illumina®

NextSeq® Series Amplicon Sequencing Solution

A fast, easy, and accurate way to detect variants in a gene of interest across various sample types and species.

Highlights

- **Fastest Sample-to-Data Amplicon Solution**
  Simple workflow with the lowest hands-on time
- **Accurate Variant Detection**
  NextSeq Series and TruSeq® Custom Amplicon assay deliver high levels of multiplexing, excellent specificity and uniformity, and high-quality data even through homopolymer regions
- **Simple Assay Customization**
  DesignStudio™ enables selection of fixed or custom panels of up to 1536 amplicons in a single reaction, across a range of amplicon sizes and reference genomes
- **Easy, Flexible Data Analysis**
  Analysis performed in BaseSpace®, with VariantStudio offering a robust method to enrich variant data with biological context
- **End-to-End Illumina Support**
  Illumina scientists are there every step of the way, providing installation, training, and applications support

Introduction

The NextSeq Series Amplicon Sequencing Solution enables researchers to investigate targeted gene regions, identifying variants rapidly and efficiently. It leverages industry-leading Illumina next-generation sequencing (NGS) technology, delivering the best data quality and highest accuracy for detecting variants in genes of interest. The NextSeq Series Amplicon Sequencing Solution includes a web-based sequencing assay design tool for easy target region selection and optimization, fully customizable library preparation assays for targeted resequencing, and user-friendly data analysis software to identify and classify disease-relevant variants quickly. With a seamless sample-to-answer workflow, the NextSeq Series Amplicon Sequencing Solution offers the flexibility to target expert-defined or custom content for cancer, genetic disease, and population genetics studies, and supports the discovery of somatic and germline variants.

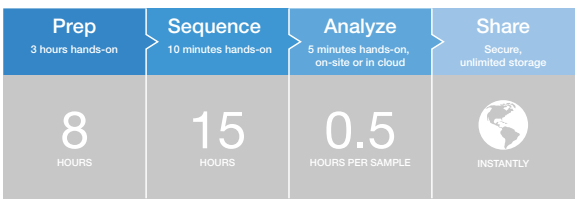

Figure 1: NextSeq Series Sample-to-Answer Amplicon Sequencing Workflow—The simple NextSeq Series workflow delivers highly accurate sequencing data. Data analysis includes alignment and variant calling.

A Simple, Customizable Amplicon Sequencing Workflow

The NextSeq Series amplicon sequencing workflow simplifies amplicon sequencing, enabling researchers to maximize their productivity (Figure 1). Researchers can create a custom amplicon panel using DesignStudio and generate libraries with TruSeq Custom Amplicon (TSCA) in 1 day. The dual sequencing output modes of the NextSeq Series and accompanying reagents deliver 20–120 Gb, enabling researchers to scale their amplicon panels and study sizes accordingly.

Base calls are generated on the NextSeq Series and data analysis (including alignment and variant calling) can be performed with the TruSeq Amplicon App in BaseSpace, the Illumina genomics computing environment (Figure 2). With its intuitive interface accessed through a common web browser, BaseSpace provides researchers with access to a rich ecosystem of commercial and open-source DNA data analysis software tools designed primarily to analyze Illumina data. For example, variants can be further explored, interpreted, and reported using VariantStudio, enabling researchers to identify variants associated with a given phenotype.

The NextSeq Series also offers cross-application flexibility, enabling researchers to transition easily between sequencing projects (Figure 3). The series is fully compatible with the industry's widest range of library preparation kits from Illumina and third parties, enabling an easy transition between Illumina amplicon or larger resequencing studies using TruSight® fixed panels,* transcriptome (RNA-Seq), and whole-genome sequencing (WGS). For example, researchers can pair amplicon sequencing with RNA-Seq to assess whether identified variants alter transcript expression or expand their studies with larger enrichment panels to explore areas beyond genes of interest. With the NextSeq 550 System, researchers can perform NGS and array scanning on the same platform for further exploration or confirmation of copy number variants detected through sequencing.

Figure 2: NextSeq System—The NextSeq Series of sequencing systems leverages the latest advances in sequencing by synthesis (SBS) chemistry and the industry's simplest workflow.

* TruSight panels comprise oligo probes targeting genes and regions thought to be relevant for particular diseases or conditions.

