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AmpliSeq™ for Illumina Childhood Cancer Panel

Targeted panel for investigating somatic variants associated with childhood and young adult cancer types.

Highlights

- **Relevant gene content**
  Target multiple variant types across 203 genes, including hotspots, SNVs, indels, CNVs, and gene fusions
- **Fast, streamlined workflow**
  Prepare sequencing-ready libraries in a single day from as little as 10 ng high-quality DNA or RNA
- **Accurate data**
  Detect somatic mutations down to 5% frequency using local or cloud-based analysis

Introduction

The AmpliSeq for Illumina Childhood Cancer Panel enables comprehensive genomic profiling of variants associated with childhood and young adult cancer types (Table 1). The Childhood Cancer 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.

The AmpliSeq for Illumina Childhood Cancer Panel requires as little as 10 ng high-quality DNA or RNA and is compatible with various sample types, including blood, bone marrow, and 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 in 203 genes, affording researchers the potential to unlock a wealth of genomic information from many tumor types.

Relevant gene content

Content for the AmpliSeq for Illumina Childhood Cancer Panel has been developed with leading scientists and pediatric oncologists. It includes 97 gene traslocations/fusions (> 1700 fusion isoform variants), 82 DNA variants with known relevance, 44 targets with full exon coverage (specifically tumor suppressor genes), and 24 copy number variation (CNV) targets across 203 genes (Figure 1). 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

Table 1: AmpliSeq for Illumina Childhood Cancer Panel at a glance

Parameter	Specification
No. of genes	203
Targets	97 gene translocations/fusions, 82 DNA variants, 44 full exons, and 24 CNV targets
Variant types	SNVs, indels, CNVs, translocations, and gene fusions[a]
Amplicon size	DNA: 114 bp on average, RNA: 122 bp on average
No. of amplicons	DNA: 3069, RNA: 1701
Input DNA/RNA requirement	20 ng high-quality DNA or RNA (10 ng recommended per pool)
No. of pools per panel	DNA panel: 2 pools, RNA panel: 2 pools
Supported sample types	Blood, bone marrow, FFPE tissue
Percent targets covered at minimum 500× at recommended throughput	> 95%
Coverage uniformity (percent of targets with >0.2× mean coverage)	> 90%
Percent on-target aligned reads	> 80%
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

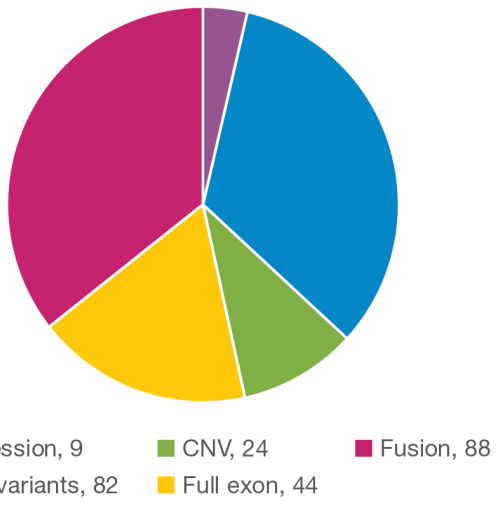

Figure 1: Overview of AmpliSeq for Illumina Childhood Cancer Panel content

Simple, streamlined workflow

The AmpliSeq for Illumina Childhood Cancer Panel is part of an integrated 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 5-6 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 a compatible 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 Integrative Genomics Viewer (IGV) app for sequence alignment and visualization. Resulting data files can be imported diretly into BaseSpace Variant Interpreter for rapid interpretation and reporting of variant data.

For FFPE samples, the AmpliSeq for Illumina Direct FFPE DNA accessory product eliminates the need for deparaffinization of FFPE tissue sections before DNA purification while minimizing sample loss. The protocol takes only two steps and requires just 30 minutes with 10 minutes of hands-on time.

Learn more about Illumina sequencing systems

Learn more about AmpliSeq for Illumina informatics

Accurate data

The AmpliSeq for Illumina Childhood Cancer Panel provides the ability to assess 203 genes per sample simultaneously, while maintaining excellent specificity and uniformity.

