

# IGH Assays

## Assay Uses

These next-generation sequencing assays identify clonal *IGH* framework (FR) 1, 2 & 3  $V_H - J_H$  rearrangements, the DNA sequences of these rearrangements, and provide the distribution frequency of  $V_H$  and  $J_H$  segment utilization using the Illumina® MiSeq® platform. In addition, the LymphoTrack *IGH* FR1 master mixes provide the degree of *IGHV* somatic hypermutation (SHM).

## Background

The immunoglobulin heavy chain (*IGH*) gene locus on chromosome 14 (14q32.3) includes 46–52 functional and 30 non-functional variable ( $V_H$ ) gene segments, 27 functional diversity ( $D_H$ ) gene segments, and 6 functional joining ( $J_H$ ) gene segments spread over 1250 kilobases. The  $V_H$  gene segments contain three conserved framework (FR) and two variable complementarity-determining regions (CDRs). Targeting all three FRs significantly reduces the risk of not detecting the presence of clonality due to SHM in the primer binding sites thus impeding DNA amplification (Evans PA, *et al.*, 2007).

During development of lymphoid cells, the antigen receptor genes undergo somatic gene rearrangement (Tonegawa S. *et al.*, 1983). For example, during B-cell development, genes encoding the *IGH* molecules are assembled from multiple polymorphic gene segments that undergo rearrangements and selection, generating  $V_H - D_H - J_H$  combinations that are unique in both length and sequence. 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 *IGH* clonal rearrangements can be useful in the study of B- and T-cell malignancies.

## Specimen Requirement

50 ng of high quality genomic DNA per framework master mix.



For illustrative purposes only.

## Method

Each multiplex master mix for *IGH* targets one of the conserved framework regions (FR1, FR2, or FR3) within the  $V_H$  and the  $J_H$  regions described in lymphoid malignancies. Primers included in the master mixes are designed with Illumina adapters and up to 24 different indices per framework assays. This allows for a one-step PCR reaction to generate sequence ready amplicons and the pooling of amplicons from several different samples and targets (generated with other LymphoTrack Assays for the Illumina MiSeq instrument) onto one MiSeq flow cell. Multiplexing allows for up to 24 samples per target to be analyzed in parallel in a single run.

## References

1. AW Langerak, *et al.* *Leukemia* 26:2159-71 (2012).
2. JE Miller, *et al.* *Molecular Genetic Pathology* (2nd ed.). Springer Science & Business Media. 2013:30.2.7.13 and 30.2.7.18.
3. KJ Trainor, *et al.* *Blood* 75:2220-2222 (1990).
4. LC Lawnickie, *et al.* *JMD* 5:82-87 (2003).
5. PA Evans, *et al.* *Leukemia* 21:207-14 (2007).
6. S Tonegawa. *Nature* 302:575-581 (1983).
7. Y Sandberg, *et al.* *Leukemia* 21:21 (2007).

## Ordering Information

CATALOG #	PRODUCTS	QUANTITY
7-121-0139	LymphoTrack® <i>IGH</i> FR1/2/3 Assay Panel - MiSeq®	24 + 24 + 24 indices - 5 sequencing reactions each
7-121-0129	LymphoTrack® <i>IGH</i> FR1/2/3 Assay Kit A - MiSeq®	8 + 8 + 8 indices - 5 sequencing reactions each
7-121-0039	LymphoTrack® <i>IGH</i> FR1 Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
7-121-0009	LymphoTrack® <i>IGH</i> FR1 Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
7-121-0099	LymphoTrack® <i>IGH</i> FR2 Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
7-121-0089	LymphoTrack® <i>IGH</i> FR2 Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
7-121-0119	LymphoTrack® <i>IGH</i> FR3 Assay Panel - MiSeq®	24 indices - 5 sequencing reactions each
7-121-0109	LymphoTrack® <i>IGH</i> FR3 Assay Kit A - MiSeq®	8 indices - 5 sequencing reactions each
7-500-0009*	LymphoTrack® 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 Assay for the MiSeq. These products are sold for Research Use Only; not for use in diagnostic procedures.

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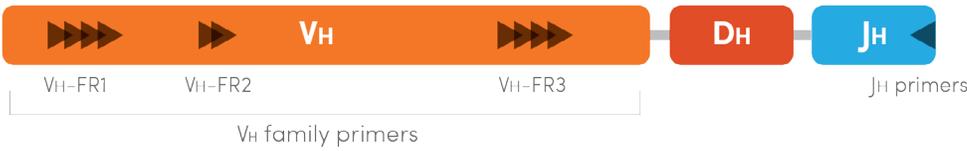


Figure 1: Simplified representation of the organization of the immunoglobulin heavy chain (IGH) gene on chromosome 14. Depicted are the variable region (V<sub>H</sub>) genes and downstream consensus joining region genes (J<sub>H</sub>) that are involved in rearrangements.

