# MiSeq<sup>™</sup> System

### Speed and simplicity for targeted resequencing and small-genome sequencing

- Exceptional data quality demonstrated through peerreviewed, scientific comparison
- Highly automated system with simple, intuitive instrument workflow
- Rapid sequencing and variant detection for time-critical studies
- Adjustable read length and flow cell options provide flexibility across a broad range of applications

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#### Introduction

The MiSeq System offers the first DNA-to-data sequencing platform integrating cluster generation, amplification, sequencing, and data analysis into a single instrument. Its small footprint—approximately two square feet—fits easily into virtually any laboratory environment (Figure 1). The MiSeq System leverages Illumina sequencing by synthesis (SBS) chemistry, a proven next-generation sequencing (NGS) technology cited in over 300,000 peer-reviewed publications—5× more than all other NGS technologies combined.<sup>1</sup> With the power of NGS delivered in a compact footprint, the MiSeq System is the ideal platform for rapid and cost-effective genetic analysis.

#### Simple, intuitive NGS workflow

The MiSeq System offers straightforward, easy-to-follow instrument control software. Perform instrument operations with an intuitive touch screen interface, use plugand-play reagent cartridges with radio frequency identifier (RFID) tracking, consult on-screen video tutorials, and enjoy step-by-step guidance throughout each sequencing workflow. All MiSeq Systems include onboard data analysis and access to BaseSpace<sup>™</sup> Sequence Hub, the Illumina



Figure 1: MiSeq System—The compact MiSeq System is well suited for rapid, cost-effective next-generation sequencing.

genomic cloud-computing platform. BaseSpace Sequence Hub provides real-time data uploading, simple data analysis tools, internet-based run monitoring, and a secure, scalable storage solution. A suite of data analysis tools and a growing list of third-party analysis apps empower researchers to perform their own informatics. BaseSpace Sequence Hub also enables fast and easy data sharing with colleagues or customers.



Figure 2: MiSeq System workflow—The streamlined workflow of the MiSeq System enables rapid turnaround time for next-generation benchtop sequencing. Libraries can be prepared with any compatible library preparation kit. Five-and-a-half hour sequence time includes cluster generation, sequencing, and quality-scored base calling with dual surface scanning for a 2 × 25 base pair run on a MiSeq System with MiSeq Control Software.

#### Fast turnaround time

For results in hours rather than days, the combination of rapid library preparation and the MiSeq System delivers a simple, accelerated turnaround time (Figure 2). Prepare your sequencing library in as few as three hours with Illumina DNA Prep library prep reagents, then move to automated clonal amplification, sequencing, and qualityscored base calling in as few as 5.5 hours on the MiSeq System (Table 1). Sequence alignment can be completed directly on the onboard instrument computer with MiSeq Local Run Manager software or through BaseSpace Sequence Hub within three hours.



Illumina SBS chemistry achieves exceptional data quality. This proprietary reversible terminator–based method enables the massively parallel sequencing of billions of DNA fragments, detecting single bases as they are incorporated into growing DNA strands. Fluorescent terminator dyes are imaged as each dNTP is added and then cleaved to allow incorporation of the next base. With all four reversible terminator–bound dNTPs present during each cycle, natural competition minimizes incorporation bias. Base calls are made directly from signal intensity measurements during each cycle, greatly reducing raw error rates compared to other technologies. The result is highly accurate base-by-base sequencing that virtually eliminates sequence context–specific errors, even within repetitive sequence regions or homopolymers (Figure 3).<sup>2</sup>



Figure 3: MiSeq System quality score distribution—Quality scores for a PhiX control library, 2 × 300 base pair run on a MiSeq System with MiSeq Control Software v2.4. This example shows 90% of bases sequenced above Q30.

