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NovaSeq X Sequencing
Systems



Illumina NovaSeq X Sequencing Systems User Manual

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Illumina X Sequencing Systems User Manual



Extraordinary throughput and transformative economics, more sustainably than ever.

- Access exceptional throughput and accuracy to perform larger studies, more ambitious projects, and more data-intensive methods
- Shrink total cost of ownership with breakthrough innovations in chemistry and informatics, operational simplicity, and streamlined workflows
- 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, deeper sequencing to identify rare genetic events, and broader sequencing methods and multiomics for a more comprehensive view.

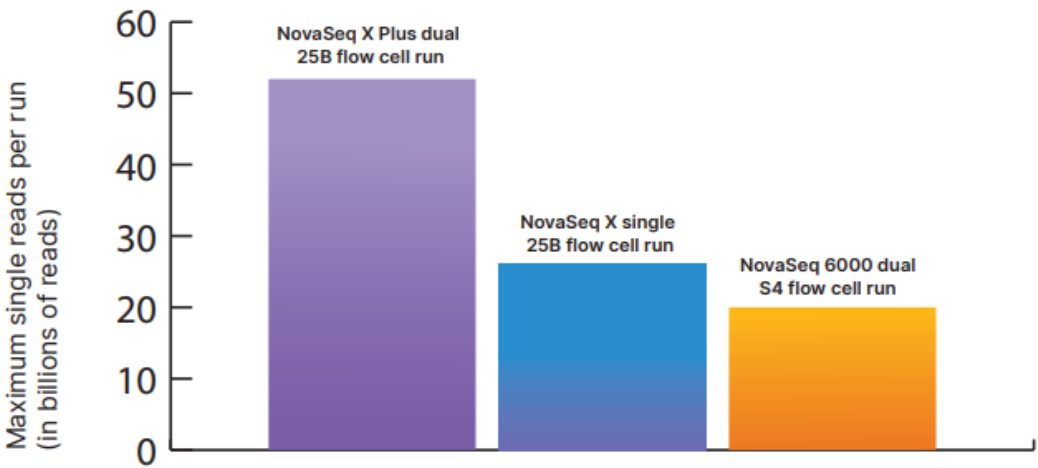
The technological advances built into the NovaSeq X and NovaSeq X Plus Sequencing Systems provide massive throughput and productivity gains to enable the sequencing of up to tens of thousands of genomes per year. These transformational sequencing economics will empower genomic scientists to realize projects 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.

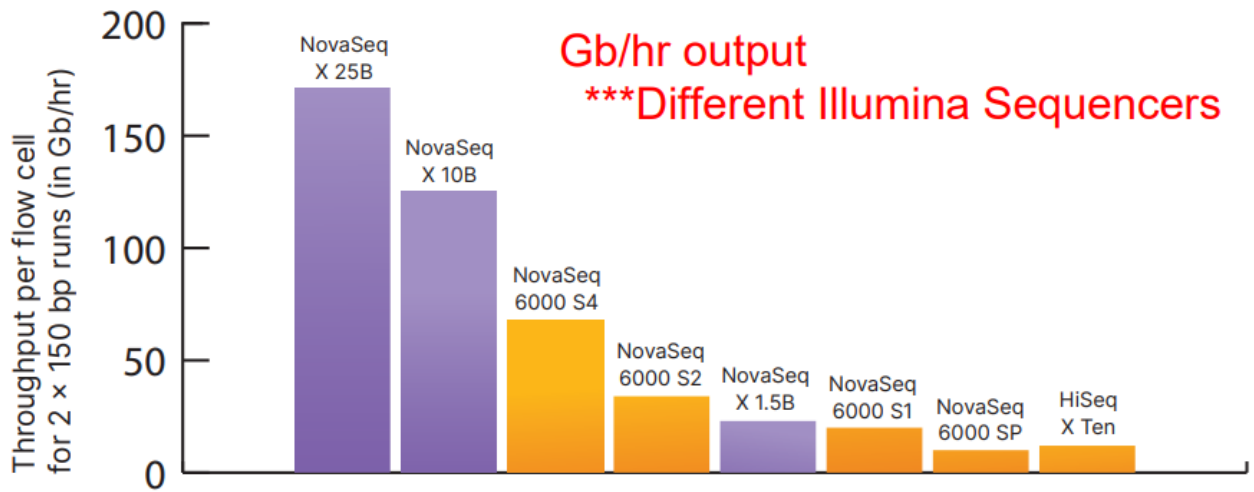


Figure 2: Maximize sequencing output with the NovaSeq X



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

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 From the first \$1000 genome to today, Illumina continues to transform the economics of high-throughput sequencing.