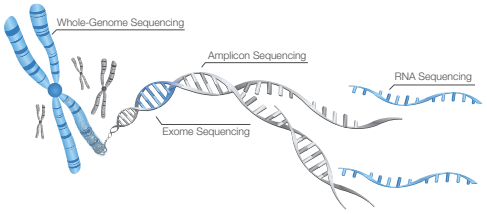

Figure 3: NextSeq Series Sequencing Applications—The flexible NextSeq Series enables researchers to transition seamlessly between sequencing applications to advance their research.

Easy Amplicon Panel Customization With DesignStudio

The NextSeq Series Amplicon Sequencing Solution supports targeted resequencing of single exons, and fixed or custom panels. TruSight fixed panels expedite research and conserve limited budgets by assessing multiple genes simultaneously at approximately the same cost as a single gene assay. For example, hematological cancers can be studied using the TruSight Myeloid Sequencing Panel, a fixed panel that employs TSCA assay chemistry and consists of 54 genes mutated frequently in myeloid malignancies.

Researchers can also use DesignStudio to create customized panels with optimized content for their studies (Figure 4). This personalized, easy-to-use, web-based assay design tool provides dynamic feedback to enhance target region coverage, reducing the time required to design custom projects. By building a custom panel, users can focus their sequencing coverage on regions of interest and gain more confidence in detection of germ line and somatic variants. Using DesignStudio, researchers can create panels with a range of amplicon sizes optimized for different sample types (eg, formalin-fixed, paraffin-embedded) and reference genomes (eg, human, mouse, rat, and bovine).

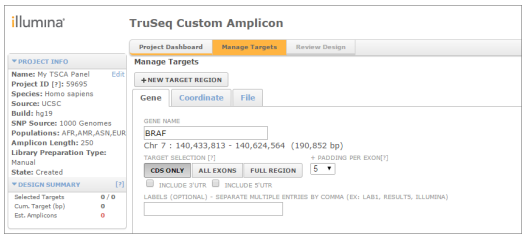

Figure 4: DesignStudio Enables Easy Creation of Custom Amplicon Sequencing Panels—Building custom amplicon panels in DesignStudio enables researchers to sequence regions of interest to detect germline and somatic variants.

Integrated Library Preparation

TSCA is used to prepare custom amplicon panels for sequencing. Following panel creation using DesignStudio, oligonucleotide probes are synthesized at Illumina and pooled into a Custom Amplicon Tube. The TSCA assay kit generates up to 1536 amplicons per reaction, incorporating integrated indexes to support up to 96 samples per run. It allows researchers to sequence hundreds of genomic regions covering as little as 2 kb or up to 650 kb of cumulative sequence.

Fast, Accurate Variant Detection

Leveraging Illumina NGS technology, the NextSeq Series delivers industry-leading sequencing accuracy of > 75% of sequenced bases over Q30† at 2 × 150 bp. NextSeq Systems can successfully sequence even the most difficult regions (GC-rich, homopolymers), yielding a higher percentage of true variants than other high-throughput desktop sequencers (Figure 5). Its low false-positive and false-negative rates drastically reduce the time and cost of downstream validation. New NextSeq v2 reagent kits are optimized to improve base calling and data quality even further.

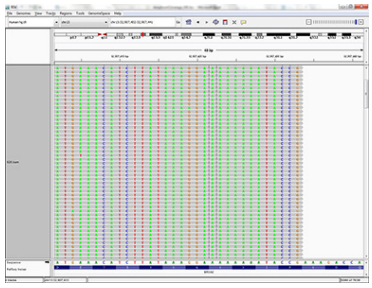

Figure 5: NextSeq Series Delivers High-Quality Sequencing Through Homopolymer Regions—The NextSeq Series can sequence successfully through GC-rich and homopolymer regions to capture the full value of amplicon sequencing.

The easy workflow of the NextSeq Series simplifies amplicon sequencing. It takes less than 20 minutes to load and initiate the system. Sequencing of up to 96 samples is completed in as little as 26 hours. The flexible, scalable NextSeq Series with its dual sequencing output modes (Mid and High) and ability to handle a range of sample sizes enables researchers to tune and optimize their amplicon studies easily (Table 1).

