MiSeq™ i100 and MiSeq i100 Plus Sequencing Systems

Sole source specifications

With the MiSeq i100 and MiSeq i100 Plus Sequencing Systems (collectively known as the MiSeq i100 Series), Illumina continues to set new standards for next-generation sequencing (NGS) simplicity and 2. speed. Breakthrough advancements in system design, sequencing chemistry, and integrated data analysis deliver increased operational simplicity, higher data accuracy, and exceptional processing speed, making NGS easier to adopt for virtually any lab.

The MiSeq i100 and MiSeq i100 Plus Systems are powered by XLEAP-SBS™ chemistry, a faster, higher quality, and more robust update to Illumina sequencing by synthesis (SBS) chemistry, providing significant improvements in stability, speed, and performance. Reformulated reagents enable groundbreaking room-temperature shipping and storage of consumables. These and other sustainability innovations, including significant reductions in packaging and plastic, result in a 35% reduction in total carbon footprint compared that of the MiSeq System.[†]

The instruments provide same-day results, with a run time as fast as 4 hours. There are up to 10 reagent configurations across multiple flow cells supporting varying study sizes with flexible batching requirements.

Every aspect of the MiSeq i100 Series workflow is optimized to minimize hands-on time and expertise required to perform sequencing. Load-and-go consumables, onboard denaturation, onboard cluster generation, and no-wash maintenance enable sequencing runs set up in only three steps in under 20 minutes. Integration with DRAGEN™ secondary analysis offers accurate, comprehensive, and efficient secondary analysis both onboard and in the cloud on BaseSpace™ Sequence Hub and Illumina Connected Analytics. The MiSeq i100 Series has sample-to-analysis workflows, including library prep kits, targeted gene panels, and DRAGEN secondary analysis. Comprehensive data summaries can be generated with analysis software in two hours or less for most applications.

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Key features

Groundbreaking sustainability	- Consumables are stored at room temperature, do not require thawing, and are ready to use immediately, expediting sequencing setup time and saving freezer space - Room-temperature shipping requires no ice packs or dry ice for less waste - Lightweight reagents, buffer cartridges, and waste containers that disassemble without special tools for simple disposal and ease of handling - CO_2 emissions from shipping reagents reduced by 52% compared to those of the MiSeq System* - Total carbon footprint reduced by 35% compared to that of the MiSeq System* - Packaging waste reduced by 85%, based on shipping weight, compared to that of the MiSeq System - Reagents are formulated free of formamide, simplifying disposal
Fast, flexible sequencing	- Powered by XLEAP-SBS chemistry, a faster, higher quality, and more robust update to proven Illumina SBS chemistry - Multiple flow cell configurations enable sequencing of 1.5 Gb to 30 Gb and up to 100 million single reads per run, providing adjustable output to meet scaling project needs - Provides same-day results, with run times as low as 4 hours - Integrated onboard and cloud-based data analysis pipelines powered by DRAGEN software - Analysis software generate data analysis summaries in two hours or less for most applications
Simplified operations and intuitive analysis	- Simplified workflow with run setup that requires only three steps completed in under 20 minutes - Sample-to-analysis workflows with preselected library prep kits and probe panels streamline experimental planning and simplify data analysis - Data analysis pipelines powered by DRAGEN secondary analysis enable accurate, comprehensive, and efficient analysis of NGS data without the need for bioinformatics expertise

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*Based on comparison of MiSeq reagent kits to MiSeq i100 reagent kits per one Gb of genetic code, measured in Global Warming Potential through an internal streamline life cycle assessment (LCA) study, aligned with the methodological requirements and guidelines of the International Organization for Standardization (ISO) standards ISO 14040 (2006a) and ISO 14044 (2006b) on LCA and the Greenhouse Gas (GHG) Protocol Product Life Cycle Accounting and Report Standard (WRI/WBCSD, 2011). As a streamlined LCA study, it does not fulfill all of the reporting requirements of these standards, including third-party review.

Instrument output

The following specifications are applicable for the MiSeq i100 and MiSeq i100 Plus Systems.

