Adaptive immune receptor repertoire profiling for biomarker discovery

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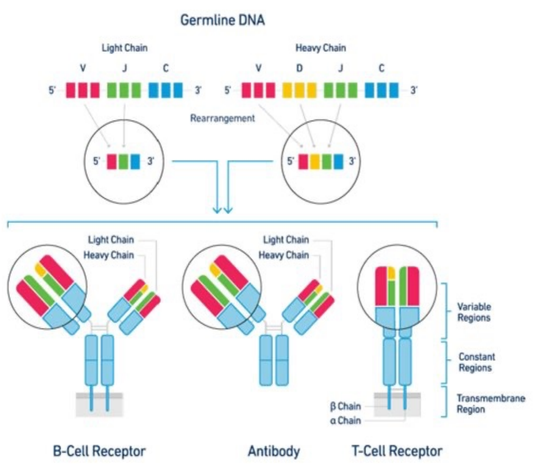
Abstract

TCR and BCR repertoire profiling referred to as adaptive immune repertoire (AIR) hold great potential for understanding disease mechanisms and for the development of new therapeutics in infectious disease, autoimmunity, and immuno-oncology. This potential could be greatly improved by combining information about receptor clonotypes with immunophenotypes of T and B cells. To facilitate these studies, we developed a novel technology for combined profiling of all human TCR and BCR variable regions and phenotypic characterization of immune cells. The developed TCR/BCR immunophenotyping method involves multiplex RT-PCR amplification and sequencing of CDR3 regions of TCR and BCR genes and a set of the most informative T- and B-cell phenotyping genes. Bioinformatic analysis of NGS data allows profiling of TCR/BCR clonotypes, and identification of major immune cell subtypes and their activation status. Preliminary studies indicate the assay has unparalleled throughput, sensitivity, and improved cost-effectiveness for high-throughput immunity biomarker discovery applications.

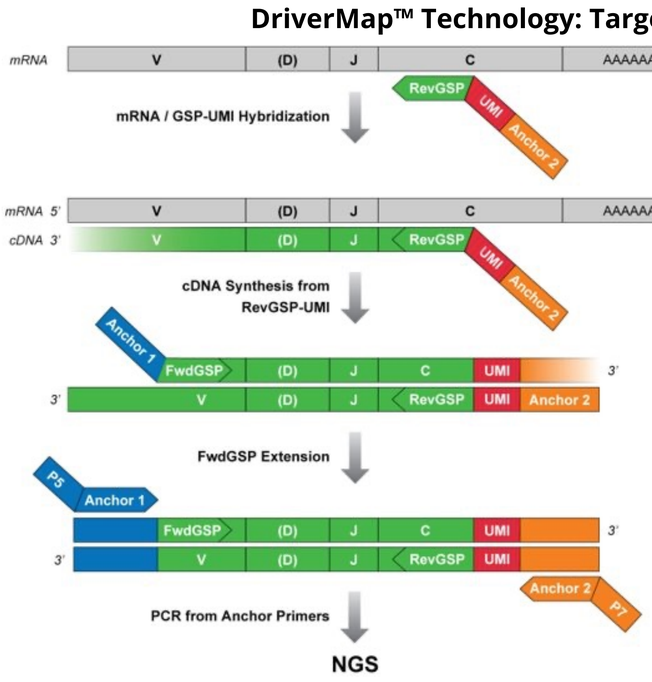
Introduction



- 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 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 to disease mechanism and define strategies for effective immunotherapies.

