AmpliSeq™ for Illumina Targeted Resequencing Solution

A streamlined, scalable amplicon sequencing solution, producing high-confidence data from low-input DNA and RNA samples.

Highlights

- **Accurate Data Across Various Sample Types**
  Proven AmpliSeq chemistry and Illumina sequencing technology combine to achieve high data quality, even from low-quality starting materials, such as FFPE tissues
- **Comprehensive Content Portfolio**
  Extensive menu of tested ready-to-use content and options for custom designs
- **Fast, Simple Workflow**
  Increased lab efficiency with a quick library preparation workflow needing < 1.5 hours of hands-on time
- **Scalable Solution**
  Multiple options for sequencing platforms, data analysis, and support meet virtually all throughput needs

Introduction

AmpliSeq for Illumina offers fast, accurate targeted resequencing that enables researchers to go from DNA or RNA to variant calls in 2.5 days. The supported solution includes curated panel content, a PCR-based library preparation assay, proven Illumina next-generation sequencing (NGS) technology, and user-friendly data analysis (Figure 1, Table 1). Delivered results provide the high on-target and uniform coverage needed to detect low-frequency variants with consistency and reliability.

Leveraging high-performance AmpliSeq chemistry, researchers can use the AmpliSeq for Illumina assay to focus their studies on specific genes, regions, or variants of interest with high accuracy. Users can take advantage of expertly selected content in ready-to-use panels or create custom panels to meet specific needs. Custom options include on-demand gene panels for human disease research, preconfigured community panels, and custom DNA panels to target unique areas of interest. The PCR-based library preparation assay uses oligonucleotides to amplify regions of interest to investigate single nucleotide variants (SNVs), insert/deletions (indels), copy number variations (CNVs), and gene fusions. AmpliSeq chemistry can multiplex 12 to > 24,000 amplicons, enabling simultaneous capture of multiple targets in a single reaction. AmpliSeq for Illumina works with RNA and DNA samples, requiring as little as 1 ng of high-quality DNA or RNA and the flexibility to accommodate formalin-fixed, paraffin-embedded (FFPE) samples, such as preserved tumor tissue.

The AmpliSeq for Illumina solution is optimized for use with Illumina sequencing by synthesis (SBS) chemistry. Prepared libraries can be sequenced on any Illumina sequencing system, including the iSeq™ 100, MiniSeq™, MiSeq™, and NextSeq™ Sequencing Systems.

