illumina®

AmpliSeq™ for Illumina Cancer Hotspot Panel v2

Fast, accurate investigation of hotspot regions in 50 genes with known cancer associations.

Highlights

- **Relevant gene content**

Target ~2800 COSMIC mutations from 50 oncogenes and tumor suppressor genes

- **Fast, streamlined workflow**

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

- **Accurate data**

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

Introduction

The AmpliSeq for Illumina Cancer Hotspot Panel v2 is a targeted resequencing assay for researching somatic mutations across the hotspot regions of 50 genes with known associations to cancer (Table 1). Starting with as little as 1 ng high-quality DNA (recommend 10 ng DNA from FFPE tissue), the panel enables the study of genes associated with different cancer types, including lung, colon, breast, ovarian, melanoma, and prostate. The low-input requirement enables use with various sample types, including formalin-fixed, paraffin-embedded (FFPE) tissues.

Relevant gene content

The AmpliSeq for Illumina Cancer Hotspot Panel v2 targets ~2800 mutations in the hotspot regions of 50 oncogenes and tumor suppressor genes as identified in the Catalogue of Somatic Mutations In Cancer (COSMIC)[1] database (Table 2). This ready-to-use panel saves researchers the time and effort of identifying targets, designing amplicons, and optimizing performance.

Simple, streamlined workflow

The AmpliSeq for Illumina Cancer Hotspot Panel v2 is part of a DNA-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, 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 compatible Illumina sequencing system (Table 3).

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.

Table 1: AmpliSeq for Illumina Cancer Hotspot Panel v2 at a glance

Parameter	Specification
No. of genes	50
Targets	Hotspot regions within oncogenes and tumor suppressor genes
Cumulative target size	22 kb
Variant types	SNVs, indels[a]
Amplicon size	106 bp on average
No. of amplicons	207
Input DNA requirement	1-100 ng (10 ng recommended)
No. of pools per panel	1
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	> 80%
Total assay time[b]	5 hours
Hands-on time	< 1.5 hours
DNA-to-data time	2.5 days

a. SNVs: single nucleotide variations; indels: insertions/deletions

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

Data on file at Illumina, Inc. 2017

Table 2: AmpliSeq for Illumina Cancer Hotspot Panel v2 Genes

ABL1	*EGFR*	*GNAS*	*KRAS*	*PTPN11*
AKT1	*ERBB2*	*GNAQ*	*MET*	*RB1*
ALK	*ERBB4*	*HNF1A*	*MLH1*	*RET*
APC	*EZH2*	*HRAS*	*MPL*	*SMAD4*
ATM	*FBXW7*	*IDH1*	*NOTCH1*	*SMARCB1*
BRAF	*FGFR1*	*JAK2*	*NPM1*	*SMO*
CDH1	*FGFR2*	*JAK3*	*NRAS*	*SRC*
CDKN2A	*FGFR3*	*IDH2*	*PDGFRA*	*STK11*
CSF1R	*FLT3*	*KDR*	*PIK3CA*	*TP53*
CTNNB1	*GNA11*	*KIT*	*PTEN*	*VHL*

Accurate data

To demonstrate assay capabilities and sensitivity, an AcroMetrix control sample, Horizon Discovery (HD) samples, and FFPE samples were evaluated using the AmpliSeq for Illumina Cancer Hotspot Panel v2 and the MiniSeq™ and MiSeq™ Systems. Results showed high coverage uniformity and on-target percentage of aligned reads, even with varying sample quality and tissue type (Figure 1). In addition, HD samples of varying quality were

