

AmpliSeq™ for Illumina Immune Response Panel

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

- Focus studies on 395 genes carrying cancer biomarkers indicative of immunotherapy response
- Prepare sequencing-ready libraries in one day from 1 ng high-quality RNA or 10 ng RNA from FFPE tissue
- 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.

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.

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 |
| Compatible sample types | FFPE tissue |
| 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.



Access a complete [list of genes on the AmpliSeq for Illumina Immune Response Panel](#)

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 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 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.

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 |



Learn more about Illumina sequencing systems
illumina.com/systems



Learn more about AmpliSeq for Illumina informatics,
illumina.com/products/by-brand/ampliseq/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 hrs |
| MiniSeq System (high output) | 24 | 24 hrs |
| MiSeq System (v2 chemistry) | 16 | 24 hrs |
| MiSeq System (v3 chemistry) | 24 | 32 hrs |
| NextSeq 550 System (mid output) | 96 | 26 hrs |

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).

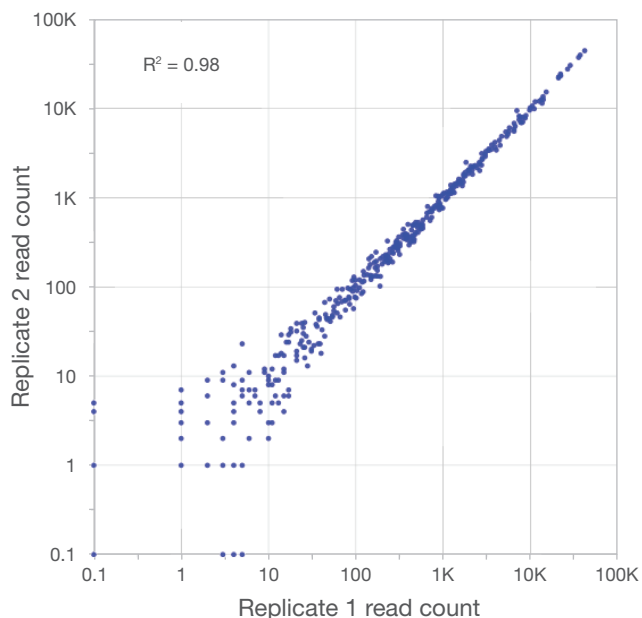


Figure 1: High concordance between replicates—Libraries were prepared using RNA isolated from lung tissue and the AmpliSeq for Illumina Immune Response Panel and sequenced on the NextSeq System. A reproducibility plot shows high correlation between two replicates of the same RNA sample. R² is a statistical measurement of data correlation.

Learn more

AmpliSeq for Illumina Immune Response Panel, illumina.com/products/by-type/sequencing-kits/library-prep-kits/ampliseq-immune-response-panel.html

AmpliSeq for Illumina targeted sequencing solution, illumina.com/products/by-brand/ampliseq.html



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Ordering information

Order AmpliSeq for Illumina products online at 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 |

References

- Masucci GV, Cesano A, Hawtin R, et al. Validation of biomarkers to predict response to immunotherapy in cancer: Volume 1 - pre-analytical and analytical validation. *J Immunother Cancer*. 2016;4:76. Published 2016 Nov 15. doi:10.1186/s40425-016-0178-1