

AmpliSeq™ for Illumina Custom RNA Fusion Panel

Fusion Panel

Custom panel of 12-1200 targets selected from a menu of > 1000 fusion genes and > 20,000 human RefSeq genes for fusion detection and gene expression profiling in a single assay.

Highlights

- **Fast fusion detection and gene expression profiling**

Prepare libraries in 6 hours with < 1.5 hours hands-on time; results available in < 2 days

- **Low-quality, low-quantity sample input**

Obtain high-quality data even when starting with minimal input from FFPE tissues

- **RNA-to-analysis solution**

Take advantage of a comprehensive workflow that spans library preparation, sequencing, and analysis

Introduction

The AmpliSeq for Illumina Custom RNA Fusion Panel is a targeted resequencing assay that enables detection of fusion genes and quantitation of gene expression levels (Table 1). It requires as little as 1 ng high-quality RNA, and is compatible with various sample types, including blood and formalin-fixed, paraffin-embedded (FFPE) tissues. The Custom RNA Fusion 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. Taking advantage of this streamlined workflow, researchers can focus their studies on gene fusions, decreasing input requirements while obtaining highly sensitive, accurate results.

Relevant gene content

The AmpliSeq for Illumina Custom RNA Fusion Panel can be customized with 12–1200 targets selected from > 1000 well-annotated fusion genes and > 20,000 quantitative gene expression assays for human RefSeq genes. This custom-designed panel saves researchers the time and effort of designing amplicons and optimizing performance.



Access a [complete list of genes on the AmpliSeq for Illumina Custom RNA Fusion Panel](#) >

Table 1: AmpliSeq for Illumina Custom RNA Fusion Panel at a glance

Parameter	Specification
No. of genes	Customize from > 1000 well-annotated gene fusions and > 20,000 quantitative gene expression assays
Variant types	Differential gene expression, gene fusions
Target insert size	150 bp
No. of amplicons	12-1200
Input RNA requirement	1-100 ng RNA (10 ng recommended per pool)
No. of pools per panel	1-2 pools
Compatible sample types	Blood, FFPE tissue, cell lines
Total assay time ^a	6 hours
Hands-on time	< 1.5 hours
RNA-to-data time	2.5 days

a. 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 Custom RNA Fusion Panel is part of a comprehensive solution that offers streamlined content, easy-to-perform library preparation, push-button sequencing systems, and simplified data analysis.

Library preparation begins with the conversion of total RNA to cDNA, followed by a straightforward, PCR-based protocol that can be completed in as little as 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 RNA Amplicon analysis workflow for gene fusion detection and gene expression profiling.

Table 2: Illumina sequencing systems recommended for use with the AmpliSeq for Illumina Custom RNA Fusion Panel

Instrument	No. of Samples per Run ^a	Run Time
iSeq™ 100 System	13	17 hours
MiniSeq System (high output)	83	24 hours
MiSeq System (v3 chemistry)	83	32 hours
NextSeq System (mid output)	384	26 hours

a. The number of samples per run for each system is based on an estimated 300,000 reads per sample for robust fusion detection.

Learn more about [Illumina sequencing systems](#)Learn more about [AmpliSeq for Illumina informatics](#)

Accurate data

The AmpliSeq for Illumina Custom RNA Fusion Panel provides the ability to detect gene fusions and analyze differential gene expression across selected target genes. To demonstrate the assay's ability to detect fusions, the Seraseq Fusion RNA Mix v3 reference was evaluated using two independent AmpliSeq for Illumina Custom RNA Fusion Panels and the MiSeq™ System. Data were analyzed using the RNA Amplicon analysis workflow in BaseSpace Sequence Hub. Results showed a high fusion call rate for both panels (Table 3).

Table 3: High call rate for gene fusions^a

Fusion ^a	Custom RNA Fusion Panel A (+12 gene expression targets)	Custom RNA Fusion Panel B (+28 gene expression targets)	Call rate
<i>EML4-ALK</i>	✓	✓	100%
<i>KIF5B-RET</i>	✓	✓	100%
<i>NCOA4-RET</i>	—	✓	100%
<i>CD74-ROS1</i>	✓	✓	100%
<i>SLC34A2-ROS1</i>	✓	✓	100%
<i>TPM3-NTRK1</i>	✓	✓	100%
<i>FGFR3-BAIAP2L1</i>	✓	✓	100%
<i>PAX8-PPARG1</i>	—	✓	100%
<i>FGFR3-TACC3</i>	—	—	—
<i>ETV6-NTRK3</i>	✓	✓	100%
<i>LMNA-NTRK1</i>	—	—	—
<i>SLC45A3-BRAF</i>	✓	✓	100%
<i>TPRSS2-ERG</i>	✓	✓	100%
<i>EGFR-SEPT14</i>	✓	✓	100%
Reference fusions detected (expected/observed)	10/10	12/12	

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

Ordering information

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

Product	Catalog No.
AmpliSeq for Illumina Custom RNA Fusion Panel	designstudio.illumina.com
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 CD Indexes Set B (96 indexes, 96 samples)	20019106
AmpliSeq for Illumina CD Indexes Set C (96 indexes, 96 samples)	20019107
AmpliSeq for Illumina CD Indexes Set D (96 indexes, 96 samples)	20019167
AmpliSeq for Illumina CD Indexes Set A–D (384 indexes, 384 samples)	20031676
AmpliSeq for Illumina UD Indexes (24 indexes, 24 samples)	20019104
AmpliSeq for Illumina cDNA Synthesis (96 reactions)	20022654
AmpliSeq for Illumina Library Equalizer	20019171

Learn more

Learn more about [the AmpliSeq for Illumina Custom RNA Fusion Panel](#)Learn more about [the AmpliSeq for Illumina targeted sequencing solution](#)