Expanded output

- Up to 25 million single read per MiSeq i100 Series 25M flow cell on the MiSeq i100 System or up to 100 million single reads per MiSeq i100 Series 100M flow cell on the MiSeq i100 Plus System
- Up to 15 Gb output per 25M flow cell with 2 × 300 bp on the MiSeq i100 System and up to 30 Gb output per 100M flow cell with 2 × 150 bp (or per 50M flow cell with 2 × 300 bp) on the MiSeq i100 Plus System

Instrument throughput

- MiSeq i100 Plus System: Generate 30 Gb per run using a 2 × 150 bp read length and 100M flow cell or a 2 × 300 bp read length and 50M flow cell 9.
- MiSeq i100 System: Generate 15 Gb per run using a 2 × 300 bp read length and 25M flow cell
- Tunable output using multiple flow cell configurations available on each system; all flow cell configurations can be run in a single day

Scalability

- ~1.5–15 Gb on the MiSeq i100 System and ~1.5–30 Gb per single flow cell run on the MiSeq i100 Plus System
- MiSeq i100 Plus System: Four flow cell types ranging in output from 5 million to 100 million single reads (10 million to 200 million paired-end reads) and from 100 to 600 cycles
- MiSeq i100 System: Two flow cell types ranging in output from 5 million to 25 million single reads (10 million to 50 million paired-end reads) and from 100 to 600 cycles

Sequencing flexibility

- Two flow cell options for the MiSeq i100 System and four flow cell options for the MiSeq i100 Plus System
- Choose from multiple read length kit configurations; 5 reagent configurations for the MiSeq i100 System and 10 reagent configurations for the MiSeq i100 Plus System

Max output per flow cell

Flow cell type	5M	25M	50M	100M	
1 × 100 bp	N/A	2.5 Gb	5 Gb	10 Gb	8.
2 × 150 bp	1.5 Gb	7.5 Gb	15 Gb	30 Gb	7.
2 × 300 bp	3 Gb	15 Gb	30 Gb	N/A	6.

Reagent cartridge configurations

- Reagent cartridges are shipped and stored at room temperature, eliminating cold storage requirements; no need to wait for reagents to thaw before starting a run, minimizing hands-on time for streamlined operations
- Multiple reagent kit configurations accommodate various sequencing runs:

Flow cell type	Reagent kit configurations
5M	300-cycle, 600-cycle
25M	100-cycle, 300-cycle, 600-cycle
50M	100-cycle, 300-cycle, 600-cycle
100M	100-cycle, 300-cycle

Instrument run time

- Run times, including automated onboard cluster generation, onboard denaturation, and sequencing, vary according to flow cell and read length:

Run configuration	Flow cell type			
	5M	25M	50M	100M
1 × 100 bp	N/A	~ 4 hr	~ 4.5 hr	~ 5 hr
2 × 150 bp	~ 7 hr	~ 7 hr	~ 7.5 hr	~ 8 hr
2 × 300 bp	~ 15 hr	~ 15 hr	~ 15.5 hr	N/A

Quality score (Q30)

- MiSeq i100 Series has a minimum specification of > 90% of bases above Q30 at 2 × 150 bp
- Performance may vary based on library type and quality, insert size, loading concentration, and other experimental factors:

Run configuration	Quality scores	
1 × 100 bp	≥ 90% of bases higher than Q30	11.1.
2 × 150 bp	≥ 90% of bases higher than Q30	11.2.
2 × 300 bp	≥ 85% of bases higher than Q30	11.3.

Method diversity

Sequencing flexibility

- Up to 100 million single reads passing filter (200 million paired-end reads) for MiSeq i100 Plus System and up to 25 million single reads passing filter (50 million paired-end read) for MiSeq i100 System, multiple flow cell configurations, and variable read lengths enable a broad range of methods 10.
- Flexible data output enables researchers to increase sample throughput and perform deeper sequencing based on sequencing and project needs
- Compatible with both Illumina and external library prep kits without additional conversion steps, streamlining operation and supporting a wide range of sequencing methods

Example sequencing methods

- 3’ gene expression
- RNA sequencing (RNA-Seq), including mRNA-Seq and total RNA-Seq (coding and noncoding)
- Targeted RNA panels
- Pathogen detection
- 16S amplicon sequencing
- Shotgun and shallow shotgun metagenomics
- Small whole-genome sequencing
- Amplicon-based targeted sequencing
- Enrichment-based targeted sequencing
- Genome editing
- Immune repertoire
- Library QC
- Additional methods and applications as developed

