APPLICATION NOTE

NextSeq™ 1000 & NextSeq 2000 exome sequencing solution

Integrated workflow for efficient exome analysis and accurate variant calling

- Streamlined library preparation and exome enrichment for highly uniform coverage of coding regions
- Flexible, scalable benchtop sequencing systems for exceptional data quality
- Onboard data analysis pipeline with award-winning performance for calling common mutations and rare somatic variants

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Introduction

The NextSeq 1000 and NextSeq 2000 exome sequencing solution offers a streamlined DNA-to-results workflow to investigate the protein-coding regions of the genome. The solution leverages industry-leading Illumina next-generation sequencing (NGS) technology and optimized sequencing by synthesis (SBS) XLEAP-SBS™ chemistry to deliver exceptional data quality. This high-accuracy exome coverage enables identification of true coding variants for a broad range of applications, including population genetics, genetic disease research, and cancer studies. The integrated workflow provides streamlined library preparation and exome enrichment, push-button sequencing, and rapid, accurate data analysis (Figure 1). With minimal hands-on time, the NextSeq 1000 and NextSeq 2000 exome sequencing solution is a highly flexible, efficient method for interrogating the exome.

Figure 2: NextSeq 1000 and NextSeq 2000 Sequencing Systems—The NextSeq 1000 and NextSeq 2000 Systems harness XLEAP-SBS chemistry and onboard secondary analysis to streamline sequencing workflows.

Simple, efficient workflow

The NextSeq 1000 and NextSeq 2000 exome sequencing solution offers a simplified, integrated workflow, enabling researchers to maximize their productivity. It begins with library preparation and exome enrichment using a library kit such as Illumina DNA Prep with Exome 2.5 Enrichment. Prepared libraries are loaded onto a flow cell and then onto the NextSeq 1000 or NextSeq 2000 System for sequencing (Figure 2). The NextSeq 1000 and NextSeq 2000 Systems feature multiple sequencing flow cell configurations that enable researchers to scale their exome studies according to their needs. Data analysis, including alignment and variant calling, is easily performed with the DRAGEN™ Enrichment pipeline onboard the instrument or in the cloud with BaseSpace™ Sequence Hub or Illumina Connected Analytics.

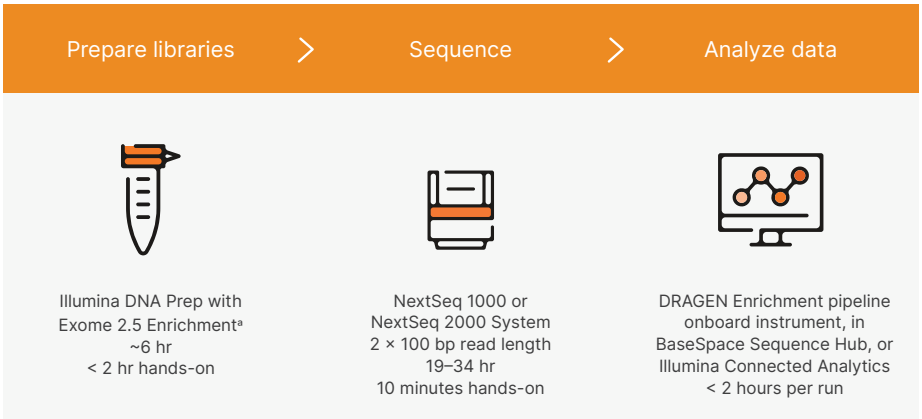

Figure 1: NextSeq 1000 and NextSeq 2000 exome sequencing workflow—The NextSeq 1000 and NextSeq 2000 Systems are part of a simple, integrated NGS workflow that delivers highly accurate exome sequencing data. Times vary by experiment and assay type.

a. Kit configuration includes Illumina DNA Prep with Enrichment and Twist Bioscience for Illumina Exome 2.5 Panel.

