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

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INTRODUCTION

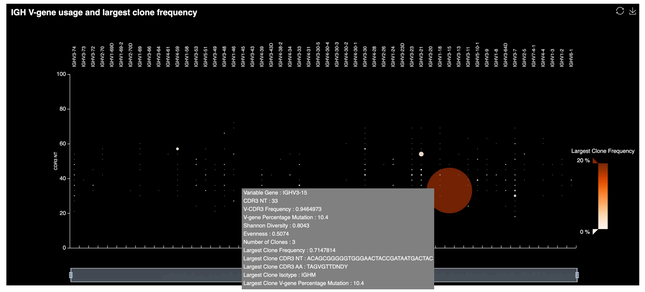
T-cell and B-cell repertoire analysis is used in oncology research, to understand the etiology of complex disease phenotypes, for the identification of biomarkers predictive of disease burden, outcome, and response to treatment, and for research in diagnosis and recurrence monitoring. Key predictors 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.

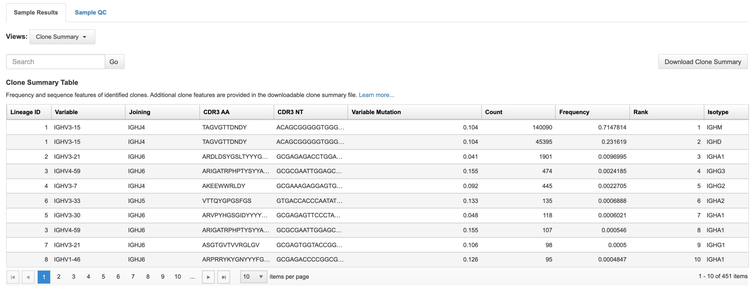
KEY ANALYSIS FEATURES

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

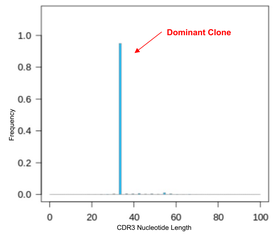
RESULTS



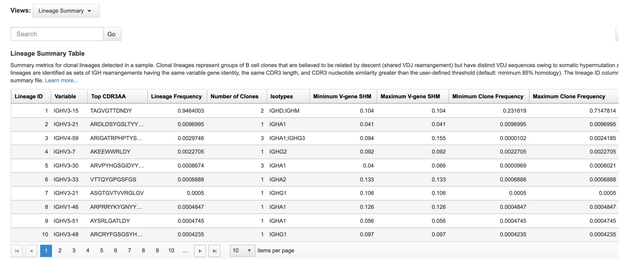
Spectra-typing plot simultaneously assesses sample clone frequencies as a function of V-gene usage and CDR3 length combinations



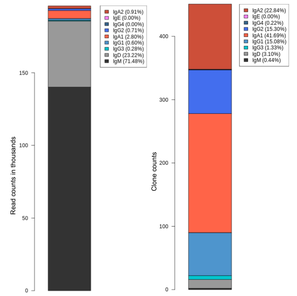
Sample level table listing all detected clones, assigned VDJ labels, CDR3 sequence, counts, frequencies, somatic hypermutation levels, and other computed attributes



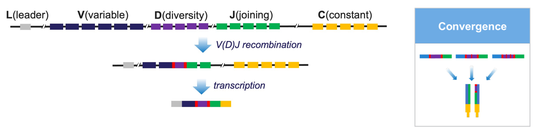
CDR3 length histogram

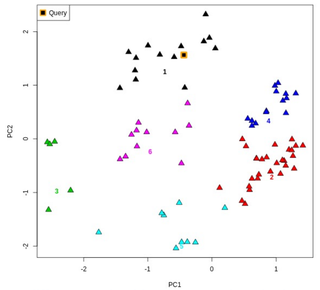


Lineage Summary table listing B-cell lineage groupings (clones related by descent, w/ differing SHM rates) of all identified clonal populations in the sample



Total reads and clones represented by B-cell Isotype





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

CONCLUSION

The Oncomine™ 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

1) Looney TJ et al. (2020) TCR Convergence in Individuals Treated With Immune Checkpoint Inhibition for Cancer. *Front. Immunol.* 10:2985.
2) 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
3) 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.

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