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, Table 1).

To ensure future scalability, customers who purchase a NovaSeq X system can fully upgrade to the NovaSeq X Plus system for dual flow cell capability as needs evolve. 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 technological innovations make this new level of sequencing possible.

- Ultrahigh-density patterned flow cells with tens of billions of nanowalls at fixed locations for up to 26 billion single reads (52 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™ Bio-IT Platform—available onboard or in the cloud—for award-winning accuracy and speed^{3,4}
- The NovaSeq X series is powered by Illumina XLEAP-SBSTM 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 and used SBS chemistry, XLEAP-SBS chemistry provides significant performance improvements.
- 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 pre-phasing. 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.⁵

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).⁶ XLEAP-SBS chemistry is also compatible with paired-end sequencing, facilitating the 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.

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 multi-omic 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.

Table 1: NovaSeq X series performance parameters

Flow cell type ^b	1.5B	10B	25B
Output per single flow cell run ^a	NovaSeq X		
2 × 50 bp	~165 Gb	1 Tb	–
2 × 100 bp	330 Gb	2 Tb	–
2 × 150 bp	500 Gb	3 Tb	8 Tb
Output per dual flow cell run ^{a,c}	NovaSeq X Plus		
2 × 50 bp	~330 Gb	2 Tb	–
2 × 100 bp	660 Gb	4 Tb	–
2 × 150 bp	1 Tb	6 Tb	16 Tb
Reads passing filter per flow cell ^a			
Single reads	1.6 billion	10 billion	26 billion
Paired-end reads	3.2 billion	20 billion	52 billion
Instrument run time ^{a,d}			
2 × 50 bp	~13 hr	~18 hr	–
2 × 100 bp	~18 hr	~22 hr	–
2 × 150 bp	~21 hr	~24 hr	~48 hr
Quality scores ^{a,e}			
2 × 50 bp	≥ 90% of bases higher than Q30		

2 × 100 bp ≥ 85% of bases higher than Q30

2 × 150 bp ≥ 85% of bases higher than Q30

- Specifications based on Illumina PhiX control library at supported cluster densities. Performance may vary based on library type and quality, insert size, loading concentration, and other experimental factors. NovaSeq X Plus system will be launched in Q1 2023. NovaSeq X system available later in 2023. Performance metrics are subject to change.
- 10B flow cell available Q1 2023. 1.5B and 25B flow cells available H2 2023.
- Dual flow cell runs only apply to the NovaSeq X Plus system.
- Run times include automated onboard cluster generation, sequencing, automated post-run wash, and base calling.
- 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.

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). The ultimate user experience Every aspect of the NovaSeq X series workflow is optimized to minimize the time and labor required to complete projects. The NovaSeq X and NovaSeq X Plus systems incorporate thoughtful ergonomic design and usability innovations such as:

- Extra-large 4K-resolution touch screen to view run progress at a glance or read detailed sequencing performance metrics on the instrument (Figure 5)
- 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 ultradeep 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
- Other details like a retractable keyboard and lighted prompts on consumable drawers for guided loading Take a virtual tour, illumina.com/TourNovaSeqX † Compared to the NovaSeq 6000 system onboard workflow.

Table 2: Estimated sample throughput for key applications:

Flow cell type ^b	Per single flow cell run			Per dual flow cell run ^c		
	1.5B	10B	25B	1.5B	10B	25B
Human genomes	~4	~24	~64	~8	~48	~128
Exomes	~41	~250	~750	~82	~500	~1500
Transcriptomes	~30	~200	~520	~60	~400	~1040

- All sample throughputs are estimates. Human genome estimates assume > 120 GB of data per sample to achieve 30× coverage. Exomes 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. NovaSeq X Plus system will be launched in Q1 2023.
- NovaSeq X system available later in 2023. Performance metrics are subject to change.
- 10B flow cell available Q1 2023. 1.5B and 25B flow cells available H2 2023.
- Dual flow cell runs only apply to the NovaSeq X Plus system.

Streamlined, comprehensive informatics

Onboard DRAGEN chips speed up computing and feature built-in lossless data compression algorithms. DRAGEN ORA (original read archive) can automate the compression of FASTQ 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 multigenome (graph) reference uses scalable 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 Enrichment for whole-exome sequencing
- DRAGEN RNA for whole-transcriptome sequencing
- These key applications are supported by comprehensive library-to-analysis workflows (Table 3).