Streamlined library preparation and exome enrichment

Illumina DNA Prep with Exome 2.5 Enrichment combines rapid library preparation using Illumina bead-linked transposome chemistry and exome enrichment with the Twist Bioscience for Illumina Exome 2.5 Panel. Providing comprehensive exome coverage from only 10 ng of input, Illumina DNA Prep with Exome 2.5 Enrichment allows labs to analyze precious DNA samples, while still producing high coverage uniformity and enrichment rates. The highly sensitive detection of low-frequency variants enables labs to identify true coding variants and rare somatic mutations.

On-bead tagmentation eliminates the need for mechanical shearing to fragment DNA. This streamlines the workflow to a total time of about six hours with less than two hours of hands-on time. Researchers can also take advantage of these workflow and data quality benefits using Illumina DNA Prep with Enrichment with other exome panels. Choose panel content from various vendors, including Agilent, Twist Bioscience, and Integrated DNA Technologies (IDT) (Table 1).

NextSeq 1000 and NextSeq 2000 Systems

The NextSeq 1000 and NextSeq 2000 Systems provide power and versatility to streamline and simplify the exome sequencing workflow. With no washes required, it takes less than 10 minutes to load and initiate the system. For the NextSeq 2000 System, P4 reagents enable sequencing of ~45 samples in approximately 34 hours with paired-end 100-bp read lengths.*

The NextSeq 1000 and NextSeq 2000 Systems are compatible with a wide range of library preparation kits from Illumina and third parties, and offer cross-application flexibility. Researchers can transition easily between sequencing projects, such as exome, bulk, and single-cell RNA sequencing (RNA-Seq), and other methods. For example, researchers can pair exome sequencing with transcriptome sequencing to assess whether identified variants alter the expression of transcripts.

Table 1: Exome panel specifications

Panel features[a]	Illumina Exome 2.5 Panel[b]	Agilent	Twist	IDT
Panel size	37.5 Mb	36 Mb	33 Mb	39 Mb
Probe size	120 bp	N/A	120 bp	120 bp
Probe type	dsDNA	RNA	dsDNA	ssDNA
Enrichment (Hyb) time	1.5 hr	16 hr	1.5 hr	1.5–16 hr
Databases used for exome panel design[c]				
RefSeq[1]	99.1%	99.88%	99.08%	99.45%
GENCODE[2]	98.02%	97.29%	96.01%	96.82%
CCDS[3]	99.90%	99.91%	99.76%	99.67%
UCSC Known Genes[4]	99.89%	98.72%	97.63%	98.13%
ClinVar[5]	98.60%	73.41%	72.56%	72.90%

a. Panel size = the total length of sequence in the target regions; probe size = length of enrichment hybridization (Hyb) probe; probe type = probe oligonucleotides can be RNA, DNA, single stranded (ss), or double stranded (ds).
b. Twist Bioscience for Illumina Exome 2.5 Panel.
c. Percentages refer to how much of the databases each exome panel covers.

A wide range of customizable Illumina targeted resequencing solutions are also available to validate variants discovered from any sequencing application.

Delivers “true coding variant” calls

A true coding variant is an accurate base call that differs from the consensus sequence within a coding region. It is not a false positive (where a variant is called but does not truly exist) or a false negative (where a variant that truly exists is not called). A system with a high false positive call rate requires extensive downstream validation, increasing costs and experimental time. A system with a high false negative call rate is failing to detect potentially important findings, often in regions that are highly repetitive or that contain homopolymer stretches. Obtaining true coding variant calls is a function of high-quality library preparation and enrichment, sequencing accuracy, and secondary analysis accuracy.

* Throughput may vary based on many factors, including exome panel size and library preparation kit used.

