illumina®

AmpliSeq™ for Illumina Comprehensive Panel v3

Starting from DNA or RNA, quickly and accurately investigate variants across 161 genes associated with various cancer types.

Highlights

- **Relevant gene content**

Target 161 unique cancer-associated genes

- **Fast, streamlined workflow**

Prepare sequencing-ready libraries in a single day from as little as 1 ng high-quality DNA or RNA, or 10 ng DNA or RNA from FFPE tissue

- **Accurate data**

Detect somatic mutations down to 5% frequency using local or cloud-based analysis

Introduction

The AmpliSeq for Illumina Comprehensive Panel v3 enables the use of targeted resequencing to study somatic mutations across 161 genes with known associations to cancer (Table 1). The Comprehensive Panel v3 is part of a streamlined workflow that includes AmpliSeq for Illumina PCR-based library preparation, Illumina sequencing by synthesis (SBS) next-generation sequencing (NGS) technology, and automated analysis.

The AmpliSeq for Illumina Comprehensive Panel v3 requires as little as 1 ng high-quality DNA or RNA and is compatible with various sample types, including formalin-fixed, paraffin-embedded (FFPE) tissues. The high gene content and low DNA/RNA input requirement enable a single, streamlined workflow for the quick assessment of cancer-related genetic variations, affording researchers the potential to unlock a wealth of genomic information from many tumor types.

Relevant gene content

The AmpliSeq for Illumina Comprehensive Panel v3 offers coverage of 161 cancer-associated genes, including kinases and genes involved in DNA repair. The panel content spans hotspots, full-length genes, copy number variations, intergenic gene fusions, and intragenic rearrangements. Experienced scientists assisted with content selection, assuring coverage of key targets and alignment with published evidence. This ready-to-use panel saves researchers the time and effort of identifying targets, designing amplicons, and optimizing performance.

Access a complete list of genes on the AmpliSeq for Illumina Comprehensive Panel v3

Table 1: AmpliSeq for Illumina Comprehensive Panel v3 at a glance

Parameter	Specification
No. of genes	161
Targets	Hotspots and full-length genes
Cumulative target size	DNA: 397 kb, RNA: 86 kb
Variant types	SNVs, indels, CNVs, and gene fusions[a]
Amplicon size	DNA: 105 bp on average, RNA: 99 bp on average
No. of amplicons	DNA: 3781, RNA: 867
Input DNA/RNA requirement	1-100 ng (10 ng recommended per pool)
No. of pools per panel	DNA panel: 2 pools, RNA panel: 2 pools
Compatible sample types	FFPE tissue
Percent targets covered at minimum 500× at recommended throughput	> 95%
Coverage uniformity (percent of targets with >0.2× mean coverage)	> 95%
Percent on-target aligned reads	> 95%
Total assay time[b]	5-6 hours
Hands-on time	< 1.5 hours
DNA/RNA-to-data time	2.5 days

a. SNVs: single nucleotide variations; indels: insertions/deletions, CNVs: copy number variations

b. Time represents library preparation only and does not include library quantification, normalization, or pooling.

Data on file at Illumina, Inc. 2017

Simple, streamlined workflow

The AmpliSeq for Illumina Comprehensive Panel v3 is part of a DNA/RNA-to-variant solution that offers streamlined content, easy-to-perform library preparation, push-button sequencing systems, and simplified data analysis.

Library preparation follows a straightforward, PCR-based protocol that can be completed in as little as 5 hours (DNA) or 6 hours (RNA), with < 1.5 hours hands-on time. Resulting libraries can be normalized, pooled, and then loaded on to a flow cell for sequencing. Prepared libraries are sequenced using proven SBS chemistry on any Illumina sequencing system (Table 2).

Resulting data can be analyzed locally with Local Run Manager or easily streamed into BaseSpace™ Sequence Hub. Local Run Manager and BaseSpace Sequence Hub can access the DNA Amplicon analysis workflow to perform alignment and variant calling. BaseSpace Sequence Hub provides access to BaseSpace Variant Interpreter, which assists with turning variant call data into annotated results.