Coverage and Sensitivity

To demonstrate assay capabilities, an AcroMetrix control, Horizon Discovery (HD), and formalin-compromised samples were evaluated using the AmpliSeq for Illumina Childhood Cancer Panel 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 2). HD samples of varying quality were tested for variant calling accuracy. Data showed high concordance between expected and detected variant frequency (VF) (Figure 3).

Gene Fusion Detection

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

Figure 2: High Coverage Uniformity and On-Target Alignment—DNA extracted from Coriell and HD samples of varying quality was evaluated using the AmpliSeq for Illumina Childhood Cancer Panel and sequenced on the NextSeq System. NA18278 and HD701: high-quality gDNA; HD798: formalin-compromised (mild); HD799: formalin-compromised (moderate); HD803: formalin-compromised (severe).

Table 2: Illumina sequencing systems recommended for use with the AmpliSeq for Illumina Childhood Cancer Panel

Instrument	DNA samples per run	RNA samples per run	Max no. of combined DNA/RNA samples per run[a]	Recommended combined DNA:RNA pooling volume ratio[b]	Run Time
MiniSeq System (mid output)	1	8	1	5:1	17 hours
MiniSeq System (high output)	5	25	4	5:1	24 hours
MiSeq System (v2 chemistry)	3	15	2	5:1	24 hours
MiSeq System (v3 chemistry)	5	25	4	5:1	32 hours
NextSeq System (mid output)	27	96	22	5:1	26 hours
NextSeq System (high output)	83	96	48	5:1	29 hours

a. For combined DNA/RNA samples, paired DNA and RNA from the same source sample are used to generate two libraries, one from each nucleic acid, that are separately indexed.
b. Recommended DNA to RNA pooling ratio is based on the read coverage ratio.

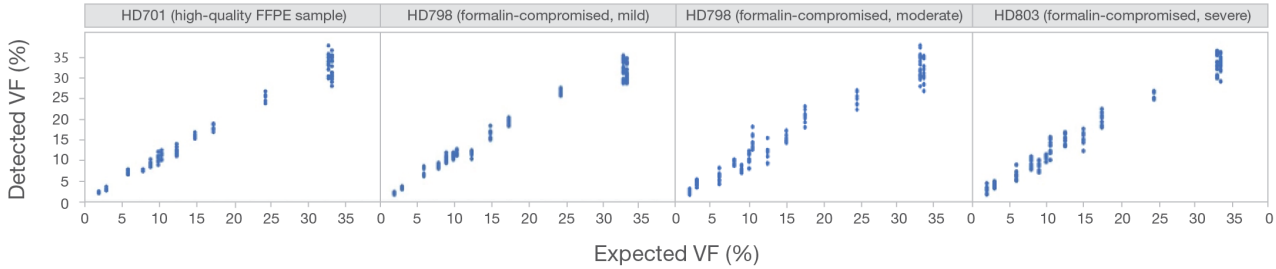

Figure 3: High Concordance Between Expected and Detected Variant Frequency—DNA from HD samples of varying quality was prepared using the AmpliSeq for Illumina Childhood Cancer Panel and sequenced on the NextSeq System.

Table 3: High call rate for gene fusions[a]

Fusion	No. samples NOT detected	No. samples detected	Call rate
TPM3-NTRK1	0	16	100%
LMNA-NTRK1	0	16	100%
SLC45A3-BRAF	0	16	100%
EML4-ALK	0	16	100%
FGFR3-TACC3	0	16	100%
FGFR3-BAIAP2L1	0	16	100%
SLC34A2-ROS1	0	16	100%
CD74-ROS1	0	16	100%
EGFR-SEPT14	0	16	100%
KIF5B-RET	0	16	100%
NCOA4-RET	0	16	100%
ETV6-NTRK3	0	16	100%
MET exon 14 skipping	0	16	100%
EGFR variant III	0	16	100%

a. Seraseq Fusion RNA Mix v3, a fusion-positive RNA sample, was used to generate RNA libraries with the AmpliSeq for Illumina Childhood Cancer Panel and sequenced on the MiniSeq System.

Ordering information

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

Product	Catalog No.
AmpliSeq for Illumina Childhood Cancer Panel (24 reactions)	20028446
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 Childhood Cancer Panel at www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/ampliseq-childhood-cancer-panel.html

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

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