow – The iSeq 100 System is part of a streamlined DNA-to-data workflow.

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

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Run configuration	Reads passing filter (PF)/run	Output	Quality scores <sup>b</sup>	Run time <sup>c</sup>
$1 \times 36$ bp	4M	144 Mb	> 85%	~ 9 hours
1 × 50 bp	4M	200 Mb	> 85%	~ 9 hours
1 × 75 bp	4M	300 Mb	> 80%	~ 10 hours
2 × 75 bp	4M	600 Mb	> 80%	~ 13 hours
2 × 150 bp	4M	1.2 Gb	> 80%	~ 19 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.

### Fast library preparation

A The iSeq 100 System is compatible with the full suite of Illumina library preparation kits. With the Nextera<sup>™</sup> 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<sup>™</sup> for Illumina Targeted Resequencing Solution offers expertly designed content. AmpliSeq for Illumina targeted panels are available 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.

#### Sequencing on the iSeq 100 System

After library preparation, libraries are loaded into a thawed, prefilled reagent cartridge for the iSeq 100 System. Starting a run 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 setup time.

# High analytical sensitivity and accuracy with SBS technology

The iSeq 100 System employs proven Illumina SBS technology, which is used to generate > 90% of the world's sequencing data,<sup>3</sup> 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>5</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. <sup>1,2</sup>

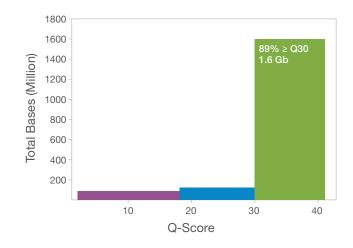


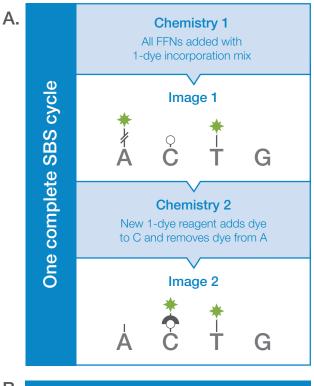
Figure 3: iSeq 100 System quality scores – A quality score (Q-Score) is a prediction of the probability of an error in base calling. A Q-Score of 30 (Q30) is widely considered a benchmark for high-quality data.<sup>4</sup> A microbial pool run on the iSeq 100 System at  $2 \times 151$  bp delivers over 89% bases at  $\geq$  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.

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).

<sup>23</sup> Read more about one-channel SBS chemistry in the Illumina CMOS Chip and One-Channel SBS Chemistry technical note.



В.	Image 1	Image 2	Result
	ON	OFF	А
	OFF	ON	С
	ON	ON	т
	OFF	OFF	G

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.

### 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 applicationspecific 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<sup>™</sup> 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 wholegenome, exome, transcriptome, and targeted resequencing data for alignment, variant detection, annotation, visualization, and more.

# Versatile to support a wide range of applications

With a maximum output of 2.0 Gb, the iSeq 100System offers rapid, multiplexed sequencing for a range of applications:

- Small whole-genome sequencing
- Targeted resequencing
  - AmpliSeq for Illumina Targeted ResequencingLong-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 1: 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	~ 19 hours
Targeted gene expression profiling		
Up to 500 targets 1 × 50 bp	1-48	~ 9 hours
Targeted amplicon sequencing		
Up to 3000 amplicons 2 × 250 bp	1-48	~ 19 hours

### 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

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

Read about more applications for the iSeq 100 System at www.illumina.com/systems/sequencing-platforms/iseq/learn

For a review of Frequently Asked Questions, visit the iSeq 100 System FAQs Page

### Ordering information

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

### References

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- 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., 2017.
- 4. Illumina (2011) Quality Scores for Next-Generation Sequencing. Accessed December 1, 2017.
- 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	Specification	
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 <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	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	
WLAN specifications	Frequency: 2.4 GHz and 5 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.

## **Innovative NGS platforms**

Illumina offers a full range of powerful NGS sequencing system, providing high data quality and accuracy tailored to meet the needs of diverse applications. Find comparison tables for benchtop to production-scales systems and tools to help you choose the right platform for your needs at

www.illumina.com/systems

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