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AmpliSeq™ for Illumina Focus Panel

Targeted DNA and RNA panel investigating 52 genes with known relevance to solid tumors.

Highlights

- **Relevant Gene Content**
  Target biomarkers across 52 genes relevant to solid tumors
- **Fast, Streamlined Workflow**
  Prepare sequencing-ready libraries in a single day from as little as 1 ng high-quality DNA and RNA, or 10 ng DNA and RNA from FFPE tissue
- **Accurate Data**
  Detect somatic mutations down to 5% frequency using local or cloud-based analysis

Introduction

The AmpliSeq for Illumina Focus Panel is a targeted resequencing assay for biomarker analysis of 52 genes with known relevance to solid tumors (Table 1). Using the Focus Panel, researchers can analyze both DNA and RNA concurrently. The Focus Panel 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.

Starting with as little as 1 ng high-quality DNA and RNA (recommend 10 ng DNA and RNA from FFPE tissue), the panel enables the study of genes associated with multiple cancer types, including lung, colon, breast, ovarian, melanoma, and prostate. The low-input requirement allows use with various sample types, including formalin-fixed, paraffin-embedded (FFPE) tissues. As part of the AmpliSeq for Illumina targeted resequencing solution, the Focus Panel enables quick and accurate assessment of genomic variation for translational and clinical oncology research.

Relevant Gene Content

The AmpliSeq for Illumina Focus Panel targets hundreds of mutations across 52 key genes associated with solid tumors (Table 2). Gene content for this panel was selected based on published literature, current guidelines (National Comprehensive Cancer Network [NCCN], Association for Molecular Pathology [AMP], College of American Pathologists [CAP], European Society for Medical Oncology [ESMO], etc.), and relevant clinical trials. Researchers can use the panel to investigate single nucleotide variants (SNVs), insertion/deletions (indels), and copy number variants (CNVs) in DNA samples, or gene fusions in RNA samples. This ready-to-use panel saves researchers the time and effort of identifying targets, designing amplicons, and optimizing performance.

Table 1: AmpliSeq for Illumina Focus Panel At A Glance

Parameter	Specification
No. of Genes	52
Targets	Genes relevant to solid tumors
Cumulative Target Size	DNA: 29 kb, RNA: 26 kb
Variant Types	SNVs, indels, CNVs, gene fusions[a]
Amplicon Size	DNA: 107 bp on average RNA: 93 bp on average
No. of Amplicons	DNA: 269, RNA: 284
Input DNA/RNA Requirement	1–100 ng (10 ng recommended per pool)
No. of Pools per Panel	1 pool for DNA panel, 1 pool for RNA panel
Supported Sample Types	FFPE tissue, blood
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	5–6 hours[b]
Hands-On Time	< 1.5 hours
DNA-to-Data Time	2.5 days

a. SNVs: single nucleotide variations, indels: insertions/deletions, CNVs: copy number variants
b. Time represents library preparation only and does not include library quantification, normalization, or pooling

Data on file at Illumina, Inc. 2017

Table 2: Gene List for the AmpliSeq for Illumina Focus Panel

DNA pool				
AKT1	*EGFR*	*FGFR4*	*JAK3*	*MYCN*
ALK	*ERBB2*	*GNA11*	*KIT*	*NRAS*
AR	*ERBB3*	*GNAQ*	*KRAS*	*PDGFRA*
BRAF	*ERBB4*	*HRAS*	*MAP2K1*	*PIK3CA*
CCND1	*ESR1*	*IDH1*	*MAP2K2*	*RAF1*
CDK4	*FGFR1*	*IDH2*	*MET*	*RET*
CDK6	*FGFR2*	*JAK1*	*MTOR*	*ROS1*
CTNNB1	*FGFR3*	*JAK2*	*MYC*	*SMO*
DDR2				
RNA pool				
ABL1	*EGFR*	*ETV5*	*NTRK1*	*PPARG*
ALK	*ERBB2*	*FGFR1*	*NTRK2*	*RAF1*
AKT3	*ERG*	*FGFR2*	*NTRK3*	*RET*
AXL	*ETV1*	*FGFR3*	*PDGFRA*	*ROS1*
BRAF	*ETV4*	*MET*		

Simple, Streamlined Workflow

The AmpliSeq for Illumina Focus Panel 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.

If starting with RNA, the first step is to convert RNA to cDNA. Library preparation, beginning with cDNA or DNA, follows a straightforward, PCR-based protocol. These steps can be completed in as little as 5 hours (DNA) or 6 hours (RNA), with < 1.5 hours (DNA) or < 2 hours (RNA) hands-on time. Resulting libraries are 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 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 and the RNA Amplicon analysis workflow for fusion 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 at www.illumina.com/systems

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

Table 3: Illumina Sequencing Systems Recommended for Use with the AmpliSeq for Illumina Focus Panel

Instrument	No. of Samples per Run (DNA and RNA combined)	Run Time
iSeq™ 100 System	8	17 hours
MiniSeq System (mid output)	16	17 hours
MiniSeq System (high output)	48	24 hours
MiSeq System (v2 chemistry Micro)	8	19 hours
MiSeq System (v2 chemistry)	30	24 hours
MiSeq System (v3 chemistry)	48	32 hours

Accurate Data

Using the AmpliSeq for Illumina Focus Panel, researchers can analyze biomarkers across 52 key solid tumor genes using DNA and RNA.

Coverage and Sensitivity

To demonstrate assay capabilities and sensitivity, an AcroMetrix control sample, Horizon Discovery (HD) samples, and FFPE tissues were evaluated using the Focus Panel on 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 evaluated for variant calling accuracy. Data showed high concordance between expected and detected variant frequency (Figure 2).

