SPECIFICATION SHEET

NextSeq™ 550Dx Instrument

An FDA-regulated, CE-marked, high-throughput sequencing platform

- Support clinical and research demands with flexible read lengths and multiple output configurations
- Accelerate research studies with fast turnaround times and user-friendly data analysis
- Access a growing pipeline of laboratory developed tests backed by Illumina science and expertise

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For *In Vitro* Diagnostic Use. Not available in all regions and countries.

Introduction

The NextSeq 550Dx instrument is the first high-throughput FDA-regulated and CE-marked platform to deliver the power of next-generation sequencing (NGS) to the clinical laboratory (Figure 1). With dual boot functionality, the NextSeq 550Dx platform includes a Diagnostic Mode (Dx Mode)* and a Research Mode. These dual modes provide the flexibility to perform IVD testing, LDT development, and clinical research on a single instrument.† For large clinical laboratories, the NextSeq 550Dx instrument offers a validated high-throughput platform and provides access to an ever-expanding pipeline of clinical applications in the fields of oncology, reproductive health, and more.

While the NextSeq 550Dx instrument can generate more than 90 Gb of data in less than two days, it also delivers the consistency of a regulated platform and includes robustness improvements in software and instrument design. In addition, running in Research Mode supports all currently available research applications including exome sequencing, transcriptome profiling, customer-designed targeted panels, and microarray scanning. With the NextSeq 550Dx instrument, clinical laboratories can run in regulated Dx Mode for IVD testing or run in Research Mode to accelerate clinical studies without sacrificing the speed and power of a high-throughput sequencer.

Figure 1: The NextSeq 550Dx Instrument—By leveraging the latest advances in SBS chemistry and user-friendly, regulated workflows, the NextSeq 550Dx instrument delivers high-quality results for both clinical and research applications.

Illumina SBS chemistry delivers exceptional accuracy

At the core of the NextSeq 550Dx instrument is proven Illumina sequencing by synthesis (SBS) chemistry—the most widely adopted NGS chemistry in the world.[1] This reversible, terminator–based method detects single bases as they are incorporated into growing DNA strands and enables the parallel sequencing of millions of DNA fragments. Illumina SBS chemistry employs natural competition among all four labeled nucleotides, which reduces incorporation bias and allows more robust sequencing of repetitive regions and homopolymers.[2]

Compared to capillary electrophoresis-based Sanger sequencing, NGS can detect a broader range of DNA variants, including low-frequency variants and adjacent phased variants, with a faster time to result and fewer hands-on steps.[3,4] Furthermore, NextSeq sequencing reagents deliver improved signal intensities and a lower number of false positives and false negatives.[5] With NextSeq SBS chemistry, the NextSeq 550Dx instrument provides exceptional accuracy for clinical tests and research applications.

Easy, three-step workflow

Assays run on the NextSeq 550Dx instrument follow a simple, three-step process that includes library preparation, sequencing, and data analysis (Figure 2). The three-step process is a fully integrated Illumina supported workflow.

* Runs performed in Dx Mode are processes regulated by US and EU regulatory bodies.

† Contact an Illumina representative for more information about IVD development partnerships.

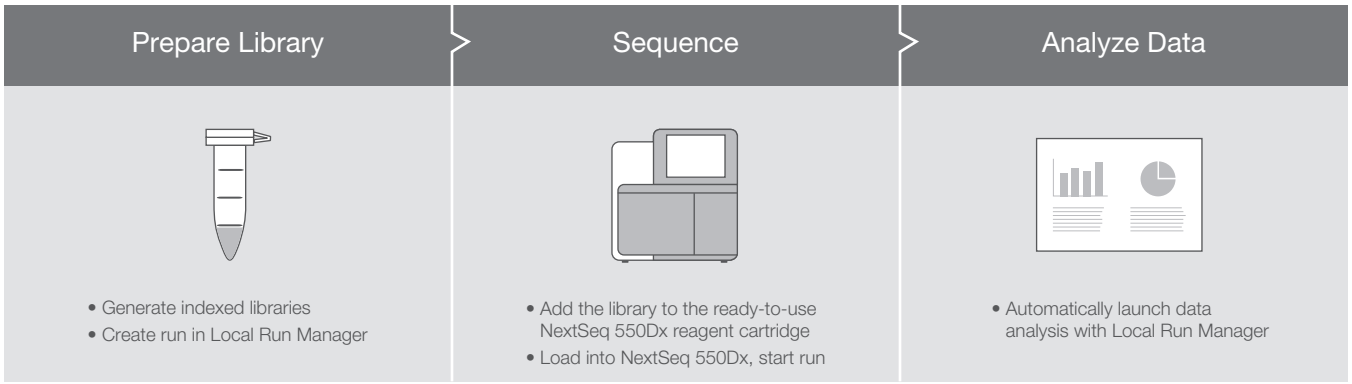

Figure 2: Three-step NextSeq 550Dx assay process— NextSeq 550Dx instrument is part of an integrated, three-step process. Detailed results reports are available with the TruSeq™ Custom Amplicon Dx Assay.

