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Analysis with the AmpliSeq™ for Illumina Immune Repertoire Plus, TCR beta Panel

Targeted RNA panel enables detecti on and quantitation of TCR beta expressing clones with exceptional accuracy and specificity.

Introduction

The AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel is a highly multiplexed targeted resequencing panel designed to measure T cell diversity and clonal expansion by sequencing T cell receptor (TCR) beta chain rearrangements (Figure 1).[1] Using a single pool of multiplex PCR primers, library reagents, and sample barcodes, libraries can be generated from RNA extracted from whole blood, fresh/frozen tissue, or fluorescence activated cell sorting (FACS)-sorted cells for sequencing on compatible Illumina sequencing systems.

Resulting data can be easily streamed into BaseSpace™ Sequence Hub for analysis. The third-party MiXCR Immune Repertoire Analyzer App[2] in BaseSpace Sequence Hub enables fast and accurate processing of sequencing data from T and B 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.

This application note demonstrates the exceptional performance, accuracy, and specificity of the AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel in detecting and quantitating TCR beta expressing clones, enabled by the MiXCR Immune Repertoire Analyzer App.

The MiXCR Immune Repertoire Analyzer App

Output from the MiXCR App includes a tab-delimited clonotype table with the following information:

- Detailed information on germline segment assignments

- Alignment and mutations

- CDR3 sequences

- Clonotype abundances

Output also includes various interactive reports displaying variable and joining segment usage, spectratype (measuring global TCR diversity), and sample clonality.
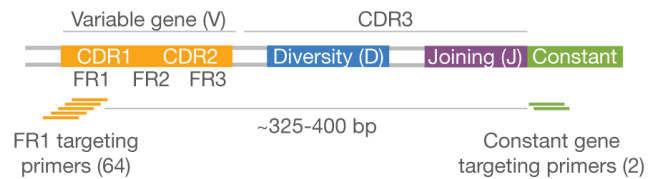
Figure 1: The AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel assay— The AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel targets the FR1 region of the variable gene and the constant gene region of TCR beta-VDJ rearrangements, producing a 330 bp amplicon.[3]

Accurate data

Detection and quantitation of TCR beta clones

To demonstrate the exceptional analytical sensitivity of the AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel, Jurkat cell RNA was serially diluted into peripheral blood mononuclear cell (PBMC) RNA from four different donors in ratios from 1:10-1:10,000. AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel libraries were prepared and sequenced on the MiSeq™ System at 2 × 301 bp. Resulting data were analyzed with the MiXCR App to determine TCR beta clone counts. Results showed that the limit of detection for this assay is 0.01%, or 10 pg of RNA (Figure 2). In a similar serial dilution experiment of Jurkat RNA into donor PBMC RNA, TCR beta clonotypes were quantitated for each dilution point. Results showed that the relative percentages of each TCR clone remained unchanged across dilution points (Figure 3).
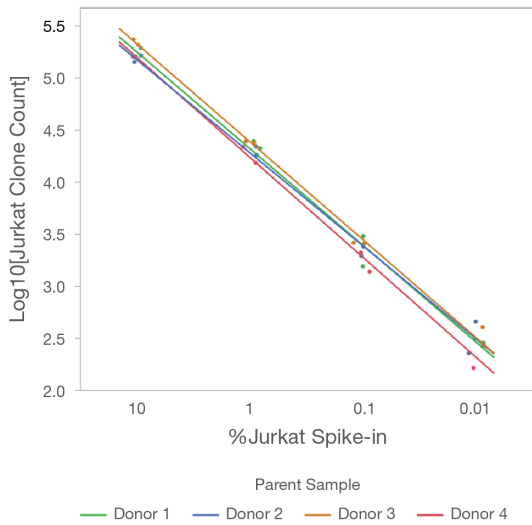
Clonal diversity as a function of input and read depth

