APPLICATION NOTE

NextSeq™ 550 amplicon sequencing solution

Streamlined workflow for rapid detection of disease-associated variants

- Comprehensive targeted panels offer optimized content for clinical cancer and genetic disease research
- Proven sequencing platform delivers excellent specificity and high-quality data
- Easy data analysis and annotation tools enrich variant data with biological context

illumina®

Introduction

The NextSeq 550 amplicon sequencing solution offers deep coverage of targeted polymerase chain reaction (PCR) products (amplicons) to detect disease-associated variants. The solution includes multiplexed PCR-based library preparation and comprehensive gene panels, push-button sequencing on the proven NextSeq 550 System (Figure 1), and simplified amplicon analysis and variant detection tools (Figure 2). It combines AmpliSeq™ for Illumina chemistry with industry-leading Illumina next-generation sequencing (NGS) technology[1] to offer high-confidence data in cancer and genetic disease applications. The NextSeq 550 amplicon sequencing solution can detect somatic mutations down to 5% frequency in complex samples (such as tumors mixed with germline DNA). With minimal hands-on time and tunable output on the NextSeq 550 System, this integrated solution delivers rapid and accurate data for rare variant calling.

Figure 1: NextSeq 550 System—Proven platform offering the accuracy of SBS chemistry as part of a simplified amplicon sequencing workflow.

Simple workflow for comprehensive amplicon panels

The NextSeq 550 amplicon sequencing solution begins with AmpliSeq for Illumina PCR-based library preparation with the AmpliSeq for Illumina Library PLUS kit and targeted gene panels. The AmpliSeq for Illumina Comprehensive Cancer Panel and the AmpliSeq for Illumina Comprehensive Panel v3 are ideal for use on the NextSeq 550 System. Resulting libraries are loaded onto a reagent cartridge and then onto the NextSeq 550 System for sequencing. The NextSeq 550 System features dual output modes (mid and high) that enable labs to scale amplicon studies according to coverage and sample throughput needs. Data analysis, including alignment and variant calling, is easily performed in the cloud using the DNA Amplicon App on BaseSpace™ Sequence Hub or locally with Local Run Manager. BaseSpace Variant Interpreter helps labs put variant data in biological context for annotation and reporting.

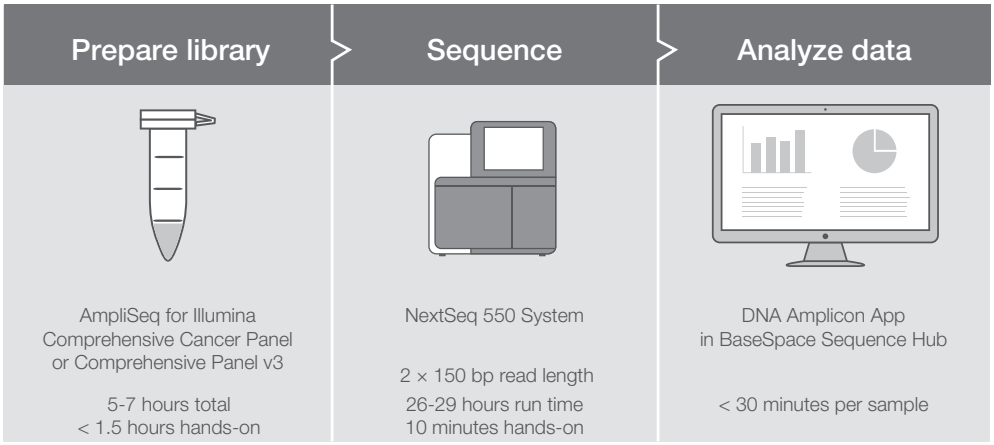

Figure 2: NextSeq 550 amplicon sequencing workflow—A simple workflow with minimal hands-on time and tunable output. The workflow includes library prep with AmpliSeq for Illumina comprehensive gene panels, push-button sequencing, and streamlined data analysis. The AmpliSeq for Illumina Comprehensive Cancer Panel and Comprehensive Panel v3 are ideal for sequencing on the NextSeq 550 System.

AmpliSeq for Illumina panels

AmpliSeq for Illumina is a suite of AmpliSeq chemistry products that are compatible with Illumina benchtop sequencing systems. As part of the NextSeq 550 amplicon sequencing solution, AmpliSeq for Illumina allows users to analyze hundreds of genes simultaneously with ultrahigh multiplexed PCR (Figure 3). Users achieve complete coverage of large targets using multiple primer pools to create overlapping amplicons.

AmpliSeq for Illumina works with DNA and RNA samples and requires as little as one nanogram of input. AmpliSeq panels can accommodate high-quality samples such as blood, cell culture, or fresh-frozen tissue and also challenging samples such as formalin-fixed paraffin-embedded (FFPE) tissue. The fast, streamlined workflow enables users to prepare sequencing-ready libraries in as few as five hours, with < 1.5 hours hands-on time.