Table 1: NextSeq 550Dx instrument performance parameters—Dx Mode[a,b]

Flow cell configuration	Read length	Output	Run time	Data quality[c]
High-Output flow cell	2 × 150 bp	≥ 90 Gb	< 35 hours	> 75% ≥ Q30

a. Libraries generated with TruSeq Custom Amplicon Kit Dx.
b. For performance parameters in Research Mode, see the NextSeq 550 System specifications.
c. A quality score of Q30 corresponds to a 0.1 percent error rate in base calling.

Library preparation

Library preparation begins with the addition of primers to genomic DNA (gDNA) samples, generating indexed libraries for simultaneous capture and amplification of hundreds of targeted regions. For a range of clinical applications, the TruSeq™ Custom Amplicon Dx Library Preparation kit supports user-defined oligo panels. The fast, efficient library prep kit requires just 50 ng gDNA or 10 µl of qualified formalin-fixed, paraffin-embedded (FFPE) tissue-derived DNA to produce high-quality sequencing libraries in less than two days.

FFPE DNA can be qualified by the TruSeq Custom Amplicon Dx–FFPE QC Kit, which uses a simple qPCR reaction to determine FFPE DNA quality. The results of the FFPE QC Kit are used to provide guidance on the quality and amount of input FFPE DNA.

Sequencing on the NextSeq 550Dx Instrument

With prefilled reagent cartridges, starting a run on a NextSeq 550Dx instrument is as easy as thaw, load, and go in roughly 30 minutes of total hands-on time. The intuitive user interface allows researchers to perform various sequencing applications with minimal user training or instrument set-up time. Furthermore, the NextSeq 550Dx instrument performs cluster generation and SBS sequencing on a single instrument, and offers a simple transition to onboard or cloud-based data analysis. As with the NextSeq 550 System, the NextSeq 550Dx instrument delivers high-quality data with over 75%of bases sequenced with a quality score of Q30 or higher (Table 1).

The NextSeq 550Dx instrument is easily configured, providing researchers with scalability to handle low- to high-throughput projects. In Research Mode, researchers can choose between two flow cell configurations (Mid-Output and High-Output), easily shifting from high-throughput to lower throughput as needed. With an expanding portfolio of Dx solutions, researchers can easily scale from the MiSeqDx to the NextSeq 550Dx instrument (Figure 3).

Integrated system software

The NextSeq 550Dx instrument offers fully integrated onboard analysis software with modular software architecture to support current and future assays. Instrument control software is accessed through a user-friendly touch screen interface. Local Run Manager software supports planning sequencing runs, tracking libraries and runs with audit trails, and integration with onboard data analysis modules. While Local Run Manager runs on the instrument computer, users can monitor run progress and view analysis results from other computers connected to the same network. After a sequencing run is completed, Local Run Manager automatically starts data analysis using one of the application-specific analysis modules.

NextSeq™ 550Dx MiSeq™Dx

Figure 3: Illumina NGS Dx instruments portfolio—Illumina NGS systems offer solutions for a broad range of applications, sample types, and sequencing scales. Each delivers high-quality data and high accuracy with flexible throughput and simple, streamlined workflows.

Available Dx applications

In Dx Mode, the NextSeq 550Dx instrument currently supports the following applications, which are performed with the TruSeq Custom Amplicon Dx assay:

- **Somatic VariantCalling**— With library preparation from FFPE-derived gDNA, the Somatic Variant Calling workflow delivers qualitative results for somatic variant calling with a limit of detection of 0.05.[1] The Somatic Variant Calling workflow supports 4–48 multiplexed samples on the NextSeq 550Dx instrument.
- **Germline VariantCalling**— With library preparation from peripheral whole blood gDNA, the Germline Variant Calling workflow delivers qualitative results for homozygous or heterozygous germline variant calling. The Germline Variant Calling workflow supports 8–96 multiplexed samples on the NextSeq 550Dx instrument.

