# NovaSeq™ X and NovaSeq X Plus Sequencing Systems

## Extraordinary throughput and transformative economics, more sustainably than ever


### Exceptional throughput and accuracy

Access exceptional throughput and accuracy to perform larger studies, more ambitious projects, and more data-intensive methods

### Lower total cost

Shrink total cost of ownership with breakthrough innovations in chemistry and informatics, operational simplicity, and streamlined workflows

### Sustainable innovation

Minimize environmental impact with lyophilized reagents for significant reductions in packaging size, weight, plastic mass, and waste

## Introduction

Genomics visionaries are stretching the limits of what next-generation sequencing (NGS) can do. Answering the most complex biological questions requires increased statistical power enabled through larger studies and deeper sequencing to identify rare genetic events. A more comprehensive view also requires a broader range of sequencing methods and multiomics. The technology advances built into the NovaSeq X and NovaSeq X Plus Sequencing Systems (the NovaSeq X Series) provide massive throughput and productivity gains to enable sequencing of up to tens of thousands of genomes per year. These transformational sequencing economics will empower genomic scientists to realize projects previously thought out of reach (Figure 1).

With the NovaSeq X Series, Illumina continues to set the standard for accuracy and usability. Breakthrough advancements in chemistry, optics, and software combine to deliver exceptional speed, data quality, and sustainability. Users can have outstanding throughput and scalability without sacrificing flexible, streamlined, and easy-to-use workflows.


**Figure 1: NovaSeq X and NovaSeq X Plus Sequencing Systems**
Illumina innovation continues to broaden access to high-throughput genomics that will drive novel scientific insights.

## Innovation to drive large-scale genomics with exceptional accuracy

The NovaSeq X and NovaSeq X Plus Systems offer the throughput and accuracy needed to enable more data-intensive applications and deliver meaningful insights at scale. Performance on the NovaSeq X Series reduces the cost per gigabase (Gb) by up to 60% compared to the NovaSeq 6000 System.[1] The NovaSeq X Plus System is our most powerful sequencing system yet with up to 16 terabases (Tb) output (or up to 52 billion single reads) per dual flow cell run.* The NovaSeq X System features a single flow cell configuration with an output range of 165 Gb to 8 Tb (or up to 26 billion single reads) per run* (Figure 2, Figure 3, and Table 1).†

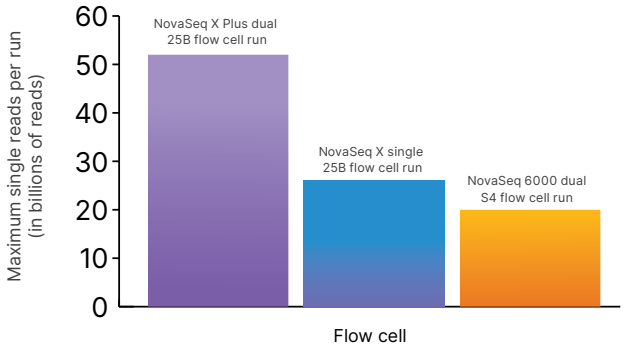


**Figure 2: Maximize sequencing output with the NovaSeq X Plus System**
Comparison of maximum output per single run in billions of reads for the NovaSeq X Plus System (dual 25B flow cell run), the NovaSeq X System (single 25B flow cell run), and the NovaSeq 6000 System (dual S4 flow cell run).[1] Actual output may be higher depending on library type, user optimization, and run performance.

* Actual output for the NovaSeq X Series may be higher, depending on library type, user optimization, and run performance
† To ensure future scalability, customers purchasing a NovaSeq X System can upgrade to the NovaSeq X Plus System for dual flow cell capability as needs evolve.

