

Fully Automated Workflows Quantify and Report Key T-Cell and B-Cell Receptor Biomarkers Relevant to Immuno-Oncology and Heme-Oncology Research

Shrutii Sarda¹, Geoffrey M. Lowman², Michelle Toro², Loni Pickle², Timothy Looney¹, Fiona Hyland¹

[1] Clinical Sequencing Division, Thermo Fisher Scientific, South San Francisco, CA 94080, USA [2] Clinical Sequencing Division, Thermo Fisher Scientific, Carlsbad, CA 92008, USA

INTRODUCTION

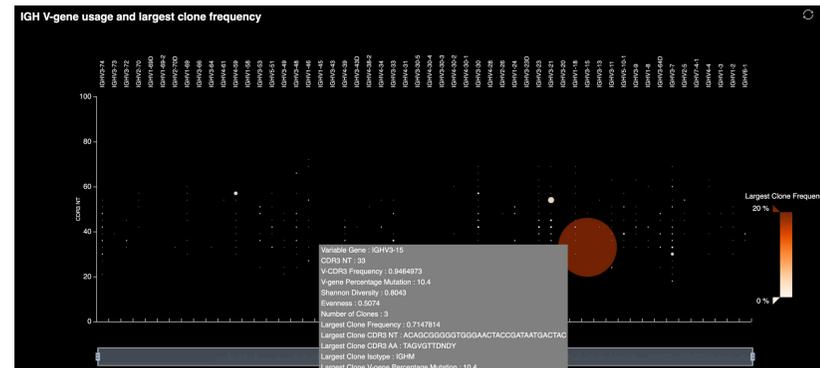
T-cell and B-cell repertoire analysis is used in oncology research, to understand the etiology of complex disease phenotypes, for the identification of biomarkers predictive of disease burden, outcome, and response to treatment, and for research in diagnosis and recurrence monitoring. Key predictors include secondary and tertiary repertoire features not reported by existing sequencing software solutions. For example, due to ongoing somatic hypermutation in mature B-cell receptors, the underlying sequence of a given clone can accumulate base differences and appear as several distinct clones with smaller frequencies, thereby hampering the ability of analysis software to detect its presence as a single dominant clone with the highest frequency. This has particularly detrimental implications for research in disorders such as follicular lymphoma and may require clonal lineage analysis for proper mitigation.

To aid the downstream analytics of biomarker identification and the study of complex disease, we developed fully automated analysis solutions that directly compute and report several key features (clonal lineage, amongst several others described below) pertinent to this area of research.

KEY ANALYSIS FEATURES

B-cell & T-cell Repertoire	1	VDJ assignment and CDR3 analysis
	2	Clonal Expansion and Diversity analysis
B-cell Repertoire	1	Somatic Hypermutation and Clonal Lineage analysis
	2	Isotype analysis
T-cell Repertoire	1	T-cell Convergence analysis
	2	T-cell Haplotype analysis

RESULTS



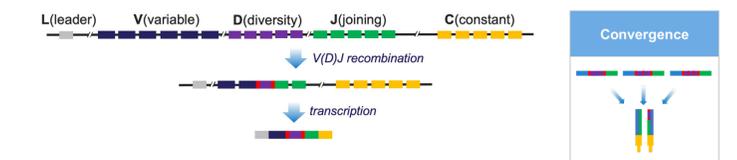
Spectra-typing plot simultaneously assesses sample clone frequencies as a function of V-gene usage and CDR3 length combinations

Lineage Summary Table

Summary metrics for clonal lineages detected in a sample. Clonal lineages represent groups of B cell clones that are believed to be related by descent (shared VDJ rearrangement) but have distinct VDJ sequences owing to somatic hypermutation. Lineages are identified as sets of IGH rearrangements having the same variable gene identity, the same CDR3 length, and CDR3 nucleotide similarity greater than the user-defined threshold (default: minimum 85% homology). The lineage ID column summary file. Learn more...

Lineage ID	Variable	Top CDR3AA	Lineage Frequency	Number of Clones	Isotypes	Minimum V-gene SHM	Maximum V-gene SHM	Minimum Clone Frequency	Maximum Clone Frequency
1	IGHV3-15	TAGVGTNDNDY	0.8464873	2	IGHM,IGHM	0.104	0.104	0.231619	0.7147814
2	IGHV3-21	ARDLDSYGLTY...	0.0096995	1	IGHA1	0.041	0.041	0.0096995	0.0096995
3	IGHV4-59	ARIGATRRPHTSY...	0.0029745	3	IGHA1,IGHG3	0.094	0.155	0.0001102	0.0024185
4	IGHV3-7	AKEEWRLDY	0.0022705	1	IGHG2	0.092	0.092	0.0022705	0.0022705
5	IGHV3-30	ARVPHGSGIDYY...	0.0008674	3	IGHA1	0.04	0.066	0.0000969	0.0006021
6	IGHV3-33	VTTQYGPQSGFS	0.0006888	1	IGHA2	0.133	0.133	0.0006888	0.0006888
7	IGHV3-21	ASGTQVTVRGLGV	0.0005	1	IGHG1	0.106	0.106	0.0005	0.0005
8	IGHV1-46	ARPRRYKGYNY...	0.0004847	1	IGHA1	0.126	0.126	0.0004847	0.0004847
9	IGHV5-51	AYSRLGATLDY	0.0004745	1	IGHA1	0.056	0.056	0.0004745	0.0004745
10	IGHV3-48	ARCRYFSGSYH...	0.0004235	1	IGHG1	0.097	0.097	0.0004235	0.0004235

