TECHNICAL NOTE

Superior performance with the NextSeq™ 2000 System

Side-by-side evaluation of the NextSeq 2000 System versus the Element AVITI System

illumina®

Introduction

Benchtop sequencing systems give labs the power of next-generation sequencing (NGS) with flexible throughput and an accessible design. They enable novice and experienced users to bring NGS in house and perform sequencing across a range of methods and applications.

The NextSeq 1000 and NextSeq 2000 Systems have been at the forefront of NGS innovation. With more than 2400 instruments shipped globally, these systems offer advanced chemistry, simplified bioinformatics, and an intuitive workflow to enable the widest range of methods, applications, and flexibility of scale in a benchtop format.[1]

To demonstrate the superior performance of the NextSeq 2000 System, an internal analysis was conducted that evaluated the NGS workflow, sequencing capabilities, informatics software, and performance specifications on the NextSeq 2000 System and the AVITI System (Element Biosciences). This technical note presents the findings of that internal analysis and concludes that the NextSeq 2000 System provides a more comprehensive and streamlined end-to-end workflow with improved variant calling accuracy and performance above specification (Table 1).

Efficient, sustainable operations

The NextSeq 2000 System is less than half the size of the AVITI System, taking up less valuable laboratory bench space. Similarly, NextSeq 2000 sequencing reagents have a significantly smaller consumable footprint, freeing up valuable freezer space (Figure 1). The NextSeq 2000 reagent cartridge has an integrated waste holder for easy disposal and is roughly 10× smaller in volume than the AVITI System, reducing environmental impact (Table 1).[1,2]

Figure 1: Efficient lab operations—Twice as many NextSeq 2000 reagent kits compared to AVITI kits can be stored in the same footprint, freeing up valuable freezer space (image is for illustrative purposes only).

Table 1: The NextSeq 2000 System offers a streamlined, optimized workflow

Workflow step	Feature	AVITI System	NextSeq 2000 System
Lab operations	Instrument footprint	(H × W × D) 29.5 in × 37.6 in × 28.5 in	(H × W × D) 23.6 in × 21.7 in × 25.6 in
	Consumable footprint	Store half as many kits in the same footprint as NextSeq 2000 kits	Store twice as many kits in the same footprint as AVITI kits
	Volume of waste generated	≤ 3200 ml per run	≤ 311 ml per run
Library prep	Third-party kit support	Limited	Virtually all
	Library conversion required?	Depends	No
Sequencing	Dry instrument?	No	Yes
	Wash cycles required?	Yes, multiple	No
	Run configurations	Narrower output range with 7 kit configurations	Broader output range with 14 kit configurations
Analysis	FASTQ generation	Off-instrument, manually or via paid subscription	Automated on-instrument
	Secondary/tertiary analysis options	Third-party software only	Illumina Connected Software with popular secondary analysis options available onboard

Flexible library preparation

Illumina continues to develop an extensive portfolio of library preparation kits, backed by a global team of technical support specialists. In addition, Illumina NGS technology has long been recognized as an industry standard, with third-party vendors making sure that their library prep kits are compatible with Illumina NGS platforms, including the NextSeq 2000 System. The AVITI System offers limited compatibility with third-party kits, often requiring users to include a conversion step to prepare sequencing-ready libraries (Table 1).[3]

Streamlined sequencing

Featuring an integrated reagent cartridge, the NextSeq 2000 System simplifies library loading and instrument use. Reagents never leave the cartridge, resulting in a dry instrument design that does not require washing, streamlines maintenance, and eliminates the risk of run-to-run contamination. In contrast, on-instrument fluidics on the AVITI System result in wash maintenance requirements and the potential for run-to-run contamination, which could lead to instrument downtime and the need to repeat sequencing runs (Table 1).[2]

Broad method and application support

Offering more kit configurations with multiple read options and a broader output range, the NextSeq 2000 System supports a wider variety of applications and study sizes, compared to the AVITI System (Table 2). With the introduction of Illumina XLEAP-SBS™ chemistry, the NextSeq 2000 System enables increased output on a single flow cell for deeper, broader sequencing with significantly shorter run times, compared to the AVITI System (Table 2).