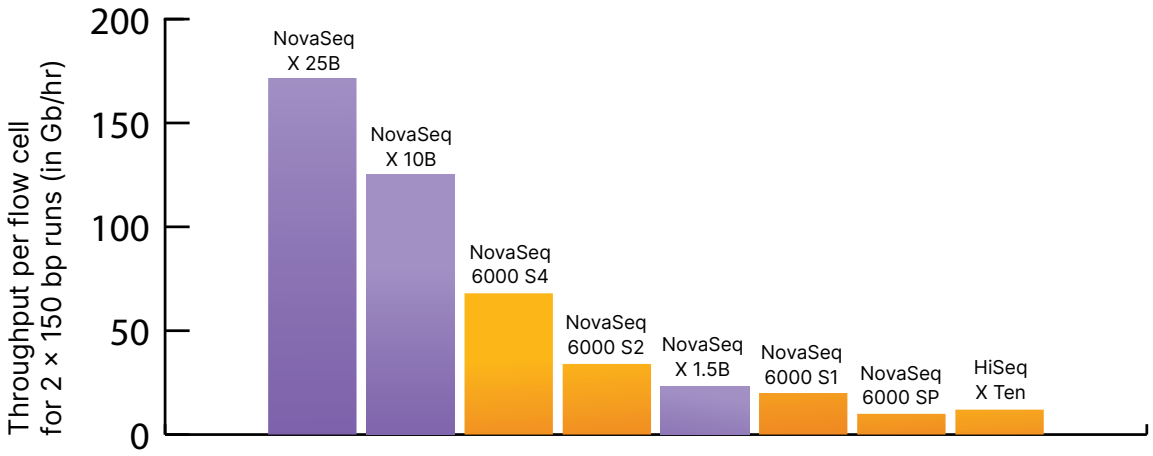


**Figure 3: The NovaSeq X Series offers maximum sequencing throughput**

Comparison of output per single flow cell per hour for NovaSeq X Series 1.5B, 10B, 25B flow cells; NovaSeq 6000 SP, S1, S2, S4 flow cells;[1] and the HiSeq™ X Ten.[2] Actual output may be higher depending on library type, user optimization, and run performance.

Three flow cell types support scalable throughput of more than 128 human genomes at 30× coverage, up to 1500 exomes, or over 1000 transcriptomes per dual flow cell run (Table 2). Numerous technology innovations make this new level of sequencing possible:

- Ultrahigh-density patterned flow cells with tens of billions of nanowells at fixed locations for up to 26–35 billion single reads (52–70 billion paired-end reads) per flow cell‡
- High numerical aperture, custom high-speed camera, and blue-green optics for ultrahigh-resolution imaging to maximize throughput and data quality
- Advanced base calling algorithms and integrated DRAGEN™ secondary analysis—available onboard or in the cloud—for award-winning accuracy and speed[3,4]

The NovaSeq X Series is powered by Illumina XLEAP-SBS™ chemistry, our fastest, highest quality, and most robust sequencing by synthesis (SBS) chemistry to date. Built from the proven foundation of the most widely adopted SBS chemistry,[5] XLEAP-SBS chemistry provides significant improvements in performance. XLEAP-SBS nucleotides use novel dyes, linkers, and blocks that are more resistant to heat, 50× more stable in solution, and 500× more stable when lyophilized. A 50× reduction in hydrolysis and 3× faster block cleavage greatly improve accuracy by reducing phasing and prephasing. The new XLEAP-SBS polymerase is engineered to incorporate nucleotides faster and with higher fidelity than ever before. Together, these innovations deliver up to 2× faster cycle times and up to 3× greater accuracy than standard SBS.[6]

‡ Performance at the higher range of output specification is not guaranteed. Actual output is dependent on library type, sample type and run optimization.

## Proven accuracy

XLEAP-SBS chemistry uses reversible-terminator nucleotides for true base-by-base sequencing that greatly reduces errors and missed calls associated with strings of repeated nucleotides (homopolymers).[7] XLEAP-SBS chemistry is also compatible with paired-end sequencing, facilitating detection of genomic rearrangements, repetitive sequence elements, gene fusions, and novel transcripts. 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.[8]

Table 1: NovaSeq X Series performance parameters[a]

| Flow cell | 1.5B | 10B | 25B |
|---|---|---|---|
| Output per single flow cell run[a,b] | | | |
| 2 × 50 bp | ~165–238 Gb | ~1–1.3 Tb | ~2.6–3.5 Tb |
| 2 × 100 bp | ~330–476 Gb | ~2–2.7 Tb | ~5.3–7 Tb |
| 2 × 150 bp | ~500–716 Gb | ~3–4 Tb | ~8–10.5 Tb |
| Output per dual flow cell run[a,b,c] | | | |
| 2 × 50 bp | ~330–476 Gb | ~2–2.6 Tb | ~5.2–7 Tb |
| 2 × 100 bp | ~660–952 Gb | ~4–5.4 Tb | ~10.6–14 Tb |
| 2 × 150 bp | ~1–1.4 Tb | ~6–8 Tb | ~16–21 Tb |
| Reads passing filter per flow cell[a,b] | | | |
| Single reads | ~1.6–2.4 billion | ~10–13 billion | ~26–35 billion |
| PE reads | ~3.2–4.8 billion | ~20–26 billion | ~52–70 billion |
| Instrument run time[a,d] | | | |
| 2 × 50 bp | ~17 hr | ~18 hr | ~25 hr |
| 2 × 100 bp | ~20 hr | ~22 hr | ~38 hr |
| 2 × 150 bp | ~23 hr | ~25 hr | ~48 hr |
| Quality scores[a,e] | | | |
| 2 × 50 bp | ≥ 90% of bases higer than Q30 | | |
| 2 × 100 bp | ≥ 85% of bases higer than Q30 | | |
| 2 × 150 bp | ≥ 85% of bases higer than Q30 | | |