Table 1: NextSeq Series Supports Various Study Sizes

Panel Size	Average Coverage Depth	Sequencing Output	Samples / Run
270 amplicons	≥ 5000×	2 × 150 (Mid Output)	96
1300 amplicons	≥ 1000×	2 × 150 (Mid Output)	96
1300 amplicons	≥ 5000×	2 × 150 (Mid Output)	24
1300 amplicons	≥ 5000×	2 × 150 (High Output)	72

Based on industry-leading Illumina sequencing by synthesis chemistry, the NextSeq Series enables researchers to compare and integrate the data it generates with data from studies performed on other Illumina systems. For example, NextSeq Series amplicon sequencing data can be integrated with data generated on the Illumina MiSeq® or HiSeq® Systems (Table 2).

† Q30 = 1 error in 1,000 base calls or an accuracy of 99.9%

Selecting Sequencing Depth for Amplicon Sequencing

Sequencing coverage (sensitivity) describes the average number of reads that align to, or "cover," known reference bases. Coverage level often determines whether variant discovery can be made with a certain degree of confidence at particular base positions. At higher levels of coverage, each base is covered by a greater number of aligned sequence reads, so base calls can be made with a higher degree of confidence. For example, higher coverage could enable detection of somatic variants that might be present at low levels.

The following are coverage guidelines to achieve appropriate detection levels for certain studies:

- Heterozygote detection—40× coverage
- 5% variation of single-base changes and multi-base deletions—1000× coverage
- 1% variation of single-base changes and multi-base deletions— ≤ 5000× coverage
- Single-base indels might require additional depth

Table 2: Illumina Amplicon Sequencing Solutions

Illumina Sequencing System	Read Length	No. of Samples	Average Coverage Per Sample
MiSeq System	2 × 150 bp	16	1600×
NextSeq Series Mid-Output Flow Cell	2 × 150 bp	48	4300×
NextSeq Series Mid-Output Flow Cell	2 × 150 bp	96	2150×
NextSeq Series High-Output Flow Cell	2 × 150 bp	96	6500×
HiSeq 2500 System Rapid-Run Mode	2 × 150 bp	96	9700×

Simplified Bioinformatics in BaseSpace

The Illumina BaseSpace environment removes much of the complexity from the typical informatics workflow. Bases generated on the NextSeq Series are instantly and securely transferred, analyzed, and stored in BaseSpace (Cloud or Onsite), delivering annotated variants in 30 minutes per sample. Researchers can choose to analyze DNA data using the industry-standard BWA/GATK method or the fast and accurate Illumina Isaac pipeline.[1] Analysis results, including coverage statistics and annotated SNPs and indels, are presented in intuitive, easy-to-interpret reports.

Clinical researchers can use the VariantStudio application in BaseSpace to identify and classify disease-relevant variants quickly. Using a cascade of filtering options, researchers can rapidly isolate the key variants that are consequential to the phenotype under study, combining their expertise with provided annotations for rich variant reports. Classified variants and associated information entered by the user are saved to a local database so the information can be applied easily to variants observed in other samples. VariantStudio offers powerful report generation capabilities to communicate significant, actionable findings concisely.

The BaseSpace environment also includes BaseSpace apps, a growing community of software solutions for visualization, analysis, and sharing. Because Illumina NGS technology is the most established and broadly adopted sequencing solution, researchers can take advantage of a large collection of commercial and open-source sequencing analysis software designed primarily for processing Illumina data. Through its intuitive user interface and this rich ecosystem of third-party tools and apps, BaseSpace enables researchers to identify known disease variants quickly.

Summary

The NextSeq Series Amplicon Sequencing Solution offers a fast and easy sample-to-answer solution for identifying variants in gene regions. Offering the flexibility of expert-defined fixed TruSight panels or custom TruSeq panels created with DesignStudio, this unique solution enables researchers to identify, evaluate, and classify gene variants accurately. Rapid library preparation, industry-leading sequencing accuracy, and simple data analysis enable researchers to identify variants with clinical relevance and move their research forward faster.

Join the Illumina Community

With a NextSeq System in their laboratory, researchers join a worldwide community of over 60,000 scientists using Illumina technology for their research studies. Illumina schedules community events throughout the year, bringing researchers together to share ideas. User group meetings, scientific symposiums, and blog forums provide venues to discuss new research methods and breakthrough studies.