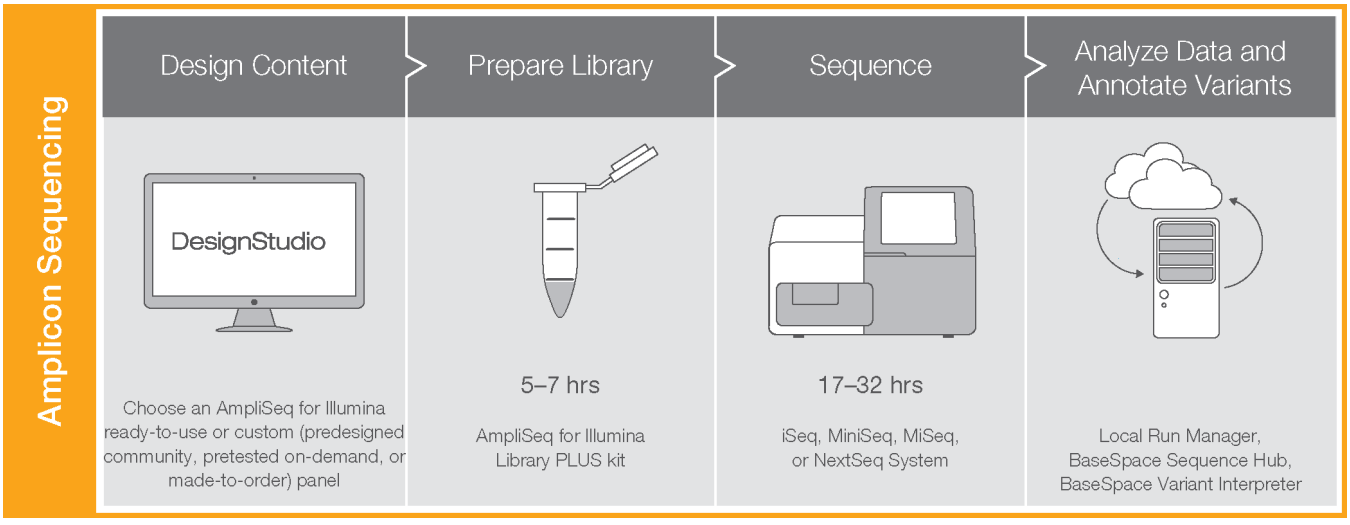

Figure 1: AmpliSeq for Illumina Targeted Resequencing Solution

Table 1: AmpliSeq for Illumina At A Glance

Parameter	Specification
Assay	PCR-based workflow
Sequencing	SBS chemistry
Sample Type	FFPE tissue, blood
Input Type	DNA, RNA
Input Amount	1–100 ng; recommended 10 ng per pool
Variant Types	SNVs, indels, CNVs, gene fusions[a]
Hands-On Time (assay)	< 1.5 hours[b]
Total Time (assay)	5–7.5 hours (panel dependent)
Options for Content	
Ready-to-Use Panel	Predesigned panels targeting specific areas of interest
Community Panel	Designs based on input from leading researchers in the respective application areas
Custom Panel	Targeted panels for specific regions of interest; most designs accommodated in 1–4 pools, 3072 amplicons per pool and up to 12,288 amplicons per panel; on-demand menu includes > 5000 genes in inherited disease research areas
Time to Order	Made to order (on-demand, community, custom): 3–4 weeks In-stock (ready-to-use panels, Library PLUS, Indexes): available in inventory
Compatible Systems	All Illumina sequencing systems

a. SNVs: single nucleotide variants, indels: insertions/deletions, CNVs: copy number variations
b. Hands-on time is for library preparation and does not include library quantification, normalization, or pooling

Order AmpliSeq for Illumina online at www.illumina.com

Fast, Simple Workflow

The AmpliSeq for Illumina solution provides a fast, efficient method for analyzing suspected variants in oncology and genetic disease studies. Library preparation takes as little as 5 hours (when starting with DNA), with < 1.5 hours hands-on time. Sequencing and data analysis times vary depending on the panel and number of samples. Simply order a panel, follow the PCR-based workflow for library preparation, sequence, and analyze (Figure 1).

Panel Design

Researchers can choose from a growing menu of content design, including ready-to-use, on-demand, community, and custom panels.

Ready-to-Use Panels

Ready-to-use panels offer predesigned content targeting variants commonly seen in an area of interest. Variants are chosen based on input from field experts, curated databases, and peer-reviewed publications. These panels remove the time and effort of selecting targets and optimizing performance. Panels are currently available for oncology and genetic disease. The menu of panels is continuously growing. Ready-to-use panels are available in small pack sizes (usually 24 reactions) for scalable studies.

Learn more about AmpliSeq for Illumina ready-to-use panels at www.illumina.com/products/by-brand/ampliseq/ready-to-use-panels.html

Made-to-Order Panels

On-Demand Panels

On-demand panels provide a choice of > 5000 pretested genes with known content relevant for inherited disease research, including hereditary cancer, primary immunodeficiency, hearing loss, muscular dystrophy, and more. Easily design and order an on-demand panel online using Illumina DesignStudio™ software. DesignStudio software is a free, user-friendly tool that creates panels optimized for the specific genomic content of interest. On-demand panels are available for 1 (24 amplicons) to 500 (15,000 amplicons) genes and come in 24- and 96-reaction sizes.

Learn more about AmpliSeq for Illumina on-demand panels at www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/ampliseq-on-demand-panel.html

Community Panels

Community panels contain content selected and designed with input from leading researchers. These panels provide an easy, fast way to start targeted resequencing studies in a specific disease area. For maximum flexibility, the panels are fully customizable. Community panels are made to order and available in large pack sizes.

Learn more about AmpliSeq for Illumina community panles at www.illumina.com/products/by-brand/ampliseq/community-panels.html

Custom Panels

If the content available in ready-to-use or community panel does not meet your needs, custom content can be easily designed and ordered online through DesignStudio software. Targets can be selected based on several preloaded reference genomes. After logging on to a personalized account, researchers can select coordinates for targeting genomic regions of interest. An optimized algorithm that considers a range of factors, including GC content, specificity, and coverage, automatically designs amplicons. Candidate amplicons are visualized and assessed using estimated success scores. Amplicons can be filtered and then added to, or removed from, the design. After visualization and QC, the panel can be ordered. DesignStudio software can also be used to personalize content.

