RepertoireID™

Comprehensive Profiling of the Immune Repertoire within the Tumor Microenvironment

Characterization of the TCR and BCR Repertoire

T cells and B cells are active cellular participants in the adaptive immune system. T cells and B cells express specialized receptors, TCR and BCR respectively, on their surfaces that recognize and respond to an incredibly diverse range of antigens. Although TCRs and BCRs are functionally distinct, they are similarly organized in structure; TCRs are heterodimer molecules composed of two distinct chains, most commonly TCRα and TCRβ chains, while BCR is composed of a pair of heavy and light chains (**Figure 1**). The diversification process of these receptors is facilitated by genetic rearrangements that occur in gene regions, known as variable (V), diversity (D), and joining (J) gene segments, that combine to form the highly polymorphic complementarity determining region 3 (CDR3), responsible for their antigen-binding specificities.[1] Variations in V, D, and J gene segments coupled with random nucleotide additions/deletions (N-diversity mechanisms) contribute to the length and expansive sequence heterogeneity of CDR3 regions (**Figure 2**). A particular TCR or BCR clone can be identified by its unique CDR3 sequence, and a population of T or B cells with identical CDR3 sequence is referred to as a clonotype. Subsequently, the overall total number of CDR3 variations in a host can be collectively referred to as their immune repertoire.

Figure 1: Schematic of BCR and TCR

*Modified from The Immune System, 4th edition by Peter Parham

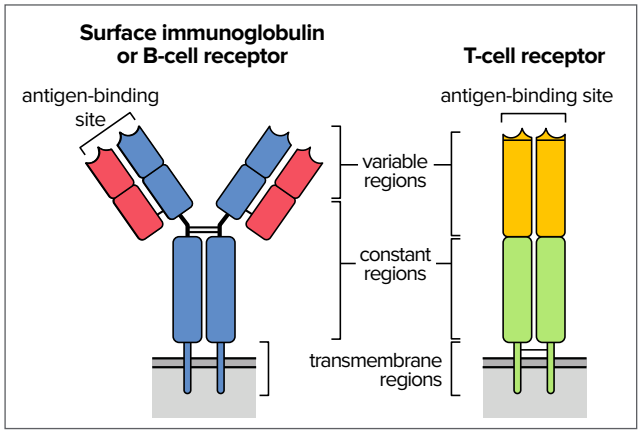
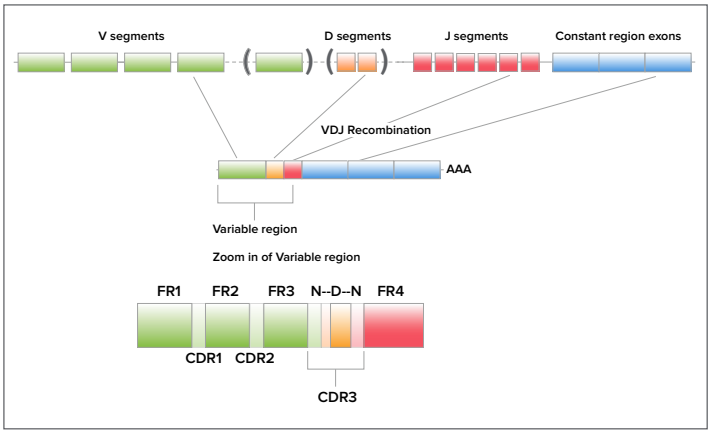


Figure 2: Schematic of VDJ recombination to form hypervariable CDR3 regions



Personalis®

Explore TCR & BCR specific characteristics as a Biomarker

RepertoireID, an analytics module of ImmunoID NeXT™, leverages RNA expression profiles to enable the detection and analysis of the top TCRα and TCRβ clonotypes, together with BCR heavy chain (BCRh) clonotypes found in the tumor microenvironment (TME) of patients' tumors. Additionally, RepertoireID also characterizes the isotype composition of BCRs (secreted form mature B cells as antibodies) within the TME. This analysis is included due to its importance in carrying various effector functions that are crucial for a host's overall immune response against tumors.[2] Ultra-deep RNA sequencing data derived from the NeXT Transcriptome™ facilitates the detailed profiling of the top BCR and TCR clonotypes present within a tumor sample. RepertoireID analytics module provides a report with key metrics and plots such as:

- Clonality
- CDR3 nucleotide and amino acid sequences
- Clonotype quantitation, distribution and frequency
- V, D and J gene segment usage
- CDR3 nucleotide sequence length
- Isotype composition and isotype-specific CDR3 clonal frequencies for BCRs

The characterization of the top clones in the repertoire and aggregate metrics like clonality are ideal information for researchers exploring the potential utility of the underlying immune repertoire as a predictive biomarker of response to treatment regimens involving immunotherapies. As the literature demonstrates, it is these aggregate metrics that are of most interest to researchers examining the TCR and BCR repertoire in the TME of patients' tumors.[3,4,5]

A New Class of NGS Platform

Purpose-built for precision oncology applications, ImmunoID NeXT merges multiple biomarker assays into one. The NeXT Platform™ provides ultra-deep coverage across specific regions of the genome that are associated with key functional areas and biomarkers in the cancer ecosystem. These areas, including the gene regions responsible for the expression of TCRs and BCRs, cannot be comprehensively characterized with the use of conventional exome-scale assays; however, the ultra-deep coverage that the NeXT Platform™ achieves across these TCR and BCR related regions provides the deep raw data needed to thoroughly profile the top clonotypes of the immune repertoire via RepertoireID.

Sophisticated Downstream Analytics

The RepertoireID analytics module is driven by an optimized algorithm that processes raw NGS data to deliver quantitated clonotypes and additional, related information as part of a detailed report. Merged seamlessly into Personalis' framework of analytical pipelines, the RepertoireID analytics solution elucidates the underlying immune repertoire, focusing on the

features discussed below.

TCR and BCR Clonotype Overview

The core data of the repertoire analysis consists of the absolute number of sequencing reads assigned to all TCRα ,TCRβ, and BCRh clonotypes present in a patient's tumor, the number of nucleotide and amino acid clonotypes, and also the repertoire's clonality score. The clonality score is a measurement from 0 to 1, with scores approaching 1 representative of a highly clonal repertoire and scores approaching 0 representing a more diverse, evenly distributed repertoire. In the case of BCRh clonotypes, clonotype quantification metrics at isotype-specific level is reported as well.

BCRh Isotype Composition

RepertoireID provides the relative proportion of read fractions assigned to 4 out of the 5 BCR isotypes, IgM, IgD, IgG, IgA as shown in **Figure 3**. IgE isotype is excluded from the reporting output.

Top Clonotypes

The report provides a table that highlights the top 10 TCRα, TCRβ, and BCRh clones, along with isotype-specific clones, identified in the sample and includes key metrics such as clone read count, clone frequency, nucleotide and amino acid sequence, as well as top V, D, and J gene segment hits per clone.

Clonal Frequency and Distribution Plots

Multiple charts, as shown in **Figure 4** and **Figure 5**, are employed to visualize the relative frequency and distribution of the top clonotypes in the repertoire.

CDR3 Length Distribution

The length of the CDR3 regions is one of the most significant determinants of diversity in the immune repertoire. Longer CDR3s not only have greater potential for sequence variation, but can also potentially reach into narrow antigenic pockets that CDR3s of reduced length simply can't access.[6] Thus, including the distribution of CDR3 nucleotide sequence length (**Figure 6**) is a key output of the analytical report that can help in providing an overall view of the immune repertoire.

V, D and J Gene Segments Usage and Overlap

The CDR3 region acts as the unique clonotype identifier in the analysis of the immune repertoire. Therefore, it is essential to characterize and understand the usage of the individual gene segments (V, D, and J regions) and the way in which these segments are recombined to produce unique CDR3 nucleotide sequences. The RepertoireID report also includes plots that chart the proportion of total reads assigned to the various V, D and J gene segment types that form part of any CDR3 region. Lastly, the report demonstrates the overlap between all V, D and J regions (i.e. those that are part of the same CDR3 sequence), clearly demonstrating which regions are most commonly combined in the repertoire.