Learn more about Illumina sequencing systems

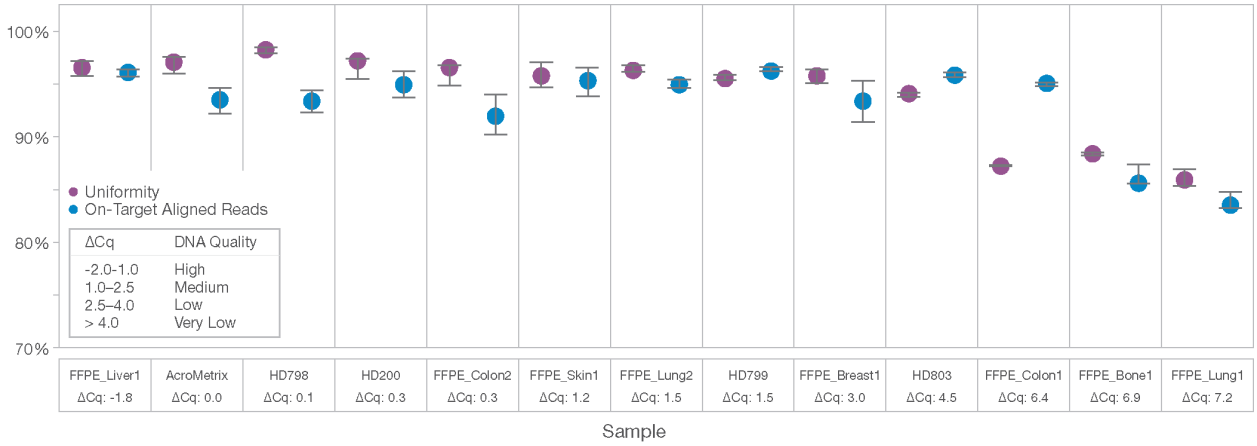

Figure 1: High Coverage Uniformity and On-Target Alignment—DNA extracted from FFPE and HD samples of varying quality was prepared using the AmpliSeq for Illumina Comprehensive Panel v3 and sequenced on the Instrument. Error bars indicate variability of technical replicates. ΔCq is an indicator of the quality of DNA isolated from FFPE tissues.

Learn more about AmpliSeq for Illumina informatics

Table 2: Illumina sequencing systems recommended for use with the AmpliSeq for Illumina Comprehensive Panel v3

Instrument	No. of Samples per Run	Run Time
MiniSeq System (high output)	3	24 hours
MiSeq System (v3 chemistry)	3	32 hours
NextSeq System (mid output)	16	26 hours
NextSeq System (high output)	48	29 hours

Accurate data

The AmpliSeq for Illumina Comprehensive Panel v3 provides the ability to assess > 160 genes per sample simultaneously, while maintaining excellent specificity and uniformity.

Coverage and Sensitivity

To demonstrate assay capabilities, an AcroMetrix control, Horizon Discovery (HD), and FFPE samples were evaluated using the AmpliSeq for Illumina Comprehensive Panel v3 and sequenced on the NextSeq™ System. Results showed high coverage uniformity and on-target percentage of aligned reads, even with varying sample quality and tissue type (Figure 1). HD samples of varying quality were tested for variant calling accuracy. Data showed high concordance between expected and detected variant frequency (VF) (Figure 2).

Gene Fusion Detection

To demonstrate the assay's ability to detect structural variants within RNA transcripts, HD samples and the Seraseq Fusion RNA Mix v2 reference were evaluated using the AmpliSeq for Illumina Comprehensive Panel v3 and the MiniSeq™ and MiSeq™ Systems. Results showed a 100% call rate for the gene fusions within these samples (Table 3).