To examine the effects of input and read depth on TCR beta clonal diversity, AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel libraries were prepared from 10–1000 ng of peripheral blood leukocyte (PBL) RNA and sequenced on the NextSeq™ 550 System at 2 × 151 bp to generate 400M total paired end (PE) reads and 30M PE reads per sample. Resulting data were then downsampled to evaluate the effect of read depth on clonotype diversity. Results showed that at low inputs, the clonotype diversity count reaches saturation at lower sequencing depth than high input samples, indicating more clonal diversity with higher input (Figure 4 and Table 1). Therefore, shallow read depth is more suited for lower input samples than higher input samples. Based on this analysis, it was also determined that sequencing depth will impact the number of clones reported. Suggested inputs, read depths, and recommended multiplexing for both the MiSeq and NextSeq 550 Systems are indicated (Table 1).
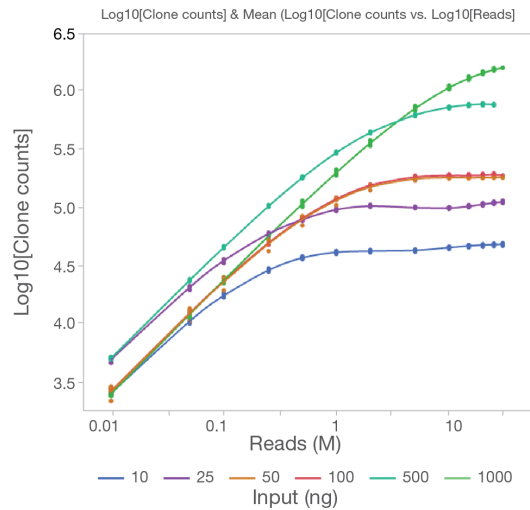

Table 1: TCR beta clone counts and recommended run settings

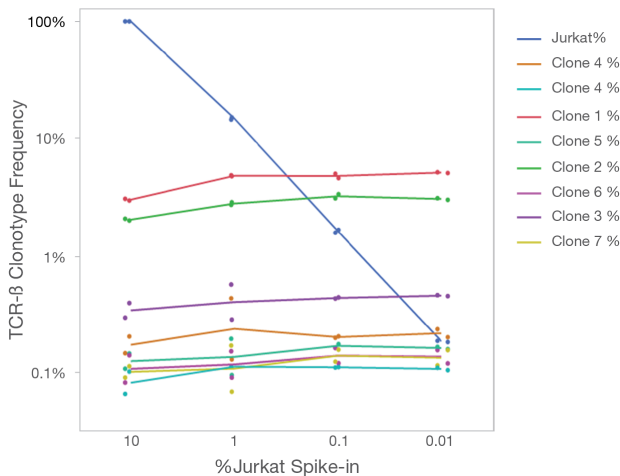| PBL input amount	Number of reads													Libraries per MiSeq run	Libraries per NextSeq run
	10KPE	50KPE	100KPE	250KPE	500KPE	1MPE	2MPE	5MPE	10MPE	15MPE	20MPE	25MPE	30MPE		
10 ng	2528	10,502	17,375	28,742	36,755	**40,947**	41,923	42,537	44,968	46,485	47,246	47,728	47,888	20	—
25 ng	4922	20,512	34,670	59,012	78,207	**95,088**	101,979	98,733	97,942	101,681	105,647	108,744	110,997	20	—
50 ng	2618	12,316	22,840	48,518	78,599	114,282	148,865	**174,563**	178,789	178,415	178,870	179,508	180,103	4	80
100 ng	2641	12,364	23,196	49,359	80,306	117,390	153,865	181,106	**186,098**	186,172	187,691	189,394	186,354	2	40
500 ng	5043	23,610	44,953	101,671	178,817	293,048	434,518	615,991	718,552	**752,406**	765,531	757,701		1	27
1000 ng	2493	12,055	23,562	56,348	106,549	197,589	354,726	701,768	1,056,648	1,270,864	1,410,682	1,508874	**1,565,544**	—	13

Data generated from a 2 × 150 bp sequencing run. The AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel is compatible with read lengths up to 2 × 300 bp.

