Adaptive immune receptor repertoire profiling for biomarker discovery

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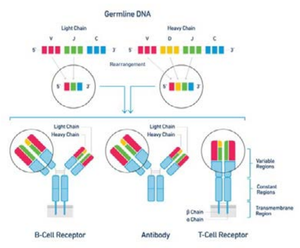
Abstract

T-cell receptor (TCR) and B-cell receptor (BCR) repertoire profiling, also referred to as adaptive immune receptor repertoire (AIRR) profiling, holds great potential for the understanding of disease mechanisms and for new treatments in infectious disease, autoimmunity, immuno-oncology and other diseases. This potential could be greatly improved by combining information about receptor clonotypes with immunophenotypes of T- and B- cells. A new technology we developed that combines profiling of all human TCR and BCR variable regions with phenotypic characterization of immune cells using the same workflow could be particularly useful. The TCR and BCR immunophenotyping method proposed involves RT-PCR amplification and sequencing of the CDR3 regions of the TCR and BCR genes, and subsequently determining the gene expression levels of the most informative T- and B-cell phenotyping genes. Preliminary results show that this method allows for comprehensive profiling of all seven TCR and BCR chains from a single sample, in a highly reproducible manner, directly from micro-samples including cancer tissue, whole blood, sorted cells and more. Bioinformatic analysis of the next-generation sequencing (NGS) data from the TCR and BCR clonotypes profile combined with RNA expression profiling of the same samples results in a richer data set that includes the identification of major immune cell subtypes and their activation status. Data from human cancer tissues and whole blood samples will be presented.

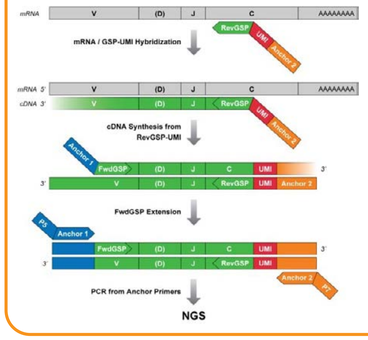
Introduction



- Genetic recombination in T and B cells generates diverse repertoires of TCR, BCR, and antibodies.
- Variable part (CDR3) of TCR and BCR recognizes foreign antigens presented by Major Histocompatibility Complex (MHC).
- Millions of different T and B cells with unique TCRs and BCRs define differences in our immune responses.
- Understanding the complex TCR-BCR repertoire can provide insights into disease mechanisms and suggest strategies for effective immunotherapies.

Method

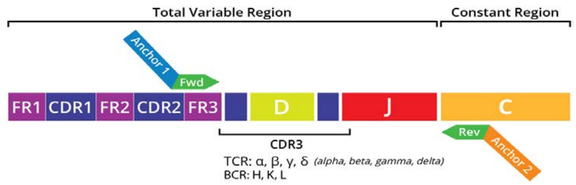
DriverMap™ Technology: Targeted Multiplex RT-PCR



- Universal assay for targeted RNA-seq profiling of all TCR/BCR and key T/B biomarker genes
- Single-cell sensitivity, 10-fold increase in sensitivity versus RNA-seq and SMART technology
- Could be run directly in cell lysate (single cell, sorted cells)
- Doesn't require rRNA, mitochondrial, or globin RNA depletion

Full-length or CDR3 receptor coverage

DriverMap ™ Adaptive Immune Repertoire (AIR) Profiling Assay



- Comprehensive AIR repertoire coverage for all seven TCR and BCR chains in a single multiplex RT-PCR reaction from total RNA
- Detection of only functional AIR clonotypes without pseudogenes and non-rearranged genes
- Improved coverage and unbiased amplification of CDR3 regions with a highly validated primer set based on DriverMap™ technology
- Quantitative clonotype analysis with AIR RNA calibration standards and UMI (unique molecular identifiers)
- Integrated with MiXCR software package for immune repertoire analysis

Integrated AIR profiling and immunophenotyping

Integrated AIR profiling and Immunophenotyping

Fig 1: DriverMap™ AIR and DriverMap™ IMP assays allow the characterization of TCR repertoire in CD8 naive, CD8 effector, CD4 naive, CD4 effector, and T reg cell fractions.

- Integrated AIR profiling and Immunophenotyping directly in sorted cells without RNA purification is possible using the DriverMap™ technology
- High-resolution immunophenotyping (matching) data from top TCR/BCR clonotypes based on the expression of 300 key cell typing and activation T/B markers.
- Candidates selected from a set of 3000 candidate genes described in >100 public databases, commercial assays, and publications

View all 7 TCR/BCR chains in a single reaction

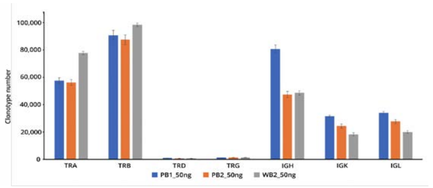


Fig 2: Number of clonotypes for 7 TCR/BCR chains identified in 50ng of normal PBMC or whole blood RNA ($10x10^6$ reads per sample, triplicates).

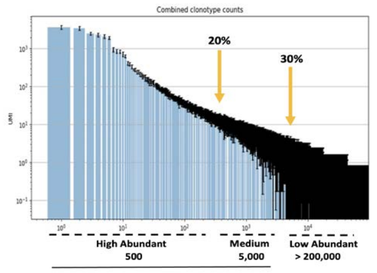


Fig 3: TCR clonotype repertoire analysis in 50 ng of whole blood in triplicate.

