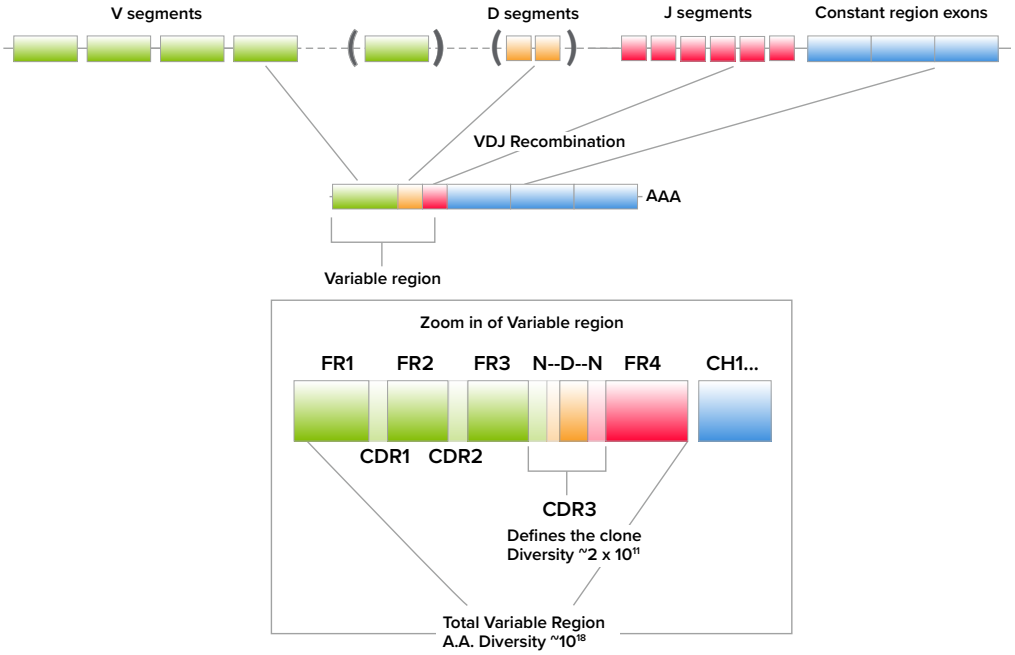
RepertoireID™

An analytical module of the ImmunoID NeXT Platform™

Comprehensive Profiling of the TCR Repertoire

T-cells are one of two types of lymphocytes (the other being B-cells) that are active cellular participants in the adaptive immune system. T-cells express T-cell receptors (TCRs) on their surface, and these receptors function to recognize and bind to antigens that are presented by host cells. Since humans come into contact with a multitude of endogenous and exogenous antigens throughout their lives, the human immune system has had to evolve to match the structural diversity of these antigenic peptides to maintain health. This ability 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 complementarity determining region 3 (CDR3) responsible for the expression of specific TCR clonotypes. Variations in the particular V, D, and J gene segments used, precise points of recombination, and random nucleotide additions (N-diversity mechanisms) all contribute to the length and expansive sequence heterogeneity of CDR3 regions. Every TCR clonotype has a unique CDR3, which largely determines the antigen specificity of each TCR, and the total number of TCR CDR3 variations in a host is referred to as the TCR repertoire.

Figure 1: TCRβ Variable and CDR3 Regions



Personalis®

Explore TCR Repertoire as a Biomarker

RepertoireID, an analytics module of the ImmunoID NeXT Platform, enables the detection and analysis of the top TCRα and TCRβ clonotypes in the TCR repertoire found in the tumor microenvironment (TME) of patients' tumors. The RNA sequencing data derived from the NeXT assay is processed by our TCR Analytics Engine, which allows for the generation of a report providing key metrics and plots such as clonality; CDR3 nucleotide and amino acid sequences; clonotype quantitation, distribution, and frequency; V, D (for TCRβ only), and J gene segment usage; CDR3 nucleotide sequence length; amongst others.

The characterization of the top clones in the repertoire and aggregate metrics like clonality are ideal deliverables for researchers exploring the potential utility of the TCR 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 repertoire in the TME of patients' tumors[1,2]. This, combined with Personalis' expertise in maximizing data extracted from challenging formalin-fixed paraffin-embedded (FFPE) tumor samples, makes us the ideal partner for your oncology biomarker discovery studies.

A New Class of NGS Platform

Purpose-built for precision oncology applications, the ImmunoID NeXT Platform merges multiple biomarker assays into one. The NeXT assay 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 in T-cells, cannot be comprehensively characterized with the use of conventional exome-scale assays. The ultra-deep coverage that the NeXT assay achieves across these TCR-related regions provides the deep raw data needed to thoroughly profile the top TCRα and TCRβ clonotypes of the TCR repertoire via RepertoireID.

Sophisticated Downstream Analytics

The RepertoireID analytics module is driven by an advanced 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 solution elucidates the TCR repertoire, focusing on the features discussed below.

Footnotes

1. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014;515:568-71.

2. Roh W, Chen PL, Reuben A, et al. Integrated molecular analysis of tumor biopsies on sequential CTLA-4 and PD-1 blockade reveals markers of response and resistance. Sci Transl Med 2017;9.