Sequencing chemistry

Sequencing chemistry	• Illumina SBS chemistry uses reversible terminators and an engineered, highly efficient DNA polymerase modified to accept reversible terminator nucleotides • Reversible terminator chemistry allows only one base addition at every cycle, greatly reducing homopolymer errors[1] • Reversible terminators are removed after base addition to allow addition of the next base during chain extension • Fluorescent dyes on the nucleotides are cleaved after imaging • Presence of all four reversible terminator nucleotides during enzymatic extension enhances incorporation of correct base, resulting in higher raw accuracy • Generation of high-quality data with > 90% of bases with Q-scores ≥ 30 at 2 × 150 bp read length
XLEAP-SBS chemistry	• A faster, higher quality, and more robust SBS chemistry built on the proven foundation of standard Illumina SBS chemistry • XLEAP-SBS nucleotides use state-of-the-art dyes and novel linkers and blockers that are more resistant to heat and show greater than 50× improvement in hydrolysis and prephasing. The XLEAP-SBS polymerase is engineered to be roughly 4× faster leading to lower levels of phasing and shorter run times • These innovations deliver 4× faster turnaround time (TAT) compared to the MiSeq System, providing same-day results and up to 3× greater accuracy than standard Illumina SBS
Paired-end read capability	• Paired-end capability allows resynthesis of the reverse strand of DNA followed by selective excision of the forward strand directly in the flow cell without additional intervention • Paired-end capability produces valuable sequence data from both ends of a template, increasing overall data quality • Sequences aligned as read pairs enable more accurate read alignment and the ability to detect insertion–deletion (indel) variants, which is more difficult with single-read data[2]

Amplification

Cluster generation	• Onboard (on-instrument) cluster generation provides automated clonal amplification of tens of billions of single-molecule DNA templates, producing dense clusters of each original DNA strand for sequencing • Estimated run times include cluster generation
Sample denaturation	• Sample denaturation is performed onboard the instrument • Estimated run times include sample denaturation
Amplification method	• Automated solid-phase isothermal amplification produces clonal clusters without user intervention • ExAmp technology delivers a high percentage of wells with single template clusters in high-density patterned flow cell substrates • Proprietary ExAmp process allows fully automated onboard clonal amplification (ie, no need for emulsion PCR or other labor-intensive methods of clonal amplification) • Automated amplification is performed directly on the instrument without the need for additional equipment

Instrumentation and operation

Fully contained sequencing system

- Intuitive interface with HD touch screen for at-a-glance viewing of run progress or reading detailed sequencing performance metrics on the instrument
- Automated onboard cluster generation; does not require specialized equipment for clonal amplification
- Automated onboard denaturation; performed directly on instrument reduces hands-on time and expedites run setup
- Automated onboard reconstitution of lyophilized reagents that does not require hookup to an external water line
- Concealed waste container allows users to perform multiple runs without having to empty
- Integrated instrument status light bar that guides the loading process and reflects the status of the instrument during setup and sequencing runs
- Sample-to-analysis workflows with onboard DRAGEN secondary analysis provide data analysis summaries for various applications on the instrument or in the cloud, minimizing the need for bioinformatic expertise

Optical system

- LED-based illumination module that provides blue and green excitation to excite the DNA fluorophores
- Includes a complimentary metal-oxide semiconductor (CMOS) flow cell for exceptional transmission efficiency and lower signal loss

Instrument control computer

- Integrated compute system composed of two computers: a Linux-based single-board computer (SBC) that displays the control software and a Linux-based compute engine (CE) that runs real-time analysis and processes the data
- Sequence output contains accurate base calls and qualities derived from real-time intensity data
- Advanced base calling algorithms improve data accuracy

Physical specifications

- Dimensions (W × D × H): 40.2 cm × 44.8 cm × 47.3 cm 4.
- Dry weight of instrument: 79.4 lb (36 kg) 5. 3.
- Crated weight of instrument: 108.1 lb (49 kg)

Site preparation

- Self-installable without relying on Field Service Engineers to expedite onboarding and begin sequencing straight out of the box
- Compatible with standard lab environments, without the need for direct water plumbing or drainage
- Requires 100–240 VAC 50/60 Hz, 300 W single phase electrical source
- USB-C available to support external storage devices
- Network speeds required:

Per instrument	Primary bandwidth (two channels, cloud, local)
Local network uploads	50 Mb/sec
BaseSpace™ Sequence Hub uploads	50 Mb/sec
Instrument operation data uploads	5 Mb/sec

- Sequencing runs can be performed without a network connection, if desired. External hard drive required if not connecting to local network; ≥ 256 Gb hard drive recommended to hold two large runs (2 × 150 bp for 100M flow cell)
- Temperature: 15–30°C; < 2°C change per hour
- Relative humidity: 20–80% relative humidity, noncondensing

