CELLECTA

TISSUE 2 TARGET

Services by **Cellecta** & **Cureline**

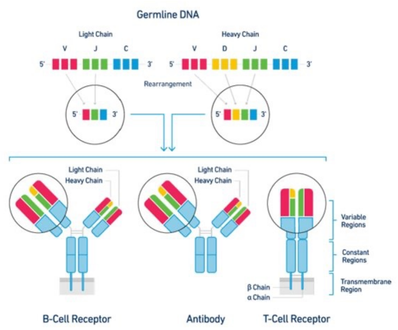
T-cell and B-cell receptor repertoire profiling for biomarker discovery

Alex Chenchik, Mikhail Makhanov, Tianbing Liu, Dongfang Hu, Khadija Ghias, Paul Diehl, Lester Kobzik

Abstract

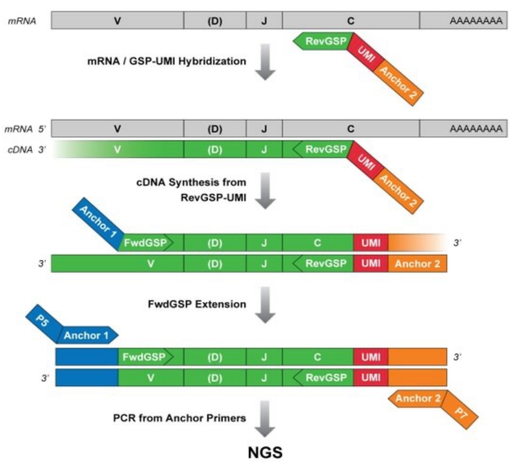
T-cell receptor (TCR) and B-cell receptor (BCR) repertoire profiling, also referred to as adaptive immune receptor repertoire (AIRR) profiling, holds great potential for the understanding of disease mechanisms and for the development of new treatments in infectious disease, autoimmunity and immuno-oncology. This potential could be greatly improved by combining information about receptor clonotypes with immunophenotypes of T- and B-cells. We developed a new technology for combined profiling of all human TCR and BCR variable regions and phenotypic characterization of immune cells in the same workflow. The TCR and BCR immunophenotyping method involves RT-PCR amplification and sequencing of the CDR3 regions of the TCR and BCR genes, as well as determining the expression levels of the most informative T- and B-cell phenotyping genes. 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 microsamples including cancer tissue, whole blood, sorted cells and more. Bioinformatic analysis of the next-generation sequencing (NGS) data allows profiling of the TCR and BCR clonotypes and identifcation of major immune cell subtypes and their activation status. Preliminary data from Rheumatoid Arthritis (RA) patient samples treated with TNFα immunotherapy shows that there is significant patient-specific variability in activation of B-cells response in anti-TNFα treated RA samples whereas there is no significant activation of T-cells in patient samples. Combined expression profiling with AIRR profiling also revealed key candidate biomarkers for responders and non-responders in RA immune response.

Introduction



- Genetic recombination in T- and B-cells generates diverse repertoires of TCR, BCR, and antibodies.
- Variable region (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.

Methods

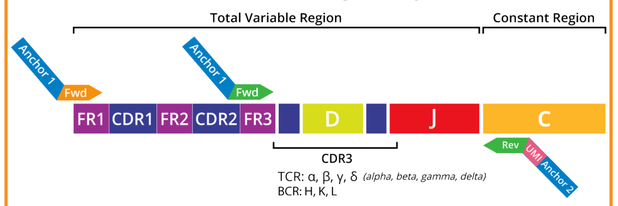


DriverMap™ Technology: Targeted Multiplex RT-PCR

- 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)

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 multiplex RT-PCR reaction from total RNA or DNA
- Improved coverage and unbiased amplification of CDR3 or full-length receptor region obtained 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