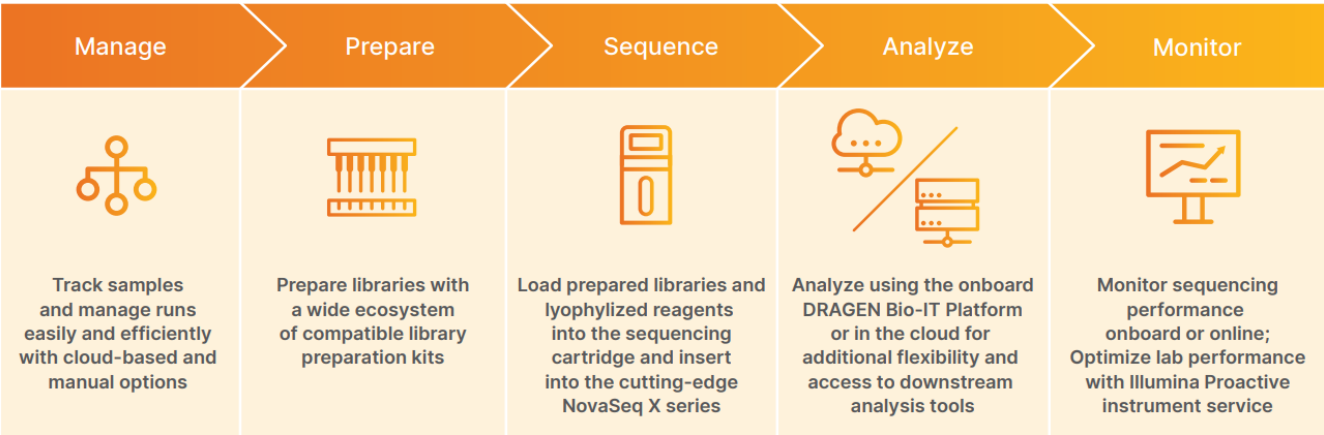


Figure 4

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

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, allow for shipping and storage in lyophilized form. This key innovation delivers remarkable benefits in terms of sustainability and user experience:

- Reagent kits shipped at ambient temperature (with no dry ice and no ice packs) for less waste and reduced time spent unpacking
- Ability to use consumables in a sequencing run immediately upon receipt (or after thawing, if previously stored) minimizing the hands-on time for streamlined operation
- Over 50% reduction in cartridge volume‡ to optimize freezer and storage space
- Weight per kit reduced by ~90%‡ for easier handling (only 10 lb total, shipped in one box)
- Simple disposal of consumables with recyclable components that disassemble without special tools and ~90% reduction in packaging waste‡
- Plastic mass was reduced by over 50%† using recyclable plastics and buffer cartridges made with plant-based biopolymer (biodegradable 96% sugar cane)
- In comparison to NovaSeq 6000 reagent kits.

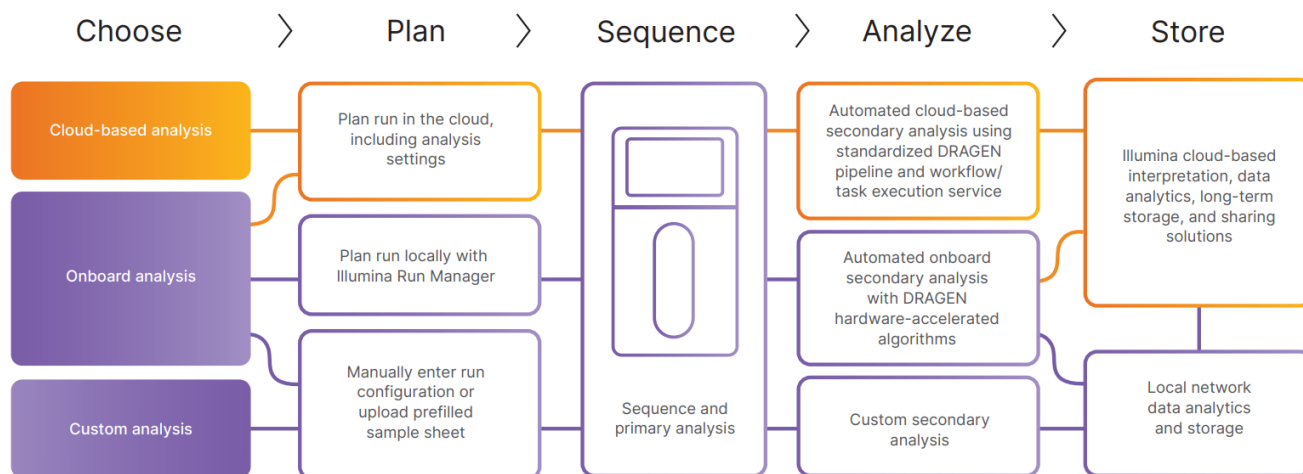


Figure 5: Straightforward operation—Many features of the NovaSeq X and NovaSeq X Plus systems are designed to simplify the sequencing workflow, including a high-resolution touchscreen interface and cartridges containing ready-to-use reagents for load-and-go operation.