Discover more with XLEAP-SBS chemistry

The NextSeq 1000 and NextSeq 2000 Systems are powered by XLEAP-SBS chemistry, the fastest, highest quality, most robust Illumina sequencing chemistry to date. XLEAP-SBS chemistry on the NextSeq 1000 and NextSeq 2000 Systems enables the highest read output and lowest price per read of any Illumina benchtop sequencer. The systems deliver accuracy of at least 90% of bases higher than Q30† at 2 × 100 bp (Table 2), and has high accuracy even in highly difficult regions (eg, GC-rich regions or homopolymers), yielding a high percentage of true coding variants. The low false positive and false negative rates drastically reduce the time and cost of downstream validation. By offering exceptional data quality, the NextSeq 1000 and NextSeq 2000 Systems offer the ideal option for comprehensive study of the exome.

Using proven Illumina NGS technology, the NextSeq 1000 and NextSeq 2000 Systems enable researchers to compare and integrate data generated across systems. For example, NextSeq 1000 and NextSeq 2000 exome sequencing data can be integrated with data from follow-up studies performed with targeted panels or large-scale exome sequencing studies run on the NovaSeq™ X Series (Table 3).

† Q30 = 1 error in 1000 base calls or an accuracy of 99.9%.

Simplified analysis with DRAGEN secondary analysis

Labs can perform exome sequencing data analysis using Illumina DRAGEN secondary analysis, a suite of accurate, comprehensive, and efficient data analysis pipelines available onboard the NextSeq 1000 and NextSeq 2000 Systems.‡ This PrecisionFDA award-winning informatics solution§ uses optimized, hardware-accelerated algorithms to help users overcome bottlenecks in data analysis and reduce reliance on external informatics experts.

The DRAGEN Enrichment pipeline analyzes output from the NextSeq 1000 and NextSeq 2000 Systems and performs accurate variant calling in less than two hours after a sequencing run is complete (Figure 3). The pipeline provides industry-leading accuracy in mapping and small variant calling and is available in Germline and Somatic

‡ DRAGEN hardware is included onboard the NextSeq 1000 and NextSeq 2000 Systems. A DRAGEN license is included with the instrument and does not need to be purchased separately.

§ DRAGEN secondary analysis was awarded Best Performance for difficult-to-map regions and Best Performance for all benchmark regions on Illumina sequencing data in the 2020 PrecisionFDA Truth Challenge V2.[7,8]

Table 2: NextSeq 1000 and NextSeq 2000 performance parameters for exome sequencing

	Single-end reads[a]	Read length	Output[a]	Run time[b]	Data quality[c]
NextSeq 1000/2000 P1 XLEAP-SBS Reagents[d]	100M	2 × 150 bp	30 Gb	17 hr	≥ 90% bases above Q30
NextSeq 1000/2000 P2 XLEAP-SBS Reagents[d]	400M	2 × 100 bp	80 Gb	19 hr	
NextSeq 2000 P3 XLEAP-SBS Reagents[d,e]	1.2B	2 × 100 bp	240 Gb	31 hr	
NextSeq 2000 P4 XLEAP-SBS Reagents[e]	1.8B	2 × 100 bp	360 Gb	34 hr	

a. Output specifications based on a single flow cell using Illumina PhiX control library at supported cluster densities.
b. Run time includes cluster generation, sequencing, and base calling on the NextSeq 1000 and NextSeq 2000 Systems.
c. 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. The percentage of bases > Q30 is averaged over the entire run.
d. XLEAP-SBS reagents for P1, P2, and P3 flow cells available Q2 2024.
e. P3 and P4 reagents are available for the NextSeq 2000 System only.

modes.[6-8] With the onboard DRAGEN Enrichment app, analysis can be set up during run planning, streamlining the workflow from sample to answer. The DRAGEN Enrichment app on BaseSpace Sequence Hub and Illumina Connected Analytics features advanced results visualization and table sorting capabilities packaged in an intuitive interface suitable for both new and experienced users.

Output from the DRAGEN Enrichment pipeline can be directly input into a broad range of available downstream analysis tools in BaseSpace Sequence Hub and Illumina Connected Analytics. Beyond DRAGEN analysis, both cloud solutions include a growing community of software tools for visualization, analysis, and sharing.