Variant calling and correlation with MiSeqDx

Variant calling accuracy with the NextSeq 550Dx instrument was tested using the TruSeq Custom Amplicon Variant Panel (TSAVP), a representative assay designed to query a variety of genes covering 12,588 bases across 23 different chromosomes. The TSAVP assay also contains a wide range of GC content (18% – 87%), indels (up to 25 base pairs), and homopolymers (7 – 13 nucleotides). Accuracy was measured using the Platinum Genome samples, a pedigree of individuals previously sequenced to high depth with variant calls confirmed with high confi-

Table 2: Variant calling in Dx Mode

	No. of samples	Analysis time[a]	PPA[b]			NPA[b]	OPA[b]
			SNVs	Ins	Dels		
Somatic variant caller	48	7.5 hours	99.9%	99.9%	99.9%	99.99%	99.98%
Germline variant caller	96	7.0 hours	> 99.9%	98.9%	100.0%	100.00%	99.99%

a. Average onboard analysis times in two 9-run studies.
b. Lowest observed positive percent agreement (PPA) for single nucleotide variants (SNVs), insertions (Ins), deletions (Dels), negative percent agreement (NPA), and overall percent agreement (OPA) across each 9-run study that included three reagent lots, three instruments, and three operators. Platinum Genome samples were tested using a representative assay, TruSeq Custom Amplicon Variant Panel (TSAVP). Somatic: N = 378. Germline: N = 819.

dence.[6] Positive, negative, and overall percent agreement were very close to 100%, indicating a high degree of variant calling accuracy (Table 2).

In a separate study, agreement between samples tested with both MiSeqDx and NextSeq 550Dx instruments was assessed. Somatic results were from TSAVP and TruSight™ Tumor 26 assays (N = 8,599), and germline results were from TSAVP and TruSight Myeloid assays (N = 13,828). Somatic and germline variant allele frequency R2 correlation with MiSeqDx were 0.997 and 0.989, respectively. Qualitative variant calling agreement with MiSeqDx was ≥ 99.8% for somatic variants and ≥ 99.6% for germline variants with a lower bound 95% confidence interval for both variant types. Altogether these data indicate a high variant calling correlation with MiSeqDx data.

Array scanning in Research Mode

In Research Mode, the NextSeq 550Dx instrument enables experimental flexibility by supporting both sequencing and microarray scanning of Illumina BeadChips.‡ With microarray scanning, researchers have instant access to a highly complementary technology for follow up studies, confirmation of copy number variants detected through sequencing, or other avenues of research (Table 3). The integrated DNA-to-data workflows enable rapid sequencing of exomes, targeted sequencing panels, and transcriptomes with Mid-Output or High-Output run configurations. For more information about the broad range of sequencing and microarray applications supported by the NextSeq 550Dx instrument in Research Mode, see the NextSeq 550 System Specifications.

‡ The NextSeq 550Dx instrument in Research Mode supports microarray scanning of the Infinium CytoSnp-850K, HUmanCytoSNP-12, and HUmanKaryomap-12 DNA BeadChips.

Table 3: NextSeq 550Dx instrument array scanning parameters in Research Mode

BeadChip	Scan time per BeadChip	Scan time per sample
Infinium MethylationEPIC BeadChip	40 minutes	5 minutes
Infinium HumanCytoSNP-850K BeadChip	40 minutes	5 minutes
Infinium CytoSNP-12 BeadChip	40 minutes	3.3 minutes
Infinium HumanKaryomap-12 BeadChip	40 minutes	3.3 minutes

Summary

The NextSeq 550Dx instrument is a transformative instrument that brings high-throughput FDA-regulated and CE-marked NGS capabilities to the clinical lab for research and diagnostic applications. The NextSeq 550Dx instrument features an easy 3-step workflow and the flexibility to deliver time-sensitive clinical IVD data or pursue the latest questions in clinical research. The NextSeq 550Dx instrument offers rapid, regulated workflows for somatic and germline variant calling applications and provides access to the growing menu of NGS-powered Illumina clinical assays.

