**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 multiple 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 immune biomarker discovery applications.

**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 lyses (single cell, sorted cells)
- High-resolution immunophenotyping (matching) of top TCR clonotypes across various sample types
- Comprehensive AIR repertoire coverage for all seven TCR/BCR chains in a single multiplex RT-PCR reaction
- 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 AIR profiling and Immunophenotyping

**Integral AIR profiling and Immunophenotyping**

- Amplification of CDR3 regions with a highly validated primer set
- 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**

- High-resolution immunophenotyping (matching) of top TCR clonotypes based on the expression of 300 key cell typing and activation T/B markers
- Amplification of CD81 regions with a highly validated primer set based on DriverMap™ technology
- DriverMap™ AIR and DriverMap™ IMP assay allows the characterization of TCR repertoire in CD8 naive, CD8 effector, CD4 naive, CD4 effector and T reg cell fractions

**Results: Reproducible clonotype repertoire analysis**

- High reproducibility of TRB repertoire profiling for top 500-1000 clonotypes in RNA samples with at least 5-10 TRB mRNA molecules
- Stochastic, reproducible profiling of rare clonotypes (hundred thousand) present in whole blood/Peripheral RNA samples at the single-molecule level

**Results: Sensitive clonotype detection**

- Similar V gene usage for TRB genes in whole blood, whole blood micro samples (30 ul direct blood), and PBMC samples, log2 (V gene usage percentage) in triplicates

**Discussion**

- DriverMap™ Adaptive Immune Repertoire (AIR) Profiling assay available as kit and custom service

**Adaptive Immune Receptor repertoire profiling for biomarker discovery**

Alex Chenchik, Mikhail Makhonov, Tianbing Liu, Dongfang Hu, Paul Diehl, Lester Kobzik; Cellecta, Inc., Mountain View, CA

**Fig 1:** Number of clonotypes for 7 TCR/BCR chains identified in 5ng of normal PBMC or whole blood RNA (10x10 reads per sample, triplicates).

**Fig 2:** TCR clonotype repertoire analysis in 50 ng of whole blood in triplicate.

**Fig 3:** Comparison of 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. (Barrennes et al., 2020)

**Fig 4:** Comparison of clonotype repertoire analysis in various sample types

**Fig 5:** Detection of Cancer-Activated CDR3 Clones in mRNA based DriverMap AIR assay (normalized to gDNA based ImmunoSeq assay)

**Fig 6:** TCR profile analysis across various sample types

**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 (Gudmew et al., 2021)

**Fig 8:** Sensitive clonotype detection

**Table 1:** Comparison of clonotype repertoire analysis across various sample types

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Clonotype Detection</th>
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<tbody>
<tr>
<td>Whole Blood</td>
<td>High Reproducibility</td>
</tr>
<tr>
<td>Whole Blood Micro</td>
<td>Moderate Reproducibility</td>
</tr>
<tr>
<td>PBMC</td>
<td>Low Reproducibility</td>
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**References:**


Integrated AIR profiling and Immunophenotyping as a kit and custom service

**AIR and Immunophenotyping assays available as kit and custom service**