Watch a demo of how to design custom panels at www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/ampliseq-custom-dna-panel.html

Amplicon Assay and Library Preparation

AmpliSeq for Illumina library preparation is fast and simple. A multiplexed, highly specific, PCR-based workflow results in libraries with high on-target alignment and high coverage uniformity (Figure 2). Up to 24,000 amplicons can be multiplexed in a single assay, allowing for simultaneous assessment of multiple genes.

Library prep starts with amplifying specific regions in the starting DNA or cDNA . Remaining primer sequences are digested and sequencing adapters are added to the resulting amplicons. The library is amplified and ready to be quantified, normalized, and pooled before sequencing. Multiple libraries can be prepared in as little as 5 hours.

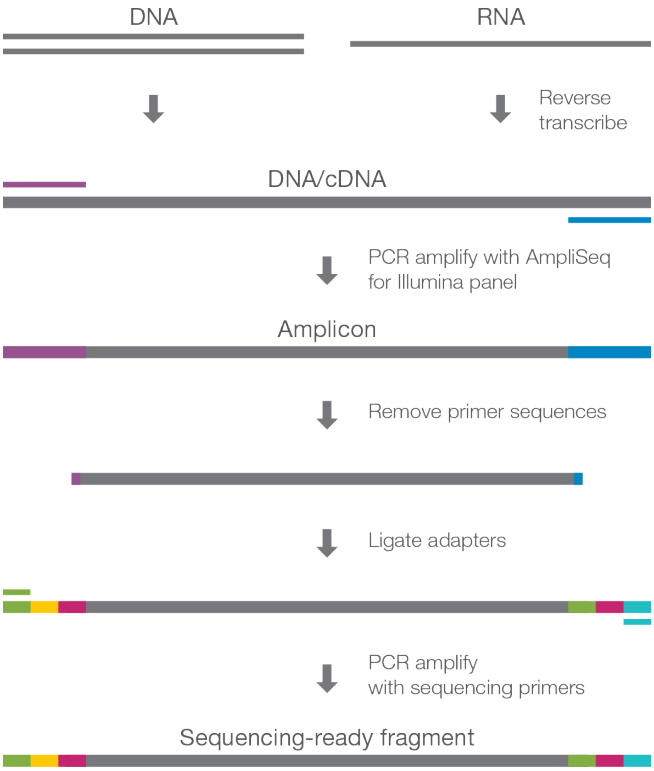

Figure 2: AmpliSeq for Illumina Library Preparation—The highly multiplexed, PCR-based workflow amplifies up to 24,000 amplicons in a single assay.

Sequencing

More than 90% of the world's sequencing data is generated by Illumina SBS chemistry.* Through massively parallel sequencing using a proprietary reversible terminator-based method, SBS enables detection of single bases as they are incorporated into growing DNA strands. A fluorescently labeled terminator is imaged as each dNTP is added and then cleaved to allow incorporation of the next base. Because all four reversible terminator-bound dNTPs are present during each sequencing cycle, natural competition minimizes incorporation bias. The result is base-by-base sequencing for highly accurate data even in difficult regions, such as homopolymers.

Watch the video to see SBS technology in action at www.illumina.com/science/technology/next-generation-sequencing.html

Multiple Systems for Scalable Throughput

Illumina sequencing systems offer user-friendly, intuitive interfaces for easy run setup and operation at every sequencing scale (Figure 3).

Data Analysis

Analyzing data generated with AmpliSeq for Illumina is simple and does not require highly trained bioinformatics support or a dedicated compute infrastructure. Informatics solutions for both DNA and RNA amplicon panels are available for processing raw sequencing data into meaningful results. With DNA amplicon panels, the secondary analysis workflow aligns reads against reference genomes and calls small variants. Additional analysis can be performed on variant calls from human data to provide annotations and generate reports. For RNA amplicon panels, the secondary analysis workflow performs differential expression analysis and gene fusion calling. Both analysis workflows generate output files that can be used for further downstream analysis and processing using third-party tools.

BaseSpace™ Informatics Suite

After streaming sequencing data directly from the sequencing system to BaseSpace Sequence Hub, the Illumina platform for genomics cloud computing, raw sequencing data are analyzed through either the DNA Amplicon or RNA Amplicon BaseSpace Apps. Analysis results can be stored or easily shared with other investigators. Further analysis can be performed on any variant calls using BaseSpace Variant Interpreter, an interpretation and reporting platform designed to decrease the time and effort required to extract biological insight from genomic data while maximizing operational efficiency.