Figure 2: Detection of TCR beta clones with high sensitivity— The AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel enables detection of TCR beta clones with a limit of detection of 0.01% (10 pg RNA).


Figure 3: Quantitation TCR beta clones with high accuracy— Varying levels of Jurkat RNA do not affect quantitation of TCR beta clones in a donor PBMC RNA sample with the AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel.

Figure 4: Clonal diversity as a function of input and read depth— At low input amounts TCR beta clonal diversity (y-axis) reaches saturation at lower sequencing depths (x-axis) than higher input amounts.

High specificity to TCR beta-expressing cells

To demonstrate specificity of the AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel to TCR beta-expressing cells, T cells (TCR beta positive cells) and B cells (TCR beta negative cells) were isolated from PBMCs by FACS based on their cell surface marker expression (CD4+/CD8+ and CD19+, respectively). RNA was extracted from the cells and AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel libraries were prepared from 10ng RNA input. Libraries were sequenced on the MiSeq System at 2 × 250 bp, and the data were analyzed with the MiXCR App. The results showed a high percent of successfully aligned reads for TCR beta positive cells, compared to low percent of aligned reads for TCR beta negative cells (Figure 5), demonstrating proper generation and utilization of library reads to the targeted TCR beta chain.
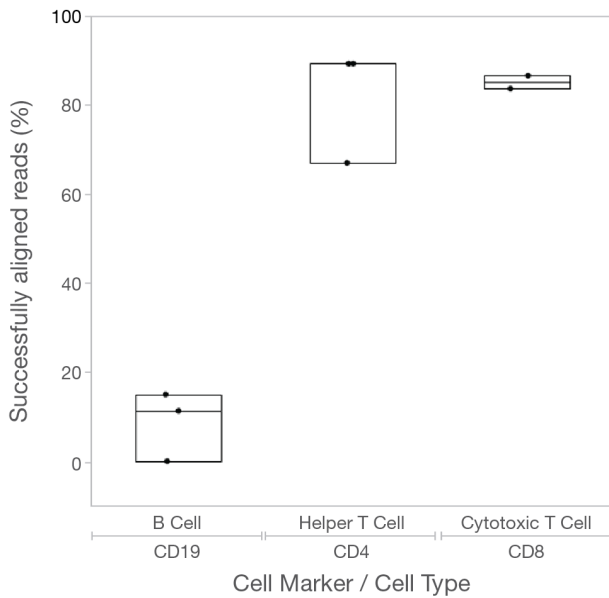
Figure 5: Detection of TCR beta-expressing cells with high specificity— The AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel is highly specific to TCR beta expressing cells, as a high percentage of aligned reads are produced from T cells (TCR beta positive) but not B cells (TCR beta negative).

Summary

The AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel is designed to measure T cell diversity and clonal expansion by sequencing T cell receptor (TCR) beta chain rearrangements. Data analysis with the MiXCR Immune Repertoire Analyzer App enables fast and accurate processing of sequencing data from T cell receptor libraries. This application note demonstrates the performance, accuracy, and specificity of the AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel in detecting and quantitating TCR beta clones.

Ordering Information

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

Product	Catalog no.
AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel (24 reactions)	20024479
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

Learn More

To learn more about the AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel, visit www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/ampliseq-immune-repertoire-panel.html

References

1. AmpliSeq for Illumina Immune Repertoire Plus, TCR beta Panel Data Sheet. www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/ampliseq-immune-repertoire-panel-data-sheet-770-2018-015.pdf. Accessed September 6, 2018.
2. Bolotin DA, Poslavsky S, Mitrophanov I, et al. MiXCR: software for comprehensive adaptive immunity profiling. Nat Methods. 2015;12 (5):380–381.
3. Thermo Fisher. Ion AmpliSeq Immune Repertoire Assay Plus, TCRβ User Guide. Accessed October 1, 2018.

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