FREQUENTLY ASKED QUESTIONS

NextSeq™ 1000 and NextSeq 2000 Systems FAQ

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Introduction

Advancing next-generation sequencing (NGS) technology is essential to unlocking the power of the genome. Over the last 20 years, Illumina has been a leader in NGS technologies across the entire workflow, increasing performance and driving down costs. This innovation continues with the NextSeq 1000 and NextSeq 2000 Sequencing Systems. For labs considering a new or updated NGS system, the following information is provided for frequently asked questions.

How are the NextSeq 1000 and NextSeq 2000 Systems different from other instruments?

The NextSeq 1000 and NextSeq 2000 Systems were designed from the ground up, with more than 75 innovations that enable our highest density, lowest flow cell costs per million reads on a benchtop system. The NextSeq name was chosen to convey continuity within the mid-throughput portfolio of Illumina NGS systems.

How do you decide whether to purchase the NextSeq 550, NextSeq 1000, or NextSeq 2000 System?

NextSeq 1000 and NextSeq 2000 Systems are highly flexible, robust, and scalable benchtop systems that offer anywhere from 100 million to 1.2 billion reads per sample, enabling various applications, including targeted panels, shotgun metagenomics, single-cell RNA expression, and spatial transcriptomics. NextSeq 1000 and NextSeq 2000 Systems are a good fit for labs looking for a versatile benchtop sequencing system.

The NextSeq 550 System is a good fit for customers who already own that system and want to expand capacity with existing workflows, or labs that want to have microarray scanning capabilities on a sequencing instrument.

How is the integrated dry cartridge different from the reagents of the MiSeq™ or NextSeq 550 Systems?

The NextSeq 1000 and NextSeq 2000 Systems are referred to as dry instruments because they use cartridges that integrate all fluidic components necessary for amplification and sequencing. In contrast, MiSeq and NextSeq 550 systems contain separate fluidics within the instrument. The integrated cartridge helps to reduce possibilities of cross-contamination. Fluid waste can be purged back into reagent cartridges for easy disposal and multiple plastic components in the cartridge can be easily separated from the waste components and recycled.

Do I need to know the specific flow cell cluster density?

Patterned flow cells consist of a nanowell substrate with billions of ordered wells. The NextSeq 1000 and NextSeq 2000 Systems use patterned flow cells, which result in a fixed cluster density, even if all nanowells are not occupied. Compared to nonpatterned flow cells, the uniform cluster sizes enable optimal spacing and increased cluster density. In these systems, monitoring the cluster percent passing filter is a better measure of potential cluster occupancy.

What are the considerations for switching to the patterned flow cell technology?

The patterned flow cell technology used by the NextSeq 1000 and NextSeq 2000 Systems is a large improvement over previous technologies due to the ability to avoid overclustering. However, some assay optimization will likely need to take place, especially for custom and third-party library preparation methods. Illumina support specialists are prepared to help every step of the way, from establishing a robust workflow and evaluation process, to offering a 1:1 consultation with a field application specialist, or the ability to send samples to an Illumina customer solutions lab.

What are the benefits of the onboard DRAGEN™ platform?

The DRAGEN (Dynamic Read Analysis for GENomics) Bio-IT Platform software onboard the NextSeq 1000 and NextSeq 2000 Systems allows labs to seamlessly integrate sequencing and analysis functions in their run setup at no additional cost. The onboard DRAGEN software enables labs to get FASTQ files and vCard files (VCF) for downstream applications directly from the instrument as the run completes, saving file transfer and analysis time. Onboard DRAGEN pipeline algorithms help novice and expert users complete common analysis functions and reduce reliance on external informatics experts (Table 1).