On-instrument Analysis

For researchers who are not BaseSpace Sequence Hub users, the same secondary analysis workflows are available for on-premise use through the Local Run Manager software. Local Run Manager is an on-instrument software used to create a run, monitor status, and analyze sequencing data. Local Run Manager is available both on-instrument for select sequencing systems, and off-instrument for installation on a separate computer.

Learn more about BaseSpace Sequence Hub at www.illumina.com/products/by-type/informatics-products/basespace-sequence-hub.html

Learn more about informatics solutions at www.illumina.com/products/by-brand/ampliseq/informatics.html

*Data calculations on file. Illumina, Inc. 2015.

	iSeq 100 System	MiniSeq System	MiSeq System	NextSeq 550 System
Max Output	1.2 Gb	8 Gb	15 Gb	120 Gb
Max Read Number	4 million	25 million	25 million	400 million
Estimated No. of Samples per Run[a]	3	6–20	12–20	104–320

a. Based on a panel with 1000 amplicons and a 1000× mean coverage

Figure 3: Illumina Sequencing Systems—Solutions for every application, sample type, and sequencing scale. Learn more at www.illumina.com/systems

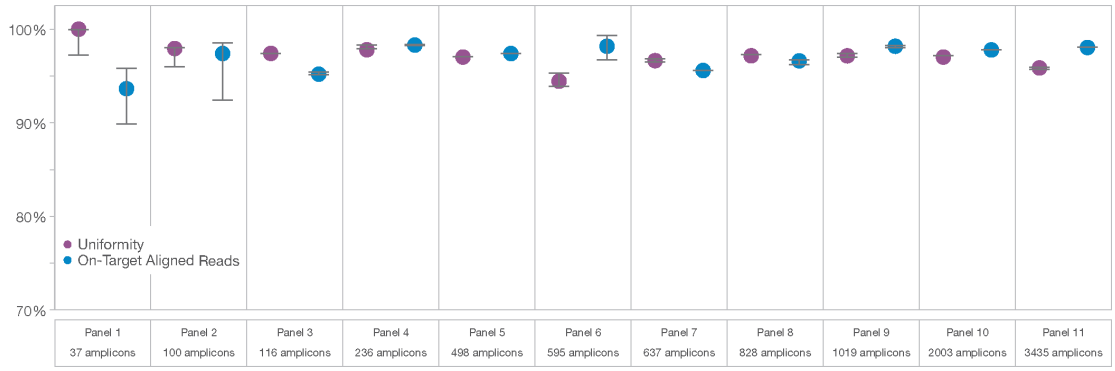

Figure 4: High Coverage Uniformity and On-Target Alignment in AmpliSeq for Illumina Custom Panels—Coriell and Horizon Discovery DNA samples were used to evaluate the performance of 11 AmpliSeq for Illumina custom panels of varying size (number of amplicons). Libraries were sequenced on the MiSeq System. Error bars indicate variability of technical replicates.

High-Quality Data

To demonstrate the capabilities of the AmpliSeq for Illumina targeted resequencing solution, 11 AmpliSeq for Illumina custom libraries were made and evaluated using Coriell and Horizon Discovery (HD) samples. Panels of varying sizes (number of amplicons) were designed using online DesignStudio software. Sequencing-ready libraries were prepared using the Coriell and HD samples following the AmpliSeq for Illumina Library PLUS protocol and then sequenced on the MiSeq System. Data analysis was performed with the DNA Amplicon analysis workflow. Results show high coverage uniformity and on-target percentage of aligned reads with all levels of plexity (Figure 4).

Summary

The AmpliSeq for Illumina Targeted Resequencing Solution provides quick, accurate reporting of different variants across various sample types, including FFPE tissues. The comprehensive solution combines the ease of design and extensive menu of AmpliSeq panels, a highly multiplexed PCR-based library preparation assay, proven Illumina sequencing, and simplified bioinformatics options to help researchers discover a wealth of genomic information.

Learn More

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

Ordering Information

To order AmpliSeq for Illumina custom panels, visit www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/ampliseq-custom-dna-panel.html

Fully Supported Solution

From design support and data analysis, to bioinformatics and training, Illumina offers a range of support options to help researchers achieve their goals. Illumina Concierge offers advanced content design to help improve coverage and enable the use of add-on or nonhuman species designs. Assistance assessing AmpliSeq for Illumina on an Illumina sequencing system before investing is also available. Bioinformatics experts can assist with custom app and pipeline design, and trained and certified scientists offer library prep training at customer sites.

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