a. Specifications based on an Illumina PhiX control library or a TruSeq™ DNA library created with human reference DNA (Coriell, Catalog no. NA12878) at supported cluster densities. Performance may vary based on library type and quality, insert size, loading concentration, and other experimental factors. Performance metrics are subject to change.
b. Performance at the higher range of output specification is not guaranteed. Actual output is dependent on library type, user optimization, and run performance.
c. Dual flow cell runs only apply to the NovaSeq X Plus System.
d. Run times include automated onboard cluster generation, sequencing, automated post-run wash, and base calling.
e. A quality score (Q-score) is a prediction of the probability of an error in base calling. The percentage of bases ≥ Q30 is averaged across the entire run.

### Delivering meaningful insights at scale

With unrivaled application breadth and revolutionary performance, the NovaSeq X Series redefines the limits of high-throughput sequencing to propel genomics research forward. Faster run times can mean faster answers for critical samples. With greater throughput, projects can be completed more efficiently. Scientists can increase statistical power via broader study design and larger sample cohorts. Labs can study more samples under different conditions or time points to reveal the dynamic properties of cells and biological systems. Single-cell, spatial, proteomic, or other multiomic studies can expand in scope to include more cells, higher resolution, or multiple modalities. Users can maximize read numbers and increase sequencing depth for the highest resolution view to detect low-frequency signals and variants.

## Transformational economics and productivity gains

The NovaSeq X Series supports the best total cost of ownership equation for high-throughput sequencing. Beyond the significant reduction in cost per Gb, the NovaSeq X Series incorporates cost-efficiency throughout the workflow, including operational simplicity, integrated data analysis, sustainability advancements, and world-class support (Figure 4).

Table 2: Estimated sample throughput for key applications[a]

| | Per single flow cell run | | | Per dual flow cell run[b] | | |
|---|---|---|---|---|---|---|
| Flow cell type | 1.5B | 10B | 25B | 1.5B | 10B | 25B |
| No. of human genomes | ~4 | ~24 | ~64 | ~8 | ~48 | ~128 |
| No. of exomes | ~41 | ~250 | ~750 | ~82 | ~500 | ~1500 |
| No. of transcriptomes | ~30 | ~200 | ~520 | ~60 | ~400 | ~1040 |

a. All sample throughputs are estimates based on the lower end range of the flowcell output. Human genome estimates assume > 120 Gb of data per sample to achieve 30× coverage. Exome estimates assume ~8 Gb per sample to achieve 100× coverage. Transcriptome estimates assume ≥ 50M reads. Throughput may vary based on the library preparation kit used. Performance metrics are subject to change.
b. Dual flow cell runs only apply to the NovaSeq X Plus System.