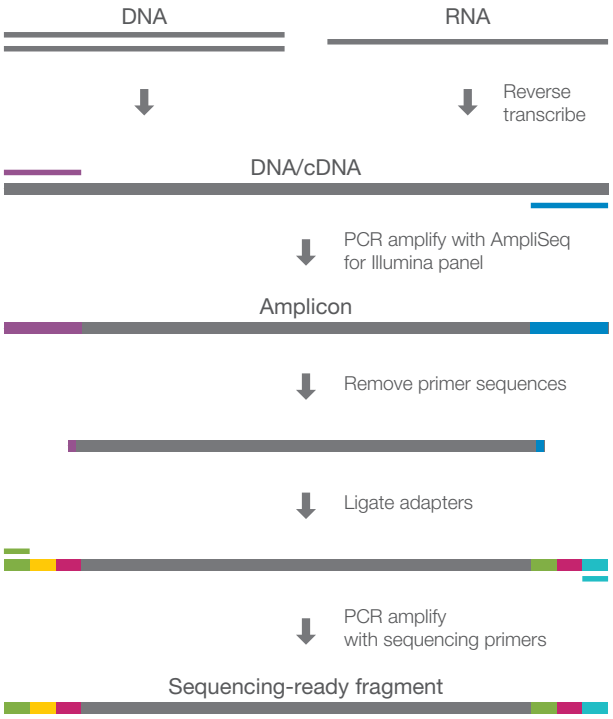

Figure 3: AmpliSeq for Illumina library preparation—The highly multiplexed, PCR-based workflow amplifies up to 24,000 amplicons in a single targeted resquencing assay. Resulting libraries are ready for sequencing on the NextSeq 550 System.

Recommended panels for the NextSeq 550 System

The NextSeq 550 amplicon sequencing solution supports targeted resequencing of fixed or custom gene panels. Ready-to-use panels save users the time and effort of identifying targets, designing primers, and optimizing panels. The AmpliSeq for Illumina Comprehensive Cancer Panel and the AmpliSeq for Illumina Comprehensive Panel v3 contain 409 and 161 targets, respectively, making them ideal for use on the NextSeq 550 System (Table 1).

Table 1: NextSeq 550 amplicon project recommendations

Amplicon Panel	No. of samples per run[a]		Read length
	Mid output	High output	
AmpliSeq for Illumina Comprehensive Cancer Panel	4	12	2 × 150 bp
AmpliSeq for Illumina Comprehensive Panel v3	16	48	2 × 150 bp

a. Assumes minimum coverage of 500×; run time for 2 × 150 bp sequencing is 26 hours at mid output and 29 hours at high output.

AmpliSeq for Illumina Comprehensive Cancer Panel

The AmpliSeq for Illumina Comprehensive Cancer Panel supports targeted resequencing for analyzing somatic mutations across 409 genes with known associations to cancer. This panel detects variants within cancer types including lung, colon, breast, ovarian, melanoma, and prostate malignancies.

AmpliSeq for Illumina Comprehensive Panel v3

The AmpliSeq for Illumina Comprehensive Panel v3 offers coverage of 161 unique cancer-associated genes, including kinase domains and genes involved in DNA repair. The panel content spans hotspot regions, full-length genes, copy number genes, and inter- and intragenic gene fusions.

Easy amplicon panel customization

If ready-to-use panels do not include genes of interest, Illumina offers several made-to-order panel options. AmpliSeq for Illumina Community Panels are curated by experts to address a broad range of disease research areas. Users can also create an AmpliSeq for Illumina Custom Panel for their specific targeted resequencing studies using DesignStudio™ software. DesignStudio Sequencing Assay Designer is a free online tool for custom panel creation with robust algorithms and a simple user interface. DesignStudio software provides dynamic feedback to optimize probe designs and enhance target region coverage. Illumina also offers concierge panel design support.

Fast, accurate variant detection

Push-button operation of the NextSeq 550 System simplifies amplicon sequencing. It takes less than 10 minutes to load and initiate the system. Sequencing of up to 48 samples is completed in as few as 29 hours. With its dual sequencing output modes (mid and high) and ability to handle a range of sample sizes, the NextSeq 550 System enables users to tune and optimize their amplicon studies easily and effectively (Table 2).

At the core of the NextSeq 550 System is proven Illumina sequencing by synthesis (SBS) chemistry, which is used to generate > 90% of the world's sequencing data.[1] The NextSeq 550 System delivers industry-leading sequencing accuracy of > 75% of sequenced bases over Q30* at 2 × 150 bp. The NextSeq 550 System can successfully sequence even the most difficult regions (GC-rich, homopolymers), yielding a high percentage of true variants. Its low false-positive and false-negative rates drastically reduce the time and cost of downstream validation.

User-friendly data analysis

With the NextSeq 550 amplicon sequencing solution, processing the raw sequencing data into meaningful results is straightforward. Automated data analysis software helps users identify and classify disease-relevant variants quickly and easily. The tools are designed for biologists and do not require dedicated computing infrastructure or bioinformatics expertise.