Read length	Total time <sup>a</sup>	Output	Quality scores <sup>b</sup>	Single reads <sup>c</sup>	Paired-end reads <sup>c</sup>
MiSeq Reagent Kit v2					
2 × 25 bp	~5.5 hours	750-850 Mb	> 90% bases higher than Q30	12-15M	24-30M
2 × 150 bp	~24 hours	4.5-5.1 Gb	> 80% bases higher than Q30		
2 × 250 bp	~39 hours	7.5-8.5 Gb	> 75% bases higher than Q30		
MiSeq Reagent Kit v3					
2 × 75 bp	~21 hours	3.3-3.8 Gb	> 85% bases higher than Q30	22-25M	44-50M
2 × 300 bp	~56 hours	13.2-15 Gb	> 70% bases higher than Q30		
MiSeq Reagent Kit v2	Micro				
2 × 150 bp	~19 hours	1.2 Gb		4M	8M
MiSeq Reagent Kit v2 Nano					
2 × 150 bp	~17 hours	300 Mb		1M	2M
2 × 250 bp	~28 hours	500 Mb			
2 × 75 bp 2 × 300 bp MiSeq Reagent Kit v2 2 × 150 bp MiSeq Reagent Kit v2 2 × 150 bp 2 × 250 bp	~21 hours ~56 hours Micro ~19 hours Nano ~17 hours ~28 hours	3.3-3.8 Gb 13.2-15 Gb 1.2 Gb 300 Mb 500 Mb	> 85% bases higher than Q30 > 70% bases higher than Q30	22-25M 4M 1M	44-50M 8M 2M

#### Table 1: MiSeq System performance parameters

a. Total time includes cluster generation, sequencing, and base calling on a MiSeq System enabled with dual surface scanning.

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

c. Install specifications based on Illumina PhiX control library at supported cluster densities between 865–965 k/mm² clusters passing filter for v2 chemistry and

1200–1400 k/mm<sup>2</sup> clusters passing filter for v3 chemistry. Actual performance parameters may vary based on library type, library quality, and clusters passing filter.

#### Extensive suite of applications

Explore an ever increasing range of sequencing applications. With faster turnaround time and simplified workflows, the MiSeq System offers a cost-effective alternative to sequencing by capillary electrophoresis and qPCR for applications such as targeted resequencing, clone checking, amplicon sequencing, and RNA expression. Local Run Manager Software and BaseSpace Sequence Hub offer optimized analysis workflows for small genome sequencing, 16S metagenomics, RNA sequencing, targeted resequencing, and preimplantation genetic screening (PGS), as well as highly multiplexed applications such as amplicon sequencing with AmpliSeq<sup>™</sup> for Illumina panels. Adjustable read lengths, flow cell options, and choice of single or paired-end reads allow flexibility for matching data output to a broad range of experimental needs.

#### Learn more

#### **MiSeq System**

#### References

- 1. Data calculations on file. Illumina, Inc., 2022.
- 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

#### MiSeq System specifications

Parameter	Specification			
Instrument configuration	RFID tracking for consumables MiSeq Control Software Local Run Manager Software			
Instrument control computer (Internal)ª	Base Unit: Intel Core i7-2710QE 2.10 GHz CPU Memory: 16 GB RAM Drive: 1 TB Solid State Drive Operating System: Windows 10 Enterprise LTSC			
Operating environment	Temperature: 22°C ± 3°C Humidity: Noncondensing 20%-80% Altitude: Less than 2,000 m (6,500 ft) Air Quality: Pollution degree rating of II Ventilation: Maximum of 1,364 BTU/h For Indoor Use Only			
Light emitting diode (LED)	520 nm, 660 nm			
Dimensions	W × D × H: 68.6 cm × 56.5 cm × 52.3 cm (27.0 in × 22.2 in × 20.6 in) Weight: 57.2 kg (126 lb) Crated Weight: 93.6 kg (206 lb)			
Power requirements	90-264 VAC @ 50/60 Hz, 10 A, 400 W			
Radio frequency identifier (RFID)	Frequency: 13.56 MHz Power: 100 mW			
Product safety and compliance	NRTL certified IEC 61010-1 CE marked FCC/IC approved			
a. Computer specifications are subject to change.				

### Ordering information

Product	Catalog no.
MiSeq System	SY-410-1003
MiSeq Reagent Kit v2 (50-cycles)ª	MS-102-2001
MiSeq Reagent Kit v2 (300-cycles) <sup>a,b</sup>	MS-102-2002
MiSeq Reagent Kit v2 (500-cycles) <sup>a,b</sup>	MS-102-2003
MiSeq Reagent Kit v3 (150-cycle)⁵	MS-102-3001
MiSeq Reagent Kit v3 (600-cycle)⁵	MS-102-3003
MiSeq Reagent Micro Kit v2 (300-cycles) <sup>b</sup>	MS-103-1002
MiSeq Reagent Nano Kit v2 (300-cycles) <sup>b</sup>	MS-103-1001
MiSeq Reagent Nano Kit v2 (500-cycles)	MS-103-1003

a. 20-packs available.

b. Illumina Advantage products available; TG-labeled consumables have features that help customers reduce the frequency of revalidation. These consumables are available only under supply agreement and require customers to provide a binding forecast. Contact your account manager to learn more.

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