Comprehensive Illumina technical support

Illumina provides a world-class support team comprised of experienced scientists who are experts in library preparation, sequencing, and analysis. This dedicated team includes highly qualified field service engineers (FSE), technical applications scientists (TAS), field applications scientists (FAS), system support engineers, bioinformaticians, and IT network experts, all deeply familiar with NGS and the applications that Illumina customers perform around the globe. Technical support is available via phone five days a week or via online support 24/7, worldwide and in multiple languages.

With this unmatched service and support, Illumina helps users maximize efficacy of their NextSeq 1000 and NextSeq 2000 Systems, train new employees, and learn the latest techniques and best practices.

Table 3: Illumina exome sequencing throughput by system

Sequencing system	Sequencing reagents	No. of exomes per run[a]
NextSeq 1000 and NextSeq 2000 Systems	P1 300 cycles	~2
	P2 200 cycles	10
	P3[b] 200 cycles	30
	P4[b] 200 cycles	45
NovaSeq X Series[c]	1.5B 200 cycles	~41
	10B 200 cycles	~250
	25B 300 cycles	~750[d]

a. No. of exomes calculations assume ~8 Gb per sample to achieve 100× coverage. Throughput may vary based on many factors including exome panel size and library preparation kit used.
b. P3 and P4 reagents are available for the NextSeq 2000 System only.
c. The NovaSeq X Plus System is capable of single flow cell runs or dual flow cell runs. The NovaSeq X System is capable of single flow cell runs.
d. A maximum of 384 unique dual indexes is available. For NovaSeq X Series, independent lane loading allows for multiplexing of more samples.

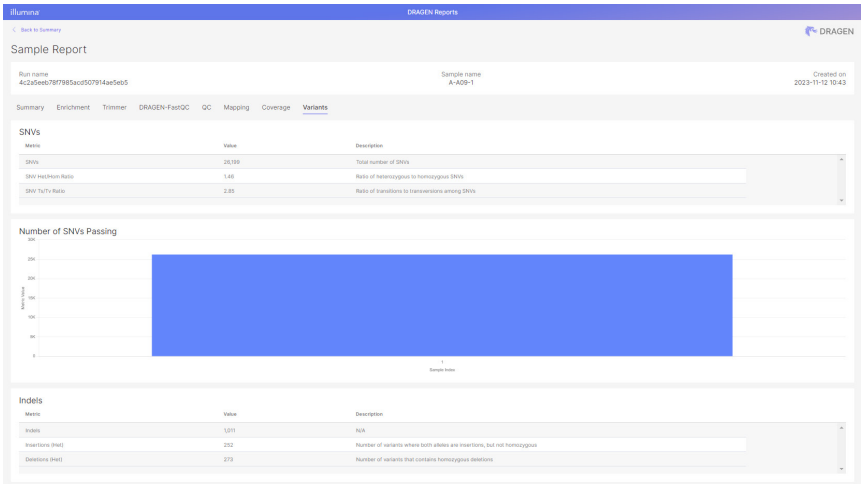

Figure 3: DRAGEN Enrichment pipeline—Screenshot example of industry-leading accuracy in mapping and small variant calling with DRAGEN Enrichment pipeline, available onboard the NextSeq 1000 and NextSeq 2000 Systems or on cloud with BaseSpace Sequence Hub or Illumina Connected Analytics.

Summary

The NextSeq 1000 and NextSeq 2000 exome sequencing solution offers an integrated, scalable workflow for identifying variants in coding regions. The solution combines the power, speed, and flexibility of the NextSeq 1000 and NextSeq 2000 Sequencing Systems with highly robust XLEAP-SBS chemistry, high-quality library preparation and enrichment options and rapid, user-friendly analysis software.