Table 1: DRAGEN Bio-IT Platform applications onboard NextSeq 1000 and NextSeq 2000 Systems

Application	Description
BCL conversion	Converts BCL files produced by Illumina sequencing systems to FASTQ files
DRAGEN ORA compression	Produces lossless, reference-based compression of FASTQ files
DRAGEN FASTQ + MultiQC	Performs hardware-accelerated FastQC metrics with no additional run time
Whole genome	Performs human genome map, align, and small variant calling—germline samples types only
Enrichment (including exome)	Performs germline small-variant calling or somatic low-frequency variant calling—germline and somatic sample types
DNA amplicon	Analyzes genetic variation in specific genomic regions Uses the DRAGEN DNA pipeline with an additional step to soft-clip primers and rewrite alignments, ensuring that primer sequences do not contribute to variant calls
RNA	Offers an RNA-Seq (splice-aware) aligner with optional rRNA filtering during alignment, reducing run time and file size
Single-cell RNA	Processes a wide range of single-cell RNA-Seq data sets from reads to cell-by-gene expression matrices
Differential expression	Runs the DESeq2 algorithm on RNA quantification data produced by the DRAGEN RNA pipeline Outputs genes and transcripts that are differentially expressed between two sample groups
nanoString GeoMx NGS	Streamlines analysis for customers using both GeoMx NGS and Illumina instruments for their spatial genomics workflows
Methylation	Handles bisulfite and Tet-assisted pyridine sequencing (TAPS) methylation data

Can you run human whole-genome sequencing (WGS) trios on NextSeq 1000 or NextSeq 2000 Systems?

The NextSeq 2000 System can run WGS trios at 30x using the P3 300 cycle flow cell.

Can you run TruSight™ Oncology 500 on NextSeq 1000 and NextSeq 2000 Systems?

Currently, TruSight Oncology 500 is not validated by Illumina on the NextSeq 1000 and NextSeq 2000 Systems.

How reliable are the NextSeq 1000 and NextSeq 2000 systems?

To verify the quality of our systems, Illumina tracks many things, including acceptance rates for new instruments, case rates for support issues, and instrument performance.

- Acceptance rates: A perfect acceptance rate means that there were no issues with the instrument, delivery, setup, consumables, installation, or training from the time the instrument was manufactured to the time the instrument is accepted by the customer. The NextSeq 1000 and NextSeq 2000 Systems have a perfect acceptance rate percentage that is comparable to the established acceptance rates of MiSeq and NextSeq 550 systems.
- Case rates: Case rates represent the number of service cases logged by customer support divided by shipment quantity since 2020. Case rates per instrument on the NextSeq 1000 and NextSeq 2000 systems have also been comparable to case rates on MiSeq and NextSeq 550 systems.
- Run data: The percentage of bases with a quality score of 30 or higher is "% Q30 ≥ 30," and error rate is determined by the PhiX alignment. We analyzed the average % Q30 ≥ 30 and error rates across MiSeq, NextSeq 550, NextSeq 1000, and NextSeq 2000 systems using run data shared with Illumina by third parties. We observed that NextSeq 1000 and NextSeq 2000 systems % Q30 ≥ 30 and error rates are comparable to MiSeq and NextSeq 550 systems. NextSeq 1000 and NextSeq 2000 instruments also demonstrated comparable run success rates, or % of runs that meet stated specifications for Q30 and output, to the NextSeq 550 (Table 2, Table 3).

Table 2: Performance parameters for NextSeq 1000 and NextSeq 2000 systems

Read length	NextSeq 1000/2000 P1 Reagents	NextSeq 1000/2000 P2 Reagents	NextSeq 1000/2000 P3 Reagents
		Data output per flow cell[a]	
1 × 50 bp (P3 only)	–	–	60 Gb
2 × 50 bp (P2 and P3 only)	–	40 Gb	120 Gb
2 × 100 bp (P2 and P3 only)	–	80 Gb	240 Gb
2 × 150 bp	30 Gb	120 Gb	360 Gb
Reads CPF	100M	400M	1.2B
		Quality scores[b]	
1 × 50 bp		≥ 90% of bases > Q30	
2 × 50 bp		≥ 90% of bases > Q30	
2 × 100 bp		≥ 85% of bases > Q30	
2 × 150 bp		≥ 85% of bases > Q30	
		Run time	
1 × 50 bp	–	–	~11 hours
2 × 50 bp	–	~13 hours	~19 hours
2 × 100 bp	–	~21 hours	~33 hours
2 × 150 bp	~19 hours	~29 hours	~48 hours