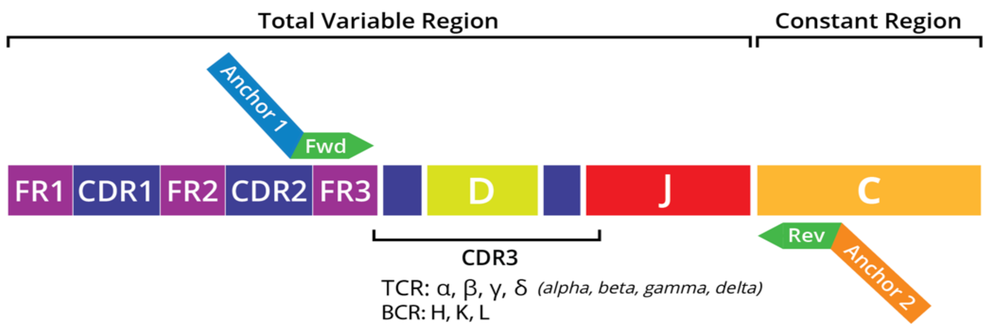
Method

DriverMap™ Technology: Targeted Multiplex RT-PCR



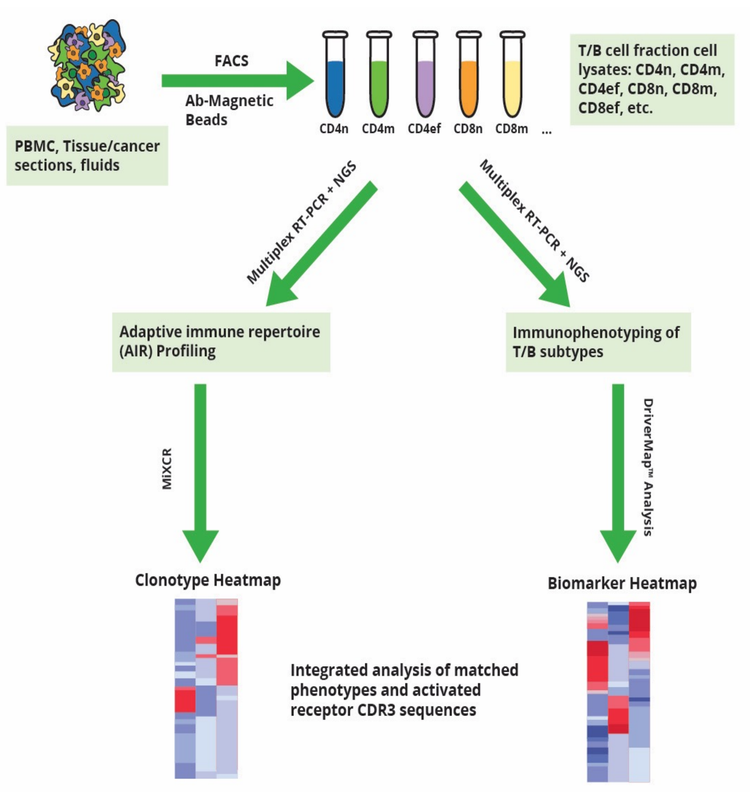
- Universal assay for targeted expression profiling of all TCR/BCR and key T/B biomarker genes
- Single-cell sensitivity, 10-fold increase in sensitivity versus RNAseq and SMART technology
- Could be run directly in cell lysate (single cell, sorted cells)
- Doesn't require rRNA, mitochondrial, globin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

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
- 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
- Integrated with MiXCR software package for immune repertoire analysis

Integrated AIR profiling and Immunophenotyping



- Integrated AIR profiling and Immunophenotyping directly in sorted cells without RNA purification using the DriverMap™ technology
- High-resolution immunophenotyping (matching) of 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

Results: All 7 TCR/BCR chains in a single reaction

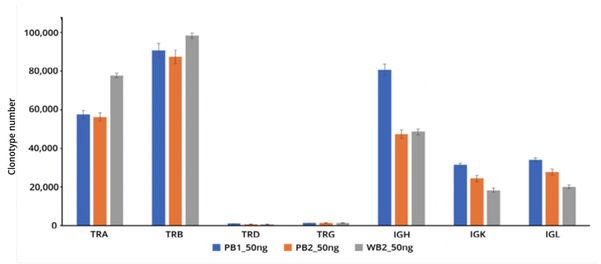
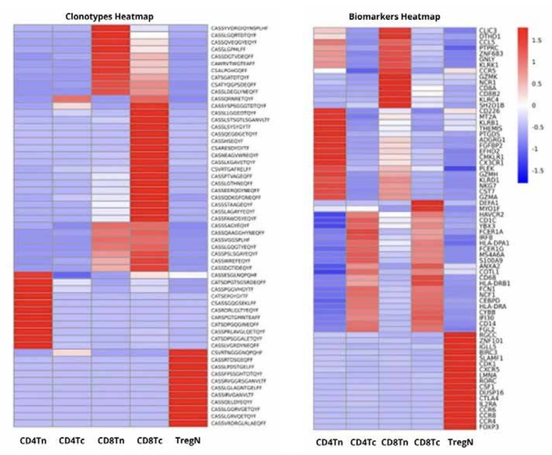
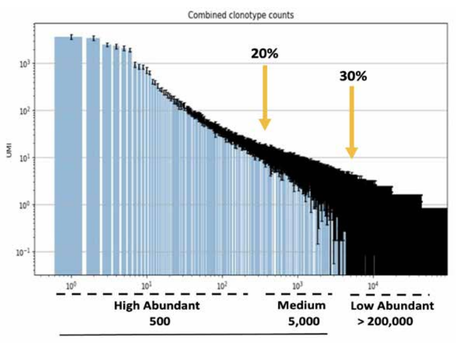


Fig 1: Number of clonotypes for 7 TCR/BCR chains identified in 50ng of normal PBMC or whole blood RNA (10x10^6 reads per sample, triplicates).



- High-resolution immunophenotyping (matching) of top TCR clonotypes based on the expression of 300 key cell typing and activation of T/B markers
- Amplification of CDR3 regions with a highly validated primer set based on DriverMap™ technology

Fig 2: DriverMap ™ AIR and DriverMap ™ IMP assay allows the characterization of TCR repertoire in CD8 naïve, CD8 effector, CD4 naïve, CD4 effector and T reg cell fractions.



- 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 thousand) present in whole blood/PBMC RNA samples at the single-molecule level.