Table 2: Enhanced sequencing parameters

Parameter	AVITI System	NextSeq 2000 System
Kit configurations	7	14
Read lengths	2 × 75 bp, 2 × 150 bp, 2 × 300 bp	1 × 50 bp, 2 × 50 bp, 2 × 100 bp, 2 × 150 bp, 2 × 300 bp
Single flow cell output	75–300 Gb	10–540 Gb
Single flow cell reads	100M–1000M	100M–1800M
Run time	20–60 hr	8–44 hr

Analysis and informatics solutions

NGS data analysis begins with conversion of binary base call (BCL) files generated by the sequencing instrument to FASTQ files that are used for downstream analyses. The NextSeq 2000 System prepares FASTQ files as part of the sequencing run automatically at no additional cost and without the need for user intervention. The AVITI System does not offer onboard FASTQ generation, forcing users to convert files off-instrument (Table 1).[2]

Downstream NGS data analysis involves aligning FASTQ files to a reference genome for variant calling (secondary analysis) and interpretation to derive biological insights (tertiary analysis). The NextSeq 2000 System integrates with Illumina Connected software, an extensive suite of bioinformatics solutions that offer flexible and integrated workflows to support research, scalable analysis, report generation, and discoveries (Figure 2). Analysis solutions are available to support on-premises and cloud environments, or onboard the system with commonly used secondary analysis pipelines. Access to onboard DRAGEN™ pipelines is included with purchase of the NextSeq 2000 System, enabling out-of-the-box secondary analysis on-instrument and access to ORA compression. Featuring industry-leading innovations such as multigenome technology and machine learning algorithms like PrimateAI-3D and SpliceAI, DRAGEN software is the most accurate and comprehensive secondary analysis on the market.[4]

For maximum scalability, Illumina cloud software provides out-of-the-box workflows for report generation and rich data visualization for methods and applications spanning whole-exome sequencing (WES), single-cell RNA sequencing (scRNA-Seq), multiomics, and more. The Illumina software portfolio is powered by DRAGEN secondary analysis and offers multiple options to support tertiary analysis, from multiomic data visualization to single-sample reporting.

The AVITI System can connect with ElemBio Cloud, a cloud-based software platform that provides real-time run management and integration with third-party software for secondary and tertiary analysis, via paid subscription only (Figure 2).[2]

Illumina informatics offerings

Lab operations	Sequencing	Secondary analysis	Tertiary analysis
Clarity LIMS	Instrument software (onboard FASTQ generation)	DRAGEN secondary analysis	Autolaunch, centralized data storage across the entire workflow	Illumina software for single sample reporting, data visualization, aggregation, and discovery

Element informatics offerings

ElemBio Cloud	Instrument software (no onboard FASTQ generation)	Third-party software required for secondary and tertiary analysis

Figure 2: More informatics options—The NextSeq 2000 System integrates with Illumina Connected Software, a suite of informatics software powered by DRAGEN analysis, while the AVITI System relies on third-party software for data analytics and insights.

Specifications and performance

Quality scores

Sequencing quality scores are a common way to assess sequencing accuracy. While different manufacturers may use proprietary methods and models to calculate quality scores, generally they measure the probability that a base is called incorrectly. A quality score of 30 (Q30) represents an error rate of 1 in 1000, with an inferred call accuracy of 99.9%. With this limited chance for errors or ambiguities, Q30 is considered a benchmark for quality for most NGS applications.

