

TRB Assay

Assay Uses

This CE-marked T Cell Receptor Beta (*TRB*) Assay identifies clonal *TRB* (V-(D)-J) rearrangements, the associated (V-(D)-J) region DNA sequences, and provides the frequency distribution of V, D, and J segment utilization using the Illumina® MiSeq® platform.

Analysis of the *TRB* locus increases the probability of identifying T cell receptor gene rearrangements, as compared to testing for *TRG* gene rearrangements only. As a result, combining the analysis of *TRB* and *TRG* loci increases the sensitivity of clonality detection.

Background

The LymphoTrack Dx *TRB* Assay represents a significant improvement over clonality assessment by fragment analysis by providing two important and complementary uses:

1. Detection of initial clonal populations.
2. Identification of sequence information required to track clonal rearrangements in subsequent samples.

The human *TRB* gene locus on chromosome 7 (7q34) includes 65 V_{β} (variable) gene segments, followed by two separate clusters of genes each containing a D_{β} (diversity) gene, several J_{β} (joining) genes, and a C_{β} (constant) region spread over 685 kilobases. The two C_{β} genes, *TRBC1* and *TRBC2*, encode highly homologous products with no functional difference.¹

During lymphoid cell development, antigen receptor genes undergo somatic gene rearrangements. Specifically, during T cell development genes encoding TRB molecules are assembled from multiple polymorphic gene segments that generate (V-(D)-J) combinations unique in both length and sequence.²

Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, all leukemias and lymphomas generally share one or more cell-specific or "clonal" antigen receptor gene rearrangements. Therefore, tests that detect *TRB* clonal rearrangements can be useful in the study of B- and T-cell malignancies.



For illustrative purposes only.

Specimen Requirement

50 ng of high-quality genomic DNA.

Method

In contrast to conventional fragment analysis assays for clonality that utilizes two master mixes, this Next Generation Sequencing (NGS) assay contains a single multiplex master mix to target conserved V, D, and J regions of the *TRB* gene locus described in lymphoid malignancies. This reduces DNA sample requirements and simplifies the testing workflow. The LymphoTrack Dx *TRB* master mix primers are also designed with Illumina® adapters and 8 indices (Kit A) or 24 indices (Panel). This allows amplicons generated from different indexed *TRB* master mixes to be pooled into a single sequencing library.

The associated LymphoTrack Dx Software* is capable of sorting complex NGS data by gene target. This offers a second layer of multiplexing to reduce per sample testing costs by allowing amplicons from any LymphoTrack Dx Assay (e.g. *IGH*, *TRG*, *IGK*) to be sequenced on the same flow cell. In addition, the LymphoTrack Dx Software provides easy visualization of data; and the LymphoTrack MRD Software** allows identified sequences to be tracked and monitored in subsequent samples.

References

1. JE Miller et al., *Molecular Genetic Pathology* (2nd Ed., sections 30.2.7.13 and 30.2.7.18). New York, USA: Springer Science & Business Media (2013).
2. EP Rock et al., *J Exp Med* 179 (1): 323-8 (1994).

Ordering Information

CATALOG #	PRODUCTS	QUANTITY
9-225-0009	LymphoTrack® Dx <i>TRB</i> Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
9-225-0019	LymphoTrack® Dx <i>TRB</i> Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
9-500-0009	LymphoTrack® Dx Software* - MiSeq®	1 CD complimentary with purchase
7-500-0008	LymphoTrack® MRD Software**	1 CD complimentary with purchase

*Only available with purchase of a LymphoTrack Dx Assay for the MiSeq.

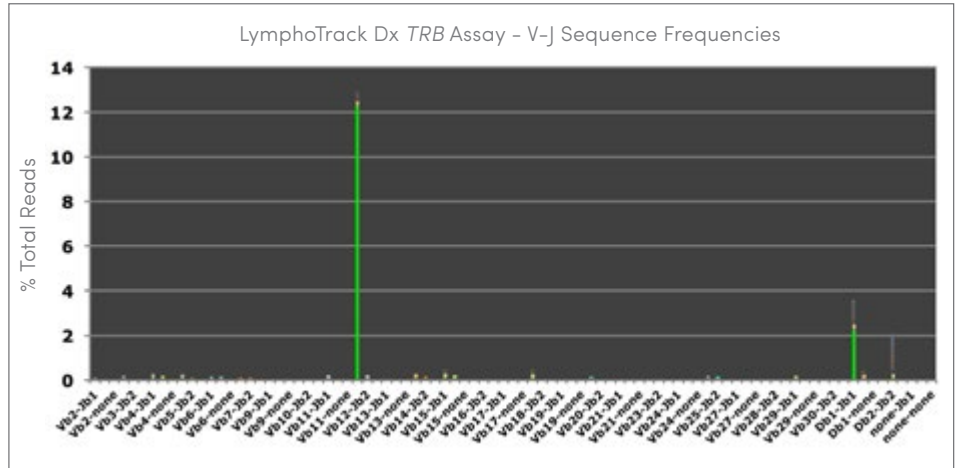
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Figure 1: Depicted are the variable (V_{β}), diversity (D_{β}), and joining (J_{β}) gene regions involved in *TRB* gene rearrangements, in addition to the downstream consensus (C_{β}) gene regions.

Figure 2: The LymphoTrack Dx Software - MiSeq[®] provides a stacked bar graph depicting the relative frequencies for the 200 most prevalent V_{β} - J_{β} rearrangements sequenced and identified in the sample.



Reagents - MiSeq[®] detection

Kit A components	
Master Mix Name	Index #
TRB MiSeq 01	A001
TRB MiSeq 02	A002
TRB MiSeq 03	A003
TRB MiSeq 04	A004
TRB MiSeq 05	A005
TRB MiSeq 06	A006
TRB MiSeq 07	A007
TRB MiSeq 08	A008
Controls	
TRB POS (+) Qty. 1	NGS NEG (-) Qty. 1

Panel components (includes all master mixes from Kit A plus the items below)			
Master Mix Name	Index #	Master Mix Name	Index #
TRB MiSeq 09	A009	TRB MiSeq 18	A018
TRB MiSeq 10	A010	TRB MiSeq 19	A019
TRB MiSeq 11	A011	TRB MiSeq 20	A020
TRB MiSeq 12	A012	TRB MiSeq 21	A021
TRB MiSeq 13	A013	TRB MiSeq 22	A022
TRB MiSeq 14	A014	TRB MiSeq 23	A023
TRB MiSeq 15	A015	TRB MiSeq 25	A025
TRB MiSeq 16	A016	TRB MiSeq 27	A027
Controls			
TRB POS (+) Qty. 3		NGS NEG (-) Qty. 3	

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