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

Table 3: Example library-to-analysis workflows for high-intensity sequencing applications on 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
Whole-exome sequencing	Illumina DNA Prep with Exome 2.0 Plus Enrichment	NovaSeq X 1.5B or 10B flow cell 200-cycle kit	DRAGEN Enrichment
Transcriptome sequencing	Illumina Stranded Total RNA Prep Illumina Stranded mRNA Prep Illumina RNA Prep with Enrichment	NovaSeq X 1.5B or 10B flow cell 200-cycle kit	DRAGEN RNA

Trusted technology, trusted partner

As a preferred NGS platform provider, Illumina has shipped over 20,000 sequencing systems globally. Illumina NGS technology is cited in over 300,000 peer-reviewed publications—5× more than all other NGS technologies combined.⁸ Building on decades of expertise, Illumina has a relentless commitment to innovation and building future NGS capabilities and applications (Figure 3). The NovaSeq X series demonstrates our continued leadership in genomics technologies.

Committed to customer success

To have confidence in your investment, achieve peak performance, and minimize interruptions, Illumina provides a world-class support team comprised of experienced scientists who are experts in library prep, sequencing, and analysis. Technical support is available via phone five days a week or 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 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, illumina.com/NovaSeqX DRAGEN Bio-IT Platform,

illumina.com/DRAGEN

Instrument specifications

Parameter	Specification
Instrument configuration	Computer and 4K touch screen display Installation setup and accessories Data collection and analysis software
Instrument control computer	Base Unit: iEi custom board with AMD V1605b CPU Memory: 2 × 16 GB DDR4 SODIMM Hard drive: None Solid-state drive: 480GB M.2 Operating system: Oracle 8
Instrument compute engine	Base Unit: iEi custom board with dual AMD 7552 CPU Memory: 8 × 64 GB + 8 × 128 GB DDR4 RDIMM Hard drive: None Solid-state drive: 480GB M.2 + 5 × 12.8TB U.2 Operating system: Oracle 8
Operating environment	Temperature: 15°C-30°C, <2°C change per hour Humidity: 20%-80% relative humidity, non-condensing Altitude: Below 2000 meters (6500 feet) Ventilation: Maximum heat output for instrument is 9200 BTU/hr and average heat output is 7507 BTU/hr For Indoor Use Only
Laser	532 nm (4.5 Watt maximum power), 457 nm (6 Watt maximum power), and third laser at 655 nm for focus tracking
Dimensions	W × D × H: 86.4 cm × 93.3 cm × 158.8 cm Dry weight (not including UPS): 1171 lb Dry weight (including UPS): 1253 lb Crated weight: 1591 lb (722 kg) Accessories pallet weight: 525 lb (238 kg)

Power requirements	208–240 VAC 50/60 Hz, 16A, single phase Illumina provides a region-specific uninterruptible power supply (UPS)
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	<i>For primary analysis data:</i> 800 MB/s/instrument for local network uploads 800 MB/s/instrument for BaseSpace Sequence Hub/Illumina Connected Analytics uploads 15 MB/s/instrument for instrument operational data uploads <i>For primary and secondary analysis data:</i> 3.2 GB/s/instrument for local network uploads 3.2 GB/s/instrument for BaseSpace Sequence Hub/Illumina Connected Analytics uploads 15 MB/s/instrument for instrument operational data uploads

Ordering information

System	Catalog no.
NovaSeq X Sequencing System	20084803
NovaSeq X Plus Sequencing System	20084804
Sequencing reagent kits ^a	Catalog no.
NovaSeq X Series 1.5B Reagent Kit (100 cycles)	Coming soon
NovaSeq X Series 1.5B Reagent Kit (200 cycles)	Coming soon
NovaSeq X Series 1.5B Reagent Kit (300 cycles)	Coming soon
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 (300 cycles)	Coming soon
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