- High reproducibility of TRB repertoire profiling for top 500-1,000 clonotypes in RNA samples with at least 5-10 TRB mRNA molecules
- Stochastic, unreproducible profiling of rare clonotypes (hundred thousands) present in whole/PBMC RNA samples at the single-molecule level.

Reproducible clonotype repertoire analysis

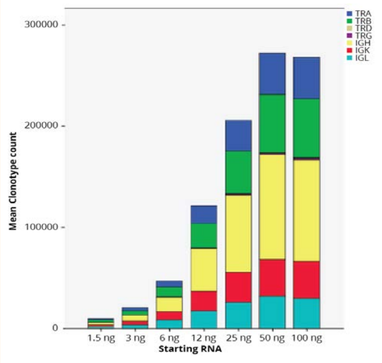


Fig 4: Reproducible clonotype distribution of TCR and BCR chains across different input amounts ranging from 1.5 ng to 100 ng of starting total RNA of all seven chains in a single reaction. (5 x 10^6 reads/sample) The recommended optimal starting amount is 50-100 ng of total RNA.

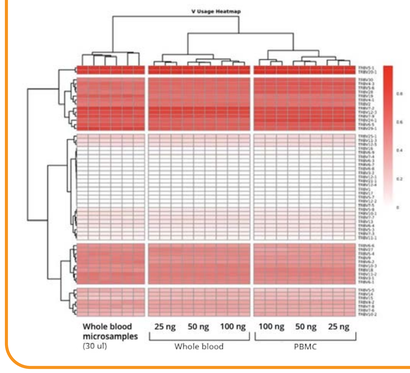


Fig 5: Similar V gene usage for TRB genes in whole blood, whole blood micro-samples (30 ul dried blood), and PBMC samples. log^{10} (V gene usage percentage) in triplicates.

Sensitive clonotype detection

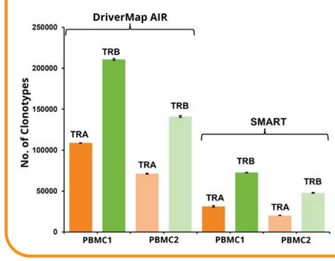
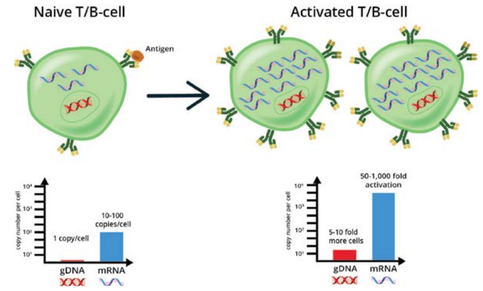
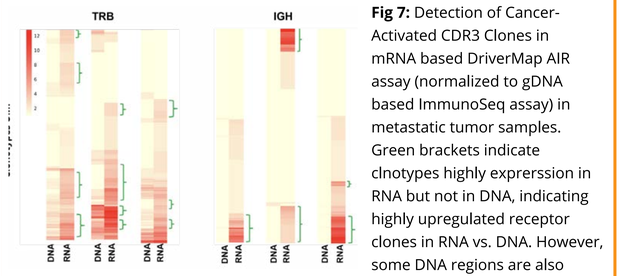


Fig 6: Comparison of TCR clonotypes detected by DriverMap™ AIR RNA vs SMART assay. Both assays were run with 50 ng of total RNA isolated from PBMC. The DriverMap™ AIR RNA assay detects ~ 3X more TCR clonotypes than the SMART assay. (Barennes et al., 2020)

Identify cancer-activated CDR3 clones

Combined AIR-RNA and AIR-DNA Analysis detects Ag-activated clones





- BCR >up-to 1,000-fold
- TCR > up to 50-fold activation

Fig 7: Detection of Cancer-Activated CDR3 Clones in mRNA based DriverMap AIR assay (normalized to gDNA based ImmunoSeq assay) in metastatic tumor samples. Green brackets indicate clnotypes highly exprerssion in RNA but not in DNA, indicating highly upregulated receptor clones in RNA vs. DNA. However, some DNA regions are also upregulated, indicating a higher copy number of cells (i.e., DNA) clonotypes in the tumor samples. (Sudmeier et al., 2021)

Discussion

- **Adaptive Immune Repertoire (AIR) Profiling assay**: Quantitative, and comprehensive TCR/BCR repertoire analysis (all seven chains) in single multiplex RT-PCR reaction in bulk RNA samples (PBMC, whole blood, cancer tissue samples).
- **Direct AIR Profiling:** High sensitivity with minimum background detection of TCR/BCR clonotypes directly in micro samples (cancer tissue, whole blood), sorted cells, and single cells using DriverMap™ technology.
- **T/B Immunophenotyping**: Integrated analysis of top TCR/BCR clonotypes and expression profiling of cell typing, activation markers in sorted T and B cell subfractions, and single cells.

Cellecta offers AIR RNA or DNA assays as kits and custom services

References:
Sudmeier, L., Hoang, K., Nduom, E., Wieland, A., Neill, S., & Schniederjan, M. et al. (2021). The CD8+ T cell landscape of human brain metastases. doi: 10.1101/2021.08.03.455000
Barennes, P., Quiniou, V., Shugay, M., Egorov, E., Davydov, A., & Chudakov, D. et al. (2020). Benchmarking of T cell receptor repertoire profiling methods reveals large systematic biases. Nature Biotechnology, 39(2), 236-245. doi: 10.1038/s41587-020-0656-3