Table 3: High call rate for gene fusions

Fusion	No. Samples NOT Detected	No. Samples Detected	Call Rate
RNA Source: HD784			
CCDC6-RET	0	16	100%
EML4-ALK	0	16	100%
SLC34A2-ROS1	0	16	100%
SLC34A2-ROS1	0	16	100%
RNA Source: Seraseq Fusion RNA Mix v2			
CD74-ROS1	0	16	100%
EGFR-SEPT14	0	16	100%
EML4-ALK	0	16	100%
ETV6-NTRK3	0	16	100%
FGFR3-BAIAP2L1	0	16	100%
FGFR3-TACC3	0	16	100%
KIF5B-RET	0	16	100%
LMNA-NTRK1	0	16	100%
MET-MET	0	16	100%
NCOA4-RET	0	16	100%
PAX8-PPARG	0	16	100%
SLC34A2-ROS1	0	16	100%
SLC45A3-BRAF	0	16	100%
TMPRSS2-ERG	0	16	100%
TPM3-NTRK1	0	16	100%

Two fusion-positive RNA samples, HD784 and Seraseq Fusion RNA Mix v2, were used to generate RNA libraries with the AmpliSeq for Illumina Comprehensive Panel v3 and sequenced on the MiniSeq and MiSeq Systems.

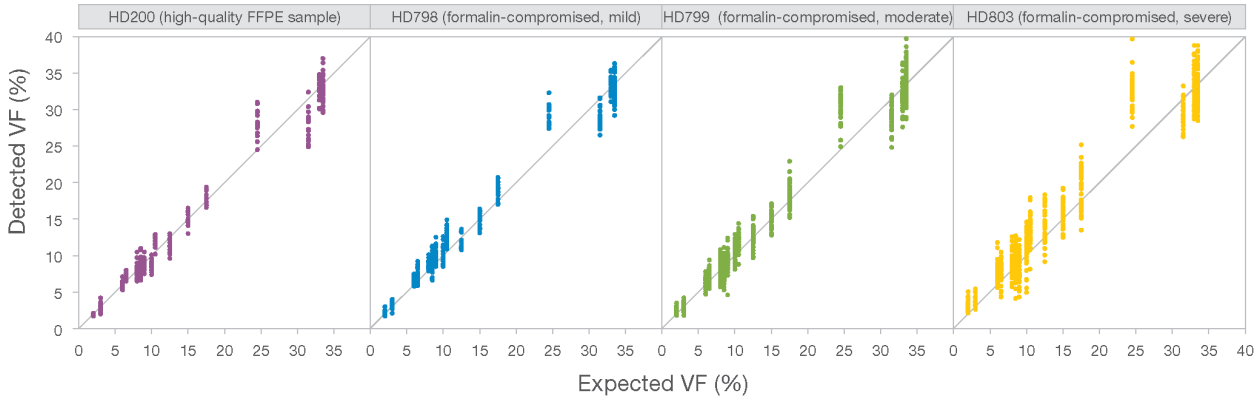

Figure 2: High Concordance Between Expected and Detected Variant Frequency—DNA from formalin-fixed HD samples was prepared using the AmpliSeq for Illumina Comprehensive Panel v3 and sequenced on the MiniSeq and MiSeq Systems. Results show that 100% of expected SNVs were detected. ΔCq values are listed in Figure 1.

Ordering information

Order AmpliSeq for Illumina products online at www.illumina.com

Product	Catalog No.
AmpliSeq for Illumina Comprehensive Panel v3 (24 reactions)	20019109
AmpliSeq for Illumina Library PLUS (24 reactions)	20019101
AmpliSeq for Illumina Library PLUS (96 reactions)	20019102
AmpliSeq for Illumina Library PLUS (384 reactions)	20019103
AmpliSeq for Illumina CD Indexes Set A (96 indexes, 96 samples)	20019105
AmpliSeq for Illumina cDNA Synthesis (96 reactions)	20022654
AmpliSeq for Illumina Direct FFPE DNA	20023378
AmpliSeq for Illumina Library Equalizer	20019171

Learn more

Learn more about the AmpliSeq for Illumina Comprehensive Panel v3

Learn more about the AmpliSeq for Illumina targeted sequencing solution

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