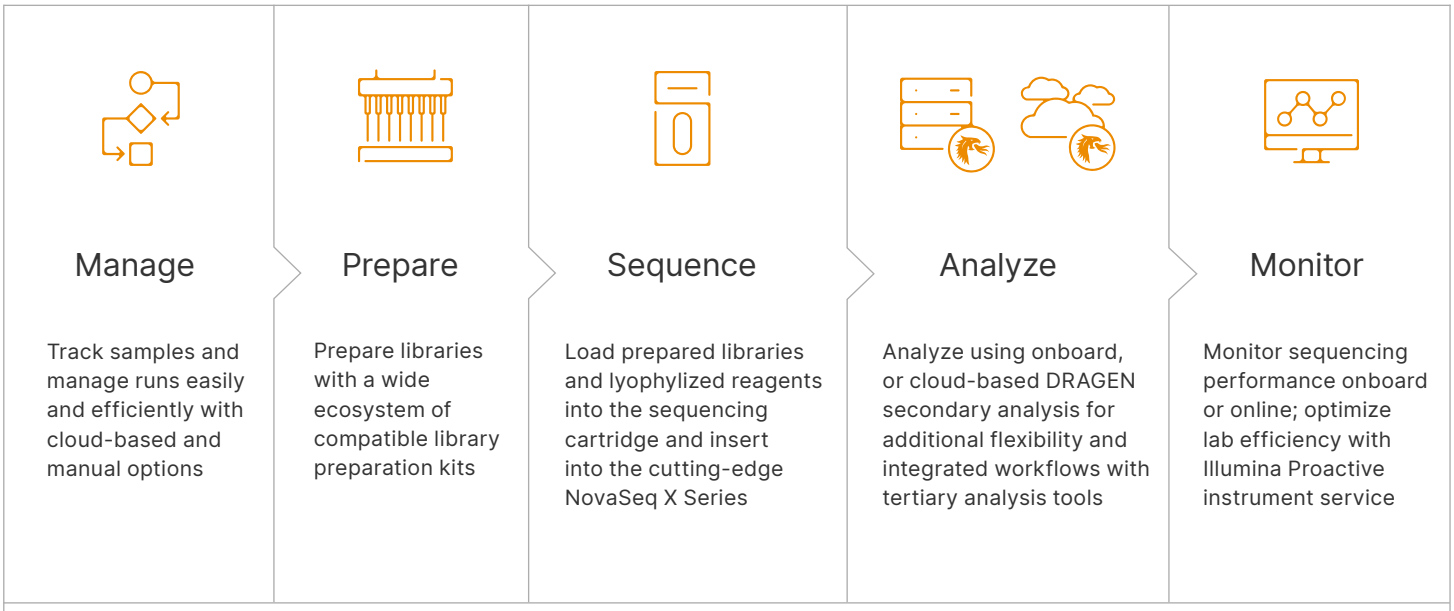


**Figure 4: Intuitive and optimized high-throughput sequencing workflow**
The NovaSeq X and NovaSeq X Plus Sequencing Systems provide a comprehensive workflow that includes user-friendly run setup, a wide ecosystem of compatible library prep kits, and integrated secondary analysis, supported by proactive monitoring of instrument performance.

## The ultimate user experience

Every aspect of the NovaSeq X Series workflow is optimized to minimize the time and labor required to complete projects (Figure 5). The NovaSeq X and NovaSeq X Plus Systems incorporate thoughtful ergonomic design and usability innovations such as:

- Extra-large 4K-resolution touch screen to clearly view run progress at a glance or read detailed sequencing performance metrics on the instrument
- Load-and-go reagent cartridges with "thaw windows" to visually verify that reagents have thawed completely
- Individually addressable flow cell lanes with automated onboard independent lane loading to easily split projects and samples across up to eight lanes
- 4× lower library input requirements§ to enable ultra-deep sequencing of precious samples and new applications for challenging sample types
- Automated onboard cluster generation and automated post-run wash to streamline the sequencing workflow
- Flexible run planning options to set up touchless secondary analysis for key applications (Figure 6)
- Lightweight reagents, buffer cartridges, and waste containers that are easy to handle
- Retractable keyboard and lighted prompts on consumable drawers for guided loading

Take a virtual tour, illumina.com/TourNovaSeqX

**Figure 5: Straightforward operation**
Many features of the NovaSeq X and NovaSeq X Plus Systems are designed to simplify the sequencing workflow, including a high-resolution touch screen interface and cartridges containing ready-to-use reagents for load-and-go operation.

§ Compared to the NovaSeq 6000 System onboard workflow.