Lineage Summary table listing B-cell lineage groupings (clones related by descent, w/ differing SHM rates) of all identified clonal populations in the sample



Analysis	Clones	Shannon_Diversity	Convergent_TCR_Frequency	Haplotype_Group
Sample1	12850	0.867	0.0218	1

Sample Metrics table listing (i) T-cell convergence level (frequencies of TCR clones derived from different nucleotide sequences but sharing the same amino acid sequence) and (ii) T-cell haplotype assignment based on V-gene allele genotyping and clustering of the sample

Sample Results Sample QC

Views: Clone Summary

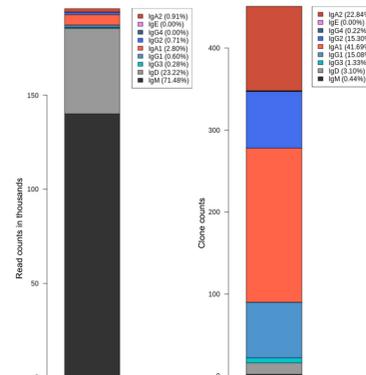
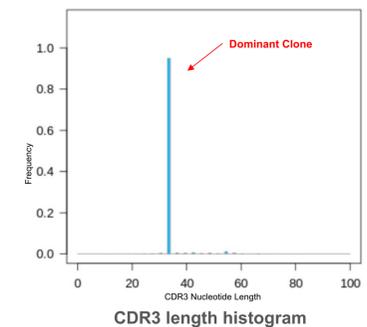
Search [] Go [] Download Clone Summary

Clone Summary Table

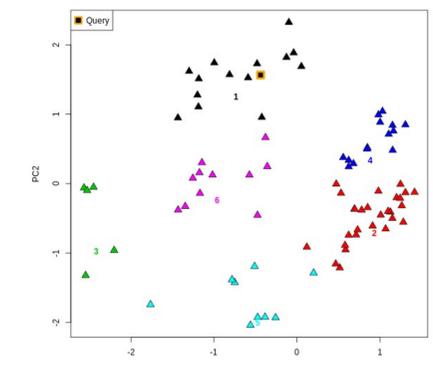
Frequency and sequence features of identified clones. Additional clone features are provided in the downloadable clone summary file. Learn more...

Lineage ID	Variable	Joining	CDR3 AA	CDR3 NT	Variable Mutation	Count	Frequency	Rank	Isotype
1	IGHV3-15	IGHJ4	TAGVGTNDNDY	ACAGCGGGGGTGGG...		0.104	140090	0.7147814	1 IGHM
1	IGHV3-15	IGHJ4	TAGVGTNDNDY	ACAGCGGGGGTGGG...		0.104	45395	0.231619	2 IGHM
2	IGHV3-21	IGHJ6	ARDLDSYGLTY...	GCGAGAGACTGGA...		0.041	1901	0.0096995	3 IGHG1
3	IGHV4-59	IGHJ6	ARIGATRRPHTSY...	GCGGAAATGGAGC...		0.155	474	0.0024185	4 IGHG3
4	IGHV3-7	IGHJ4	AKEEWRLDY	GCGAAGAGGAGTG...		0.092	445	0.0022705	5 IGHG2
6	IGHV3-33	IGHJ5	VTTQYGPQSGFS	GTGACACCCCAAT...		0.133	135	0.0006888	6 IGHG2
5	IGHV3-30	IGHJ6	ARVPHGSGIDYY...	GCGAGATTCCTCA...		0.048	118	0.0006021	7 IGHG1
3	IGHV4-59	IGHJ6	ARIGATRRPHTSY...	GCGGAAATGGAGC...		0.155	107	0.000546	8 IGHG1
7	IGHV3-21	IGHJ6	ASGTQVTVRGLGV	GCGAGTGTACCCG...		0.106	98	0.0005	9 IGHG1
8	IGHV1-46	IGHJ6	ARPRRYKGYNY...	GCGAGACCCCGCG...		0.126	95	0.0004847	10 IGHG1

Sample level table listing all detected clones, assigned VDJ labels, CDR3 sequence, counts, frequencies, somatic hypermutation levels, and other computed attributes



Total reads and clones represented by B-cell Isotype



CONCLUSION

The OncoPrint™ Immune Repertoire workflows for T-cell and B-cell receptor sequencing were designed to be of high utility in distinct areas of malignancy research, and we expect them to greatly simplify complex downstream analyses. The unique capabilities of the workflows to automatically report secondary and tertiary repertoire features such as,

- (i) BCR clonal lineages for improved dominant clone detection in blood cancers,
- (ii) TCR clone convergence for prediction of response to immune checkpoint inhibitors [1,2],
- (iii) TCR haplotype grouping for evaluation of risk factors for autoimmunity and immune-related adverse events [3], and
- (iv) isotype classification in BCRs for studying pan-cancer immune evasion mechanisms, demonstrate the clear advantages of using these automated workflows over other existing solutions.

REFERENCES

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- Naidus et al. (2021) Early changes in the circulating T cells are associated with clinical outcomes after PD-L1 blockade by durvalumab in advanced NSCLC patients. *Cancer Immunology, Immunotherapy* 70:2095–2102
- Looney TJ et al. (2019) Haplotype Analysis of the T-Cell Receptor Beta (TCRB) Locus by Long-amplicon TCRB Repertoire Sequencing. *Journal of Immunotherapy and Precision Oncology.* 2 (4): 137–143.

CORRESPONDENCE

Shrutii Sarda
shrutii.sarda@thermofisher.com