An integral part of the Illumina community is our dedicated service and support team, consisting of more than 300 people worldwide, 75% of whom have advanced degrees. Illumina technical support begins when the NextSeq System is delivered, with Illumina scientists and engineers assisting with system installation and setup, and the training of laboratory personnel. They are there 24/7 globally to answer questions every step of the way, giving researchers the peace of mind to focus on their next research study.

As researchers' needs change, new systems are brought into the laboratory, or new methods are undertaken, the Illumina support and training teams are there to provide assistance. In addition to on-site support, training courses (via webinar or at an Illumina facility) are available to bring laboratory personnel quickly up to speed.

Table 3: NextSeq Series Performance Parameters

Flow Cell Configuration	Read Length (bp)	Output (Gb)	Run Time	Data Quality	Required Input TruSeq Custom Amplicon Assay
High-Output Flow Cell Up to 400 M single reads Up to 800 M paired-end reads	2 × 150	100–120	29 hours	> 75% > Q30	≥ 50 ng High-Quality Genomic DNA ≥ 250 ng FFPE Genomic DNA
	2 × 75	50–60	18 hours	> 80% > Q30	
	1 × 75	25–30	11 hours	> 80% > Q30	
Mid-Output Flow Cell Up to 130 M single reads Up to 260 M paired-end reads	2 × 150	32.5–39	26 hours	> 75% > Q30	
	2 × 75	16.25–19.5	15 hours	> 80% > Q30	

Total times include cluster generation, sequencing, and base calling on a NextSeq 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.

Table 4: NextSeq Series Specifications

Instrument Configuration
RFID tracking for consumables
Instrument Control Computer (Internal)[a]
Base Unit: Dual Intel Xeon ES-2448L 1.8 GHz CPU
Memory: 96 GB RAM
Hard Drive: 750 GB
Operating System: Windows 7 embedded standard
Operating Environment
Temperature: 19°C to 25°C (22°C ± 3°C)
Humidity: Noncondensing 20%–80% relative humidity
Altitude: Less than 2,000 m (6,500 ft)
Air Quality: Pollution degree rating of II
Ventilation: Up to 2,048 BTU/hr @ 600 W
For Indoor Use Only
Light Emitting Diode (LED)
520 nm, 650 nm; Laser diode: 780 nm, Class IIIb
Dimensions
W×D×H: 53.3 cm × 63.5 cm × 58.4 cm (21.0 in × 25.0 in × 23.0 in)
Weight: 83 kg (183 lbs)
Crated Weight: 151.5 kg (334 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

a. Computer specifications are subject to change.

Ordering Information

System Name	Catalog No.
NextSeq 500 System	SY-415-1001
NextSeq 550 System	SY-415-1002
Output Kit Name	**Catalog No.**
NextSeq 500 Mid-Output Kit (150 cycles)	FC-102-1001
NextSeq 500 Mid-Output Kit (300 cycles)	FC-404-1003
NextSeq 500 High-Output Kit (75 cycles)	FC-404-1005
NextSeq 500 High-Output Kit (150 cycles)	FC-404-1002
NextSeq 500 High-Output Kit (300 cycles)	FC-404-1004
NextSeq 500 Mid-Output v2 Kit (150 cycles)	FC-404-2001
NextSeq 500 High-Output v2 Kit (150 cycles)	FC-404-2002
NextSeq 500 Mid-Output v2 Kit (300 cycles)	FC-404-2003
NextSeq 500 High-Output v2 Kit (300 cycles)	FC-404-2004
NextSeq 500 High-Output v2 Kit (75 cycles)	FC-404-2005
Library Preparation Kit Name	**Catalog No.**
TruSeq Custom Amplicon v1.5 Kit (96 samples)	FC-130-1001
TruSeq Custom Amplicon Index Kit (96 indexes, 384 samples)	FC-130-1003
TruSeq Index Plate Fixture Kit	FC-130-1005
TruSeq Index Plate Fixture and Collar Kit (2 each)	FC-130-1007

Reference

1. Raczy C, Petrovski R, Saunders CT, et al. Isaac: Ultra-fast whole genome secondary analysis on Illumina sequencing platforms. *Bioinformatics.* 2013;29:2041-2043.

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

For Research Use Only. Not for use in diagnostic procedures.

Pub. No. 770-2014-027 Current as of 02 June 2015

illumina®