Learn more

NextSeq 550Dx instrument, illumina.com/systems/sequencing-platforms/nextseq-dx.html

Ordering information

Product	Catalog no.
NextSeq 550Dx instrument	20005715
NextSeq 550Dx High-Output Reagent Kit v2.5 (75 cycles)[a]	20028870
NextSeq 550Dx High-Output Reagent Kit v2.5 (300 cycles)[a]	20028871
TruSeq Custom Amplicon Kit Dx[a]	20005718
TruSeq Custom Amplicon Kit Dx FFPE QC Kit[a]	20006259
NextSeq Air Filter	20022240

a. Class I sequencing consumables have single lot shipment, kit lot testing, advance change notification, and a Certificate of Analysis available for each lot. Reagents are developed under design control principles, manufactured under current good manufacturing practices (cGMP), and verified to ensure specification compliance.

References

1. Data calculations on file. Illumina, Inc. 2017.
2. Bentley DR, Balasubramanian S, Swerdlow HP, et al. Accurate Whole Human Genome Sequencing using Reversible Terminator Chemistry. *Nature*. 2008;456(7218):53-59.
3. Shokralla S, Porter TM, Gibson JF, et al. Massively parallel multiplex DNA sequencing for specimen identification using an Illumina MiSeq platform. *Sci Rep*. 2015;5:9687.
4. Precone V, Monaco VD, Esposito MV. Cracking the Code of Human Diseases Using Next-Generation Sequencing: Applications, Challenges, and Perspectives. *Biomed Res Int*. 2015;161648.
5. Illumina (2017). NextSeq 550 Sequencing System Specification Sheet. Accessed February 10, 2021.
6. Eberle MA, Fritzilas E, Krusche P, et al. A reference data set of 5.4 million phased human variants validated by genetic inheritance from sequencing a three-generation 17-member pedigree. *Genome Res*. 2017;27:157-164.

NextSeq 550Dx instrument specifications

Parameter	Specification
Instrument configuration	RFID tracking for concumables
Instrument control computer	Processor: Dual Intel Xeon E5-2648L v3 1.8 GHz CPU, Memory: 128 GB Hard Drive, Dx Mode: 2 × 2 TB (RAID 1) Hard Drive, Research Mode: 2 × 2 TB (RAID 1) Operating System: Windows 10
Operating environment	Temperature: 19°C to 25°C (22°C ± 3°C) Humidity: Noncondensing 20%–80% relative humidity Altitude: 0-2000 m (6500 ft) Ventilation: Up to 2048 BTU/hr @ 600 W For Indoor Use Only
Light emitting diode (LED)	Green 510-525 nm, Red 645-655 nm; Laser diode: 780 nm, Class IIIb
Dimensions	W × D × H: 54 cm × 69 cm × 58 cm Weight: 186 lbs, Crated Weight: 360 lbs
Power requirements	100–120 VAC 15 A, 220–240 VAC 10 A
Radio frequency identifier (RFID)	Frequency: 13.56 MHz Power: Supply current 120 mA, RF output power 200 mW
Product safety and compliance	NRTL certified IEC 61010-1 CE marked FCC/IC approved

Intended use statements

NextSeq 550Dx instrument intended use (European Union/Other)

The NextSeq 550Dx instrument is intended for sequencing of DNA libraries when used with in vitro diagnostic (IVD) assays performed on the instrument. The NextSeq 550Dx instrument is to be used with specific registered, certified or approved IVD reagents and analytical software.

NextSeq 550Dx instrument intended use (United States)

The NextSeq 550Dx instrument is intended for targeted sequencing of DNA libraries from human genomic DNA extracted from peripheral whole blood or formalin-fixed, paraffin-embedded (FFPE) tissue, when used forin vitro diagnostic (IVD) assays performed on the instrument. The NextSeq 550Dx instrument is not intended for whole genome orde novo sequencing. The NextSeq 550Dx instrument is to be used with registered and listed, cleared or approved, IVD reagents and analytical software.

TruSeq Custom Amplicon Kit Dx intended use

The Illumina TruSeq Custom Amplicon Kit Dx is a set of reagents and consumables used to prepare sample libraries from DNA extracted from peripheral whole blood and formalin-fixed, paraffin-embedded (FFPE) tissue. User-supplied analyte specific reagents are required for the preparation of libraries targeting specific genomic regions of interest. The generated sample libraries are intended for use on Illumina's high-throughput DNA sequence analyzers.

TruSeq Custom Amplicon Dx – FFPE QC Kit intended use

The Illumina TruSeq Custom Amplicon Dx – FFPE QC Kit is a set of reagents used to determine the amplification potential of genomic DNA (gDNA) extracted from formalin-fixed, paraffin-embedded (FFPE) samples.

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