Base calls are generated on the NextSeq 550 System and data can be analyzed locally with Local Run Manager or in the cloud with BaseSpace Sequence Hub, the Illumina

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

Table 2: NextSeq 550 System sequencing performance

Flow cell configuration	Read length	Output	Run time	Data quality	Required input
High-output flow cell Up to 400M single reads Up to 800M paired-end reads	2 × 150 bp	100-120 Gb	29 hours	> 80% bases above Q30 at 2 × 75 bp	AmpliSeq for Illumina: ≥ 1 ng high-quality genomic DNA ≥ 10 ng FFPE genomic DNA
	2 × 75 bp	50-60 Gb	18 hours		
	1 × 75 bp	25-30 Gb	11 hours		
Mid-output flow cell Up to 130M single reads Up to 260M paired-end reads	2 × 150 bp	32.5-39 Gb	26 hours	> 75% bases above Q30 at 2 × 150 bp	
	2 × 75 bp	16.25-19.5 Gb	15 hours		

Run time includes cluster generation, sequencing, and base calling on the NextSeq 550 System. Install specifications are based on Illumina PhiX control library at supported cluster densities (between 129 and 165 K/mm² clusters passing filter). Actual performance parameters may vary based on sample type, sample quality, and clusters passing filter. The percentage of bases > Q30 is averaged over the entire run.

genomics computing environment. The analysis workflow for amplicon panels uses the BaseSpace DNA Amplicon App to align reads against reference genomes and call small variants.

Biological interpretation and annotation

BaseSpace Sequence Hub provides access to BaseSpace Variant Interpreter, which can rapidly identify biologically significant variants associated with a given phenotype. Users can annotate data using curated sources and summarize findings in structured reports. BaseSpace Sequence Hub also includes a growing community of Illumina and third-party bioinformatics tools for visualization, analysis, and sharing.

World-class service and support

With a NextSeq 550 System in their laboratory, users join a global community of thousands of scientists using Illumina technology. 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 application 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 access online support 24/7, worldwide and in multiple languages.

With this unmatched service and support, users can maximize the effectiveness of their NextSeq 550 System, train new employees, and learn the latest techniques and best practices.

Summary

The NextSeq 550 amplicon sequencing solution delivers a streamlined workflow for rapid and accurate detection of disease-associated variants in targeted gene regions. AmpliSeq for Illumina gene panels and tunable sequencing output on the NextSeq 550 System support comprehensive amplicon analysis for a range of sample throughputs. Rapid library preparation, industry-leading sequencing accuracy, and simple data analysis empower users to identify somatic and germline variants with clinical relevance for cancer and genetic disease applications.

Learn more

To learn more about AmpliSeq for Illumina, visit illumina.com/products/by-brand/ampliseq.html.

Learn more about the NextSeq 550 System at illumina.com/systems/sequencing-platforms/nextseq.html.

References

1. Data calculations on file. Illumina, Inc., 2017.

Ordering information

Product	Catalog no.
NextSeq 550 System	SY-415-1002
NextSeq 500/550 Mid-Output v2.5 Kit (150 cycles)	20024904
NextSeq 500/550 Mid-Output v2.5 Kit (300 cycles)	20024905
NextSeq 500/550 High-Output v2.5 Kit (75 cycles)	20024906
NextSeq 500/550 High-Output v2.5 Kit (150 cycles)	20024907
NextSeq 500/550 High-Output v2.5 Kit (300 cycles)	20024908
TG NextSeq 500/550 Mid-Output Kit v2.5 (150 cycles)[a]	20024909
TG NextSeq 500/550 Mid-Output Kit v2.5 (300 cycles)	20024910
TG NextSeq 500/550 High-Output Kit v2.5 (75 cycles)	20024911
TG NextSeq 500/550 High-Output Kit v2.5 (150 cycles)	20024912
TG NextSeq 500/550 High-Output Kit v2.5 (300 cycles)	20024913

a. TG-labeled consumables have features that help customers reduce the frequency of revalidation. These consumables are available only under supply agreement and require customers to provide a binding forecast. Contact your account manager for more.

Product	Catalog no.
AmpliSeq Comprehensive Cancer Panel for Illumina (24 reactions)	20019160
AmpliSeq Comprehensive Panel v3 for Illumina (24 reactions)	20019109
AmpliSeq Library PLUS (24 Reactions) for Illumina	20019101
AmpliSeq Library PLUS (96 Reactions) for Illumina	20019102
AmpliSeq Library PLUS (384 Reactions) for Illumina	20019103
AmpliSeq UD Indexes for Illumina (24 indexes, 24 samples)	20019104
AmpliSeq CD Indexes Set A for Illumina	20019105
AmpliSeq CD Indexes Set B for Illumina	20019106
AmpliSeq CD Indexes Set C for Illumina	20019107
AmpliSeq CD Indexes Set D for Illumina	20019167
AmpliSeq CD Indexes Set A-D for Illumina (384 indexes, 384 samples)	20031676
AmpliSeq CD Indexes Large Volume for Illumina (96 indexes, 96 samples)	20019108

illumina®

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

Pub. no. 770-2014-027-C. QB11306.