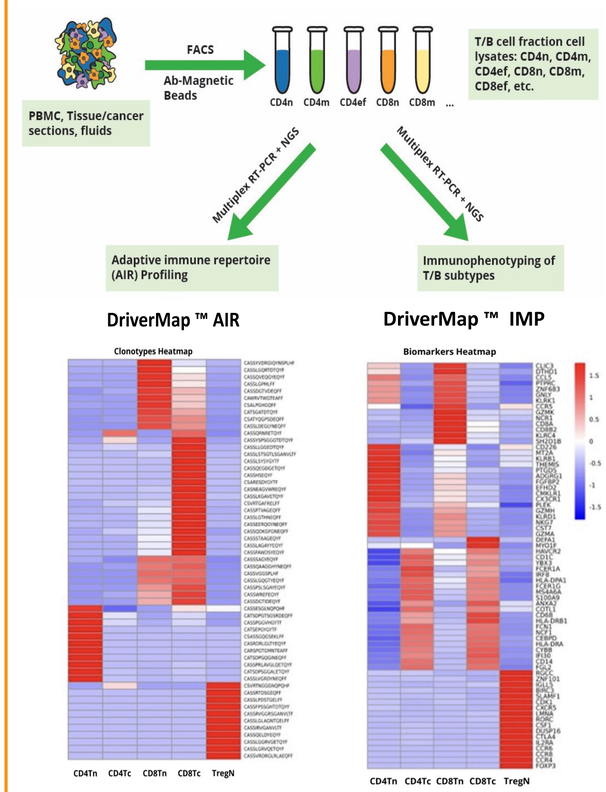


Fig 1: DriverMap™ AIR and DriverMap™ EXP assays allow the characterization of TCR repertoire in CD8 naive, CD8 effector, CD4 naive, CD4 effector, and T reg cell fractions.

- 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

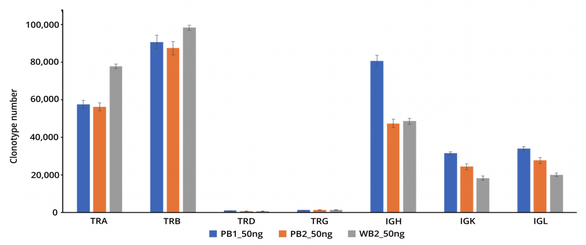


Fig 2: Number of clonotypes for 7 TCR/BCR chains identified in 50ng of normal PBMC or whole blood RNA (10x10^6 reads per sample, triplicates).

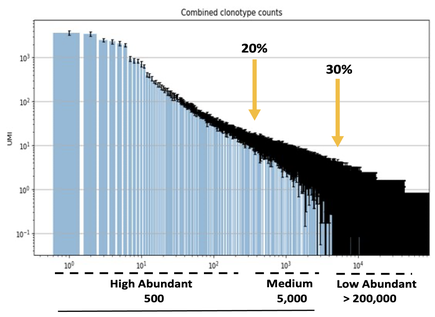


Fig 3: TCR clonotype repertoire analysis in 50 ng of whole blood in triplicate.

- 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

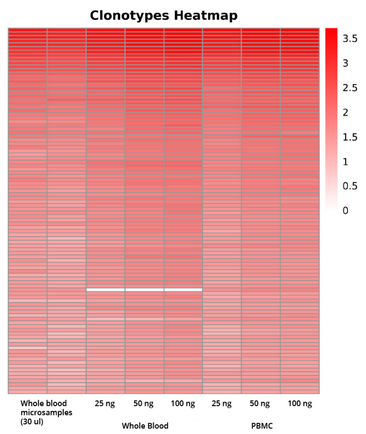


Fig 4: Reproducible clonotype profiling of TRB repertoire in 25 ng-100 ng total RNA samples from whole blood, PBMC and microsamples (30-ul of dried blood).