Learn more

Exome sequencing

Illumina DNA Prep with Exome 2.5 Enrichment

NextSeq 1000 and NextSeq 2000 Sequencing Systems

DRAGEN secondary analysis

Demo data on BaseSpace Sequence Hub

References

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4. University of California, Santa Cruz Genome Browser. UCSC Known Genes. genome.ucsc.edu/. Updated August 18, 2023. Accessed August 25, 2023.
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6. Illumina. Accuracy improvements in germline small variant calling with the DRAGEN Platform. Accessed August 25, 2023.
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8. Mehio R, Ruehle M, Catreux S, et al. DRAGEN Wins at PrecisionFDA Truth Challenge V2 Showcase Accuracy Gains from Alt-aware Mapping and Graph Reference Genomes. illumina.com/science/genomics-research/dragen-wins-precisionfda-challenge-showcase-accuracy-gains.html. Accessed August 25, 2023.

Ordering information

Product	Catalog no.
NextSeq 2000 Sequencing System	20038897
NextSeq 1000 Sequencing System	20038898
NextSeq 1000 to NextSeq 2000 upgrade	20047256
NextSeq 1000/2000 P1 XLEAP-SBS Reagent Kit (100 cycles)[a,b]	20100983
NextSeq 1000/2000 P1 XLEAP-SBS Reagent Kit (300 cycles)[a,b]	20100982
NextSeq 1000/2000 P1 XLEAP-SBS Reagent Kit (600 cycles)[a,b]	20100981
NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (100 cycles)[a,b]	20100987
NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (200 cycles)[a,b]	20100986
NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (300 cycles)[a,b]	20100985
NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (600 cycles)[a,b]	20100984
NextSeq 2000 P3 XLEAP-SBS Reagent Kit (100 cycles)[a,b]	20100990
NextSeq 2000 P3 XLEAP-SBS Reagent Kit (200 cycles)[a,b]	20100989
NextSeq 2000 P3 XLEAP-SBS Reagent Kit (300 ycles)[a,b]	20100988

a. XLEAP-SBS reagent kits for the NextSeq 1000 and NextSeq 2000 instruments are shipped and stored at the same temperature as standard SBS reagent kits.
b. XLEAP-SBS reagents for P1, P2, and P3 flow cells available Q2 2024.Kit configuration includes Illumina DNA Prep with Enrichment and Twist Bioscience for Illumina Exome 2.5 Panel.
c. Kit configuration includes Illumina DNA Prep with Enrichment and Twist Bioscience for Illumina Exome 2.5 Panel.

Ordering information

Product	Catalog no.
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (50 cycles)[a]	20100995
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (100 cycles)[a]	20100994
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (200 cycles)[a]	20100993
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (300 cycles)[a]	20100992
NextSeq 1000/2000 XLEAP-SBS Read and Index Primers[a]	20112856
NextSeq 1000/2000 XLEAP-SBS Index Primer Kit[a]	20112858
NextSeq 1000/2000 XLEAP-SBS Read Primer Kit[a]	20112859
Illumina DNA Prep with Exome 2.5 Enrichment, (S) Tagmentation Set B (96 samples, 12-plex)[c]	20077595
Illumina DNA Prep with Exome 2.5 Enrichment, (S) Tagmentation Set D (96 samples, 12-plex)[c]	20077596
Illumina DNA Prep with Enrichment, (S) Tagmentation (16 samples)	20025523
Illumina DNA Prep with Enrichment, (S) Tagmentation (96 samples)	20025524
Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples)	20091654
Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples)	20091656
Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 indexes, 96 samples)	20091658
Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 indexes, 96 samples)	20091660

a. XLEAP-SBS reagent kits for the NextSeq 1000 and NextSeq 2000 instruments are shipped and stored at the same temperature as standard SBS reagent kits.
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c. Kit configuration includes Illumina DNA Prep with Enrichment and Twist Bioscience for Illumina Exome 2.5 Panel.

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M-GL-00479 v3.0