TCR Clonotype Overview

We provide the core data of the TCR repertoire analysis; reporting out on the absolute number of sequencing reads assigned to all TCRα and TCRβ 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.

Top Clonotypes

The report provides a table that highlights the top 10 TCRα and TCRβ 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 2** and **Figure 3**, are employed to visualize the relative frequency and distribution of the top clonotypes in the repertoire.

V and J Gene Segments Usage and Overlap

The CDR3 region acts as the unique TCR clonotype identifier in the analysis of the TCR 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 includes plots (**Figure 5** and **Figure 6**) that chart the proportion of total reads assigned to the various V and J gene segment types that form part of any CDR3 region. The report also demonstrates the overlap between all V 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.

CDR3 Length Distribution

The length of the TCRα and TCRβ regions is one of the most significant determinants of diversity in the TCR repertoire. This is because 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[3]. Thus, including the distribution of the CDR3 nucleotide sequence length (**Figure 4**) is a key output of the analytical report that can help in providing an overall view of the TCR repertoire.

Get in Touch

To learn more about how we can help accelerate your biomarker discovery and translational research programs, contact us at **info@personalis.com.**

3. Wesolowski J, Alzogaray V, Reyelt J, Unger M, Juarez K, Urrutia M, Cauerhff A, Danquah W, Rissiek B, Scheuplein F, Schwarz N, Adriouch S, Boyer O, Seman M, Licea A, Serreze DV, Goldbaum FA, Haag F, Koch-Nolte F: Single domain antibodies: promising experimental and therapeutic tools in infection and immunity. Med Microbiol Immunol (Berl) 2009;198:157-174.

RepertoireID Output Example*

Figure 2: Clonal Frequency Distribution

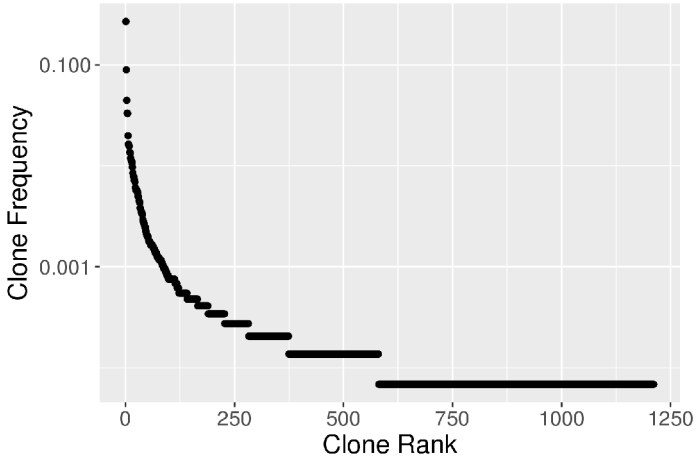


Figure 3: Top Clone Distribution

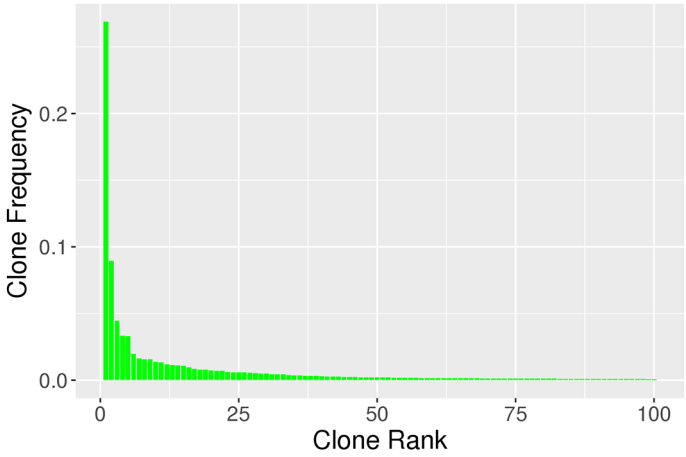
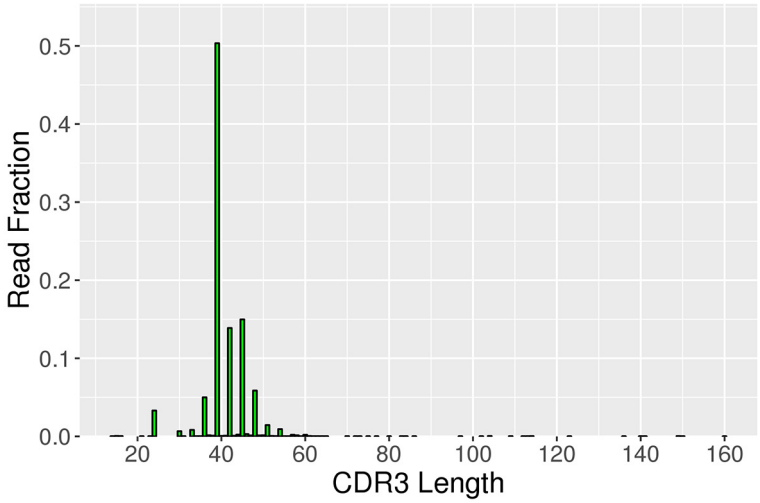


Figure 4: CDR3 Length Distribution



*This output example includes a selection of the 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.

