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AmpliSeq™ for Illumina TCR beta-SR Panel

Targeted panel for measuring T cell diversity and clonal expansion in FFPE tumor samples by sequencing T cell receptor beta chain rearrangements.

Highlights

- **High-value content**
  Target the TCR beta chain, with up to 80-bp read-length amplicons for coverage of CDR3
- **Simple, streamlined workflow**
  Prepare sequencing-ready libraries in a single day from as little as 10 ng of input DNA or RNA from FFPE tumor samples
- **Accurate data**
  Obtain high-quality data, even with minimal input from FFPE samples

Introduction

The T cell receptor (TCR) is a transmembrane heterodimer that enables T cells to recognize and respond to foreign "nonself" material. The vast majority of TCRs consist of an alpha and beta chain that contain complementary determining regions (CDRs). T cell receptor (TCR) diversity describes the potential of a small set of genes encoding the CDRs within the TCR to create in excess of 10^{12} T cell clonotypes (populations of T cells expressing identical TCRs) by recombination, random insertion, deletion, and substitution. TCR diversity plays a vital role in host defense. Investigation of this diversity may be useful in understanding immune function, autoimmune diseases, and immune-mediated adverse effects (IMAEs).

The AmpliSeq for Illumina TCR beta-SR Panel is a highly multiplexed resequencing panel designed to measure T cell diversity and clonal expansion in formalin-fixed paraffin-embedded (FFPE) tumor samples by sequencing TCR beta chain rearrangements (Table 1). Using a single pool of multiplex PCR primers, library reagents, and sample barcodes, libraries can be generated from DNA/RNA extracted from FFPE tissue, fresh/frozen tissue, whole blood, or fluorescence-activated cell sorting (FACS)-sorted cells for sequencing on compatible Illumina sequencing systems.

The AmpliSeq for Illumina TCR beta-SR 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. By taking advantage of this streamlined workflow, researchers can focus their studies on RNA coding regions, decreasing input requirements while obtaining accurate results with high analytical sensitivity.

Table 1: AmpliSeq for Illumina TCR beta-SR Panel at a glance

Parameter	Specification
No. of genes	Variable
Targets	TCR beta chain, CDR3
Amplicon size	~80 bp
No. of amplicons	Variable
Input DNA/RNA requirement	10-1000 ng
No. of pools per panel	1
Compatible sample types	Blood, fresh/frozen tissue, FACS cells, FFPE tissue
Total assay time[a]	6 hours
Hands-on time	< 1.5 hours
DNA/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

High-value content

The AmpliSeq for Illumina TCR beta-SR Panel offers coverage of the TCR beta chain, with up to 80 bp read-length amplicons for characterization of CDR3. 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 TCR beta-SR Panel is part of a DNA/RNA-to-repertoire classification 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 , 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 easily streamed into BaseSpace™ Sequence Hub for analysis. The MiXCR Immune Repertoire Analyzer app in BaseSpace Sequence Hub enables fast and accurate processing of sequencing data from T cell receptor libraries. It aligns reads against germline segments, assembles clonotypes, and corrects for PCR and sequencing errors. Output provides detailed information on germline segment assignments, alignment, and mutations.

Table 2: Illumina sequencing systems recommended for use with the AmpliSeq for Illumina TCR beta-SR Panel

Instrument
MiniSeq™ System (high output)
MiSeq™ System (v3 chemistry)
NextSeq System (mid output)
NextSeq System (high output)

Learn more about Illumina sequencing systems

Learn more about AmpliSeq for Illumina informatics

Accurate data

The AmpliSeq for Illumina TCR beta-SR Panel enables investigation of TCR diversity in FFPE samples. To demonstrate assay capabilities, samples were analyzed using the AmpliSeq for Illumina TCR beta-SR Panel, the NextSeq™ System, and the MiXCR Immune Repertoire Analyzer app. Results show that control sample (PBMCs) and melanoma FFPE samples have high levels of clone counts, (Figure 1) demonstrating compatibility for clonal detection in FFPE samples.

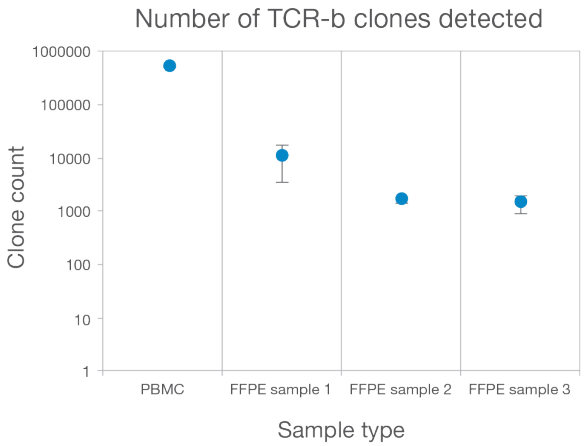

Figure 1: Clonal detection in FFPE samples—Libraries were prepared using the AmpliSeq for Illumina TCR beta-SR Panel, sequenced on the NextSeq System, and analyzed with the MiXCR app. Input RNA from 100 ng of PBMC sample or 50 ng of three individual melanoma FFPE samples show high level clone counts in each sample. Error bars indicate variability between replicates.

Ordering information

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

Product	Catalog No.
AmpliSeq for Illumina TCR beta-SR Panel (24 reactions)	20031675
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 Library Equalizer	20019171

Learn more

Learn more about the AmpliSeq for Illumina TCR beta-SR Panel at www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/ampliseq-tcr-beta-panel.html

Learn more about the AmpliSeq for Illumina targeted sequencing solution at science-docs.illumina.com/documents/LibraryPrep/ampliseq-technology-overview-data-sheet-770-2017-022/Content/Source/Library-Prep/AmpliSeq/ampliseq-technology-overview/ampliseq-technology-overview.html

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