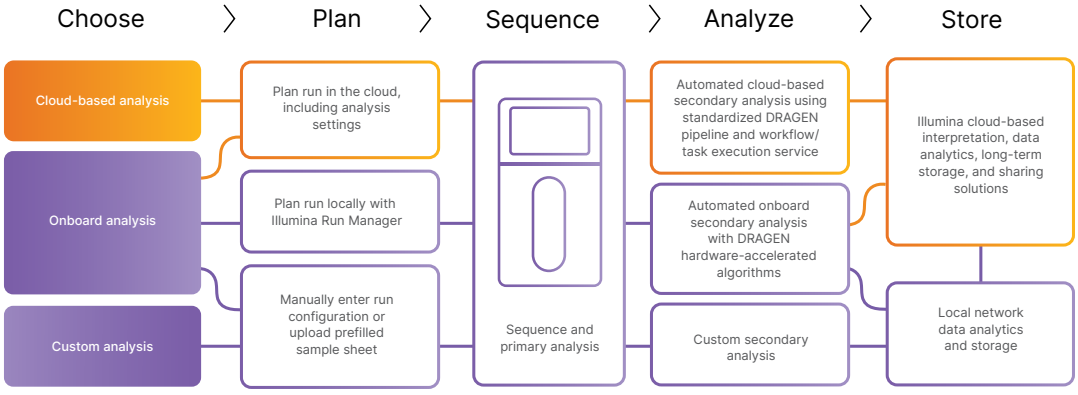


**Figure 6: Flexible informatics suite**
The NovaSeq X and NovaSeq X Plus Systems feature both local (purple) and cloud-based (orange) options for run setup, run management, and data analysis, enabling users to run their sequencing their way.

## Streamlined, comprehensive informatics

Onboard DRAGEN chips speed up computing and feature built-in lossless data compression algorithms. DRAGEN ORA (original read archive) can automate compression of FASTQ (fastq.gz) files up to 5× to enable faster data transfers and easier data management. A smaller data footprint also reduces storage and energy consumption costs.

With parallel compute structure, DRAGEN secondary analysis uses multigenome (graph) mapper and machine learning to systematically increase accuracy.[3,4] As integrated into the NovaSeq X Series, the DRAGEN platform can run multiple secondary analysis pipelines in parallel, either onboard or in the cloud. Perform up to four simultaneous applications per flow cell in a single run. Automated secondary analysis pipelines include:

- DRAGEN Germline for whole-genome sequencing
- DRAGEN Somatic for whole-genome sequencing
- DRAGEN Enrichment for whole-exome sequencing
- DRAGEN RNA for whole-transcriptome sequencing
- DRAGEN Methylation for methylation sequencing

These key applications are supported by comprehensive library-to-analysis workflows (Table 3).

## Groundbreaking sustainability innovations

The NovaSeq X and NovaSeq X Plus Systems were purposefully designed to reduce environmental impact. The improved robustness and stability of XLEAP-SBS reagents allows for shipping and storage in lyophilized form. This innovation delivers key benefits in sustainability and user experience:

- Reduces waste and unpacking time with ambient-temperature shipping that eliminates the need for dry ice or ice packs
- Streamlines workflow with consumables ready for use upon delivery or after thawing, minimizing hands-on time
- Optimizes freezer and storage space with a > 50% reduction in cartridge volume¶
- Improves handling with total kit weight reduced to 10 lb
- Simplifies disposal with recyclable components that disassemble easily and reduce packaging waste by ~90%
- Reduces plastic use > 50¶ with recyclable plastics and biopolymer-based buffer cartridges made from 96% sugar cane

¶ In comparison to NovaSeq 6000 reagent kits.

Table 3: Example library-to-analysis workfows for high-intensity sequencing applications on the NovaSeq X Series

| Application | Prepare libraries | Sequence | Analyze data |
|---|---|---|---|
| Whole-genome sequencing | Illumina DNA PCR-Free Prep | NovaSeq X 1.5B, 10B, or 25B flow cell, 300-cycle kit | DRAGEN Germline<br>DRAGEN Somatic |
| Whole-exome sequencing | Illumina DNA Prep with Exome 2.5 Enrichment | NovaSeq X 1.5B, 10B, or 25B flow cell, 200-cycle kit | DRAGEN Enrichment |
| Transcriptome sequencing | Illumina Stranded Total RNA Prep<br>Illumina Stranded mRNA Prep<br>Illumina RNA Prep with Enrichment | NovaSeq X 1.5B, 10B, or 25B flow cell, 200-cycle kit | DRAGEN RNA |
| Methylation sequencing | Illumina DNA Prep | NovaSeq X 1.5B, 10B, or 25B flow cell, 200-cycle kit | DRAGEN Methylation Pipeline |

## Trusted technology, trusted partner

As a preferred NGS platform provider, Illumina has shipped over 25,000 sequencing systems globally. With over 420,000 peer-reviewed citations, Illumina NGS is referenced five times more often than all other NGS platforms combined.[9] Building on decades of expertise, Illumina has a relentless commitment to innovation and building future NGS capabilities and applications. The NovaSeq X Series demonstrates our continued leadership in genomics technologies.