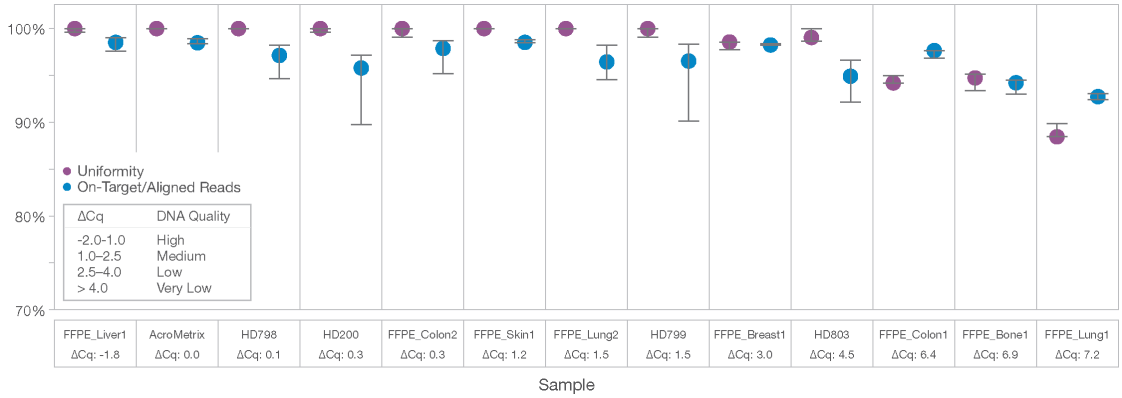

Figure 1: High Coverage Uniformity and On-Target Alignment—DNA extracted from FFPE and HD samples of varying quality was prepared using the AmpliSeq for Illumina Cancer Hotspot Panel v2 and sequenced on the Instrument. Error bars indicate variability of technical replicates. ΔCq is an indicator of the quality of DNA isolated from FFPE tissues.

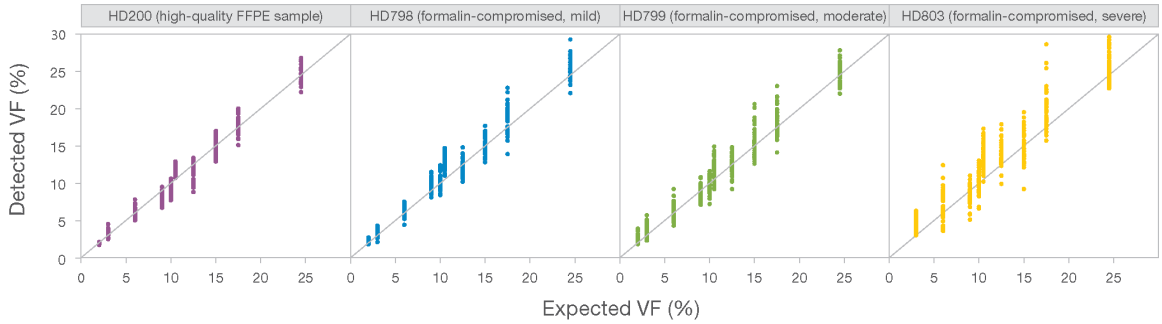

Figure 2: High Concordance Between Expected and Detected Variant Frequency—DNA from HD samples was prepared using the AmpliSeq for Illumina Cancer Hotspot Panel v2 and sequenced on the Instrument. Results show that 100% of expected SNVs were detected. ΔCq values are listed in Figure 1.

evaluated for variant calling accuracy. Data showed high concordance between expected and detected SNVs (Figure 2).

Table 3: Illumina sequencing systems recommended for use with the AmpliSeq for Illumina Cancer Hotspot Panel v2

Instrument	No. of Samples per Run	Run Time
iSeq™ 100 System	16	17 hours
MiniSeq™ System (mid output)	32	17 hours
MiniSeq System (high output)	96	24 hours
MiSeq™ System (v2 chemistry Nano)	4	17 hours
MiSeq System (v2 chemistry Micro)	16	19 hours
MiSeq System (v2 chemistry)	60	24 hours
MiSeq System (v3 chemistry)	96	32 hours

Learn more

Learn more about the AmpliSeq for Illumina Cancer Hotspot Panel v2

Learn more about the AmpliSeq for Illumina targeted sequencing solution

Ordering information

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

Product	Catalog No.
AmpliSeq for Illumina Cancer Hotspot Panel v2 (24 reactions)	20019161
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 Sample ID Panel	20019162
AmpliSeq for Illumina Direct FFPE DNA	20023378
AmpliSeq for Illumina Library Equalizer	20019171

References

1. Catalogue of Somatic Mutations in Cancer – Home Page. http://cancer.sanger.ac.uk/cosmic. Accessed October 25, 2017.

Illumina, Inc. • 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.