RepertoireID Output Example

Figure 5: V Gene Usage

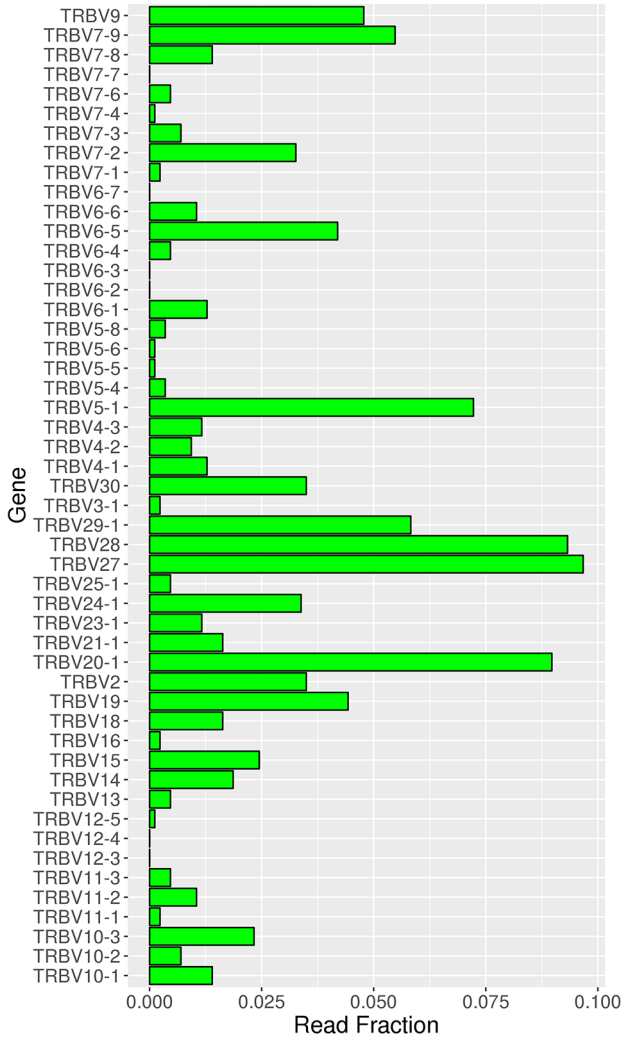
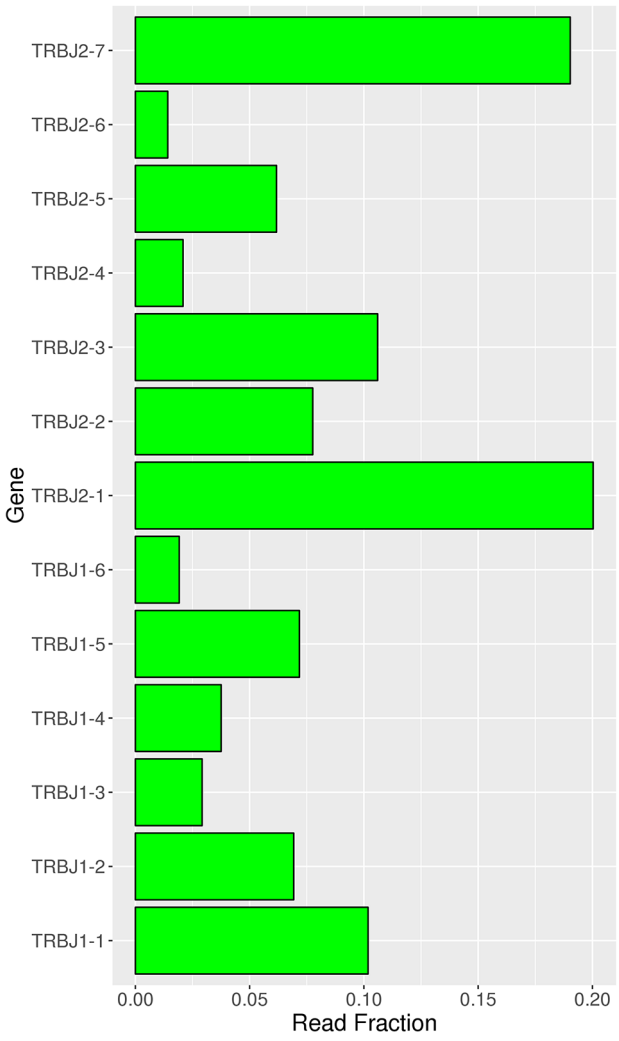


Figure 6: J Gene Usage



Sales Contact

United States

info@personalis.com

Europe

europe@personalis.com

Other Countries

info@personalis.com

Personalis, Inc.

1330 O'Brien Drive, Menlo Park, CA 94025

+1 855-GENOME4 (436-6634) | +1 650-752-1300

www.personalis.com

Personalis®

101-513-C