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

Results: Reproducible clonotype repertoire analysis

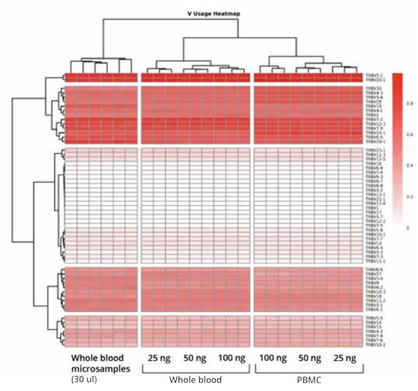


Fig 4: Similar V gene usage for TRB genes in whole blood, whole blood micro samples (30 ul dried blood), and PBMC samples. log^{10} (V gene usage percentage) in triplicates.

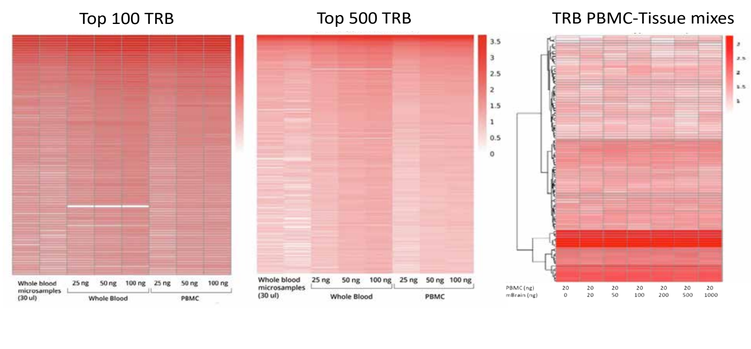


Fig 5: Reproducibility in TRB clonotype repertoire analysis across various sample types

Results: Sensitive clonotype detection

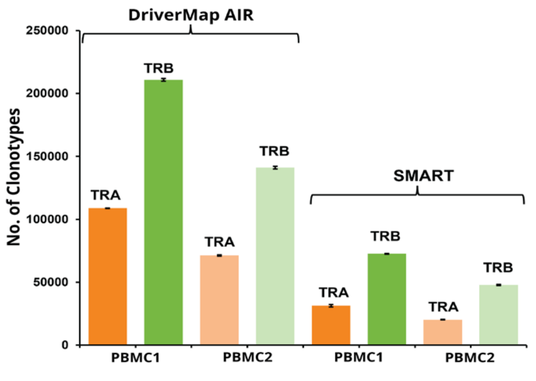


Fig 6: Comparison of TCR clonotypes detected by DriverMap™ AIR vs SMART assay. Both assays were run with 50 ng of total RNA isolated from PBMC. The DriverMap™ AIR assay detects ~ 3X more TCR clonotypes than the SMART assay. (Barennes et al., 2020)

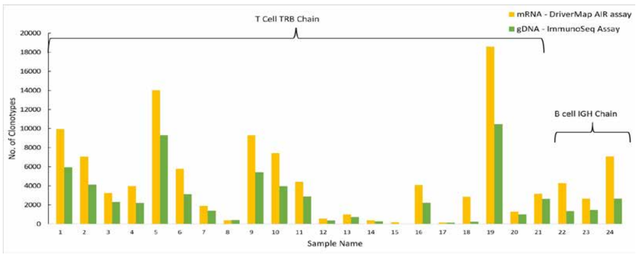


Fig 7: Comparison of clonotypes detected by DriverMap™ AIR (mRNA) vs ImmunoSEQ (gDNA) assay from patient tumor samples. DriverMap™ AIR assay from mRNA detects 1.5-2x more TCR/BCR Clonotypes than gDNA from immunoSEQ assay. (Sudmeier et al., 2021)

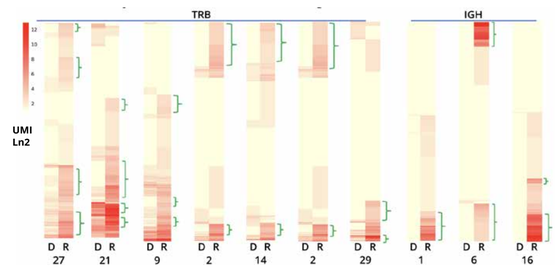


Fig 8: Detection of Cancer-Activated CDR3 Clones in mRNA based DriverMap AIR assay (normalized to gDNA based ImmunoSeq assay)

R = RNA

D = DNA

- BCR >up-to 1,000-fold
- TCR > up to 50-fold transcriptional activation of immune receptor gene

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.

AIR and Immunophenotyping assay available as kit and custom service

References:
- De Masi, C., Spitalieri, P., Murdocca, M., Novelli, G., & Sangiuolo, F. (2020). Application of CRISPR/Cas9 to human-induced pluripotent stem cells: from gene editing to drug discovery. Human Genomics, 14(1). https://doi.org/10.1186/s40246-020-00276-2
- Ye, L., Swingen, C., & Zhang, J. (2013). Induced Pluripotent Stem Cells and Their Potential for Basic and Clinical Sciences. Current Cardiology Reviews, 9(1), 63–72. https://doi.org/10.2174/157340313805076278