Illumina Proactive instrument performance service

- Connected instruments securely share system performance data for proactive monitoring to minimize unplanned downtime
- With over a decade of data and predictive analytics, Illumina support teams can preemptively detect risk failures and troubleshoot remotely

Instrument reagents and operation

- Ready-to-use cartridges containing preconfigured reagents for amplification and sequencing
- Each dry cartridge contains up to two flow cells and is paired with a separate wet cartridge
- Reagents are formulated free of formamide, simplifying disposal
- Reagent cartridges are available in the following configurations with additional cycles to support dual indexing and dual unique molecular indexes:

Flow cell type[a]	Read length
5M	2 × 150 bp, 2 × 300 bp
25M	1 × 100 bp, 2 × 150 bp, 2 × 300 bp
50M[b]	1 × 100 bp, 2 × 150 bp, 2 × 300 bp
100M[b]	1 × 100 bp, 2 × 150 bp

a. 5M and 25M flow cells are compatible with the MiSeq i100 System. 5M, 25M, 50M, and 100M flow cells are compatible with the MiSeq i100 Plus System.

b. Available in H2 2025.

Reagent loading

- System automates loading the cartridge and cartridge tray into position without manual intervention to ensure proper seating
- Sensors verify the presence and compatibility of the flow cell via instant radio frequency identification (RFID) read

Technical support

- Technical support is available worldwide and in multiple languages via phone five days a week or online 24/7

Sustainability

Lyophilized reagents	• Improved robustness and stability of XLEAP-SBS reagents and new lyophilized reagents enable room-temperature shipping and storage of all consumables • Reagent kits are shipped at room temperature, with no ice packs or dry ice for less waste and streamlined unpacking • Reagent kits are stored at room temperature and do not require thawing, expediting setup time and saving freezer space
Reduced mass for consumables	• CO_2 emissions from shipping reagents reduced by 52% compared to those of the MiSeq System[†] • Total carbon footprint reduced by 35% compared to that of the MiSeq System[†] • Packaging waste reduced by 85%, based on shipping weight compared to that of the MiSeq System

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Software

Instrument control software	• MiSeq i100 Control Software offers an intuitive, easy-to-use interface that requires minimal training to configure, launch, and monitor runs • MiSeq i100 Control Software includes real-time analysis software that automatically calls bases and generates quality scores in real time, directly on the instrument computer • Quality statistics from runs can be monitored in real time from any location using BaseSpace Sequence Hub in the cloud, allowing users to monitor runs through a web browser for remote viewing
Secondary analysis with onboard DRAGEN pipelines	• Real-time analysis software provides real-time, on-instrument image processing to produce primary base calls • Local data analysis for the MiSeq i100 Series features onboard DRAGEN secondary analysis (including onboard DRAGEN license) for on-premises analysis of base calls, including alignment, variant calling, gene counts, and differential expression • Automated analysis solutions built for the MiSeq i100 Series within DRAGEN secondary analysis (onboard or cloud), BaseSpace Sequence Hub (cloud), and Illumina Connected Analytics (cloud) provide easy-to-interpret data for various NGS applications • The MiSeq i100 Series has sample-to-analysis workflows, including library prep kits, panels, and DRAGEN secondary analysis providing comprehensive data summaries in two hours or less for most applications

Informatics

Third-party analysis software	- Data files are compatible with third-party applications and pipelines - Multiple standardized data formats (ie, FASTQ, BAM, VCF, BCL, CRAM, and *.txt) ensure compatibility with third-party downstream analysis and visualization tools
Streamlined cloud-based analysis with BaseSpace Sequence Hub and Illumina Connected Analytics	- Upon selection, automatically stream data from the MiSeq i100 Series directly to BaseSpace Sequence Hub and/or Illumina Connected Analytics to eliminate manual data transfers. BaseSpace Sequence Hub provides simplified run planning and manual or automated DRAGEN secondary analysis. Centralize your data with Illumina Connected Analytics to power bioinformatics operations and population-wide insights - A minimum 50 MB/sec of bandwidth is required for streaming individual runs to BaseSpace Sequence Hub and/or Connected Analytics

References

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2. Nakazato T, Ohta T, Bono H. Experimental design-based functional mining and characterization of high-throughput sequencing data in the sequence read archive. *PLoS One*. 2013;8(10):e77910. doi:10.1371/journal.pone.0077910

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