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AmpliSeq™ for Illumina Immune Response Panel

RNA panel for investigating 395 genes involved in tumor-immune system interactions.

Highlights

- **Relevant gene content**
  Focus studies on 395 genes carrying cancer biomarkers indicative of immunotherapy response
- **Fast, streamlined workflow**
  Prepare sequencing-ready libraries in a single day from as little as 1 ng high-quality RNA or 10 ng RNA from FFPE tissue
- **Accurate data**
  Detect expression of genes involved in tumor-immune response interactions

Introduction

Understanding expression of cancer biomarkers may be useful in predicting the success of certain immunotherapy treatments.[1] To assist with this endeavor, Illumina offers researchers the AmpliSeq for Illumina Immune Response Panel, a targeted resequencing assay for quantitating expression of cancer biomarkers in 395 genes involved in tumor–immune system interactions (Table 1).

The Immune Response panel is part of an integrated workflow that includes AmpliSeq for Illumina PCR-based library preparation, Illumina sequencing by synthesis (SBS) chemistry and next-generation sequencing (NGS) technology, and automated analysis. Starting with as little as 10 ng input RNA, the panel enables researchers to detect biomarkers associated with different leukocyte subsets, antigen presentation, checkpoint pathways, and tumor progression. The low-input requirement enables use with samples of varying quality, including formalin-fixed, paraffin-embedded (FFPE) tissues. As part of the AmpliSeq for Illumina targeted resequencing solution, the Immune Response panel enables quick and accurate assessment of gene expression for translational and clinical research studies.

Relevant gene content

Content for the AmpliSeq for Illumina Immune Response Panel was selected based on input from multiple sources, including peer-reviewed articles noting potentially predictive markers for drug response, experts at the Japan National Cancer Center, pharmaceutical companies, public data bases such as the Database for Annotation, Visualization, and Integrated Discovery (DAVID), and the registry for clinical trials (clinicaltrials.gov). The resulting panel targets 395 genes expressed in the tumor microenvironment (Table 2). This ready-to-use panel saves researchers the time and effort of identifying targets, designing amplicons, and optimizing performance.

Table 1: AmpliSeq for Illumina Immune Response Panel at a glance

Parameter	Specification
No. of genes	395
Targets	Genes associated with immune response across multiple functional genes
Cumulative target size	42 kb
Analysis types	Gene expression levels, including nonexpressing and low-expressing genes
Amplicon size	106 bp on average
No. of amplicons	398
Input RNA requirement	1-100 ng (10 ng recommended)
No. of pools per panel	1
Supported sample types	FFPE tissue, blood
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

🔗 Access a complete list of genes on the AmpliSeq for Illumina Immune Response Panel

Simple, streamlined workflow

The AmpliSeq for Illumina Immune Response Panel is part of a RNA-to-results 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 any compatible Illumina sequencing systemthe (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 RNA Amplicon analysis workflow for analysis. The RNA Amplicon analysis workflow aligns reads against regions specified in the manifest file, quantifies the relative expression of genes and isoforms between several samples, and compares abundance across samples. Output provides identification of transcript expression and differential expression results.

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Table 2: Gene list for the AmpliSeq for Illumina Immune Response Panel

Lymphocyte Regulation	Lymphocyte Markers
Antigen presentation	B cell marker
Antigen processing	Dendritic cell
Innate immune response	Dendritic cell, macrophage
Leukocyte inhibition	Helper T cells
Leukocyte migration	Macrophage
Lymphocyte activation	Myeloid marker
Lymphocyte development	Neutrophil
Lymphocyte infiltration	NK cell activation
B cell receptor signaling	NK cell marker
T cell receptor signaling	T cell differentiation
T cell regulation	**Checkpoint Pathway**
TCR coexpression	Checkpoint pathway
Cytokine Signaling	PD-1 signaling
Chemokine signaling	Drug target
Cytokine signaling	**Tumor Characterization**
Interferon signaling	Adhesion, migration
Type I interferon signaling	Apoptosis
Type II interferon signaling	Proliferation
Housekeeping	Tumor antigen
Housekeeping	Tumor marker

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🔗 Learn more about AmpliSeq for Illumina informatics

Table 3: Illumina sequencing systems recommended for use with the AmpliSeq for Illumina Immune Response Panel

Instrument	No. of Samples per Run	Run Time
MiniSeq™ System (mid output)	8	17 hours
MiniSeq System (high output)	24	24 hours
MiSeq System (v2 chemistry)	16	24 hours
MiSeq System (v3 chemistry)	24	32 hours
NextSeq System (mid output)	96	26 hours

Accurate data

The AmpliSeq for Illumina Immune Response Panel enables investigation of genes involved in the tumor microenvironment and immune response system. To demonstrate assay accuracy, RNA isolated from lung tissue was analyzed in replicate using the Immune Response panel and the NextSeq™ System. Results show high concordance ($R^2 = 0.98$) between the two samples (Figure 1).
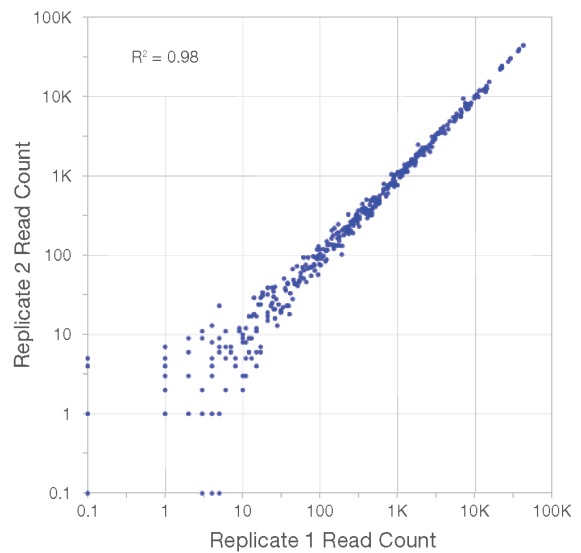

Figure 1: High Concordance Between Replicates — Libraries were prepared using RNA isolated from lung tissue and the AmpliSeq for Illumina Immune Response Panel and sequenced on the NextSeq System. A reproducibility plot shows high correlation between two replicates of the same RNA sample. R^2 is a statistical measurement of data correlation.

Ordering information

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

Product	Catalog No.
AmpliSeq for Illumina Immune Response Panel (24 reactions)	20019169
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 Sample ID Panel	20019162
AmpliSeq for Illumina Direct FFPE DNA	20023378
AmpliSeq for Illumina Library Equalizer	20019171

Learn more

Learn more about the AmpliSeq for Illumina Immune Response Panel

Learn more about the AmpliSeq for Illumina targeted sequencing solution

References

1. Masucci GV, Cesano A, Hawtin R, et al. Validation of biomarkers to predict response to immunotherapy in cancer: Volume I - pre-analytical and analytical validation. *J Immunother Cancer*. 2016;4:76.

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