The NextSeq 2000 System reports ≥ 90% of bases higher than Q30 and ≥ 80% of bases higher than Q40 (1 in 10,000 error rate or 99.99% call accuracy) using P4 XLEAP-SBS reagents (Figure 3).[1] The AVITI System also reports ≥ 90% of bases higher than Q30[5] and ≥ 90% of bases higher than Q40 using Element Cloudbreak sequencing reagents.[6]

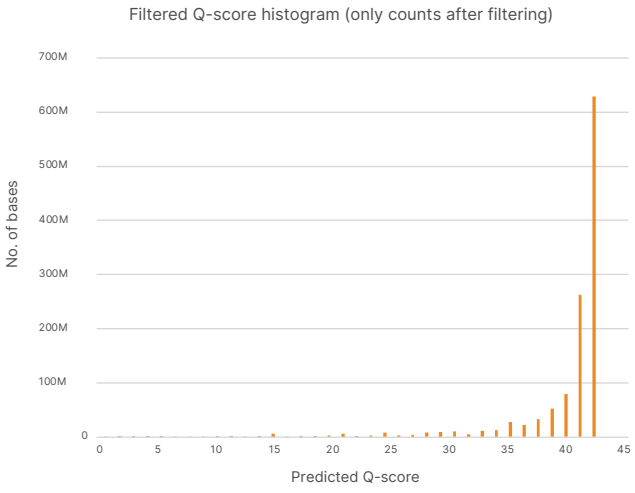

Figure 3: High-quality data—Sequencing on the NextSeq 2000 System using P4 XLEAP-SBS reagents results in 80% or more bases at or above Q40. Q40 was measured using an internal real-time analysis software that provides unbinned Q-scores.

Exceptional real-world performance

In addition to system specifications, it is important to consider real-world performance when evaluating a sequencing system. Illumina product specifications are traditionally set to guarantee performance at or above specification. When output exceeds specifications, the real-world cost per sample decreases.

Labs using both standard SBS and XLEAP-SBS kits consistently achieve data far above specifications for both yield and percentage of bases at or above Q30 (% ≥ Q30) on their NextSeq 2000 Systems. A review of hundreds of real-world runs shows that data generated using XLEAP-SBS kits consistently perform above specifications (Table 3). Similar performance above specification was observed for standard SBS kits over thousands of runs (data not shown).[1] Claims made by Element Biosciences regarding improved quality and new capabilities of the AVITI System have not been supported by publicly available data or peer-reviewed publications to date.

Variant calling accuracy

Given the importance of discerning true variants from false mismatches, variant calling accuracy was assessed on both the NextSeq 2000 System and the AVITI System using the benchmark sample HG002 (Coriell Institute for Medical Research, Catalog no. NA24385).

Data generated on the NextSeq 2000 System using P4 XLEAP-SBS reagents (300 cycles) were analyzed with DRAGEN v4.2 software onboard the instrument and DRAGEN v4.3 software in the cloud to call variants. The AVITI data set (available from Element Biosciences) was processed by Illumina personnel through the third-party Giraffe v1.54[7] and DeepVariant v1.6[8] pipelines for small variant calling.

Accuracy was determined for each system as the combined number of false positives (FP) and false negatives (FN) called for single nucleotide polymorphisms (SNPs) and insertions/deletions (indels). Results show that variant calling on the NextSeq 2000 System was ~2.6× more accurate using DRAGEN v4.3 software in the cloud and ~1.5× more accurate using DRAGEN v4.2 software onboard the NextSeq 2000 System than variant calling on

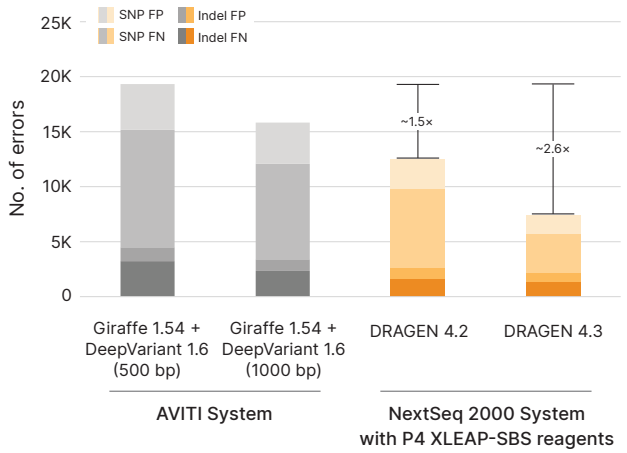

Figure 4: More accurate variant calling—The benchmark sample HG002 was sequenced at 35× coverage on the AVITI System with insert sizes of 500 and 1000 bp and on the NextSeq 2000 System using P4 XLEAP-SBS reagents (300 cycles). The number of errors (false positives plus false negatives) was determined for both systems.