RepertoireID Output Example*

Figure 3: BCRh Isotype Composition

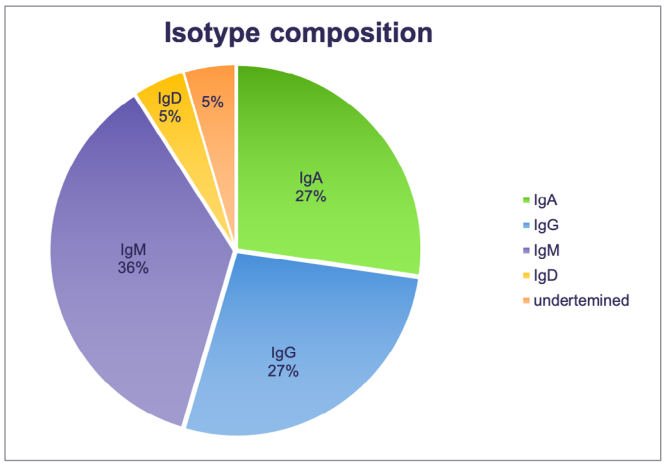


Figure 4: Clonal Frequency Distribution

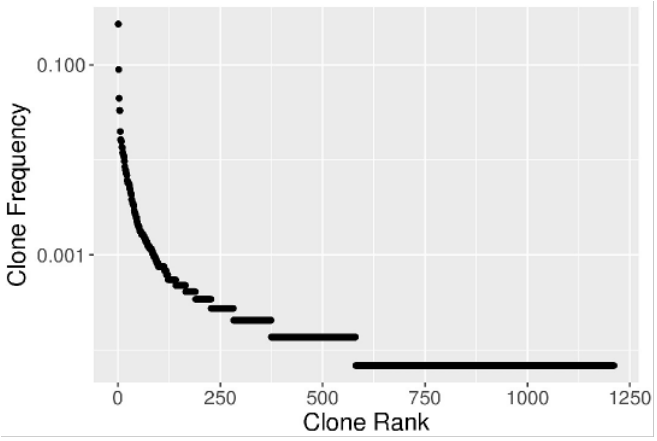


Figure 5: Top Clone Distribution

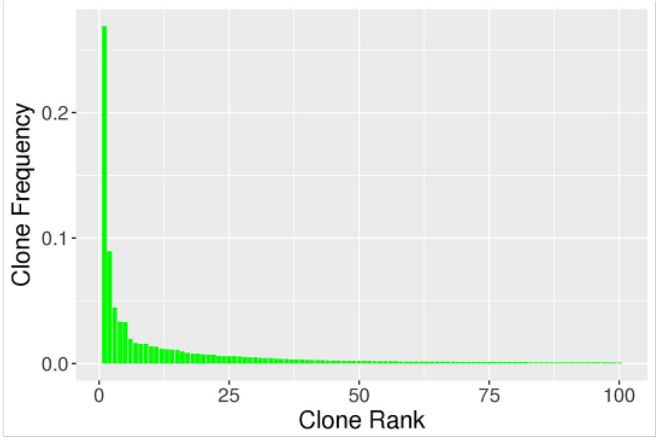
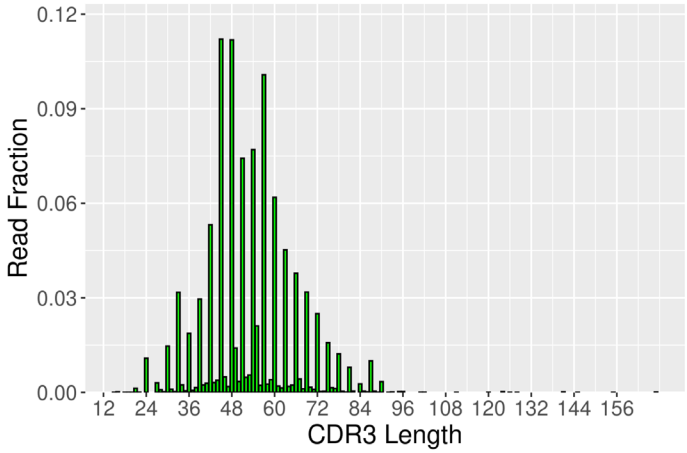


Figure 6: CDR3 Length Distribution



*This output example includes a selection of the modified charts that are delivered with RepertoireID, but not all report deliverables are shown. The data is from an FFPE tumor sample that was processed at Personalis.

References

1. Calis JJ, Rosenberg BR. Characterizing immune repertoires by high throughput sequencing: strategies and applications. Trends in Immunology. 2014.

2. Isaeva OI, Sharonov GV, Serebrovskaya EO, et al. Intratumoral immunoglobulin isotypes predict survival in lung adenocarcinoma subtypes. Journal for ImmunoTherapy of Cancer. 2019.

3. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014.

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6. Wesolowski J, Alzogaray V, Reyelt J, et al. Single domain antibodies: promising experimental and therapeutic tools in infection and immunity. Medical Microbiology and Immunology. 2009.

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