Gene Fusion Detection

To demonstrate the ability of the AmpliSeq for Illumina Focus Panel to recognize structural variants within RNA transcripts, HD samples and the Seraseq Fusion RNA Mix v2 reference were evaluated using the panel on the MiniSeq and MiSeq Systems. Results showed a 100% call rate for gene fusions within these samples (Table 4).

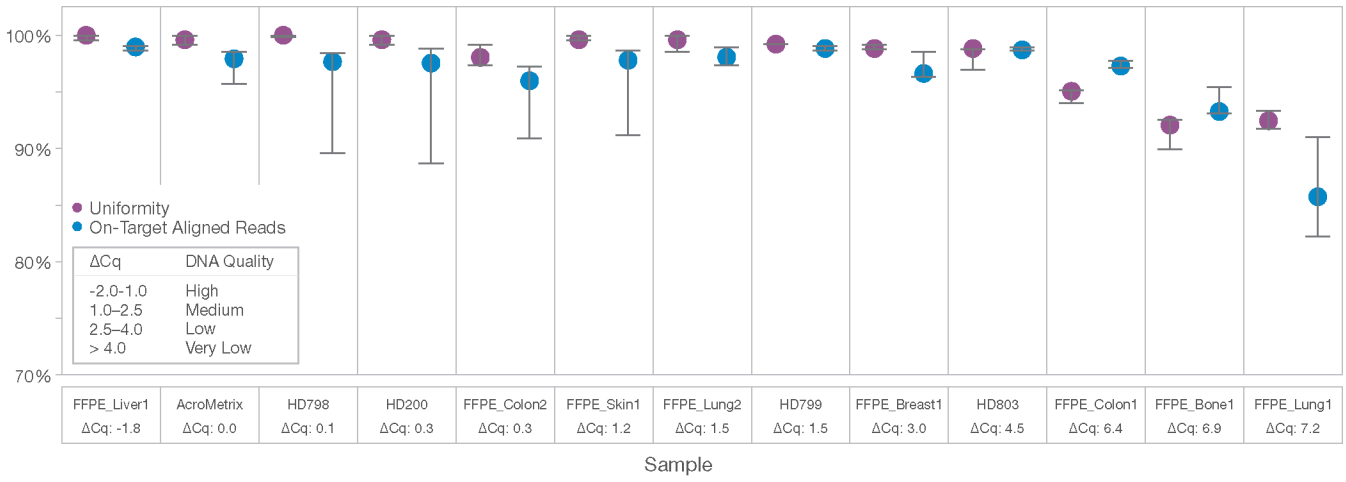

Figure 1: High Coverage Uniformity and On-Target Alignment—DNA extracted from FFPE and HD samples of varying quality was evaluated using the AmpliSeq for Illumina Focus Panel and sequenced on the MiniSeq and MiSeq Systems. Error bars indicate variability of technical replicates. ΔCq is an indicator of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

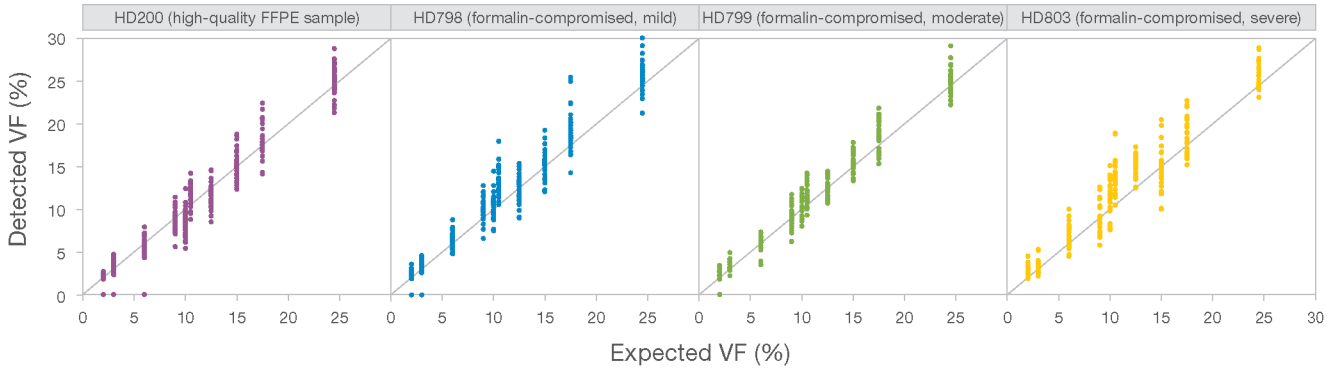

Figure 2: High Concordance Between Expected and Detected Variant Frequency—DNA from formalin-fixed HD samples was evaluated using the AmpliSeq for Illumina Focus Panel and sequenced on the MiniSeq and MiSeq Systems. Results show that 100% of expected SNVs were detected. ΔCq values are listed in Figure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

Table 4: High Call Rates 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%
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%
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 Focus Panel and sequenced on the MiniSeq and MiSeq Systems.

Learn More

To learn more about the AmpliSeq for Illumina Focus Panel, visit www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/ampliseq-focus-panel.html

To learn more about the AmpliSeq for Illumina targeted resequencing solution, read the overview at www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/ampliseq-for-illumina-targeted-resequencing-solution-data-sheet-770-2017-022.pdf

Ordering Information

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

Product	Catalog No.
AmpliSeq for Illumina Focus Panel (24 reactions)	20019164
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

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