Table 3: Examples of real-world data generated using XLEAP-SBS reagents

NextSeq 1000/2000 reagents	Parameter	Specification	Average across recent runs
NextSeq 1000/2000 P2 XLEAP-SBS Reagent kits	Clusters passing filter	400M	523M
	Q30 score	90%	93%
NextSeq 2000 P3 XLEAP-SBS Reagent kits	Clusters passing filter	1.2B	1.3B
	Q30 score	90%	94%
NextSeq 2000 P4 XLEAP-SBS Reagent kits	Clusters passing filter	1.8B	1.9B
	Q30 score	90%	93%

the AVITI System, even with a larger insert size of 1000 bp, using Giraffe v1.54 and DeepVariant v1.6 (Figure 4).

Trusted global leader

With over 25 years of expertise, Illumina has a relentless commitment to innovating NGS capabilities, building future methods, and empowering scientists to make new discoveries. Illumina has shipped over 25,000 NGS systems globally, and Illumina technology has been cited in over 421,000 peer-reviewed publications, 5× more than all other NGS technologies combined.[9] With a mature global quality and manufacturing infrastructure, Illumina provides comprehensive support and best-in-class product consistency, setting the standard for NGS solutions. Joining this community gives access to a large ecosystem of methods, protocols, and informatics that have been built in collaboration with thousands of researchers and industry thought leaders across the globe.

Summary

This technical note demonstrates the superior sample-to-answer workflow of the NextSeq 2000 System as compared to the Element AVITI System. The NextSeq 2000 System offers simplified operation for flexible and scalable sequencing in a benchtop format to support a broad range of methods and applications. It features minimal maintenance and integration with Illumina Connected software to simplify and streamline the NGS workflow. Taking advantage of the latest advancements in proven Illumina sequencing technology, the NextSeq 2000 System generates high-quality data that results in improved variant calling accuracy compared to the AVITI System. The NextSeq 2000 System expands what can be accomplished with a benchtop sequencing system.

Learn more

NextSeq 2000 Sequencing System

Illumina Connected Software

References

1. Illumina. NextSeq 1000 and NextSeq 2000 Sequencing Systems specification sheet. Published 2020. Accessed September 29, 2024.
2. Element Biosciences. Element AVITI System User Guide. Published 2024. Accessed August 21, 2024.
3. Element Biosciences. Element Adept Library Compatibility Workflow specification sheet. Published 2024. Accessed September 29, 2024.
4. Illumina. DRAGEN sets new standard for data accuracy in PrecisionFDA benchmark data. Optimizing variant calling performance with Illumina machine learning and DRAGEN graph. illumina.com/science/genomics-research/articles/dragen-shines-again-precisionfda-truth-challenge-v2.html. Accessed September 29, 2024.
5. Element Biosciences. Element AVITI System specification sheet. Published 2024. Accessed September 29, 2024.
6. Arslan S, Garcia FJ, Guo M, et al. Sequencing by avidity enables high accuracy with low reagent consumption. *Nat Biotechnol.* 2024;42(1):132–138. doi:10.1038/s41587-023-01750-7
7. Sirén J, Monlong J, Chang X, et al. Pangenomics enables genotyping of known structural variants in 5202 diverse genomes. *Science.* 2021;374(6574):abg8871. doi:10.1126/science.abg8871
8. Poplin R, Chang PC, Alexander D, et al. A universal SNP and small-indel variant caller using deep neural networks. *Nat Biotechnol.* 2018;36(10):983-987. doi:10.1038/nbt.4235
9. Data calculations on file. Illumina, Inc. 2022.

illumina®

1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

M-GL-03083 v1.0