## Committed to customer success

To provide confidence in your investment, achieve peak performance, and minimize interruptions, Illumina has a world-class support team made up of experienced scientists who are experts in library prep, sequencing, and analysis. Technical support is available via phone five days a week or access online support 24/7, worldwide and in multiple languages, with rapid response time near most major metropolitan areas. Illumina provides excellent product consistency, supply, and quality enabled by a mature global manufacturing infrastructure.

# Summary

The NovaSeq X and NovaSeq X Plus Sequencing Systems provide extraordinary sequencing power to fuel data-intensive applications like whole-genome sequencing, single-cell sequencing, and multiomics. Numerous technical innovations, including XLEAP-SBS chemistry and onboard DRAGEN secondary analysis, enable maximum throughput and accuracy for genomics scientists. The transformative economics made possible by the NovaSeq X and NovaSeq X Plus Systems will drive a new era of genomic knowledge to improve human health.

**Learn more** →

NovaSeq X and NovaSeq X Plus Sequencing Systems

DRAGEN secondary analysis

Ordering information

| **System** | Catalog no. |
|---|---|
| NovaSeq X Sequencing System | 20084803 |
| NovaSeq X Plus Sequencing System | 20084804 |
| **Sequencing reagent kits** | Catalog no. |
| NovaSeq X Series 1.5B Reagent Kit (100 cycles) | 20104703 |
| NovaSeq X Series 1.5B Reagent Kit (200 cycles) | 20104704 |
| NovaSeq X Series 1.5B Reagent Kit (300 cycles) | 20104705 |
| NovaSeq X Series 10B Reagent Kit (100 cycles) | 20085596 |
| NovaSeq X Series 10B Reagent Kit (200 cycles) | 20085595 |
| NovaSeq X Series 10B Reagent Kit (300 cycles) | 20085594 |
| NovaSeq X Series 25B Reagent Kit (100 cycles) | 20125967 |
| NovaSeq X Series 25B Reagent Kit (200 cycles) | 20125968 |
| NovaSeq X Series 25B Reagent Kit (300 cycles) | 20104706 |

NovaSeq X Series instrument specifications

| Parameter | Specification |
|---|---|
| Instrument configuration | Computer and 4K touch screen display<br>Installation setup and accessories<br>Data collection and analysis software |
| Instrument control computer | Base Unit: iEi custom board with AMD V1605b CPU<br>Memory: 2 × 16 GB DDR4 SODIMM<br>Hard drive: none<br>Solid-state drive: 480GB M.2<br>Operating system: Oracle 8 |
| Instrument compute engine | Base Unit: iEi custom board with dual AMD 7552 CPU<br>Memory: 8 × 64 GB + 8 × 128 GB DDR4 RDIMM<br>Hard drive: none<br>Solid-state drive: 480GB M.2 + 5 × 12.8 TB U.2<br>Operating system: Oracle 8 |
| Operating environment | Temperature: 15°C–30°C, < 2°C change/hr<br>Humidity: 20%–65% relative humidity, non-condensing<br>Altitude: below 2000 meters (6500 feet)<br>Ventilation: maximum heat output for instrument is 9200 BTU/hr and average heat output is 7507 BTU/hr<br>For indoor use only |
| Laser | CLASS 1 laser product<br>532 nm (4.5 watt maximum power), 457 nm (6 watt maximum power) |
| RFID | Operating frequency 13.56 MHz, 200 mW output power |
| Dimensions | W × D × H: 86.4 cm × 93.3 cm × 158.8 cm<br>Dry weight (not including UPS): 1171 lb<br>Dry weight (including UPS): 1253 lb<br>Crated weight: 1591 lb (722 kg)<br>Accessories pallet weight: 525 lb (238 kg) |
| Power requirements | 200–240 VAC 50/60 Hz, 15A, single phase<br>Illumina provides a region-specific uninterruptible power supply (UPS)<br>Minimum amperage requirements can depend on regional voltage |
| Network connection | Dedicated 10 GBE connection ((10GBASE-T) using RJ-45 from instrument) between the instrument and data management system; connect directly or through network |
| Bandwidth for network connection | *For primary analysis data:*<br>800 Mbit/s/instrument for local network uploads<br>800 Mbit/s/instrument for BaseSpace Sequence Hub/Illumina Connected Analytics uploads<br>15 Mbit/s/instrument for instrument operational data uploads<br>*For primary and secondary analysis data:*<br>3.2 Gbit/s/instrument for local network uploads<br>3.2 Gbit/s/instrument for BaseSpace Sequence Hub/Illumina Connected Analytics uploads<br>15 Mbit/s/instrument for instrument operational data uploads |

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