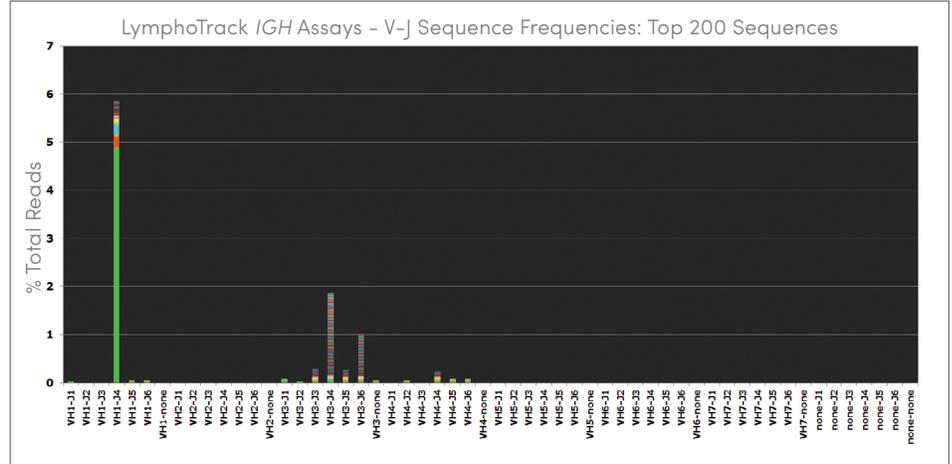


Figure 2: The LymphoTrack Software - MiSeq provides a stacked bar graph depicting the relative frequencies for the 200 most prevalent V<sub>H</sub> - J<sub>H</sub> rearrangements sequenced and identified in the sample.

## Reagents

The LymphoTrack IGH FR1/2/3 Assays contain components from respective individual FR Kit As or Panels.

LymphoTrack IGH FR1 Components		LymphoTrack IGH FR2 Components		LymphoTrack IGH FR3 Components	
Master Mix Name	Index #	Master Mix Name	Index #	Master Mix Name	Index #
IGH FR1 MiSeq 01	A001	IGH FR2 MiSeq 01	A001	IGH FR3 MiSeq 01	A001
IGH FR1 MiSeq 02	A002	IGH FR2 MiSeq 02	A002	IGH FR3 MiSeq 02	A002
IGH FR1 MiSeq 03	A003	IGH FR2 MiSeq 03	A003	IGH FR3 MiSeq 03	A003
IGH FR1 MiSeq 04	A004	IGH FR2 MiSeq 04	A004	IGH FR3 MiSeq 04	A004
IGH FR1 MiSeq 05	A005	IGH FR2 MiSeq 05	A005	IGH FR3 MiSeq 05	A005
IGH FR1 MiSeq 06	A006	IGH FR2 MiSeq 06	A006	IGH FR3 MiSeq 06	A006
IGH FR1 MiSeq 07	A007	IGH FR2 MiSeq 07	A007	IGH FR3 MiSeq 07	A007
IGH FR1 MiSeq 08	A008	IGH FR2 MiSeq 08	A008	IGH FR3 MiSeq 08	A008
IGH FR1 MiSeq 09	A009	IGH FR2 MiSeq 09	A009	IGH FR3 MiSeq 09	A009
IGH FR1 MiSeq 10	A010	IGH FR2 MiSeq 10	A010	IGH FR3 MiSeq 10	A010
IGH FR1 MiSeq 11	A011	IGH FR2 MiSeq 11	A011	IGH FR3 MiSeq 11	A011
IGH FR1 MiSeq 12	A012	IGH FR2 MiSeq 12	A012	IGH FR3 MiSeq 12	A012
IGH FR1 MiSeq 13	A013	IGH FR2 MiSeq 13	A013	IGH FR3 MiSeq 13	A013
IGH FR1 MiSeq 14	A014	IGH FR2 MiSeq 14	A014	IGH FR3 MiSeq 14	A014
IGH FR1 MiSeq 15	A015	IGH FR2 MiSeq 15	A015	IGH FR3 MiSeq 15	A015
IGH FR1 MiSeq 16	A016	IGH FR2 MiSeq 16	A016	IGH FR3 MiSeq 16	A016
IGH FR1 MiSeq 18	A018	IGH FR2 MiSeq 18	A018	IGH FR3 MiSeq 18	A018
IGH FR1 MiSeq 19	A019	IGH FR2 MiSeq 19	A019	IGH FR3 MiSeq 19	A019
IGH FR1 MiSeq 20	A020	IGH FR2 MiSeq 20	A020	IGH FR3 MiSeq 20	A020
IGH FR1 MiSeq 21	A021	IGH FR2 MiSeq 21	A021	IGH FR3 MiSeq 21	A021
IGH FR1 MiSeq 22	A022	IGH FR2 MiSeq 22	A022	IGH FR3 MiSeq 22	A022
IGH FR1 MiSeq 23	A023	IGH FR2 MiSeq 23	A023	IGH FR3 MiSeq 23	A023
IGH FR1 MiSeq 25	A025	IGH FR2 MiSeq 25	A025	IGH FR3 MiSeq 25	A025
IGH FR1 MiSeq 27	A027	IGH FR2 MiSeq 27	A027	IGH FR3 MiSeq 27	A027

Kit As only contain indices IGH FRX A001 to A008. Panels contain all master mixes listed above.

## Controls

Individual FR (1, 2, or 3) Kit A		Individual FR (1, 2, or 3) Panels		Combo FR1/2/3 Kit A		Combo FR1/2/3 Panel	
IGH POS (+)	Qty. 1	IGH POS (+)	Qty. 3	IGH POS (+)	Qty. 2	IGH POS (+)	Qty. 6
IGH NEG (-)	Qty. 1	IGH NEG (-)	Qty. 3	IGH NEG (-)	Qty. 2	IGH NEG (-)	Qty. 6

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