Sensitive clonotype detection

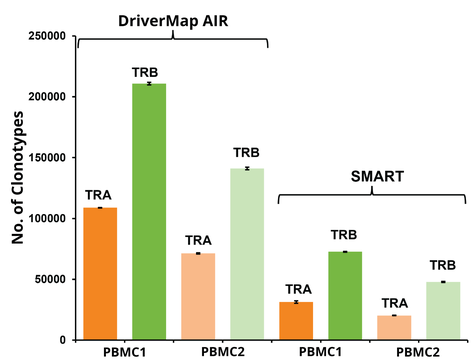
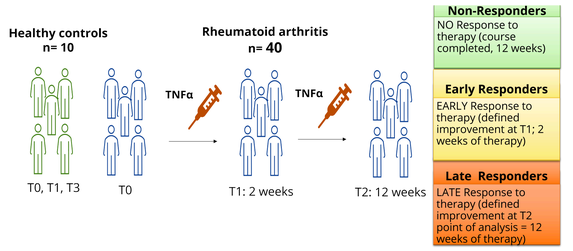


Fig 5: Comparison of TCR clonotypes detected by DriverMap™ AIR RNA vs SMART assay. Both assays were run with 50 ng of total RNA isolated from PBMC. The DriverMap™ AIR RNA assay detects ~ 3X more TCR clonotypes than the SMART assay. (Barennes et al., 2020)

Rheumatoid Arthritis Case Study



- Blood samples from 40 patients treated with TNFα immunotherapy (Humira) and healthy donors were obtained from Cureline, Inc (Biobank).
- Patients were divided into three groups based on their response to treatment (Non-responders, Early Responders and Late Responders).
- For analysis, DriverMap™ AIR TCR/BCR profiling and DriverMap™ EXP Genome-wide expression profiling was conducted.

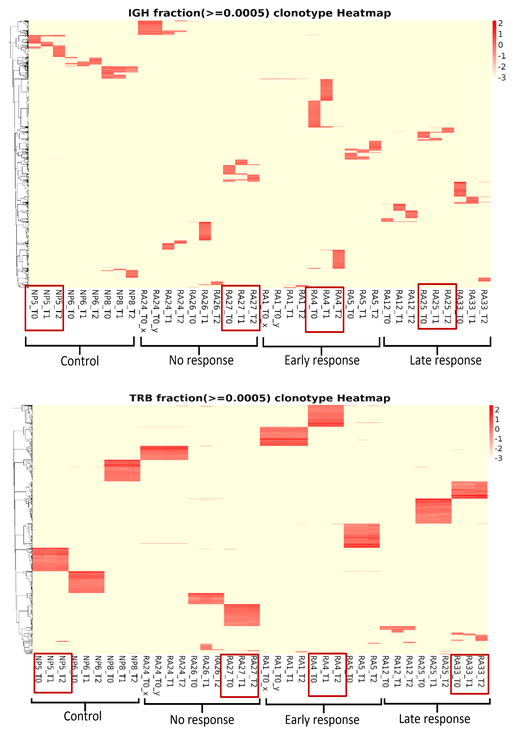
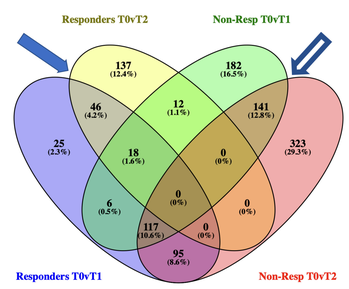


Fig 6: DriverMap™ AIR analysis reveals significant patient-specific differences in B-cells (IGH repertoire) in RA samples whereas no difference in T-cells (TRB repertoire) was observed before/after anti-TNFα therapy vs. control samples.

- DriverMap™ EXP Genome-wide Expression Profiling reveals candidate biomarkers for follow-up studies.
- 141 genes unique to non-responders.
- 46 genes unique to responders.



Discussion

- **Adaptive Immune Repertoire (AIR) Profiling assay**: Quantitative, and comprehensive TCR/BCR repertoire analysis (all seven chains) in a multiplex RT-PCR reaction in bulk RNA or DNA samples (PBMC, whole blood, cancer tissue samples).
- **Direct AIR Profiling:** High sensitivity with minimum background detection of TCR/BCR clonotypes directly in microsamples (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 service

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