a. Output specifications based on a single flow cell using Illumina PhiX control library at supported cluster densities. CPF, clusters passing filter.
b. Quality scores are based on an Illumina PhiX control library. Performance may vary based on library type and quality, insert size, loading concentration, and other experimental factors.

Table 3: Performance parameters for NextSeq 550 systems.

Read length	Mid-output flow cell	High-output flow cell
	Data output per flow cell[a]	
2 × 150 bp	32-39 Gb	100-120 Gb
2 × 75 bp	16-19 Gb	50-60 Gb
1 × 75 bp	–	23-30 Gb
	Quality scores[b]	
2 × 150 bp	> 75% of bases > Q30	> 75% of bases > Q30
2 × 75 bp	> 80% of bases > Q30	> 80% of bases > Q30
1 × 75 bp	–	> 80% of bases > Q30

a. Output specifications based on using Illumina PhiX control library at supported cluster densities (between 129 and 165 k/mm^2 clusters passing filter).
b. Quality scores are based on an Illumina PhiX control library. Performance may vary based on library type and quality, insert size, loading concentration, and other experimental factors.

What are the storage conditions for the instrument consumables?

NextSeq 550 System consumables have a 3 month shelf life. NextSeq 1000 and NextSeq 2000 System consumables have a shelf life of 6 months. An overview of storage conditions is shown in Table 4.

Table 4: Summary of NextSeq 550, NextSeq 1000, and NextSeq 2000 Systems consumables

Consumable	Storage temperature	Shelf life
NextSeq 550 system		
Reagent cartridge	-25°C to -15°C	3 months
Buffer cartridge	15°C to 30°C	3 months
HT1 Hybridization Buffer	-25°C to -15°C	3 months
Flow cell	2°C to 8°C	3 months
NextSeq 1000 and NextSeq 2000 Systems		
Reagent cartridge	-25°C to -15°C	6 months
Flow cell	2°C to 8°C	6 months
Resuspension buffer with Tween 20	2°C to 8°C	6 months

How does the upgrade process work?

While every lab is different, there are some steps that the customer can expect when undergoing an instrument upgrade or when adding a new instrument.

Step 1: Your territory account manager will coordinate with the Illumina service and support team before the sale of your instrument completes to ensure a quick and easy process.

Step 2: After your order is placed, Illumina service and support make sure that your site is ready to receive your new instrument. This includes checking the power requirements, waste disposal, benchtop space, and other related factors. There will be an introductory conversation to discuss application needs for your new system. In some cases, Illumina can also do a proof of concept evaluation before the instrument is on site.

Step 3: After the instrument is delivered to your lab, an Illumina engineer will take one to two days to make sure that the instrument is running as expected.

Step 4: The Illumina service and support team will schedule training based on your availability. During the training, our expert will work with your samples, taking into account your unique analysis and library preparation needs, to help make sure that you are comfortable with running your assays on your new system.

Summary

Labs interested in purchasing a new sequencing instrument often have questions about which sequencing instrument is right for their lab. The NextSeq 1000 and NextSeq 2000 Systems have a large and growing customer base and are an excellent choice for labs seeking a versatile benchtop sequencing system. These systems feature easy-to-use DRAGEN Bio-IT Platform software onboard, consumables for a range NGS application needs, and excellent customer support from Illumina. Visit the website to find out if a NextSeq 1000 or NextSeq 2000 System is right for you.

Learn more

NextSeq 1000 and NextSeq 2000 systems, illumina.com/systems/sequencing-platforms/nextseq-1000-2000.html

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